WEED BIOLOGY AND ECOLOGY IN AGROECOSYSTEMS

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WEED BIOLOGY AND ECOLOGY IN AGROECOSYSTEMS

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EDITORIAL
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Editorial: Weed Biology and Ecology in Agroecosystems

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Keywords: community shifts, decision support tools, evolution, herbicide resistance, hybridization, integrated weed management, seedbank, selection

Editorial on the Research Topic

Weed Biology and Ecology in Agroecosystems

SUMMARY

Novel tactics are needed to manage herbicide resistant weeds. The most successful strategies will likely incorporate multiple tactics, such as chemical, cultural, and mechanical methods, in an integrated weed management (IWM) approach. All of these methods should target weak points in the species life cycle, which are best identified through detailed knowledge of weed biology and ecological interactions. The knowledge needed to create successful IWM systems spans a wide breadth of scientific disciplines. This special topic in Weed Biology and Ecology covers aspects of weed evolution and community shifts, seedbank biology, and the combination of multiple tactics in an IWM approach, including decision support tools and the use of lasers. An examination of the role of herbicides in IWM is also included. These contributions represent various perspectives on IWM and represent a framework for considering weed management in an agroecosystem through a multidisciplinary lens focused on weed biology and ecology.

An understanding of weed biology and ecology is critical to the ability to create an effective weed management program. As the global occurrence of novel herbicide resistant biotypes continues to increase, an enhanced focus is being placed upon Integrated Weed Management (IWM), which combines multiple practices with biological and ecological considerations, including chemical, cultural, and mechanical methods (Bagavathiannan and Davis, 2018; Gage and Schwartz-Lazaro, 2019). Examples of plant traits which promote success of weedy species in agroecosystems are tolerance to disturbance and stress, genetic variation, phenotypic plasticity, variable seed dormancy, rapid seed germination and growth, prolific seed production, effective dispersal, rapid nutrient sequestration, and production of allelopathic exudates. Through an understanding of weed biology and ecology, it is possible to identify integrated methods and application timings which provide the greatest impact on the reduction of weed seeds which are returned to the system. New technological adaptations, such as harvest weed seed control, precision agriculture, robotics, herbicide tolerance traits, competitive cultivars, biocontrols, and others, are advancing the possibilities for successful weed control programs when combined with knowledge of weed biology and ecology.

We consider our special topics issue as a call to action to present new insights or perspectives in the use of weed biology and ecology to form the basis of management in agroecosystems. Therefore, in preparing this issue, we have brought together authors and reviewers from a wide array of disciplines from around the world to provide several avenues of research. From the

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resulting set of manuscripts, several overarching themes emerged: (1) weed species evolution and community shifts in response to management, (2) integrating knowledge of seedbank biology in management, (3) role of weed biology and ecology in IWM, including decision support tools and advanced technologies to enhance weed management, and (4) transitioning away from reliance upon chemical control.

The characteristic traits of weedy species which promote success in managed habitats may allow rapid evolution of weeds (e.g. changes in genotype, phenotype, geographic range, or competitive ability) in response to disturbance, stress, and management. Understanding the rate and mechanisms of weed evolution can help facilitate the design of programs that minimize undesirable adaptations through management of weed survivorship and fecundity. While management associated with crop production has been an ancient form of selection pressure contributing to weed evolution, natural selection has also continued to act upon domesticated crop species, leading to the de-domestication of crops as they evolve weedy traits (Ellstrand et al., 2010). This selection pressure has led to independent de-domestication events in weedy/red rice (*Oryza* spp.), with the confirmation by Vigueira et al. that weedy rice populations in South Korea and the United states are genetically distinct. Two traits most often cited as the basis for the evolution of weedy rice are seed shattering and dormancy, but less is known about vegetative traits (plant stature, nitrogen assimilation, photosynthetic capacity, etc.). In a study of 14 accessions of weedy rice from the United States and South Asia, Huang et al. found that there is no consistent vegetative trait or physiological mechanism that has led to de-domestication in weedy rice, which supports the idea of multiple pathways to the evolution of weediness. Hybridization events between crop species and their wild relatives has been associated with rapid adaptation and evolution of crop-wild hybrids (Campbell and Snow, 2007; Hovick et al., 2012; Hartman et al., 2013). However, in a study of 40 weedy *Raphanus* populations, Shukla et al. found that evolution rates of crop-wild hybrids were lower than those of weedy populations, but crop-wild hybrids exhibited traits associated with increased fitness that were consistently expressed across a moisture gradient. The response of individual species to management may also lead to community shifts, according to species functional traits, as was documented by Cordeau et al. in response to long-term soil nutrient management. Knowledge of the response of the weed community to management may allow future manipulation to selectively favor less economically damaging species.

Successful weed management can be observed in weed seedbank reduction. While most traditional management programs target weeds in the vegetative stage of growth, new technologies in harvest weed seed control are expanding opportunities to manage the seedbank (Walsh et al., 2013, 2018; Schwartz-Lazaro et al., 2017; Shergill et al., 2020). Once seed rain occurs, seeds may persist for long periods of time in the soil seedbank (Burnside et al., 1996; Conn et al., 2006). An example of the role of seed dormancy and fecundity in weediness can be seen in the *Echinochloa* species, in which Tahir and Burgos rated 94 accessions and determined that both factors varied greatly among and within each species. This impacts the longevity of each species in the soil seedbank. Further effects on the soil seedbank can be impacted not only by a weed's biology and ecology, but by IWM practices, such as cover crops. Nichols et al. examined fields beginning in either a corn or soybean rotation and the effects of winter cover cropping on the weed communities and changes in the soil seedbank composition over time. They found that increases in cover crop biomass did not correlate to weed suppression or reductions in the soil seedbank, which is the opposite of previous studies (Moonen and Bàrberi, 2004; Mirsky et al., 2010; MacLaren et al., 2019; Smith et al., 2020). However, it can be concluded that the combined impacts of crop rotation and cover crops, with additional weed management tactics, can reduce the weed seedbank.

Another emergent theme is understanding the importance of weed biology and ecology in improving IWM programs. Herbicides are an essential IWM tool and understanding the evolution and distribution of herbicide resistant weeds is vital. Jones et al. screened 239 samples of *Lolium perenne* across four different herbicide sites of action and confirmed some level of herbicide resistance to three of the four sites of action. This level of resistance resulted in elimination of a critical herbicide application timing. Thus, considering the increasing concern of availability and efficacy of herbicides, non-chemical weed management tactics, such as the use of cover crops, decision support tools, and advanced technologies, need to be examined. Cover crops provide several ecological services in addition to weed suppression, such as reduced soil erosion, enhanced nutrient cycling, reduction of nitrate leaching, and improved water quality of agricultural field runoff (Ruffo et al., 2004; Strock et al., 2004; Snapp et al., 2005; Hodgdon et al., 2016; Osipitan et al., 2018, 2019). Determining the proper level of cover crop biomass for weed suppression, coupled with proper cover crop termination timing, is critical to protect crop yields. Vollmer et al. found that for cereal rye, summer annual weed control was improved with delayed termination timing to allow for biomass to accumulate. Lacroix et al. developed IPSIM-Cirsium to evaluate varying infestation levels of *Cirsium arvense* as a function of farming practices, environmental conditions, and soil types. This is similar to another decision support tool, Palmer amaranth Management Model (PAM), that allows farmers to input their management practices to determine how to best drive down the soil seedbank (Lindsay et al., 2017). Additional novel non-chemical weed management tactics include the use of low energy lasers to control weed species such as *Lolium rigidum* (Coleman et al.) and the use of harvest weed seed control tactics in combination with other cultural (e.g., planting date and cover crops) weed management tactics (Beam et al.). The use of each additional IWM tactic consistently drove down weed populations over time (Thill et al., 1991; Norsworthy et al., 2012). These research findings assist in developing novel weed control options in conservation cropping systems, and the success of implementation hinges on an understanding of weed biology and ecology.

In addition to presenting novel research and technological advancements in weed biology and ecology, compelling perspectives on the future of IWM were made. For example, Colbach et al. found that although it has been well-documented that weed species contribute to crop yield loss (Cousens, 1985; Weaver et al., 1987; Blackshaw, 1993; Knezevic et al., 1994; Chikoye et al., 1995), there is a need to transition away from extended herbicide use, which rarely result in increased weed infestations if additional IWM tactics are utilized. Further specific parameters can affect this relationship between weed infestations and reduction in crop yields, such as weather and soil conditions, species combinations, and other variables (Bauer et al., 1991; Lindquist et al., 1996), and studies which isolate individual parameters and elucidate the individual role of herbicides are needed.

To understand the complexity of agroecosystems, a multidisciplinary and collaborative approach is needed. Like IWM systems, a diverse approach to weed ecology and biology can be combined to provide a larger picture of the problem at hand. It is important that this collaborative effort includes people from academia, industry, farmers, and public citizens. There have been similar calls to action (Davis et al., 2009; Ward et al., 2014; Müller-Schärer et al., 2018). One common theme is a focus on innovation in teaching and training students to solve complex problems in agroecosystem management, as well as increased networking and cooperation, technology transfer, and knowledge sharing between scientists in diverse yet complimentary fields of research (Chauhan et al., 2017). Long-term funding to support multidisciplinary approaches may be difficult to maintain, but some model outreach initiatives which incorporate weed biology education have emerged, such as the Australian Herbicide Resistance Initiative (AHRI), Getting Rid of Weeds through Integrated Weed Management (GROW) and the United Soybean Board's TakeAction campaign in the US, and the Southeast Asian Regional Centre for Tropical Biology (SEAMEO BIOTROP). As current and future agronomists, ecologists, biologists, weed scientists, social scientists, etc., it is our responsibility to engage those who will work toward creating significant and meaningful changes within agroecosystems.

AUTHOR CONTRIBUTIONS

LS-L, KG, and BC wrote and edited the article. All authors contributed to the article and approved the submitted version.

REFERENCES

Bagavathiannan, M. V., and Davis, A. S. (2018). An ecological perspective on managing weeds during the great selection for herbicide resistance. *Pest Manag. Sci.* 74, 2277–2286. doi: 10.1002/ps.4920

Bauer, T. A., Mortensen, D. A., Wicks, G. A., Hayden, T. A., and Martin, A. R. (1991). Environmental variability associated with economic thresholds for soybeans. *Weed Sci.* 39, 564–569.

Blackshaw, R. E. (1993). Downy brome (*Bromus tectorum*) density and relative time of emergence affect interference in winter wheat (*Triticum aestivum*). *Weed Sci.* 41, 551–556.

Burnside, O. C., Wilson, R. G., Weisberg, S., and Hubbard, K. G. (1996). Seed longevity of 41 weed species buried 17 years in eastern and western Nebraska. *Weed Sci.* 44, 74–86.

Campbell, L. G., and Snow, A. A. (2007). Competition alters life history and increases the relative fecundity of crop-wild radish hybrids (*Raphanus* spp.). *New Phytol.* 173, 648–660. doi: 10.1111/j.1469-8137.2006.01941.x

Chauhan, B. S., Matloob, A., Mahajan, G., Aslam, F., Florentine, S. K., and Jha, P. (2017). Emerging challenges and opportunities for education and research in weed science. *Front. Plant Sci.* 8:1537. doi: 10.3389/fpls.2017.01537

Chikoye, D., Weise, S. F., and Swanton, C. J. (1995). Influence of common ragweed (*Ambrosia artemisiifolia*) time of emergence and density in white bean (*Phaseolus vulgaris*). *Weed Sci.* 43, 375–380.

Conn, J. S., Beattie, K. L., and Blanchard, A. (2006). Seed viability and dormancy of 17 weed species after 19.7 years of burial in Alaska. *Weed Sci.* 54, 464–470. doi: 10.1614/WS-05-161R.1

Cousens, R. (1985). A simple model relating crop yield loss to weed density. *Ann. App. Biol.* 107, 239–252.

Davis, A. S., Hall, J. C., Jasieniuk, M., Locke, M. A., Luschei, E. C., Mortensen, D. A., et al. (2009). Weed science research and funding: a call to action. *Weed Sci.* 57, 442–448. doi: 10.1614/WS-09-020.1

Ellstrand, N. C., Heredia, S. M., Leak-Garcia, J. A., Heraty, J. M., Burger, J. C., Yao, L., et al. (2010). Crops gone wild: evolution of weeds and invasives from domesticated ancestors. *Evol. Appl.* 3, 494–504. doi: 10.1111/j.1752-4571.2010.00140.x

Gage, K. L., and Schwartz-Lazaro, L. M. (2019). Shifting the paradigm: an ecological systems approach to weed management. *Agriculture* 9:179. doi: 10.3390/agriculture9080179

Hartman, Y., Uwimana, B., Hooftman, D. A. P., Schranz, M. E., van de Wiel, C. C. M., Smulders, M. J. M., et al. (2013). Genomic and environmental selection patterns in two distinct lettuce crop-wild hybrid crosses. *Evol. Appl.* 6, 569–584. doi: 10.1111/eva.12043

Hodgdon, E. A., Warren, N. D., Smith, R. G., and Sideman, R. G. (2016). In-season and carry-over effects of cover crops on productivity and weed suppression. *Agron. J.* 108, 1–12. doi: 10.2134/agronj2015.0419

Hovick, S. M., Campbell, L. G., Snow, A. A., and Whitney, K. D. (2012). Hybridization alters early life-history traits and increases plant colonization success in a novel region. *Am. Nat.* 179, 192–203. doi: 10.1086/663684

Knezevic, S. Z., Weise, S. F., and Swanton, C. J. (1994). Interference of redroot pigweed (*Amaranthus retroflexus*) in corn (*Zea mays*). *Weed Sci.* 42, 568–573.

Lindquist, J. L., Mortensen, D. A., Clay, S. A., Schemnk, R., Kells, J. J., Howatt, K., et al. (1996). Stability of coefficients in the corn yield loss-velvetleaf density relationship across the north central US. *Weed Sci.* 44, 309–313.

Lindsay, K., Popp, M., Norsworthy, J. K., Bagavathiannan, M., Powles, S., and Lacoste, M. (2017). PAM: decision-support for long-term palmer amaranth (*Amaranthus palmeri*) control. *Weed Technol.* 31, 915–927. doi: 10.1017/wet.2017.69

MacLaren, C., Swanepoel, P., Bennett, J., Wright, J., and Dehnen-Schmutz, K. (2019). Cover crop biomass production is more important than diversity for weed suppression. *Crop Sci.* 59, 733–748. doi: 10.2135/cropsci2018.05.0329

Mirsky, S. B., Gallandt, E. R., Mortensen, D. A., Curran, W. S., and Shumway, D. L. (2010). Reducing the germinable weed seedbank with soil disturbance and cover crops. *Weed Res.* 50, 341–352. doi: 10.1111/j.1365-3180.2010.00792.x

Moonen, A. C., and Bàrberi, P. (2004). Size and composition of the weed seedbank after 7 years of different cover-crop-maize management systems. *Weed Res.* 44, 163–177. doi: 10.1111/j.1365-3180.2004.00388.x

Müller-Schärer, H., Sun, Y., Chauvel, B., Karrer, G., Kazinczi, G., Kudsk, P., et al. (2018). Cross-fertilizing weed science and plant invasion science to improve efficient management: a European challenge. *Basic Appl. Ecol.* 33, 1–3. doi: 10.1016/j.baae.2018.08.003

Norsworthy, J. K., Ward, S. M., Shaw, D. R., Llewellyn, R. S., Nichols, R. L., Webster, T. M., et al. (2012). Reducing the risks of herbicide resistance: best management practices and recommendations. *Weed Sci.* 60, 31–62. doi: 10.1614/WS-D-11-00155.1

Osipitan, O. A., Dille, J. A., Assefa, Y., and Knezevic, S. Z. (2018). Cover crop for early season weed suppression in crops: systematic review and meta-analysis. *Agron. J.* 110, 2211–2221. doi: 10.2134/agronj2017.12.0752

Osipitan, O. A., Dille, J. A., Assefa, Y., Radicetti, E., Ayeni, A., and Knezevic, S. Z. (2019). Impact of cover crop management on level of weed suppression: a meta-analysis. *Crop Sci.* 59, 833–842. doi: 10.2135/cropsci2018.09.0589

Ruffo, M. L., Bullock, D. G., and Bollero, G. A. (2004). Soybean yield as affected by biomass and nitrogen uptake of cereal rye in winter cover crop rotations. *Agron. J.* 96, 800–805. doi: 10.2134/agronj2004.0800

Schwartz-Lazaro, L. M., Norsworthy, J. K., Walsh, M. J., and Bagavathiannan, M. V. (2017). Efficacy of the integrated harrington seed destructor on weeds of soybean and rice production systems in the Southern United States. *Crop Sci.* 57, 2812–2818. doi: 10.2135/cropsci2017.03.0210

Shergill, L. S., Schwartz-Lazaro, L. M., Leon, R., Ackroyd, V. J., Flessner, M. L., Bagavathiannan, M., et al. (2020). Current outlook and future research needs for harvest weed seed control in North American cropping systems. *Pest Manag. Sci.* 76, 3887–3895. doi: 10.1002/ps.5986

Smith, R. G.,Warren, N. D., and Cordeau, S. (2020). Are cover crop mixtures better at suppressing weeds than cover crop monocultures? *Weed Sci.* 68, 186–194. doi: 10.1017/wsc.2020.12

Snapp, S., Swinton, S., Labarta, R., Mutch, D., Black, J., Leep, R., et al. (2005). Evaluating cover crops for benefits, costs and performance within cropping system niches. *Agron. J.* 97, 322–332. doi: 10.2134/agronj2005.0322a

Strock, J., Porter, P., and Russelle, M. (2004). Cover cropping to reduce nitrate loss through subsurface drainage in the northern US Corn Belt. *J. Environ. Qual.* 33, 1010–1016. doi: 10.2134/jeq2004.1010

Thill, D. C., Lish, J. M., Callihan, R. H., and Bechinski, E. J. (1991). Integrated weed management-a component of integrated pest management: a critical review. *Weed Technol.* 5, 648–656.

Walsh, M., Newman, P., and Powles, S. (2013). Targeting weed seeds in-crop: a new weed control paradigm for global agriculture. *Weed Technol.* 27, 431–436. doi: 10.1614/WT-D-12-00181.1

Walsh, M. J., Broster, J. C., Schwartz-Lazaro, L. M., Norsworthy, J. K., Davis, A. S., Tidemann, B. D., et al. (2018). Opportunities and challenges for harvest weed seed control in global cropping systems. *Pest Manag. Sci.* 74, 2235–2245. doi: 10.1002/ps.4802

Ward, S. M., Cousens, R. D., Bagavathiannan, M. V., Barney, J. N., Beckie, H. J., Busi, R., et al. (2014). Agricultural weed research: a critique and two proposals. *Weed Sci.* 62, 672–678. doi: 10.1614/WS-D-13-00161.1

Weaver, S. E., Smits, N., and Tan, C. S. (1987). Estimating yield losses of tomato (*Lycopersicon esculentum*) caused by nightshade (*Solanum* spp.) interference. *Weed Sci.* 35, 163–168.

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BRIEF RESEARCH REPORT
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Weedy Rice From South Korea Arose From Two Distinct De-domestication Events

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Agro-ecosystems are dominated by crop plants and the weedy species that thrive under agricultural conditions. Weedy crop relatives are some of the most difficult weeds to manage and can dramatically reduce crop yields when left unchecked. Weedy rice has resulted from multiple de-domestication events from crop rice in different rice growing regions. Interestingly, both South Korea and the United States harbor weedy rice populations that share ancestry with indica cultivars and temperate japonica cultivars. Here we compare weedy rice populations from South Korea and the United States on order to identify if they are the result of the same de-domestication events. We find that weedy rice populations in South Korea are genetically distinct from weedy rice found in the USA and are therefore the result of two unique de-domestication events. Low levels of genetic diversity among Korean weedy rice accessions (haplotype diversity = 0.0188 and 0.0324) indicate recent de-domestication events from crop relatives.

Keywords: oryza, weedy crop relatives, de-domestication, agricultural weeds, populaton genomics

INTRODUCTION

Agricultural weeds account for approximately one third of all crop yield loss (Oerke, 2006), contributing to food shortages worldwide. Understanding the population structure and mechanisms of adaption in weedy plants informs best management practices in agro-ecosystems. In particular, weedy crop relatives have played a longstanding role in agro-ecosystem dynamics, driving both the evolution of crops as well as the development of new management strategies (Kwit et al., 2011; Li and Olsen, 2020). A well-documented example is weedy rice, a conspecific weed of cultivated rice (*Oryza sativa* L.). Weedy rice has a distribution that spans nearly all rice growing regions around the world. Infestations of this species can cause up to an 80% loss in harvest for cultivated rice and is often cited as a major limiting factor for rice production. In the United States alone, estimates of production loss due to weedy rice could feed an additional 12 million people annually (Durand-Morat et al., 2018). Management efforts for weedy rice have ranged from manual removal to large scale herbicide application.

There are five main cultivated rice subtypes that are genetically and phenotypically distinguishable: *indica*, *aus*, *aromatic*, *tropical japonica*, and *temperate japonica*. Weedy rice has arisen through de-domestication events from at least 3 of the 5 cultivated subtypes (Qiu et al., 2020).

In the United States which has no wild *Oryza* species, there are three main weedy rice subgroups: (1) straw-hulled weedy rice from the southern US (SH weeds) that are genetically similar to *indica* type cultivated rice, (2) black-hulled weedy rice from the southern US (BH weeds) that are genetically similar to *aus* type cultivated rice, and (3) California weedy rice (CA weeds) that is genetically similar to *temperate japonica* cultivated rice. All three of these subgroups seem to have evolved from de-domestication events in each progenitor cultivar group (Reagon et al., 2010; Kanapeckas et al., 2016; Qiu et al., 2020). In other world regions where wild rice (*Oryza rufipogon*) is common, weedy rice populations have genetic contributions from both wild rice and cultivated rice populations (Vigueira et al., 2019; Qiu et al., 2020).

Weedy rice from South Korea (Korean weedy rice) is composed of two main subgroups based on population structure analysis: *indica*-like and *temperate japonica*-like (Vigueira et al., 2019). Using whole genome sequencing, other researchers have also placed Korean weedy rice into the same two subgroups (He et al., 2017). Like the United States, South Korea is not in the natural range of wild *Oryza* species. Therefore, all rice crops have been imported into the region for cultivation. Weedy rice populations in these countries have therefore either been introduced with cultivated rice seed or have evolved in place since rice cultivation began (Reagon et al., 2010; Kanapeckas et al., 2016; Qiu et al., 2020).

Here, we aim to more closely examine the genetic similarities of weedy rice from South Korea and the United States. We have used both candidate genes (*Rc*, controlling pericarp color; *Bh4*, controlling hull color; and *sh4,* controlling seed shattering) as well as genome-wide neutral genetic markers (Sequence Tagged Sites) to better understand the evolutionary history and population structure of weedy rice from these two regions. We find that Korean weedy rice is genetically distinct from US weedy rice populations despite their phenotypic similarities, indicating that these weeds were the result of unique de-domestication events.

METHODS

Sampling and Sequencing

Rice seeds were obtained from the International Rice Germplasm Collection (IRGC) of the International Rice Research Institute (IRRI; Los Baños, Philippines). Twenty-four accessions of weedy rice from South Korea were selected to represent the phenotypic diversity for hull color, pericarp color, and presence of awns (phenotypes are listed in **Table 1**) from a total sample of 226 accessions (**Supplementary Table 1**). Eighteen of these samples were previously included in a comparative study with weedy, wild, and cultivated rice from Southeast Asian and the United States (Vigueira et al., 2019). Seeds were germinated and grown to the young seedling stage in the greenhouse. DNA was extracted from young leaf tissue using DNeasy Plant DNA kits (QIAGEN, Hilden, Germany).

Polymerase Chain Reaction (PCR) was carried out using standard conditions to amplify 48 Sequence Tagged Sites (STS loci) as described in Reagon et al. (2010). Due to inconsistent amplification, 7 loci were excluded from the analysis. Regions of the three candidate genes (*Rc*, *Bh4*, and *sh4*) were amplified by PCR using primers and conditions as previously described (Konishi et al., 2006; Sweeney et al., 2006; Zhu et al., 2011). Successful PCR amplification was confirmed using gel electrophoresis and excess primers and dNTPs were removed using Exonuclease I and Antarctic phosphatase treatment. Direct Sanger sequencing in both the forward and reverse direction was carried out by Eurofins Genomics (Louisville, KY, USA).

Sequences were assembled into contiguously aligned sequence "contigs" and aligned using CodonCode Aligner. All sequences were inspected visually for quality and for the presence of heterozygous sites. Low quality sequences were removed from the dataset. Heterozygous base calls were randomly assigned to two pseudo-haplotypes, which were then phased using PHASE version 2.1 (Stephens et al., 2001; Stephens and Scheet, 2005). Due to very low levels of heterozygosity in the data set, haplotypes were inferred with very high probabilities and were consistently assigned across five independent runs. All sequences have been submitted to NCBI GenBank (accession numbers MT976168-MT977030).

Analysis of STS Loci

Phased haplotypes were aligned with STS sequences from 27 weedy rice accessions collected in California (Kanapeckas et al., 2016; GenBank accessions KT441140-KT443009) as well as from a diverse sampling of 206 accessions that includes Southern US weedy as well as wild and cultivated rice representing major *Oryza* varieties and species (Reagon et al., 2010; GenBank accessions GQ999668-GQ999777). Population structure was inferred using *STRUCTURE* 2.3.4 (Pritchard et al., 2000). Initial runs included all samples in the dataset. To limit the number of possible subpopulations with proposed ancestry to Korean weedy rice and therefore better resolve differences between closely related groups, we reduced the data set to include the following groups: all Korean weedy rice (24 accessions), weedy rice from California (27 accessions), weedy rice from the southern US (58 accessions of BH and SH weedy rice), and the five cultivar groups (75 accessions representing *indica*, *aus*, *aromatic*, *temperate japonica*, and *tropical japonica*). The number of populations (K) was tested with five permutations each between values of $K = 1$ to $K = 10$. Each permutation had a burn-in period of 100,000 steps and a MCMC chain length of 500,000 steps after the burn-in. *STRUCTURE HARVESTER* (Earl and vonHoldt, 2012) was used to calculate Delta K (Evanno et al., 2005) and determine the K value that maximized the marginal likelihood. *DISTRUCT* version 1.1 (Rosenberg, 2003) was used to produce the graphical display of structure results. As a complement to *STRUCTURE* analysis, principal component analysis (PCA) was run on haplotype data using the ML model in JMP. PCAs were produced for the full dataset and the subset of data used in the *STRUCTURE* analysis. The PCA produced from data in our *STRUCTURE* analysis separated California weedy rice from the rest of the groups. Therefore, a PCA was also run with California weedy rice removed from the dataset.

Summary statistics for each STS locus, including nucleotide diversity at silent sites (π) using the Juke's Cantor correction

TABLE 1 | Korean Weedy Rice Accession Phenotypes and Haplotypes.

IRGC #	Hull color	Awn presence	Pericarp color	*STRUCTURE* grouping	*Bh4* haplotype	*Rc* haplotype	*sh4* haplotype
90740	Straw	Yes	Red	Indica	22 bp deletion	No deletion	T
90818	Straw	Yes	White	Indica	22 bp deletion	No deletion	T
90835	Straw	No	Red	Indica	22 bp deletion	No deletion	T
112801	Straw	Yes	White	Indica	22 bp deletion	No deletion	T
115298	Straw	No	Red	Indica	22 bp deletion	No deletion	T
82881	Black	No	Red	Japonica	No deletion	No deletion	T
90734	Straw	No	Red	Japonica	22 bp deletion	No deletion	T
90752	Black	No	Red	Japonica	No deletion	No deletion	T
90754	Straw	Yes	Red	Japonica	22 bp deletion	No deletion	T
90758	Black	No	Red	Japonica	No deletion	No deletion	T
90759	Black	Yes	Red	Japonica	No deletion	No deletion	T
90760	Black	Yes	Red	Japonica	No deletion	No deletion	T
90785	Black	Yes	White	Japonica	No deletion	No deletion	T
90819	Straw	No	Red	Japonica	22 bp deletion	No deletion	T
90822	Black	Yes	Red	Japonica	No deletion	No deletion	T
90823	Black	Yes	Red	Japonica	No deletion	No deletion	T
112787	Black	Yes	Red	Japonica	No deletion	No deletion	T
112820	Straw	Yes	White	Japonica	No deletion	14 bp deletion	T
112832	Straw	No	Red	Japonica	22 bp deletion	No deletion	T
113866	Straw	Yes	Red	Japonica	22 bp deletion	No deletion	T
115306	Straw	Yes	Red	Japonica	22 bp deletion	No deletion	T
115580	Straw	Yes	Red	Japonica	22 bp deletion	No deletion	T
117123	Black	No	Red	Japonica	No deletion	No deletion	T
117124	Straw	No	Red	Japonica	22 bp deletion	No deletion	T

(Jukes and Cantor, 1969), Watterson's estimator of θ at silent sites (Watterson, 1975), number of segregating sites S, and haplotype diversity were calculated in DnaSP version 5.0 (Librado and Rozas, 2009). Averages for these statistics across all STS loci were calculated in Excel.

Candidate Gene Analysis

Candidate gene sequences were aligned with rice sequences from previous studies (Gross et al., 2010; Thurber et al., 2010; Vigueira et al., 2013). Genetic variants were determined by identifying haplotypes and mutations shared between Korean weedy rice and wild, weedy, or cultivated rice varieties.

RESULTS

Population Structure

Analyses of neutral STS markers grouped Korean weedy rice groups two distinct genetic subpopulations. *STRUCTURE* analysis comparing Korean weeds with the five major cultivars and weedy rice from the United States partitions Korean weeds into an *indica*-like group and a *temperate japonica*-like group (**Figure 1**). *STRUCTURE* plots are shown for $K = 4$ based on Delta K results and $K = 5$ because *temperate japonica* was distinguishable from *tropical japonica* at that number of subpopulations. Principal Component Analysis reveals the same genetic groupings as found in *STRUCTURE* analysis. PCA was performed with and without California weedy rice (**Figure 2**), as it was the most genetically distinct group. Due to consistent genetic grouping, we analyzed the Korean weedy rice as two separate populations, *indica*-like and *temperate japonica*-like weedy rice, for the remainder of the analysis.

Diversity of Korean Weedy Rice

Summary statistics indicated very low genetic diversity within Korean weedy rice sub-groups, consistent with a population bottleneck during de-domestication from cultivated rice (**Table 2**). Average haplotype diversity and nucleotide diversity is lower in weedy rice groups compared to cultivated rice. Korean weedy rice has even lower values of average haplotype diversity (0.0188 and 0.0324) than SH weedy rice (0.0515), BH weedy rice (0.1456) and California weedy rice (0.0466). In addition, Korean weedy rice had an average pairwise nucleotide diversity (π) of about half the average value found in SH and BH weedy rice from the Southern US (**Table 2**). This level of genetic diversity is similar to that found in California weedy rice. Low levels of genetic diversity in Korean weedy rice could be a result of a very recent genetic bottleneck associated with a recent de-domestication event.

Candidate Gene Alleles

Korean weedy rice phenotypes and candidate gene allele information can be found in **Table 1**. Of the 24 Korean weedy rice accessions included in our analysis, 14 had a straw colored hull and 10 had a black colored hull. All straw colored hull accessions

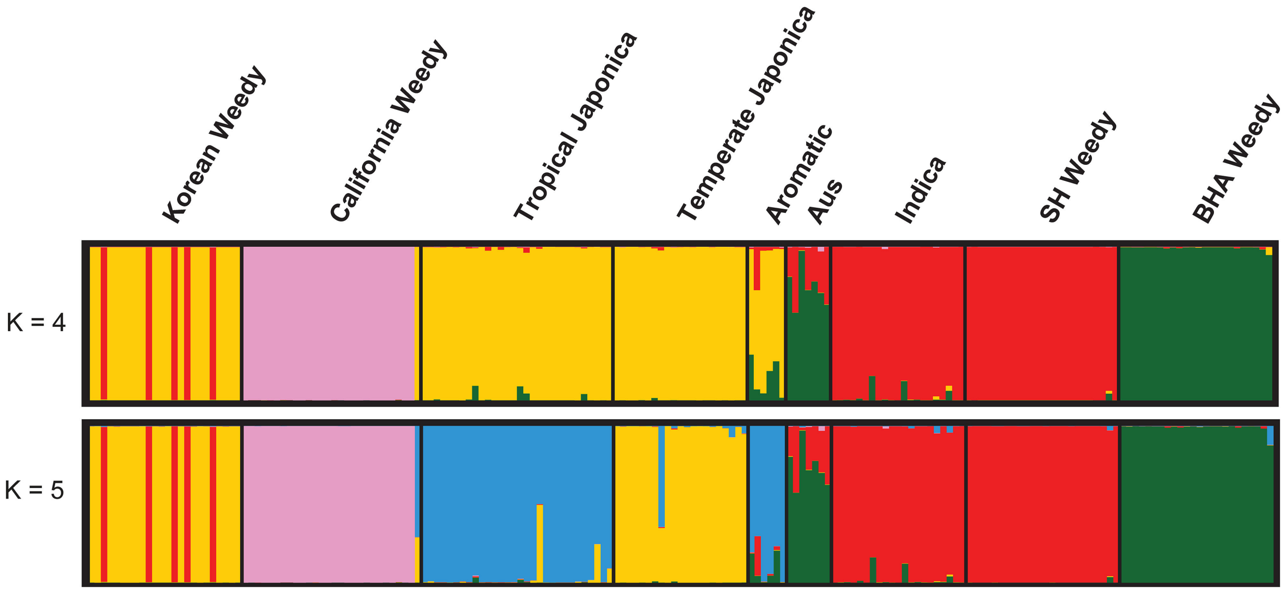

FIGURE 1 | *STRUCTURE* plot of five cultivated rice subgroups and four weedy rice groups. $K = 4$ was chosen using Delta K, while $K = 5$ was included as it separated temperate and tropical japonica. Korean weedy rice groups with *indica* (red) and *temperate japonica* (yellow) cultivars.

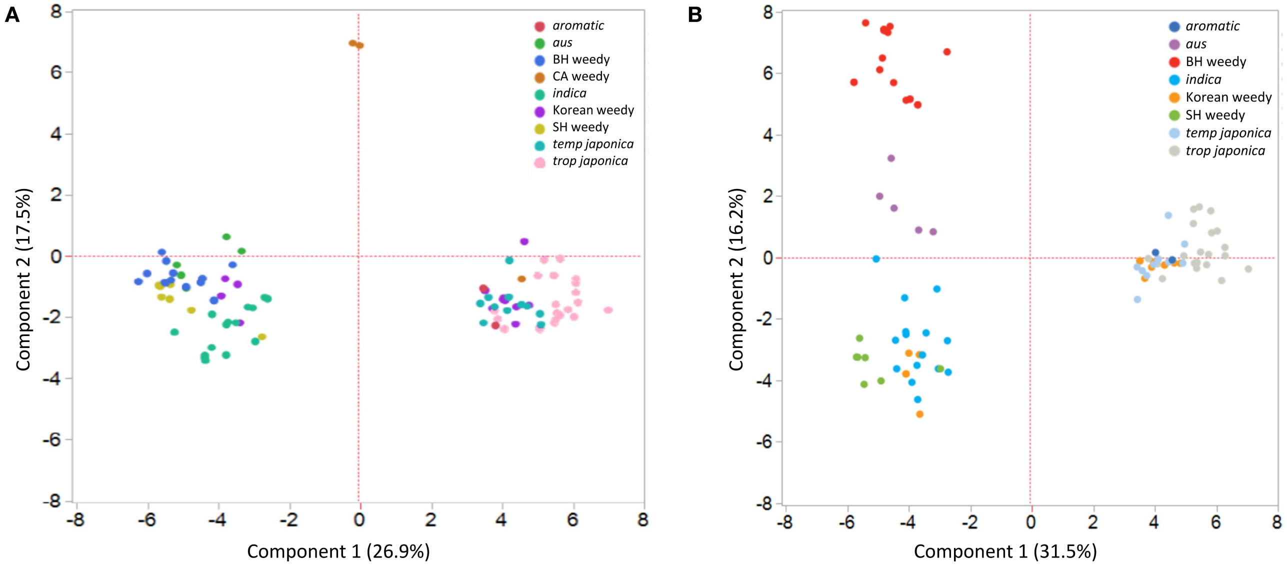

FIGURE 2 | Principal Component Analysis (PCA) of cultivated and weedy rice including California weedy rice **(A)** and without California weedy rice. **(B)** Korean weedy rice groups with temperate japonica cultivars and indica cultivars.

with the exception of one (IRGC #112820) contained the 22 bp causal deletion in *Bh4* that is found in most cultivated rice. As expected, black hulled Korean weeds carried the ancestral wild-type allele (lacking the 22 bp deletion) which is found in black hull weedy and wild rice.

Four Korean weedy rice accessions had a white pericarp color and twenty had red pericarps. Of the four, one contained a previously determined white pericarp allele (14 bp deletion) found in nearly all cultivated rice. Interestingly, this accession is the same accession (IRGC #112820) that does not have the 22 bp deletion at the *Bh4* locus despite having a straw hull. The other three had no obvious deletions or loss of function mutations.

All 24 Korean weedy rice accessions contained the "T" reduced-shattering allele found in cultivated rice at the *sh4* locus. This allele is also present in weedy rice from other world regions (Thurber et al., 2010), which further supports

TABLE 2 | Diversity Statistics Averaged Across 41 Sequence Tagged Sites.

Group	Number of haplotypes	Haplotype diversity	π (JC)*	θ-W**
Korean *indica*	1.0513	0.0188	0.0003	0.0003
Korean *japonica*	1.2564	0.0324	0.0004	0.0005
California weedy	1.6667	0.0466	0.0003	0.0009
Trop japonica	1.9744	0.1426	0.0013	0.0013
Temp japonica	1.5385	0.0851	0.0005	0.0007
Aromatic	1.5385	0.1840	0.0019	0.0016
Aus	1.6667	0.2337	0.0011	0.0009
Indica	2.4872	0.2955	0.0017	0.0015
SH weedy	1.3077	0.0515	0.0006	0.0004
BH weedy	1.6154	0.1456	0.0011	0.0009

** nucleotide diversity at silent sites (π) using the Juke's Cantor correction.*

*** Watterson's estimator of θ at silent sites.*

the gain of shattering phenotype in weedy rice was acquired during de-domestication.

DISCUSSION

Korean weedy rice groups most closely with two distinct cultivated rice subtypes: *indica* and *temperate japonica*. These weeds likely originated from two distinct de-domestication events from cultivated varieties. These de-domestication events are likely recent, given low genetic divergence from cultivated groups and low genetic diversity within weedy rice groups. We find patterns consistent with cultivated rice de-domestication at both STS loci as well as candidate genes for weedy traits (*Bh4*, *Rc*, and *sh4*). These patterns have also been found in weedy rice from the United States (Gross et al., 2010; Thurber et al., 2010; Vigueira et al., 2013).

Weedy rice subtypes from the southern US include straw-hulled (*indica*-like) and black-hulled (*aus*-like). In California, there is a distinct weedy rice population that groups most closely with *temperate japonica* cultivars based on coalescent modeling in Kanapeckas et al. (2016). Our PCA results from the California weedy rice group may better resolve this grouping. One weedy accession from California groups closely with *japonica* cultivars in our PCA, while the other accessions are genetically distinct from all other rice subtypes. This is an interesting discovery that warrants additional sampling from the California weedy rice population. Weedy rice from Korea does not seem to share recent ancestry with any of the US weedy rice populations, further supporting the previous findings that this group is the result of two distinct de-domestication events from *indica* and *japonica* cultivars.

Although this study does not provide definitive evidence for the location of Korean weedy rice de-domestication, recent studies of world-wide samples of weedy and cultivated rice points to other de-domestication events from rice cultivars found in the Korean peninsula (Qiu et al., 2020). Interestingly, kinship analysis results for Korean weeds identified closest cultivar relatives from Korea, Japan, China, India, and Egypt (Qiu et al., 2020). Taken together, it seems that Korean weedy rice may have multiple origins possibly including de-domestication events from cultivars *in situ*.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI GenBank (accession numbers MT976168-MT977030).

AUTHOR CONTRIBUTIONS

CV, PV, and KO designed the study. CV, PV, CW, and ZC collected the data. CV and PV analyzed the data. CV wrote the manuscript. All authors contributed to the article and approved the submitted version.

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The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2020.602612/full#supplementary-material

REFERENCES

Durand-Morat, A., Nalley, L. L., and Thoma, G. (2018). The implications of red rice on food security. *Glob. Food Sec.* 18, 62–75. doi: 10.1016/j.gfs.2018.08.004

Earl, D. A., and vonHoldt, B. M. (2012). Structure harvester: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour.* 4, 359–361. doi: 10.1007/s12686-011-9548-7

Evanno, G., Regnaut, S., and Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* 14, 2611–2620. doi: 10.1111/j.1365-294X.2005.02553.x

Gross, B. L., Reagon, M., Hsu, S.-C., Caicedo, A. L., Jia, Y., and Olsen, K. M. (2010). Seeing red: the origin of grain pigmentation in US weedy rice. *Mol. Ecol.* 19, 3380–3393. doi: 10.1111/j.1365-294X.2010.04707.x

He, Q., Kim, K. W., and Park, Y. J. (2017). Population genomics identifies the origin and signatures of selection of Korean weedy rice. *Plant Biotechnol. J.* 15, 357–366. doi: 10.1111/pbi.12630

Jukes, T. H., and Cantor, C. R. (1969). "Evolution of protein molecules," in *Mammalian Protein Metabolism,* ed H. N. Munro (New York, NY: Academic Press). 21–132. doi: 10.1016/B978-1-4832-3211-9.50009-7

Kanapeckas, K.imberly L., Vigueira, C. C., Ortiz, A., Gettler, K. A., Burgos, N. R., Fischer, A. J., et al. (2016). Escape to ferality: the endoferal origin of weedy rice from crop rice through de-domestication. *PLoS ONE* 11:e0162676. doi: 10.1371/journal.pone.0162676

Konishi, S., Izawa, T., Lin, S. Y., Ebana, K., Fukuta, Y., Sasaki, T., et al. (2006). An SNP caused loss of seed shattering during rice domestication. *Science* 312, 1392–1396. doi: 10.1126/science.1126410

Kwit, C., Moon, H. S., Warwick, S. I., and Stewart, C. N. (2011). Transgene introgression in crop relatives: Molecular evidence and mitigation strategies. *Trends Biotechnol.* 29, 284–293. doi: 10.1016/j.tibtech.2011.02.003

Li, L.-F., and Olsen, K. M. (2020). "Population genomics of weedy crop relatives: insights from weedy rice," in *Population Genomics*, eds O. P. Rajora (Cham: Springer). doi: 10.1007/13836_2020_77

Librado, P., and Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25, 1451–1452. doi: 10.1093/bioinformatics/btp187

Oerke, E. C. (2006). Crop losses to pests. *J. Agric. Sci.* 144, 31–43. doi: 10.1017/S0021859605005708

Pritchard, J. K., Stephens, M., and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959. doi: 10.1111/j.1471-8286.2007.01758.x

Qiu, J., Jia, L., Wu, D., Weng, X., Chen, L., Sun, J., et al. (2020). Diverse genetic mechanisms underlie worldwide convergent rice feralization. *Genome Biol.* 21:70. doi: 10.1186/s13059-020-01980-x

Reagon, M., Thurber, C. S., Gross, B. L., Olsen, K. M., Jia, Y., and Caicedo, A. L. (2010). Genomic patterns of nucleotide diversity in divergent populations of U.S. weedy rice. *BMC Evol. Biol.* 10:180. doi: 10.1186/1471-2148-10-180

Rosenberg, N. A. (2003). Distruct: a program for the graphical display of population structure. *Mol. Ecol. Notes* 4, 137–138. doi: 10.1046/j.1471-8286.2003.00566.x

Stephens, M., and Scheet, P. (2005). Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. *Am. J. Hum. Genet.* 76, 449–462. doi: 10.1086/428594

Stephens, M., Smith, N. J., and Donnelly, P. (2001). A new statistical method for haplotype reconstruction from population data. *Am. J. Hum. Genet.* 68, 978–989. doi: 10.1086/319501

Sweeney, M. T., Thomson, M. J., Pfeil, B. E., and Mccouch, S. (2006). Caught red-handed : rc encodes a basic helix-loop-helix protein conditioning red pericarp in rice. *Plant Cell* 18, 283–294. doi: 10.1105/tpc.105.038430

Thurber, C. S., Reagon, M., Gross, B. L., Olsen, K. M., Jia, Y., and Caicedo, A. L. (2010). Molecular evolution of shattering loci in U.S. weedy rice. *Mol. Ecol.* 19, 3271–3284. doi: 10.1111/j.1365-294X.2010.04708.x

Vigueira, C. C., Li, W., and Olsen, K. M. (2013). The role of Bh4 in parallel evolution of hull colour in domesticated and weedy rice. *J. Evol. Biol.* 26, 1738–1749. doi: 10.1111/jeb.12171

Vigueira, C. C., Qi, X., Song, B.-K., Li, L.-F., Caicedo, A. L., Jia, Y., et al. (2019). Call of the wild rice: Oryza rufipogon shapes weedy rice evolution in Southeast Asia. *Evol. Appl.* 12:eva12581. doi: 10.1111/eva.12581

Watterson, G. A. (1975). On the number of segregating sites in genetical models without recombination. *Theor. Popul. Biol.* 7, 256–276. doi: 10.1016/0040-5809(75)90020-9

Zhu, B.-F., Si, L., Wang, Z., Zhu, Y. Z. J., Shangguan, Y., Lu, D., et al. (2011). Genetic control of a transition from black to straw-white seed hull in rice domestication. *Plant Physiol.* 155, 1301–1311. doi: 10.1104/pp.110.168500

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Effects of Long-Term Cover Cropping on Weed Seedbanks

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Cool-season cover crops have been shown to reduce soil erosion and nutrient discharge from maize (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] production systems. However, their effects on long-term weed dynamics are not well-understood. We utilized five long-term research trials in Iowa to quantify germinable weed seedbank densities and compositions after 10+ years of cover cropping treatments. All five trials consisted of zero-tillage maize-soybean rotations managed with and without the inclusion of a yearly winter rye (*Secale cereal* L.) cover crop. Seedbank sampling was conducted in the early spring before crop planting at all locations, with three of the five trials having grown a soybean crop the preceding year, and two a maize crop. Two of the trials (both previously soybean) showed significant and biologically relevant decreases (4,070 and 927 seeds m^{-2}, respectively) in seedbank densities in cover crop treatments compared to controls. In another two trials, one previously maize and one previously soybean, no difference was detected in seedbank densities. In the fifth trial (previously maize), there was a significant, but biologically unimportant increase of 349 seeds m^{-2}. All five trials' weed communities were dominated by common waterhemp [*Amaranthus tuberculatus* (Moq.)], and changes in seedbank composition from cover-cropping were driven by changes in this species. Although previous studies have shown that increases in cover crop biomass are strongly correlated with weed suppression, in our study we did not find a relationship between seedbank changes and the mean amount of cover crop biomass produced over a 10-years period (experiment means ranging from 0.5 to 2.0 Mg ha^{-1} yr^{-1}), the stability of the cover crop biomass production, nor the amount produced going into the previous crop's growing season. We conclude that long-term use of a winter rye cover crop in a maize-soybean system has the potential to meaningfully reduce the size of weed seedbanks compared to winter fallows. However, identifying the mechanisms by which this occurs requires further research into processes such as seed predation and seed decay in cover cropped systems.

Keywords: maize (*Zea mays* L.), cover crop, sustainable weed management, corn belt, waterhemp [*Amaranthus tuberculatus* (Moq.) J. D. Sauer], germinable seed bank

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INTRODUCTION

One-third of the global maize (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] production comes from the United States (US; Food and Agriculture Organization of the United Nations, 2020). The majority of US production occurs in the Midwest region (USDA, 2020a), and 80% of the agricultural land in the two top-producing states, Iowa and Illinois, is dedicated to a rotation consisting solely of these two crops (USDA, 2020b). Maize-soybean cropping systems traditionally leave the soil fallow over the winter and early spring, resulting in high levels of nutrient and soil export that render the sustainability of the system questionable (O'Neal et al., 2005; Dold et al., 2017; Nearing et al., 2017; Jones et al., 2018). Incorporation of an over-wintering rye (*Secale cereal* L.) cover crop into these systems can significantly reduce soil erosion and nutrient leaching (Strock et al., 2004; Kaspar and Singer, 2011; Kaspar et al., 2012), and may offer additional long-term benefits to the soil (Moore et al., 2014; Basche et al., 2016a,b). Surveys indicate farmers consider cover crops to be a valuable component of an integrated approach to weed management (Arbuckle and Lasley, 2013). Moreover, ecologically-based approaches to weed management such as cover crops are becoming more critical as weeds develop herbicide resistance to multiple modes of action (Patzoldt et al., 2005; Price et al., 2011; Bunchek et al., 2020; MacLaren et al., 2020). However, the effects of over-wintering cover crops on weed dynamics in these systems is not well-understood.

There is evidence cover crops can reduce weed biomass in many production contexts (Baraibar et al., 2018; MacLaren et al., 2019; Smith et al., 2020), and specifically in midwestern maize-soybean systems (Nichols et al., 2020a). In other production systems, there is also evidence cover crops can reduce weed seed densities in the soil (Moonen and Bàrberi, 2004; Mirsky et al., 2010; Alonso-Ayuso et al., 2018) and decrease the survival success of herbicide-resistant weeds (Cholette et al., 2018; Wallace et al., 2019). However, the majority of relevant studies have been conducted in plots where cover crop treatments were in place <3 years, so the long-term effect of cover cropping on weed dynamics in these systems is unclear. The density and species composition of emerged weeds can vary greatly from year to year based on weather conditions, rendering the more subtle effects of management practices difficult to discern in short-term studies (Teasdale et al., 2018). Additionally, weed seeds can persist in the soil for several years, creating legacy effects that can overwhelm short-term changes in management. Measurements taken in long-term, replicated settings may therefore more accurately reflect management-induced changes.

Aboveground measurements of weeds are useful, but the potential for annual weed species to interfere with crop growth and yield is ultimately an expression of the weed seedbank. In the midwestern US, management practices that target weed seedbanks are particularly relevant, as the majority of problematic weeds are annual species whose persistence depends on replenishing seedbanks (Davis, 2006). While seedbank sizes are of primary concern, the seedbank composition can provide insight into weed dynamics and differences in composition can be used to assess the relative strength of the filters defining the weed community (e.g., Ryan et al., 2010). Additionally, there is some evidence that crop yield loss and weed diversity are negatively correlated (Adeux et al., 2019) and more diverse assemblages of weed seeds in the soil may reflect the impacts of more sustainable management strategies (Storkey and Neve, 2018). Information about the size and composition of weed seedbanks after two or more full crop rotation sequences may therefore provide a more complete picture of weed responses to cover cropping.

To address the lack of data concerning long-term effects of cover cropping on weed seedbanks in maize-soybean systems, we measured the size and composition of the germinable weed seedbank sampled from five trials in Iowa where rye cover crop treatments had been in place for at least 10 years. We hypothesized that long-term use of over-wintering rye cover crops in maize-soybean rotation systems would: (1) reduce the size and (2) increase the species diversity of the weed seedbank.

METHODS AND MATERIALS

Site Descriptions

Three research sites were used for this study (**Table 1**). The West and East sites were grain production fields on commercial farms, and only one phase of the maize-soybean rotation was present each year. The Central site had both a grain-based maize-soybean rotation and a silage-based rotation. In the silage rotation, the maize phase was harvested for silage at the milk stage (R3; Abendroth et al., 2011). The trials were part of a larger study (Kaspar et al., 2007, 2012) and had both phases of the rotations of both systems (grain- and silage-based) present each year, but each phase was located in a separate field.

All trials consisted of two treatments that had been in place for at least 10 years: (1) a maize-soybean rotation (either grain- or silage-based) with a winter rye cover crop planted in the fall following cash crop harvest and terminated in the spring, and (2) the same rotation without a cover crop. Every trial was arranged in a randomized complete block design with four (West and East) or five (Central) replicates. More detailed accounts of agronomic management at the Central site have been published elsewhere (Moore et al., 2014). None of the studies were originally set-up with the goal of assessing weed dynamics; as such there are unfortunately no baseline measurements of the weed seedbank available.

The plots within each trial were managed identically save for the planting of the cover crop in the fall. All sites applied herbicide 1–2 weeks before maize or soybean planting to all plots and in certain years an additional herbicide application shortly after cash crop planting (**Table 2**). The exact herbicide and nutrient programs varied by site, reflective of their particular managers and contexts (**Supplementary Material**). All sites had sub-surface tile drainage and were managed without tillage since initiation of the trials.

Weed Seedbank Sampling

Midwestern row crop production fields typically have early spring seedbank densities well-above 500 seed m^{-2} in the top 10 cm of the soil profile (Forcella et al., 1992; Felix and Owen, 2001). For these expected values, 20 soil samples 5 cm in diameter are expected to provide a high level of precision when estimating seedbank densities (Dessaint et al., 1996; Forcella et al., 2003). We used these estimates to guide our sampling protocol.

A soil sampler was constructed using PVC pipe with an inner diameter of 5.25 cm and a line indicating 10 cm sampling depth to extract a total of 52.5 cm^3 of soil per core. In no-till systems,

TABLE 1 | Summary of the four trials sampled.

Trial	Latitude, longitude	Year started	Number of replicates	Plot size	30-years annual mean		Mean cover crop biomass (Mg ha^{-1})		2018 crop	2019 sampling date
					Air temperature (°C)	Precipitation (mm)	5-years	10-years		
WEST										
1	42°03′N 94°20′W	2008	4	25 × 250 m	9.5	880	0.24	0.45	Soybean	April 17
CENTRAL SILAGE										
2	42°00′N 94°12′W	2002	5	3.8 × 55 m	9.8	907	2.38	1.98	Soybean	April 16
CENTRAL GRAIN										
3	42°00′N	2009	5	3.8 × 55 m	9.8	907	1.53	0.88	Soybean	April 8
4	94°12′W						1.93	1.34	Maize	April 9
EAST										
5	41°19′N 92°17′W	2009	4	25 × 275 m	10.2	947	1.73	1.32	Maize	April 6

TABLE 2 | Summary of herbicide active ingredients applied at each site during 2017–2019 growing seasons.

Site	Maize year			Soybean year		
	Pre-plant	At planting/post-emergence	Herbicide groups	Pre-plant	At planting/post-emergence	Herbicide groups
West	Glyphosate	Metolachlor Atrazine Mesotrione	5, 9, 15, 27	Glyphosate	Glyphosate Fluthiacet-methyl	9, 14
Central	Glyphosate	Metolachlor Atrazine Mesotrione	5, 9, 15, 27	Glyphosate	Glyphosate Hand weeding in late July	9
East	Glyphosate Acetochlor	Atrazine Acetochlor Glyphosate	5, 9, 15	Glyphosate Chlorimuron-ethyl Flumioxazin Pyroxasulfone	Dicamba Acetochlor	2, 4, 9, 14, 15

The same herbicide treatments were applied to the cover-crop and no-cover plots.

this represents a generous depth from which most midwestern US weed seedlings can emerge (Mohler, 1993), so we assumed our sampling efforts accurately recovered seeds with the potential to contribute to weed infestations in maize and soybean crops.

Sampling was done in April 2019 at all locations. Each plot was divided longitudinally into five sampling areas. Within each sampling area, four cores were taken. The East and West locations' plots were wide, so the cores were taken randomly within each of the five sampling areas. For the Central sites, which had narrower plots, the cores were taken from the middle of the sampling area to minimize edge effects. Within each sampling area, four cores were taken and the soil was emptied into a bucket, thoroughly mixed, then placed in a sealed polyethylene bag and stored for a maximum of 5 h in a cooler for transportation. Each plot had a total of 1,050 cm^3 of soil sampled (20 cores, each 52.5 cm^3). Sampling occurred before maize (West, Central-grain) or soybean (East, Central-grain, Central-silage) planting at each site. At the Central site, both phases of the grain rotation were sampled, while only one phase of the silage rotation was sampled due to time constraints (**Table 1**).

Germinable Seedbank Measurements

The germinable seedbank method was chosen over the extractable seedbank method based on practicality, and its applicability for assessing treatment differences (Reinhardt and Leon, 2018). Soil processing, as described below, occurred on the same day as collection.

The field-wet soil was weighed to ensure each plot had approximately the same mass of soil sampled (**Supplementary Material**). The soil from each plot's five sampling points was then combined and sieved through a 5 mm wire mesh screen into a bucket and transported to a greenhouse.

Plastic 25 × 50 cm trays with drainage holes were filled with vermiculite to a depth of 1 cm (Greenhouse Megastore, Danville, Illinois, US). The bulked soil from each plot was evenly distributed into three trays, creating a 1 cm soil layer covering the vermiculite. The soil was saturated using a three-hole fine-mist brass nozzle (Greenhouse Megastore). The greenhouse area had no artificial lighting and was maintained near 28°C. Germination from soil samples occurred between April and July, during a period with 13–15 h of daylight.

Trays were checked 1–3 times per day to ensure proper germination conditions. Weed seedlings were identified, counted, and pulled daily, after which the trays were randomly relocated within the greenhouse to avoid the effects of micro-environments on germination. When no new seedlings appeared for at least 3 days, the tray was allowed to dry in order to avoid conditions that would promote decay of un-germinated seeds. Once all trays were dried (∼2 months after sampling), each tray's soil was recollected, re-sieved, redistributed into the same tray, and again saturated. This process was repeated twice, and after the second soil re-sieving no seedlings emerged. The total number of emerged seedlings was reported as the seedbank density.

Cover Crop Biomass Sampling

Cover crop biomass was sampled in each trial since initiation. For the East and West experiments, four 76 × 48 cm quadrats were collected per plot before cover crop termination. For the Central experiments, cover crop biomass was sampled before cover crop termination using an 81 × 30 cm quadrat, with two quadrat samples per plot. Only two quadrats were used at this site because the plots were small and removing more biomass could affect the long-term plots' integrity. Biomass from all sites was dried at 60°C for at least 48 h and then weighed. Carbon-to-nitrogen ratios of the biomass were collected in select years, but due to the inconsistency of data collection those results are not presented here. No other cover crop metrics were collected (e.g., height, stand count, stage). Mean values at each trial for each year are available in **Supplementary Material** and in the published dataset (Nichols et al., 2020b).

Data Analysis

The raw dataset is available on Iowa State University's DataShare platform (Nichols et al., 2020b) and as an R package available on github (https://github.com/vanichols/PFIweeds2020). All data management, visualization, and statistical analyses were conducted using R version 3.6.1 (R Core Team, 2016); all code is publicly available (https://github.com/vanichols/PFIweeds2020_analysis). The *tidyverse* meta package (Wickham et al., 2019) was used for data manipulation and visualization, in addition to several other packages (Becker et al., 2018; Wickham and Bryan, 2018; Wilke, 2019). All packages used for statistical analyses are cited below.

Seedbank Size

The number of emerged seedlings was assumed to represent the seedbank density. The distribution of measured seedbank densities exhibited a high right-skewness and over-dispersion typical of count data. Several candidate statistical models were evaluated, and the detailed exploration process can be found in an online format (https://lydiae.com/2020/04/22/many-models/). We chose to use a generalized linear mixed-effect model (McCulloch and Neuhaus, 2005) using a log-linked Poisson distribution and observation-level random effects to account for overdispersion (Harrison, 2014), fit using the *glmer* function from the *lme4* package (Bates et al., 2015). We used the trial as a fixed effect, which had five levels (West, Central-grain/soybean, Central-grain/maize, Central-silage/soybean, East). Additionally, the cover crop treatment (cover, no-cover) and its interaction with the trial were included as fixed effects. In addition to the random intercept for each observation to address overdispersion, we included a random intercept term for the blocks nested within the trial. All pair-wise comparisons were conducted using the *emmeans* package (Lenth et al., 2018), which calculates the least-squares means and computes contrasts. Raw seedling counts were converted to seeds m^{-2} based on the PVC sampling tube diameter.

We ran a leave-one-out sensitivity analysis wherein the statistical model was run on datasets with one observation removed to explore the sensitivity of our results to any single experimental unit. One cover-cropped plot in the West location had a waterhemp [*Amaranthus tuberculatus* (Moq.)] seed count of more than 16,000 seeds m^{-2}, while the plot with the next highest observed waterhemp density at that site (a no-cover plot) was <10,000 seeds m^{-2}. We ran all models both with and without the outlier (**Supplementary Material**), and found it did affect the magnitude of the cover crop treatment effect in that experiment, but not the direction of the effect. We felt this large value may not be a realistic representation of the actual seed density in the plot, as the producer did not recall that plot having twice the weed biomass of other plots (*personal comm*). Due to the mixing of individual soil cores that was done in the field, it is not possible to isolate whether this large value was caused by a single core. Waterhemp plants grown in highly competitive environments can still produce 10,000 seeds (Schwartz et al., 2016), so it is conceivable we captured the seed rain from a single plant. We chose to present the results with the outlier removed as we felt it was more representative, but note the effect of the outlier when relevant throughout the results.

We used a first- and second-order stochastic dominance analysis to compare the cumulative distribution curves of seedbank size for no-cover and cover-cropped production systems (Levy, 1992). Stochastic dominance is a tool commonly used in risk-assessments to identify scenarios with a higher probability of favorable outcomes (e.g., Goplen et al., 2018). We assumed producers want to minimize the size of the weed seedbank, and therefore used the inverse of the cumulative probability distributions to assess outcomes from using a cover crop compared to no cover crop. Comparing the cumulative distributions at a given value of weed seedbank densities provides information concerning outcomes of a practice (first-order), while comparing the area under the cumulative probability curves provides information about the risk associated with a particular practice (second-order).

To quantify cover crop biomass production for each trial, we calculated different metrics to capture varying functional aspects of the cover crop that might affect weed seedbanks. Using cover crop biomass data from 2009 through 2019, we calculated the following metrics using both the previous 10 years of data and only the previous 5 years: (1) mean biomass production, (2) variance in biomass production, (3) maximum biomass production, (4) number of years with >1 Mg ha^{-1} production, (5) number of years with >2 Mg ha^{-1} production, (6) mean-to-standard-deviation ratio of biomass production (stability), and (7) biomass production the year of sampling, as well as the year prior.

We used non-parametric Spearman rank correlations to assess the association between the metrics listed above and cover crop effect on seedbank densities (relative and absolute).

Seedbank community composition

Changes in the weed seedbank community were assessed using both uni- and multivariate approaches. For the univariate approach, linear mixed-effect models with trial, cover crop treatment, and their interaction as fixed effects and random intercepts for nested blocks were used to assess the impact of cover cropping on seedbank diversity metrics. Our diversity metrics included species richness, Shannon Hill diversity and evenness (Jost, 2006) for each experimental unit (a plot) using the raw seedling counts and the following equations:

$$Shannon\ Hill\ diversity = exp(H')$$

$$Evenness = \frac{H'}{log(S)}$$

Where

$$S = species\ richness$$

$$H' = -\sum_{i}^{N} p_i log(p_i)$$

Evenness describes how a given species richness is distributed and ranges from 0 to 1, with 1 signifying all species are equally present. Shannon Hill diversity can be interpreted as the "effective" number of species; when evenness is 1, Shannon Hill diversity is equal to species richness.

For the multivariate approach, species composition was compared across trials and cover crop treatments using non-metric multidimensional scaling (NMDS) implemented through the *vegan* package (Oksanen et al., 2019). NMDS assists in visualizing and analyzing similarities between groups of individuals (Prentice, 1977). The removal of rare species from multi-variate analyses can impact interpretations (Poos and Jackson, 2012), so we performed all analyses on both the full dataset and on a dataset containing only the species comprising more than 5% of the observations and found the results did not change. Bray-Curtis dissimilarities were calculated on raw seed counts. Variation in distance matrices were partitioned into trial and cover crop contributions using permutations implemented through the *adonis* function of the *vegan* package. The *adonis* function works by creating permutations of the raw data wherein data is randomly assigned to a group. It finds the centroids and squared-deviations for each permutation, and by comparing those values to the raw data, the significance tests reflect the probability of observing the true data assuming no group structure exists.

RESULTS

Weed Seedbank Size

The West (previous crop of soybean) trial had the largest estimated mean seedbank size (5,647 seeds m^{-2}), followed by the Central-silage (previously soybean; 935 seeds m^{-2}), with the Central-grain (previously maize, soybean) and East (previously maize) locations having similarly low mean densities (382–482 seeds m^{-2}; **Figure 1**). Due to a significant interaction between trial and cover crop treatment, all results are reported on a per-trial basis.

In the three trials with a soybean crop the preceding year, the seedbank density was lower in the cover crop treatment compared to the no-cover treatment by 91 (Central-grain), 927 (Central-silage), and 4,070 (West) seeds m^{-2}, respectively, corresponding to a 17, 61, and 51% reduction. The magnitude of the West results were sensitive to the inclusion of the outlier (**Supplementary Material**), but the direction of the effect was not. In the trials previously planted to maize, seedbank densities in cover cropped plots were lower in one trial (East; reduced by 188 seeds m^{-2}, 35%) and increased in another (Central-grain; increased by 349 seeds m^{-2}, 134%).

Neither the absolute nor relative differences between the cover crop and control treatments were meaningfully related to any of the cover crop biomass metrics we calculated (**Supplementary Material**).

Weed Seedbank Community

A total of 4,677 seedlings were counted, consisting of 16 identified species (**Table 3**). Seven seedlings were identified as belonging to the *Setaria* genus, but the species was unclear. The species common to the Midwest, *Setaria faberi Herrm., Setaria viridis* (L.) P. Beauv, and *Setaria pumila* (Poir.) Roem. & Schult., for this reason we combined the *Setaria* seedlings into one category for reporting and analysis. Unidentified dicotyledon seedlings were classified as "unknown dicotyledon (UD)" and accounted for 8 of the seedlings, respectively, representing $<0.2\%$ of the data (**Table 3**). Because they made up such a small contribution to the overall community we left them in the analysis, but labeled as unknown.

We note that using the germination-method is known to cause varying underestimation of species. The method can bias counts toward species responsive to the particular conditions used, for example by specifically underestimating species with

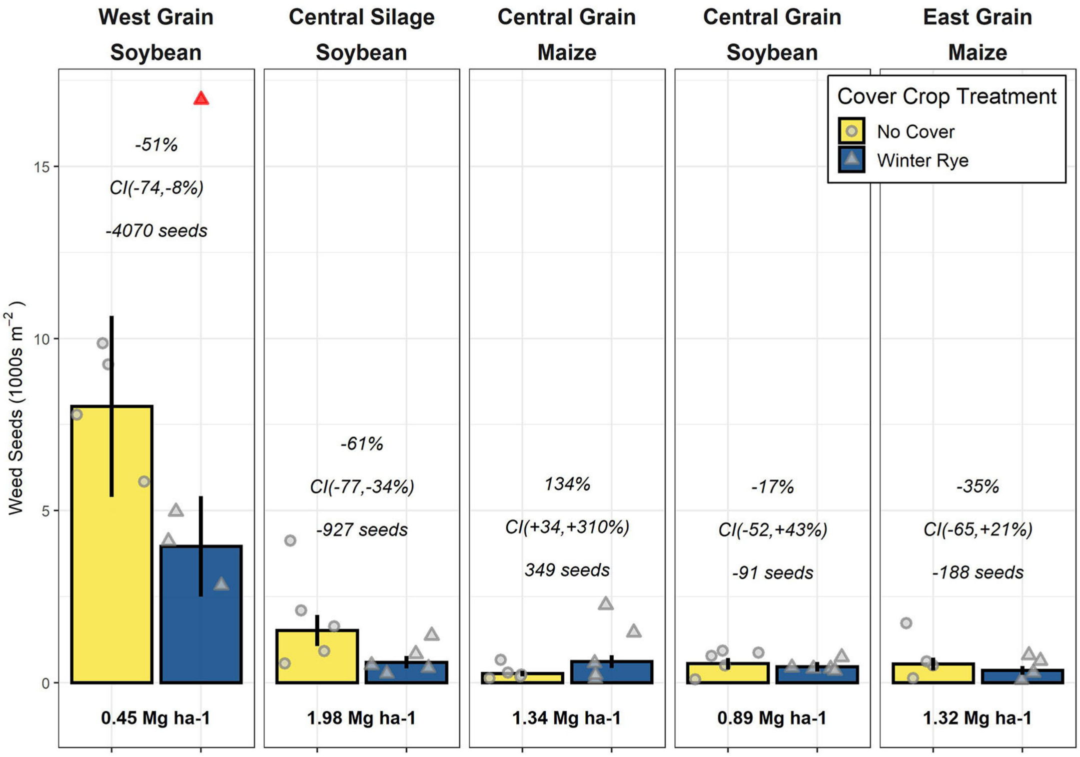

FIGURE 1 | Weed seed densities for no-cover and winter rye (*Secale cereale*) cover crop plots (points jittered for ease of viewing) with a removed outlier indicated in red; panel titles present the trial location, cropping system type, and previous year's cash crop; bars represent mean seed densities with standard errors of the means indicated by the vertical lines; italicized text presents the (*top*) estimated change from no-cover to cover treatments within a trial on a relative scale, (*middle*) 90% confidence intervals (CIs) of the relative change, and (*bottom*) the absolute difference in seeds per m^{-2}. Bold text below bars presents the 10-years mean cover crop biomass production for that trial.

long seed dormancies or seeds that were not sufficiently stratified the previous winter (Gross, 1990).

The changes in seedbanks were driven by changes in the number of waterhemp (*Amaranthus tuberculatus,* AMATU) seeds (**Figure 2**).

Differences in seedbank composition were strongest at the trial level ($p < 0.01$) and were not statistically significant for cover crop treatment. In the trials with significant cover cropping effects, community changes were again driven mainly by a decrease in waterhemp (**Figures 2**, **3**) which resulted in a slight shift toward a more grass-dominated community, but the effect was not strong. Community shifts were not consistently associated with an increase in the Shannon-Hill diversity index, species evenness, nor species richness (**Table 4**), but these results must be taken in context of the herbicide programs (**Table 2**).

Risk of Increasing Seedbanks

Results from the stochastic dominance analysis indicate that at low weed seedbank densities (<300 seeds m^{-2}), cover cropping and control treatments did not differ, whereas at higher weed seedbank densities (>300 seeds m^{-2}), cover cropping consistently exhibited lower densities than the no-cover treatments (**Figure 4**).

DISCUSSION

Cover Crop Interactions With Waterhemp

As is the case in many midwestern maize and soybean fields, waterhemp was the driver weed species in the locations sampled in this study (van Wychen, 2017, 2019). Due to the dominance of waterhemp in the weed communities of this study, the herbicide programs implemented at each trial may have provided contexts where cover crop effects on weeds would vary. Waterhemp populations with resistance to seven herbicide groups have been identified (Tranel, 2020), with populations resistant to one or more are prevalent in the midwest (Patzoldt et al., 2005; Chatham et al., 2015; Oliveira et al., 2017). While we did not measure the presence of resistance genes, resistances to herbicide groups 5, 9, 14, and 27 were likely present (Owen, 2017) with possible resistances to group 15 (Hager, 2019). The varying levels of waterhemp control via herbicides and the use of residuals in the different trials may have rendered cover cropping more or less effective (**Table 2**). The West site, where the largest absolute reduction in waterhemp with the use of cover cropping was observed (**Figure 1**), did not utilize chemistries that would reliably kill resistant waterhemp plants that had already emerged, nor a residual herbicide to suppress future waterhemp emergence. Of the sites included in this study, the West site was

TABLE 3 | Summary of weed species identified in this study in order of prevalence.

Code	Scientific name	Common name	Description	Percent of total found
AMATU	*Amaranthus tuberculatus* (Moq.) J. D. Sauer	Waterhemp	C_4 forb	88.58%
CHEAL	*Chenopodium album* L.	Lamb's quarters	C_3 forb	6.67%
POROL	*Portulaca oleracea* L.	Purslane	C_4 grass	1.28%
DIGSA	*Digitaria sanguinalis* (L.) Scop.	Large crabgrass	C_4 grass	1.00%
SETARIA[a]	*Setaria faberi* Herrm., *Setaria viridis* (L.) P. Beauv., *Setaria pumila* (Poir.) Roem. & Schult., unknown species	Foxtail	C_4 grass	0.56%
EPHMA	*Euphorbia maculata* L.	Spotted spurge	C_4 forb	0.41%
PLAMA	*Plantago major* L.	Plantain	C_3 forb	0.41%
SOPT7	*Solanum ptychanthum* Dunal	Eastern black nightshade	C_3 forb	0.34%
ERICA	*Erigeron canadensis* L.	Horseweed	C_3 forb	0.32%
UD[b]	-	-	-	0.17%
POLAV	*Polygonum aviculare* L.	Prostrate knotweed	C_3 forb	0.11%
SECCE	*Secale cereale* L.	Cereal rye	C_3 grass	<0.10%
ABUTH	*Abutilon theophrasti* Medik.	Velvet leaf	C_3 forb	<0.10%
OXAST	*Oxalis stricta* L.	Yellow woodsorrel	C_3 forb	<0.10%
RAPSR	*Raphanus sativus* L.	Radish	C_3 forb	<0.10%
TAROF	*Taraxacum officinale* F. H. Wigg.	Dandelion	C_3 forb	<0.10%

[a] *Seedlings identified as belonging to the Setaria genus were combined.*
[b] *Unknown dicot.*

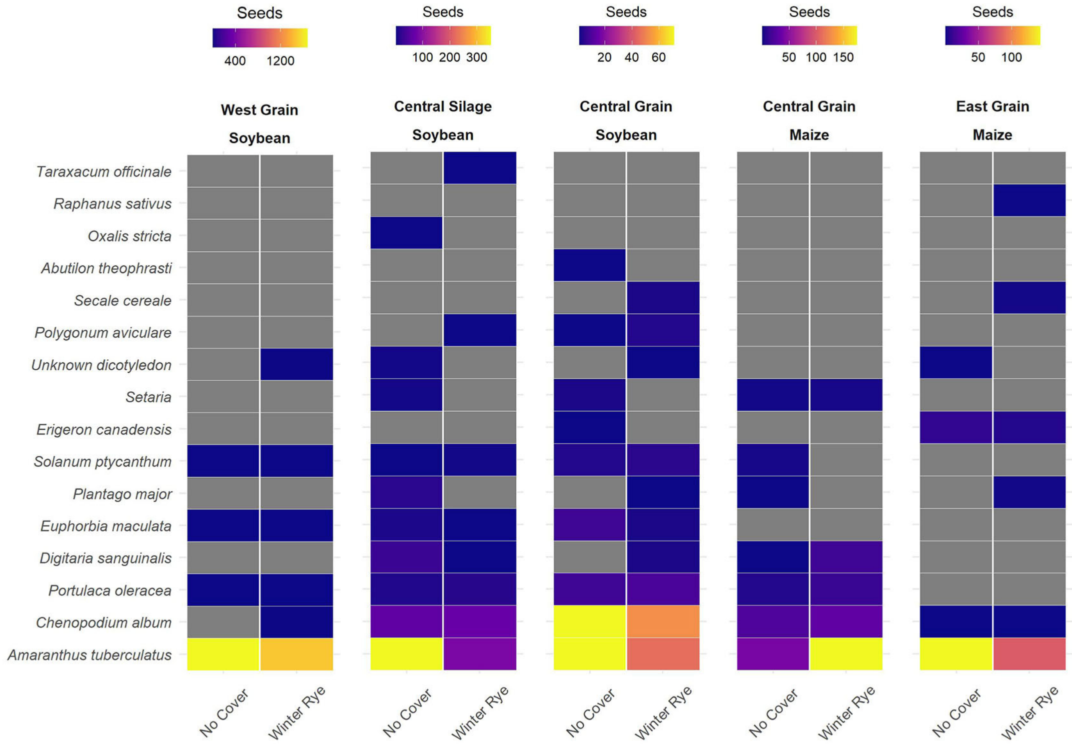

FIGURE 2 | Differences in winter rye (*Secale cereal*) cover crop and no-cover treatments were mainly observed in common waterhemp (*Amaranthus tuberculatus* [Moq.]), with the cover crop treatment having lower numbers of waterhemp seeds in four of the five trials sampled.

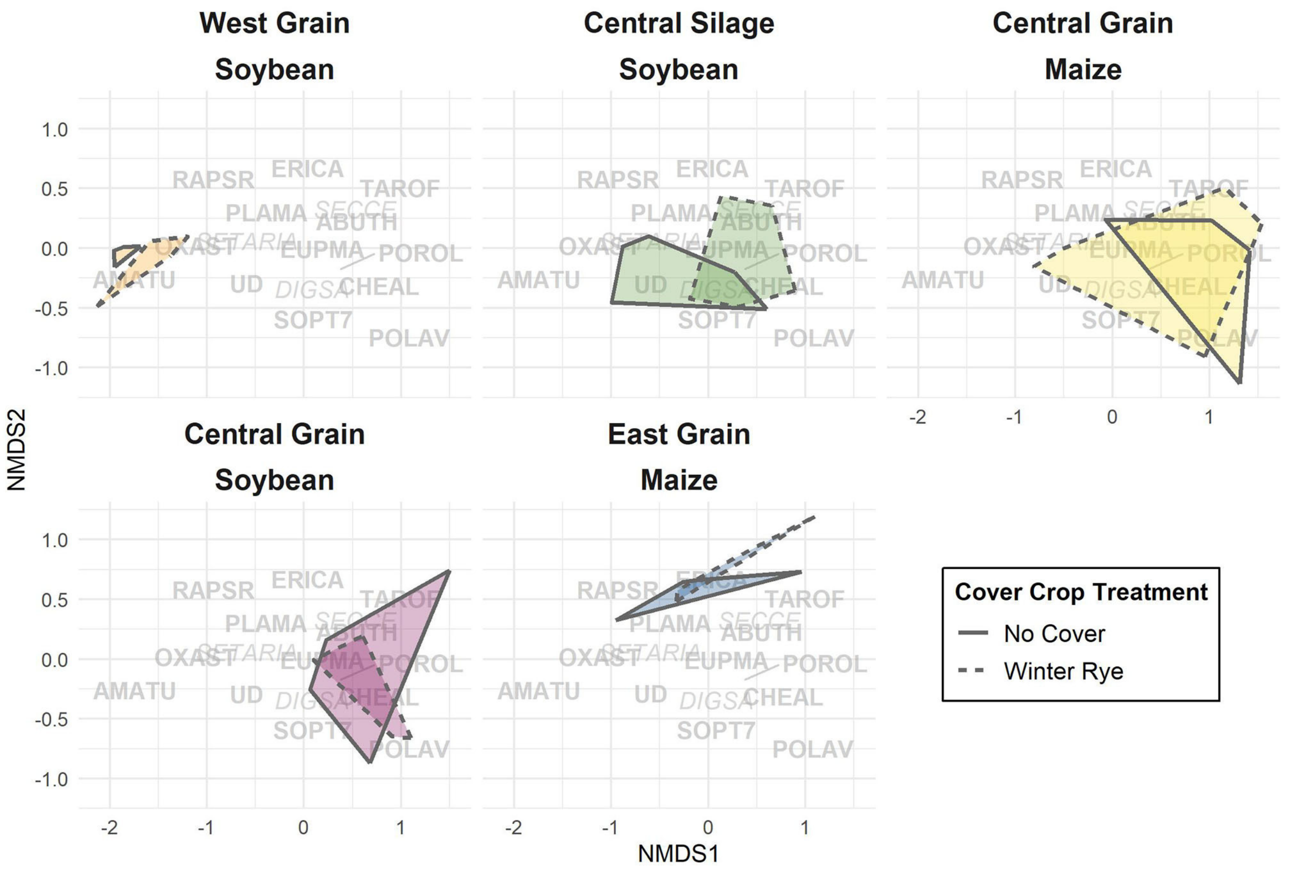

FIGURE 3 | Non-metric multidimensional scaling (NMDS, stress = 0.102) applied to weed seedbank communities. Overlap of regions indicates degree of similarity between communities. Text represents Bayer weed codes (italics: monocots, bold: dicots; **Table 3**) and their NMDS component weightings. Trials are separated for ease of viewing.

TABLE 4 | Estimated changes, standard errors (SE), and *p*-values for models of changes in diversity, richness, and evenness for each trial.

	Shannon Hill diversity		**Richness**		**Evenness**	
	Change (SE)	***P*-value**	**Change (SE)**	***P*-value**	**Change (SE)**	***P*-value**
West-grain (Soybean)	0.05 (0.49)	0.92	***1.5 (0.86)***	***0.09***	0.03 (0.13)	0.81
Central-silage (Soybean)	−0.22 (0.43)	0.51	***−2.2 (0.77)***	***0.01***	***0.18 (0.10)***	***0.10***
Central-grain (Maize)	−0.33 (0.43)	0.45	0.6 (0.77)	0.44	−0.15 (0.10)	0.13
East-grain (Maize)	0.18 (0.48)	0.38	0.8 (0.87)	0.39	0.03 (0.11)	0.82
Central-grain (Soybean)	0.42 (0.43)	0.97	0.4 (0.77)	0.60	0.03 (0.10)	0.78

Estimate values show expected changes with the inclusion of a cover crop (ex. positive values indicate that metric increased with the inclusion of a cover crop). Trials are listed in descending order of absolute change in seedbank size with cover cropping. Significant differences at $p < 0.10$ are indicated with bold italics.

therefore most susceptible to waterhemp living and setting seed, and therefore may have provided the biggest opportunities for cover cropping effects to manifest. The Central trials utilized hand-weeding in soybean phases late in the season, which may have reduced the opportunities for cover crops effects to be expressed. The East site, which had the lowest average seedbank densities observed in this study, utilized a herbicide program that would control resistant waterhemp biotypes and included residual herbicide that would also reduce/delay waterhemp emergence, perhaps leaving little room for cover cropping effects. While the previous crop of the individual trials may also play a role in dictating the weed responses to cover cropping, in the present study the previous crop is confounded with site effects, so it is difficult to draw conclusions from what may be spurious associations.

Regardless of the mechanisms involved, we believe our results regarding reductions in waterhemp seed densities are robust. Under no-till management and in the absence of new inputs to the seedbank, waterhemp seed densities can decline >99% after 5 years (Steckel et al., 2007). Our plots

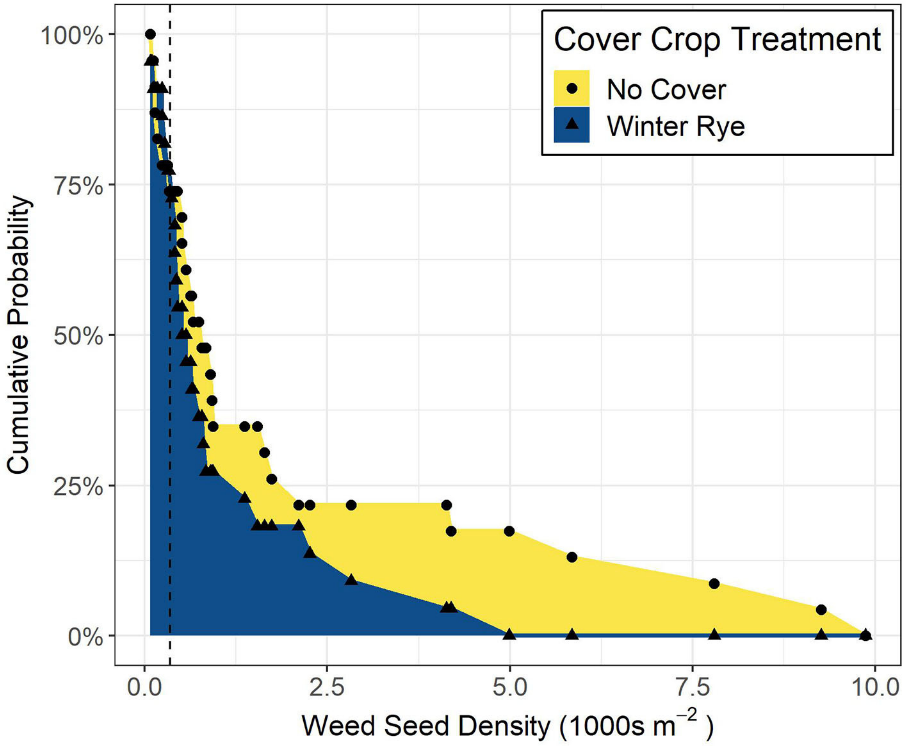

FIGURE 4 | Each point represents the cumulative probability of having a weed seedbank of that density or higher; as seedbank densities increase above 300 seeds m^{-2} (dashed line), the probability of having a larger seedbank is higher for no-cover systems compared to cover-cropped systems; the area under the curve is proportional to the risk of increasing weed seedbank densities.

have been in place long enough (>10 years) for treatment effects to be detected despite possible legacy effects of the original seedbanks.

Cover Crop Mechanisms of Weed Suppression

Previous research indicates a cover crop's potential for in-season weed suppression is strongly related to the cover crop's biomass production (Baraibar et al., 2018; MacLaren et al., 2019; Nichols et al., 2020a; Smith et al., 2020). Cover crops might reduce weed seedbank densities via several mechanisms, all of which could conceivably be intensified with increases in the quantity of cover crop biomass produced. It is thus surprising that in the present study neither the absolute nor relative effects of cover cropping on the weed seedbank was related to any of the cover crop biomass production metrics we evaluated. For example, while the West trial consistently produced <1 Mg ha^{-1} of cover crop biomass, it exhibited the largest absolute decreases in weed seedbank size from cover cropping (**Figure 1**). Even considering the herbicide program, it is surprising such small amounts of cover crop biomass could have meaningful effects on the weed seedbank.

The pattern in the present study might also be related to the emergence timing of waterhemp, which can extend well-beyond the time the cover crop is killed while maintaining high reproductive success (Wu and Owen, 2014). In other studies, weed communities may not have been dominated by late germinating weed species such as waterhemp, and the communities might have therefore been more directly responsive to cover crop biomass.

It is also possible cover crops affected weeds in ways less directly dependent upon the amount of cover crop biomass produced. The act of planting the cover crop itself may have provided some weed control. Additionally, even modest amounts of biomass present over the winter may provide enough ground cover to promote seed mortality through granivore activity (Carmona and Landis, 1999; Heggenstaller et al., 2006), and in the spring cover crop mulch can provide habitat for seed-eating invertebrates (Pullaro et al., 2006). Allelopathic compounds from rye residue may catalyze pathogen attack on seeds and reduce the vigor of germinated seeds (Barnes and Putnam, 1983; Mohler et al., 2012), and production of these compounds may be more dependent upon growing conditions compared to rye biomass production *per se* (Mwaja et al., 1995). While our study did not test these effects directly, our data suggest these mechanisms should be considered when assessing the effects of cover cropping on weed communities.

We note that with endpoint sampling, as we did in the present study, it is difficult to link the cumulative effect of 10 years of biomass production with one season's weed seedbank. Sampling weed seedbanks yearly would enable a more direct connection to be drawn between cover crop biomass production and seedbank densities. However, our data show that in the contexts we sampled, the weed suppressive potential of cover crops in the long-term was not directly related to cover crop biomass production.

Cover Crop Effects on Weed Seed Communities

The lack of a consistent and significant effect of cover cropping on the structure of the weed seed communities in the present study is consistent with the findings of other studies (Moonen and Bàrberi, 2004; Smith et al., 2015; Alonso-Ayuso et al., 2018). It is unsurprising that an over-wintering cover crop would be a weak filter in systems dominated by summer annuals that are well-adapted for regeneration in maize-soybean rotations (Tranel, 2020). In systems with more diverse cropping systems or seedbanks, cover crops might create more marked shifts in weed communities.

While the germination method may have failed to identify or underestimated weed species resulting in an underestimation of weed species richness, the number of weed species found in each plot (ranging from 1 to 8) matches field-based observations of maize-soybean rotations (Hirsh et al., 2013). Additionally, the dominance of common waterhemp rendered the Shannon Hill diversity and evenness metrics insensitive to small contributions by other species. Accordingly, our results may be due to the already-simplified nature of the communities, where random variation easily obscures subtle signals in the less prevalent species.

CONCLUSIONS

Our study is the first we are aware of that quantifies the long-term impacts of cover cropping on weeds in the midwestern United States. We found evidence that cover cropping can meaningfully reduce the size of the weed seedbank compared to a no-cover control in certain contexts. More research in long-term plots comparing cover crop effects in various cropping systems and management regimes is needed to identify conditions in which cover crops are most effective at reducing and/or preventing weed seed deposits. Endpoint sampling, used in our study, is useful in assessing whether systems merit more attention, but longitudinal samplings of weed seedbanks in long-term studies are needed to better assess the seedbank trajectories of these systems.

Changes in seedbanks were driven by change in densities of common waterhemp, a weed resistant to multiple herbicide modes of actions. We found that when weed seed densities are above 300 seeds m^{-2}, cover cropping exhibits no risk of enlarging weed seedbanks compared to no-cover systems.

In the production contexts examined, the amount of cover crop biomass produced was not associated with the magnitude of cover crop effects on weed seedbanks. The lack of relationship suggests cover crop biomass may not be the best metric for predicting long-term impacts of cover-cropping on weeds in all systems, particularly those dominated by late-germinating species such as waterhemp. Cover crops may suppress weeds through a combination of mechanisms, and the relative contribution likely varies by site and/or year. Parsing out these effects could aid in the design of systems better able to take advantage of cover crop weed suppression.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in csv format in on online repository accessed via https://doi.org/10.25380/iastate.12762011.v1 and as an R package at https://github.com/vanichols/PFIweeds2020.

AUTHOR CONTRIBUTIONS

VN, ML, and SC designed the study. SG and SC contributed data and project management. VN facilitated data collection and wrote the original draft of the manuscript. VN, ML, and LE analyzed the data. All authors contributed to editing of the final manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2020.591091/full#supplementary-material

REFERENCES

Abendroth, L., Elmore, R., Boyer, M., and Marlay, S. (2011). *Corn Growth and Development.* Available online at: https://www.researchgate.net/profile/Lori_Abendroth/publication/280092215_In_Corn_Growth_and_Development/links/5a984384a6fdccecff0d2320/In-Corn-Growth-and-Development.pdf (accessed July 20, 2020).

Adeux, G., Vieren, E., Carlesi, S., Bàrberi, P., Munier-Jolain, N., and Cordeau, S. (2019). Mitigating crop yield losses through weed diversity. *Nat. Sustain*. 211, 1018–1026. doi: 10.1038/s41893-019-0415-y

Alonso-Ayuso, M., Gabriel, J. L., García-González, I., Del Monte, J. P., and Quemada, M. (2018). Weed density and diversity in a long-term cover crop experiment background. *Crop Prot*. 112, 103–111. doi: 10.1016/j.cropro.2018.04.012

Arbuckle, J. G., and Lasley, P. (2013). *Iowa Farm and Rural Life Poll: 2013, Summary Report*. Ames, IA: Iowa State University Extension and Outreach.

Baraibar, B., Mortensen, D. A., Hunter, M. C., Barbercheck, M. E., Kaye, J. P., Finney, D. M., et al. (2018). Growing degree days and cover crop type explain weed biomass in winter cover crops. *Agron. Sustain. Dev*. 38:65. doi: 10.1007/s13593-018-0543-1

Barnes, J. P., and Putnam, A. R. (1983). Rye residues contribute weed suppression in no-tillage cropping systems. *J. Chem. Ecol*. 9, 1045–1057. doi: 10.1007/BF00982210

Basche, A. D., Archontoulis, S. V., Kaspar, T. C., Jaynes, D. B., Parkin, T. B., and Miguez, F. E. (2016a). Simulating long-term impacts of cover crops and climate change on crop production and environmental outcomes in the Midwestern United States. *Agric. Ecosyst. Environ*. 218, 95–106. doi: 10.1016/j.agee.2015.11.011

Basche, A. D., Kaspar, T. C., Archontoulis, S. V., Jaynes, D. B., Sauer, T. J., Parkin, T. B., et al. (2016b). Soil water improvements with the long-term use of a winter rye cover crop. *Agric. Water Manag*. 172, 40–50. doi: 10.1016/j.agwat.2016.04.006

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw*. 67, 1–48. doi: 10.18637/jss.v067.i01

Becker, R. A., Wilks, A. R., Brownrigg, R., Minka, T. P., and Deckmyn, A. (2018). *maps: Draw Geographical Maps.* Available online at: https://rdrr.io/cran/maps/man/map.html (accessed October 2020).

Bunchek, J. M., Wallace, J. M., Curran, W. S., Mortensen, D. A., vanGessel, M. J., and Scott, B. A. (2020). Alternative performance targets for integrating cover crops as a proactive herbicide-resistance management tool. *Weed Sci*. 68, 534–544. doi: 10.1017/wsc.2020.49

Carmona, D. M., and Landis, D. A. (1999). Influence of refuge habitats and cover crops on seasonal activity-density of ground beetles (Coleoptera: Carabidae) in field crops. *Environ. Entomol*. 28, 1145–1153. doi: 10.1093/ee/28.6.1145

Chatham, L. A., Bradley, K. W., Kruger, G. R., Martin, J. R., Owen, M. D. K., Peterson, D. E., et al. (2015). A multistate study of the association between glyphosate resistance and EPSPS gene amplification in waterhemp (*Amaranthus tuberculatus*). *Weed Sci*. 63, 569–577. doi: 10.1614/WS-D-14-00149.1

Cholette, T. B., Soltani, N., Hooker, D. C., Robinson, D. E., and Sikkema, P. H. (2018). Suppression of glyphosate-resistant Canada fleabane (*Conyza canadensis*) in corn with cover crops seeded after wheat harvest the previous year. *Weed Technol*. 32, 244–250. doi: 10.1017/wet.2018.19

Davis, A. S. (2006). When does it make sense to target the weed seed bank? *Weed Sci*. 54, 558–565. doi: 10.1614/WS-05-058R.1

Dessaint, F., Barralis, G., Caixinhas, M. L., Mayor, J. P., Recasens, J., and Zanin, G. (1996). Precision of soil seedbank sampling: how many soil cores? *Weed Res*. 36, 143–151. doi: 10.1111/j.1365-3180.1996.tb01810.x

Dold, C., Sauer, T. J., Hatfield, J. L., Prueger, J. H., and Wacha, K. M. (2017). "*Changes in soil carbon in a continuous corn-soybean rotation in the Midwest, 2005–2016*," in Proceedings of ASA-CSSA-SSSA Annual Meeting (Tampa, FL).

Felix, J., and Owen, M. D. K. (2001). Weed seedbank dynamics in post conservation reserve program land. *Weed Sci*. 49, 780–787. doi: 10.1614/0043-1745(2001)0490780:WSDIPC2.0.CO;2

Food and Agriculture Organization of the United Nations. *FAOSTAT Statistical Database.* Rome: FAO (2020).

Forcella, F., Webster, T.M., and Cardina, J. (2003). "Protocols for weed seedbank determination in agroecosystems," in *Addendum to Weed Management for Developing Countries*, Vol. 120, ed R. Labrada (Rome: FAO), 3–18.

Forcella, F., Wilson, R. G., Renner, K. A., Dekker, J., Harvey, R. G., Alm, D. A., et al. (1992). Weed seedbanks of the U.S. Corn Belt: magnitude, variation, emergence, and application. *Weed Sci*. 40, 636–644. doi: 10.1017/S0043174500058240

Goplen, J. J., Coulter, J. A., Sheaffer, C. C., Becker, R. L., Breitenbach, F. R., Behnken, L. M., et al. (2018). Economic performance of crop rotations in the presence of herbicide-resistant giant ragweed. *Agron. J*. 110, 260–268. doi: 10.2134/agronj2016.09.0536

Gross, K. L. (1990). A comparison of methods for estimating seed numbers in the soil. *J. Ecol*. 78, 1079–1093. doi: 10.2307/2260953

Hager, A. (2019) *Waterhemp Resistance to Group 15 Herbicides. Department of Crop Sciences, University of Illinois at Urbana-Champaign.* Available online at: https://farmdoc.illinois.edu/field-crop-production/weeds/waterhemp-resistance-to-group-15-herbicides.html (accessed October 2020).

Harrison, X. A. (2014). Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ* 2:e616. doi: 10.7717/peerj.616

Heggenstaller, A. H., Menalled, F. D., Liebman, M., and Westerman, P. R. (2006). Seasonal patterns in post-dispersal seed predation of *Abutilon theophrasti* and *Setaria faberi* in three cropping systems. *J. Appl. Ecol*. 43, 999–1010. doi: 10.1111/j.1365-2664.2006.01198.x

Hirsh, S. M., Mabry, C. M., Schulte, L. A., and Liebman, M. (2013). Diversifying agricultural catchments by incorporating tallgrass prairie buffer strips. *Ecol. Restor*. 31, 201–211. doi: 10.3368/er.31.2.201

Jones, C. S., Nielsen, J. K., Schilling, K. E., and Weber, L. J. (2018). Iowa stream nitrate and the Gulf of Mexico. *PLoS ONE* 13:e0195930. doi: 10.1371/journal.pone.0195930

Jost, L. (2006). Entropy and diversity. *Oikos* 113, 363–375. doi: 10.1111/j.2006.0030-1299.14714.x

Kaspar, T., and Singer, J. (2011). *The Use of Cover Crops to Manage Soil*. Lincoln, NE: University of Nebraska - Lincoln. doi: 10.2136/2011.soilmanagement.c21

Kaspar, T. C., Jaynes, D. B., Parkin, T. B., and Moorman, T. B. (2007). Rye cover crop and gamagrass strip effects on NO_3 concentration and load in tile drainage. *J. Environ. Qual*. 36, 1503–1511. doi: 10.2134/jeq2006.0468

Kaspar, T. C., Jaynes, D. B., Parkin, T. B., Moorman, T. B., and Singer, J. W. (2012). Effectiveness of oat and rye cover crops in reducing nitrate losses in drainage water. *Agric. Water Manag*. 110, 25–33. doi: 10.1016/j.agwat.2012.03.010

Lenth, R., Singmann, H., and Love, J. (2018). *Emmeans: Estimated Maringal means, Aka Least-Squares Means.* Available online at: https://rdrr.io/cran/emmeans/ (accessed October 2020).

Levy, H. (1992). Stochastic dominance and expected utility: survey and analysis. *Manage. Sci*. 38, 555–593. doi: 10.1287/mnsc.38.4.555

MacLaren, C., Storkey, J., Menegat, A., Metcalfe, H., and Dehnen-Schmutz, K. (2020). An ecological future for weed science to sustain crop production and the environment. A review. *Agron. Sustain. Dev*. 40:24. doi: 10.1007/s13593-020-00631-6

MacLaren, C., Swanepoel, P., Bennett, J., Wright, J., and Dehnen-Schmutz, K. (2019). Cover crop biomass production is more important than diversity for weed suppression. *Crop Sci*. 59, 733–748. doi: 10.2135/cropsci2018.05.0329

McCulloch, C. E., and Neuhaus, J. M. (2005). *Generalized Linear Mixed Models. Encyclopedia of Biostatistics.* Chichester, UK: John Wiley & Sons, Ltd. doi: 10.1002/0470011815.b2a10021

Mirsky, S. B., Gallandt, E. R., Mortensen, D. A., Curran, W. S., and Shumway, D. L. (2010). Reducing the germinable weed seedbank with soil disturbance and cover crops. *Weed Res*. 50, 341–352. doi: 10.1111/j.1365-3180.2010.00792.x

Mohler, C. L. (1993). A model of the effects of tillage on emergence of weed seedlings. *Ecol. Appl*. 3, 53–73. doi: 10.2307/1941792

Mohler, C. L., Dykeman, C., Nelson, E. B., and Ditommaso, A. (2012). Reduction in weed seedling emergence by pathogens following the incorporation of green crop residue. *Weed Res*. 52, 467–477. doi: 10.1111/j.1365-3180.2012.00940.x

Moonen, A. C., and Bàrberi, P. (2004). Size and composition of the weed seedbank after 7 years of different cover-crop-maize management systems. *Weed Res*. 44, 163–177. doi: 10.1111/j.1365-3180.2004.00388.x

Moore, E. B., Wiedenhoeft, M. H., Kaspar, T. C., and Cambardella, C. A. (2014). Rye cover crop effects on soil quality in no-till corn silage-soybean cropping systems. *Soil Sci. Soc. Am. J.* 78, 968–976. doi: 10.2136/sssaj2013.09.0401

Mwaja, V. N., Masiunas, J. B., and Weston, L. A. (1995). Effects of fertility on biomass, phytotoxicity, and allelochemical content of cereal rye. *J. Chem. Ecol.* 21, 81–96. doi: 10.1007/BF02033664

Nearing, M. A., Xie, Y., Liu, B., and Ye, Y. (2017). Natural and anthropogenic rates of soil erosion. *Int. Soil Water Conserv. Res.* 5, 77–84. doi: 10.1016/j.iswcr.2017.04.001

Nichols, V., English, L. E., and Liebman, M. (2020b). *Long Term Cover Cropping Effects on Weed Seedbanks* [Dataset]. Iowa State University. doi: 10.25380/iastate.12762011.v1

Nichols, V., Martinez-Feria, R., Weisberger, D., Carlson, S., Basso, B., and Basche, A. (2020a). Cover crops and weed suppression in the U.S. Midwest: a meta-analysis and modeling study. *Agric. Environ. Lett.* 5:e20022. doi: 10.1002/ael2.20022

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGline, D., et al. (2019). *vegan: Community Ecology Package*. Available online at: https://cran.r-project.org/package=vegan (accessed October 2020).

Oliveira, M. C., Jhala, A. J., Gaines, T., Irmak, S., Amundsen, K., Scott, J. E., et al. (2017). Confirmation and control of HPPD-inhibiting herbicide–resistant waterhemp (*Amaranthus tuberculatus*) in Nebraska. *Weed Tech.* 31, 67–79. doi: 10.1017/wet.2016.4

O'Neal, M. R., Nearing, M. A., Vining, R. C., Southworth, J., and Pfeifer, R. A. (2005). Climate change impacts on soil erosion in Midwest United States with changes in crop management. *Catena* 61, 165–184. doi: 10.1016/j.catena.2005.03.003

Owen, M. D. (2017). "Weed management update for 2018 and beyond: the more things change," in *Proceedings of the 29th Annual Integrated Crop Management Conference* (Ames, IA: Iowa State University). doi: 10.31274/icm-180809-242

Patzoldt, W. L., Tranel, P. J., and Hager, A. G. (2005). A waterhemp (*Amaranthus tuberculatus*) biotype with multiple resistance across three herbicide sites of action. *Weed Sci.* 53, 30–36. doi: 10.1614/WS-04-087R

Poos, M. S., Jackson, D. A. (2012). Addressing the removal of rare species in multivariate bioassessments: the impact of methodological choices. *Ecol. Ind.* 18, 82–90. doi: 10.1016/j.ecolind.2011.10.008

Prentice, I. C. (1977). Non-metric ordination methods in ecology. *J. Ecol.* 85–94. doi: 10.2307/2259064

Price, A. J., Balkcom, K. S., Culpepper, S. A., Kelton, J. A., Nichols, R. L., and Schomberg, H. (2011). Glyphosate-resistant Palmer amaranth: a threat to conservation tillage. *J. Soil Water Conserv.* 66, 265–275. doi: 10.2489/jswc.66.4.265

Pullaro, T. C., Marino, P. C., Jackson, D. M., Harrison, H. F., and Keinath, A. P. (2006). Effects of killed cover crop mulch on weeds, weed seeds, and herbivores. *Agric. Ecosyst. Environ.* 115, 97–104. doi: 10.1016/j.agee.2005.12.021

R Core Team (2016). *R: A Language and environment for statistical computing, Vienna, Austria*. Available online at: https://www.R-project.org/ (accessed October 2020).

Reinhardt, T., and Leon, R. G. (2018). Extractable and germinable seedbank methods provide different quantifications of weed communities. *Weed Sci.* 66, 715–720. doi: 10.1017/wsc.2018.56

Ryan, M. R., Smith, R. G., Mirsky, S. B., Mortensen, D. A., and Seidel, R. (2010). Management filters and species traits: weed community assembly in long-term organic and conventional systems. *Weed Sci.* 58, 265–277. doi: 10.1614/WS-D-09-00054.1

Schwartz, L. M., Norsworthy, J. K., Young, B. G., Bradley, K. W., Kruger, G. R., Davis, V. M., et al. (2016). Tall waterhemp (*Amaranthus tuberculatus*) and Palmer amaranth (*Amaranthus palmeri*) seed production and retention at soybean maturity. *Weed Technol.* 30, 284–290. doi: 10.1614/WT-D-15-00130.1

Smith, R. G., Atwood, L. W., Pollnac, F. W., and Warren, N. D. (2015). Cover-crop species as distinct biotic filters in weed community assembly. *Weed Sci.* 63, 282–295. doi: 10.1614/WS-D-14-00071.1

Smith, R. G., Warren, N. D., and Cordeau, S. (2020). Are cover crop mixtures better at suppressing weeds than cover crop monocultures? Weed Sci. 68, 186–194. doi: 10.1017/wsc.2020.12

Steckel, L. E., Sprague, C. L., Stoller, E. W., Wax, L. M., and Simmons, F. W. (2007). Tillage, cropping system, and soil depth effects on common waterhemp (*Amaranthus rudis*) seed-bank persistence. *Weed Sci.* 55, 235–239. doi: 10.1614/WS-06-198

Storkey, J., and Neve, P. (2018). What good is weed diversity? *Weed Res.* 58, 239–243. doi: 10.1111/wre.12310

Strock, J. S., Porter, P. M., and Russelle, M. P. (2004). Cover cropping to reduce nitrate loss through subsurface drainage in the northern US Corn Belt. *J. Environ. Qual.* 33, 1010–1016. doi: 10.2134/jeq2004.1010

Teasdale, J. R., Mirsky, S. B., and Cavigelli, M. A. (2018). Meteorological and management factors influencing weed abundance during 18 years of organic crop rotations. *Weed Sci.* 66, 477–484. doi: 10.1017/wsc.2018.15

Tranel, P. J. (2020) *Herbicide Resistance in Amaranthus tuberculatus.* Pest Manag Sci,.

USDA. (2020a). *National Agricultural Statistics Service Cropland Data Layer.* Published crop-specific data layer [Online]. Washington, DC: USDA-NASS. Available online at: https://nassgeodata.gmu.edu/CropScape/ (accessed September 2020).

USDA. (2020b). *National Agricultural Statistics Service.* NASS - Quick Stats. USDA National Agricultural Statistics Service. Washington, DC: USAD-NASS. (accessed September 2020).

van Wychen, L. (2017) Survey of the most common and troublesome weeds in grass crops, pasture and turf in the United States and Canada. *Weed Science Society of America National Weed Survey Dataset.* Available online at: http://wssa.net/wp-content/uploads/2017-Weed-Survey_Grass-crops.xlsx (accessed October 2020).

van Wychen, L. (2019). *WSSA Survey Ranks Most Common and Most Troublesome Weeds in Broadleaf Crops, Fruits and Vegetables.* Available online at: http://wssa.net/wp-content/uploads/2019-Weed-Survey_broadleaf-crops.xlsx (accessed October 2020).

Wallace, J. M., Curran, W. S., and Mortensen, D. A. (2019). Cover crop effects on horseweed (*Erigeron canadensis*) density and size inequality at the time of herbicide exposure. *Weed Sci.* 67, 327–338. doi: 10.1017/wsc.2019.3

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., Francois, R., et al. (2019). Welcome to the Tidyverse. *J. Open Source Softw.* 4:1686. doi: 10.21105/joss.01686

Wickham, H., and Bryan, J. (2018). *readxl: Read Excel Files*. Available online at: https://cran.r-project.org/package=readxl (accessed October 2020).

Wilke, C. (2019). *cowplot: Streamlined Plot Theme and Plot Annotations for "ggplot2"*. Available online at: https://rdrr.io/github/wilkelab/cowplot/man/cowplot.html (accessed October 2020).

Wu, C., and Owen, M. D. (2014) When is the best time to emerge: reproductive phenology and success of natural common waterhemp (Amaranthus rudis) cohorts in the Midwest United States?. *Weed Sci.* 62, 107–117. doi: 10.1614/WS-D-13-00079.1

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Hybridization Slows Rate of Evolution in Crop-Wild Compared to Wild Populations of Weedy *Raphanus* Across a Moisture Gradient

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Hybrid offspring of crops and their wild relatives commonly possess non-adaptive phenotypes and diminished fitness. Regularly, diminished success in early-generation hybrid populations is interpreted to suggest reduced biosafety risk regarding the unintended escape of novel traits from crop populations. Yet hybrid populations have been known to evolve to recover fitness relative to wild progenitors and can do so more rapidly than wild populations, although rates of evolution (for both hybrid populations and their wild progenitors) are sensitive to environmental context. In this research, we asked whether hybrid populations evolved more rapidly than wild populations in the context of soil moisture. We estimated evolutionary rates for 40 *Raphanus* populations that varied in their history of hybridization and environmental context (imposed by an experimental moisture cline) in two common gardens. After five generations of growing wild and crop-wild hybrid populations across a soil-moisture gradient, hybrid populations exhibited increased seedling emergence frequencies (∼6% more), earlier emergence (∼1 day), later flowering (∼3 days), and larger body size (15–35%)—traits correlated with fitness—relative to wild populations. Hybrid populations, however, exhibited slower evolutionary rates than wild populations. Moreover, the rate of evolution in hybrid populations was consistent across evolutionary watering environments, but varied across watering environments in wild populations. These consistent evolutionary rates exhibited in hybrid populations suggests the evolution of robust traits that perform equally across soil moisture environments—a survival strategy characterized as "jack of all trades." Although, diverse integrated weed management practices must be applied to wild and hybrid genotypes to diversify selection on these populations, evaluating the evolutionary rates of weeds in diverse environments will support the development of multi-faceted weed control strategies and effective integrated weed management policies.

Keywords: evolutionary divergence rates, haldanes, crop-wild hybrids, integrated weed management, soil moisture

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INTRODUCTION

Genes from crop and wild progenitors contribute genetic variation that may support crop-wild success in a diversity of environments (managed or unmanaged) and/or through increased competitive ability with other uncultivated populations (Warwick et al., 1986; Langevin et al., 1990). Root system structure, early flowering, and asynchronous emergence rates are examples of specific traits that wild populations can possess that better support competitive growth in multiple environments (Conner and Via, 1993; Casper and Jackson, 1997; Sahli et al., 2008). Crop plants often possess traits that are rare or absent in wild populations (e.g., salinity tolerance and herbicide resistance) which can improve crop-wild hybrid survival and reproduction in stressful environments (Gasser and Fraley, 1989; Ellstrand and Hoffman, 1990; Bagavathiannan and Van Acker, 2008). Crop-wild hybrid offspring often share morphological features with their crop progenitors and avoid eradication by farmers in the field (Ye et al., 2019); thereby enhancing their survival in agricultural fields. Furthermore, crop-derived traits such as early emergence and high seed production can contribute to crop-wild hybrid success when competing with wild populations in natural environments (Snow and Campbell, 2005; Kost et al., 2015). Thus, we predict that populations that are capable of rapidly evolving these traits may be more successful than populations that evolve these traits slowly.

Cultivated radish (*Raphanus sativus* L.) and wild radish (or jointed charlock, *Raphanus raphanistrum* L.) are annual, insect pollinated, self-incompatible, diploid species that can hybridize (Panetsos and Baker, 1967). Cultivated radish is an annual crop species that flowers late in the growing season, exhibits low rates of dormancy and rapid germination, grows large, and edible hypocotyls (i.e., roots; Snow and Campbell, 2005). In contrast, wild radish flowers early in the growing season, has a long-lived seed bank, exhibits seed dormancy, and variable germination times after soil disturbance, and develops a relatively small, inedible mature hypocotyl. Wild radish is a common weed in agricultural systems in Australia, temperate North America, Europe, and also found in disturbed and coastal sites in temperate climates (Holm et al., 1997; Ashworth et al., 2016). The success of their hybrid derivative (*R. raphanistrum* × *R. sativus*) as an aggressive weed is apparently environmentally dependent (Campbell et al., 2006, 2009a,b; Hegde et al., 2006; Campbell and Snow, 2009; Ridley and Ellstrand, 2010; Hovick et al., 2012). Moreover, hybrid radish populations tend to evolve faster than wild radish populations but this has varied with selection pressure (Campbell et al., 2009a,b). Since fitness of crop-wild hybrid radish relative to wild radish has varied with diverse moisture, temperature and ecological contexts (Campbell et al., 2006; Hovick et al., 2012), we chose to manipulate moisture conditions in field plots to explore the influence of moisture on the relative fitness of crop-wild hybrids.

The success of crop-wild hybrid populations may also depend on their environment, as certain adaptive traits unique to crop-wild hybrids may be especially advantageous in specific environments (Campbell and Snow, 2007; Arnold and Martin, 2010; Hovick et al., 2012; Hartman et al., 2013). A model example of environmentally-dependent hybrid invasive success is found in the crop-wild hybrid radish species complex. When surveyed over five decades ago, crop radish (*Raphanus sativus* L.) in California was found predominantly in coastal regions and wild radish (*Raphanus raphanistrum* L.*)* was found in inland regions, with the hybrid "wild" radish (*R. sativus* × *R. raphanistrum*) growing between the two areas (Panetsos and Baker, 1967). Subsequent surveys found predominantly hybrid populations had spread across all regions of California (Ellstrand and Marshall, 1985; Nason and Ellstrand, 1995). These hybrid populations were particularly successful in evolving invasive traits and genetically swamping both parental populations (Hegde et al., 2006). In contrast, experimental populations of crop-wild hybrid radish are also capable of persisting for up to a decade in Michigan or Ontario (Snow et al., 2010; Shukla et al., unpublished data) but have not spread as they did in California (Snow et al., 2001; Campbell et al., 2016b; Teitel et al., 2016b). This evidence suggests that hybrid populations successfully evolve competitive weed strategies in some environments and not others, prompting us to ask how the environment influences the rate of evolution in hybrid populations relative to wild populations.

Whether they differ among genotypes or environments, rates of evolution (ROE) vary among plant populations. A difference in ROE across natural environmental pressures such as temperature and water availability has been observed, with higher temperature and water availability resulting in an increased rate of evolution (Rohde, 1992; Wright et al., 2006; Goldie et al., 2010). A variety of other abiotic environmental factors such as CO_2 concentrations (Ward et al., 2000), soil pH (Snaydon and Davies, 1972; Davies and Snaydon, 1976), and soil contaminants like zinc (Antonovics and Bradshaw, 1970) and copper (Macnair et al., 1993) also have the potential to affect a population's ROE, with stronger selection pressures increasing the rate of evolution (Bone and Farres, 2001). Artificial selection and other anthropogenic activities have also been observed to affect ROE (e.g., herbicide resistance; Powles et al., 1998; Mallory-Smith et al., 1999). These examples have demonstrated evolutionary change in response to selection over a relatively short time period (i.e., contemporary evolution), anywhere between 1 to 124 years rather than thousands of years. When we can understand how various levels of climate related phenomena can influence selection and thus rates of evolution we can explore how climate change may complement other integrated weed management techniques which alter the speed of gene flow, rate of evolution, and expression of potentially weedy traits.

The growing number of studies on adaptive evolution in agricultural weeds is contributing to the growing body of literature on weed management that includes an evolutionary perspective (Dekker, 1997; Délye et al., 2013; Vigueira et al., 2013). Crop-wild hybrid weeds present a unique set of challenges to weed management, such as their potential for rapidly evolving weedy traits, and acquiring novel crop traits (Whitney et al., 2006; Schierenbeck and Ellstrand, 2009). Integrated weed management (IWM) is a component of integrated pest management (IPM), and is the strategy of applying many diverse weed control measures to diversify selection on a population at the same time including cultural, genetic, mechanical, biological, and chemical (Rodgers, 1978; Swanton and Weise, 1991). Using an IWM approach generates a plan of action to limit the success of genetically diverse weeds in agricultural contexts. When environmental selection (such as water availability) is imposed at different intensities and with differences in the consistency of selection between years, ROE may provide a metric with which we can compare various IWM control strategies.

To investigate the rate of evolution (ROE) between wild and crop-wild hybrid populations, we measured and compared ROE of fifth generation wild (*Raphanus raphanistrum)* and crop-wild hybrid (*R. raphanistrum* × *R. sativus*) radish plants from different evolutionary watering environments grown together in a common garden. First, we wanted to determine if, after five generations, mean trait responses varied between radish genotypes from different evolutionary (i.e., historical, over multiple generations) watering environments. Then, considering what we know of the genetic diversity of hybrid populations, we expect a faster rate of evolution compared to wild populations (Anderson and Stebbins, 1954; Lavergne and Molofsky, 2007; Campbell et al., 2009a,b). Given this, we ask whether hybrid radish populations always evolve weedy traits faster than wild radish populations. Due to the numerous observed cases of hybrid populations being more successful in particular environments (Ellstrand and Schierenbeck, 2006; Whitney et al., 2009), we expect to see differences in rate of evolution due to the selection environment within which these plants evolved (Campbell and Snow, 2007; Hovick et al., 2012: Hartman et al., 2013). Alternatively, hybrid success across environments may resemble a generalist approach ("jack of all trades") in which evolutionary rates are similar, suggesting the evolution of robust traits that perform equally across environments.

MATERIALS AND METHODS

Seed History of Wild and Hybrid Radish Populations Used in our Experiment

Ancestral populations (i.e., F_0 generation) of wild radish (*Raphanus raphanistrum*) were collected from greenhouse populations that were grown for several generations near Binghamton, NY, USA (Conner and Via, 1993). The crop radish (*Raphanus sativus*) cultivar used was Red Silk (Harris-Moran Seed Company, Modesto, CA, USA). As in (Campbell et al., 2016a), in 2010, both cultivated and wild plants (nine seedlings per genotype) were planted in 36 plots as part of a randomized block design at the Waterman Farm at Ohio State University in Columbus, Ohio USA, within a larger experiment (Sneck, 2012; Campbell et al., 2016a; **Figure 1**). Ancestral seedlings were planted in one of four watering treatments with one plot per treatment, per block, for a total of 10 blocks (thus we originally planted 40 populations; however four populations did not produce any F_1 seeds). Plots were ∼200 meters apart to minimize gene flow among plots; although likely negligible, some gene-flow may have occurred. In the F_0 generation, gene-flow naturally occurred within mixed plots of wild and cultivated-crop plants and gave rise to the first generation (i.e., F_1) of wild and crop-wild hybrid (*R. sativus* × *R. raphanistrum*) seeds (Teitel et al., 2016a). As previously described (e.g., Campbell et al., 2016a; Teitel et al., 2016a), we manipulated soil moisture using rain-out shelters and imposed one of four watering treatments within these plots/shelters to impose a natural selection experiment on replicated wild and crop-wild hybrid radish populations:

1. Low Rain: To create relatively dry soil conditions, water collected from low rain shelter barrels was withheld.
2. Control Unsheltered: To establish a control precipitation treatment, ambient rainwater fell on un-manipulated populations.
3. Control Sheltered: To determine the effect of a rain-out shelter (but not manipulation of moisture availability) on plant growth, ambient rainwater, collected from the shelter, was applied to the plot.
4. Double Rain: To create relatively wet soil conditions, water collected from double rain and low rain shelters was applied to double rain plots; that is, double the ambient rainfall.

The F_1 and following generations of wild and crop-wild hybrid seeds were grown at the Koffler Scientific Reserve (KSR) on Jokers Hill, King City, Ontario, Canada (lat. 44°0′ N, long. 79°3′ W; elevation 285 masl) when the Campbell lab relocated from Columbus, Ohio to Toronto, Ontario, Canada. Because we were moving locations and reconstituting 40 populations from 18 wild and 18 hybrid seeds producing populations, all F_1 wild seeds were randomly assigned to a population and all F_1 hybrid seeds were randomly assigned to a new population at KSR. As described in Teitel et al. (2016a,b), at KSR, F_1 seeds from 36 F_0 populations were grown in germination trays in a hoop-house. Wild and hybrid F_1 seedlings were grown to the two-leaf stage, at which point they were transplanted (∼117 seeds per plot) into sheltered and non-sheltered plots, plots which were scattered across KSR and were exposed to natural conditions (e.g., weather patterns, herbivory, and pollinators) and weeded to reduce interspecific competition. The plots were exposed to one of the four soil moisture conditions for an additional three generations (**Figures 1A,B**). Each of the four soil moisture treatments had five replicate populations for a total of 20 wild and 20 hybrid populations.

Rain-out shelters were 3.05 m by 2.44 m wooden frames with transparent sheet plastic stretched over the frame, acting as a roof that blocked rain and minimally reduced light transmission; new sheet plastic was applied each year. Using metal poles, frames were slanted and elevated to ∼1.2 m above ground at their lowest corner. Frames were slanted to intercept and divert natural rainfall into a 208 L plastic collection barrel via an eavestrough attached to the lowest side of the wooden frame (**Supplementary Figure 1**). Shelters were placed at least 40 m apart to reduce gene flow among plots, as in the F_0 generation. Since wild radish have long-lived seed banks and since annual regeneration of populations was a result of seeds that dropped to the ground and naturally germinated, fruits collected from the pedicels of senescing plants in 2015/2016 could have belonged to one of three generations (F_2-F_5). For simplicity, we refer to these plants as G_2-G_5 generation seeds. Small population size in the F_1, G_2, and G_3 generations meant that populations

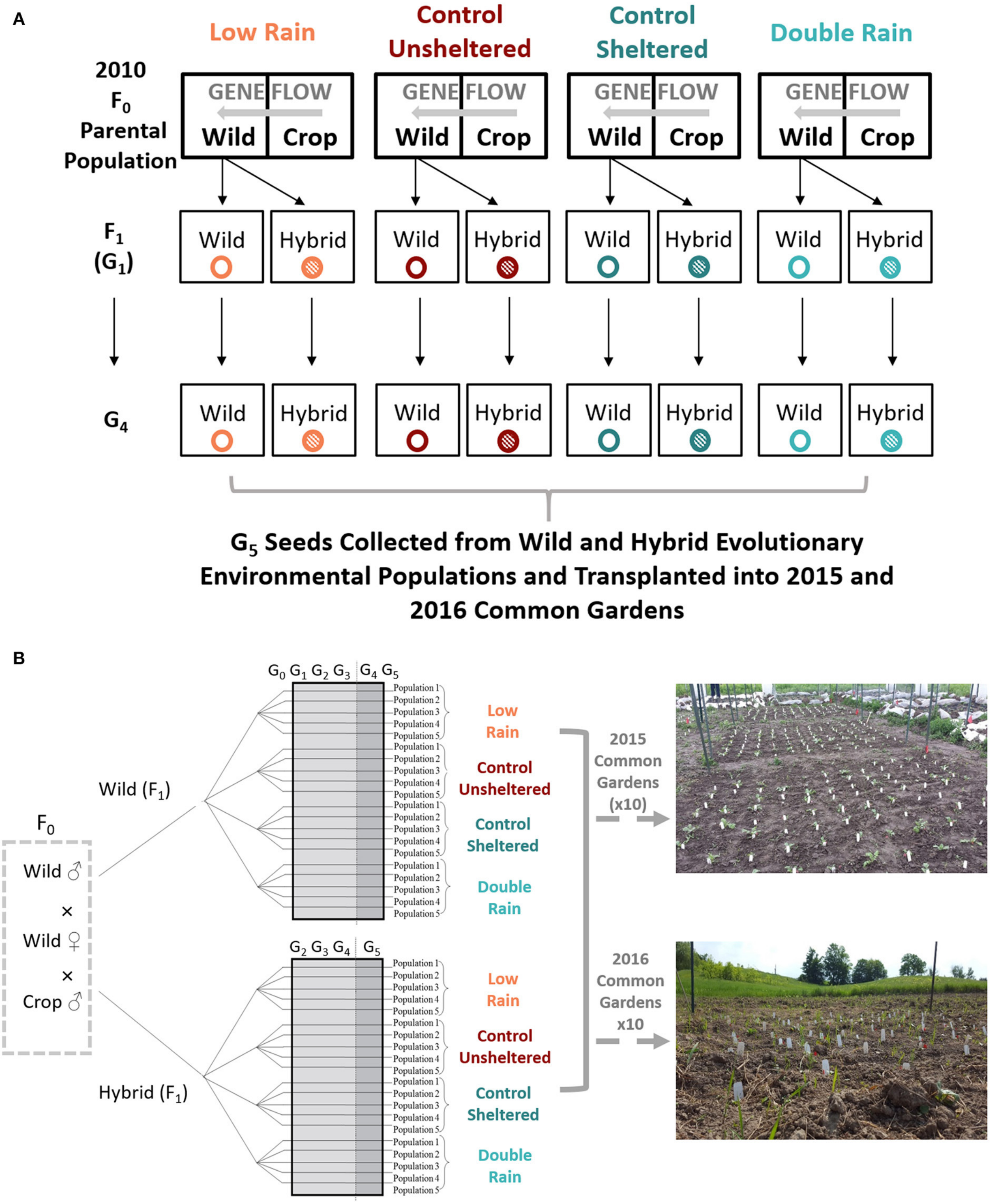

FIGURE 1 | (A) The evolutionary history of fifth generation (G_5) wild and hybrid *Raphanus* populations planted in 2015 and 2016 common gardens. In 2010, cultivated and wild *Raphanus* seeds were planted into one of four soil moisture treatments (F_0: Low-Rain, Control-Unsheltered, Control-Sheltered, and Double-Rain; Waterman Farm at Ohio State University in Columbus, Ohio, USA). Gene flow naturally occurred within mixed plots of wild and cultivated plants which gave rise to first-generation (i.e., F_1/G_1) wild (*R. raphanistrum*—open colored circles) and crop-wild hybrid (*R. sativus* × *R. raphanistrum* – filled colored circles) seeds. Succeeding wild and hybrid generations (G_2-G_4) from 2011 to 2014 were transplanted into the same four soil moisture environments as F_0 populations (Low-Rain, Control-Unsheltered, Control-Sheltered, and Double-Rain) at the Koffler Scientific Reserve (KSR), King City, Ontario, Canada. **(B)** Forty wild and hybrid 2nd generation (G_2) to 4th generation (G_4) plants (five replicate populations per watering treatment) were grown under the same watering conditions at the Koffler Scientific Reserve (KSR) in King City, Ontario, Canada. Fifth generation plants were transplanted and grown in 2015 and 2016 common gardens at KSR.

may have experienced genetic drift (Teitel et al., 2016a). However, the experimental design used allowed us to detect the consequences of genetic drift—if there was substantial genetic drift, we predicted there would be significant differentiation among the five replicate lineages, within experimental treatment combinations; which we did not find (Shukla et al., unpublished data). Wild and hybrid G_5 seeds were collected from G_4 plants in fall 2014 and used in both 2015 and 2016 experiments.

Common Garden Set-Up

To estimate the rate at which phenotypes diverged after five generations of selection, we grew two common gardens (one in the 2015 growing season and another in 2016) of G_5 wild and hybrid plants. In 2015, 10 common garden plots (3.5 m . 3.0 m), treated as blocks, were tilled and planted with a total of 120 seeds per block; from each G_5 genotype by evolutionary watering environment combination, 15 seeds were randomly selected from each of the five populations of each genotype (20 wild populations and 20 hybrid populations; **Figure 1B**). On June 1-2, 2015, we planted seeds in the soil in a 10 × 12 grid, with 30 cm spacing between plants, arranged in a randomized, complete block design. Common garden rainfall was not manipulated in either common garden and plots were weeded to reduce competition. In 2016, we replicated the 2015 experiment at a second site at KSR. The second common garden was tilled and 10 experimental blocks (3.5 m . 3.0 m) arranged in a randomized, complete block design on May 20th and May 24th, 2016. In 2016, every genotype by evolutionary watering environment combination from 2015 along with three crop seeds (*Raphanus sativus*) were planted in each block. However, these plants were removed from the analysis due to lack of replication across years. Due to limited seed stock, we planted 100 seeds per block in each common garden. We harvested the plants as they senesced, when flowering was complete and at least 10 fruits were ripe. At the end of the growing season (October 15th, 2015 and 2016), all remaining plants were harvested. Natural rainfall varied over the growing season between common garden years, with a cumulative rainfall of 307.8 mm in 2015 and 206.7 mm in 2016 (nearest weather station: Buttonville, Ontario 43°51′39.000″ N, 79°22′07.000″ W; Government of Canada, 2018).

Trait Measurement

Flower color, a simply inherited trait, differs between wild and crop radish plants and is a visual marker to track crop trait introgression at one locus in hybrid populations (Snow et al., 2001; Campbell et al., 2006). Wild radish (*Raphanus raphanistrum*) is homozygous recessive for yellow flower petal color and crop radish (*R. sativus*) is homozygous dominant for white or pink flower petal color (Panetsos and Baker, 1967; Kay, 1976; Stanton et al., 1989). In hybrid populations, the white petal color exhibits Mendelian dominance over the yellow petal color, and therefore allows us to track crop allele persistence (Panetsos and Baker, 1967; Stanton et al., 1989) into advanced populations of crop-wild *Raphanus* hybrids. Hues of pink petal color is controlled by two additional loci (Panetsos and Baker, 1967) but variation in pink hue was not tracked in this experiment.

Radish hybrids can be heterozygous for a reciprocal translocation that can affect chromosome pairing during meiosis (Panetsos and Baker, 1967; Campbell et al., 2006). This translocation can affect pollen fertility and produce up to ∼60% aborted pollen grains in F_1 hybrid progeny (Snow et al., 2001; Campbell et al., 2006). After four generations of evolution, we compared hybrid pollen fertility to that of wild populations to determine the rate of evolution in hybrid pollen fertility across environments. To assess pollen viability of G_5 hybrid populations relative to the pollen viability of wild radish, we collected a single, newly opened flower from each plant (n∼1,000 plants/year) during August, 2015 and July–August, 2016 between the hours of 8:00 am and 12:00 pm and refrigerated at 2°C until processing. At the time of staining, two anthers were collected and wiped on microscope slides (VWR VistaVision, Radnor, PA, USA). Slides were stained with Alexander stain (Alexander, 1969) and stored in slide boxes. After staining, we measured pollen fertility by categorizing at least 100 pollen grains per plant as either the number of aborted or fertile pollen grains using a compound microscope (Nikon©, H550L, Japan).

We monitored each seed daily to record the date of seedling emergence from the soil and first flower during the experimental period (June to October 15, 2015 and June to August 26, 2016). From this, the days to seedling emergence and age at first flowering (i.e., number of days between anthesis and emergence) was calculated. Additional life-history traits (e.g., longest leaf length, stem diameter) were measured at the date of first flower.

To measure the photosynthetic performance of plants in the different watering treatments, and to evaluate whether water stress or excess water influenced photosynthesis, we measured the dark-adapted quantum efficiency of photosystem II (PSII) as the ratio of variable (F_v) to maximal (F_m) chlorophyll fluorescence (Maxwell and Johnson, 2000). Because all reaction centers were fully oxidized by shading prior to measurement, F_v/F_m represents the maximum capacity of PSII to absorb light energy. In both years, we took outdoor measurements using a portable fluorescence meter (Handy PEA fluorometer, Hansatech Instruments Ltd., King's Lynn, UK). Prior to experimental measurement, we randomly sampled 100 plants to determine a standard curve of the minimum time it took reaction centers to become fully oxidized (i.e., a dark adaptation period) where fluorescence remained consistent; this occurred after ∼10 min. Then, for the experiment, we non-destructively sampled quantum efficiency of PSII on a subset of 24 plants (three plants per genotype × evolutionary watering environment combination) per block for a total of 240 plants across the whole experiment in 2015 and another 240 plants in 2016. In random order, we measured plants after a 10-min dark adaptation period. Measurements were collected on July 7, 2015 and between June 29 and July 3, 2016, between 8:00 am and noon.

Evolutionary Divergence Rate Metrics

Calculations of evolutionary rates are based upon the average phenotypic difference between two populations (usually described as Δ), relative to the time since isolation (i.e., Δt) and are commonly measured in darwins or, more recently, haldanes (Haldane, 1949; Gingerich, 1983, 1993). Because we compared independently evolving populations, we calculated synchronic rates of evolutionary divergence in haldanes (Hendry and Kinnison, 1999; Bone and Farres, 2001), using the following equation:

$$haldanes\ (h) = \frac{\left[\left(\frac{\ln\ \bar{x}_2}{s_p}\right) - \left(\frac{\ln \bar{x}_1}{s_p}\right)\right]}{t_2 - t_1} \tag{1}$$

where the mean trait values of control-sheltered wild and control-sheltered hybrid populations were represented by $\bar{x}_1$ and trait values of low rain, control-unsheltered, or double rain wild and hybrid populations were represented by $\bar{x}_2$ in Equation 1. By calculating haldanes, mean trait evolution was standardized by incorporating pooled trait variances (s_p) and measuring evolutionary change through time (t_2-t_1 = 5 generations, or F_0 – F_5) (Haldane, 1949; Gingerich, 1993, 2001). We calculated the natural log of trait values to reduce heteroscedasticity in the dataset since standard deviations are typically expected to increase with the mean, particularly for morphological traits (Wright, 1968; Hendry and Kinnison, 1999). Although haldanes are more commonly used to measure contemporary evolutionary rates, we have calculated evolutionary rates in darwins (*d*), as well, presented in **Supplementary Table 1**.

Statistical Analysis

To determine if seedling emergence and flowering frequency differed between genotypes (wild vs. hybrid) from different evolutionary watering environments (Low Rain, Control Unsheltered, Control Sheltered, and Double Rain), we ran a generalized linear mixed-model ANOVA fitted to a binomial distribution; 0 indicating no emergence/flowering and 1 indicating emergence/flowering. In our model, genotype and evolutionary watering environment were fixed effects and year a random effect; block as a random nested effect within year was not significant and, therefore, omitted from the model to increase statistical power.

To determine if four phenotypic traits (days to emergence, days to first flower, longest leaf length, and stem diameter) differed between genotypes (wild vs. hybrid) from different evolutionary watering environments (Low Rain, Control Unsheltered, Control Sheltered, and Double Rain), we ran a linear nested mixed-model ANOVA. Considering our traits of interest are highly correlated (**Supplementary Table 2**), we performed a principal components analysis (PCA) which allows correlated variables to be condensed into two or fewer composite proxy variables (Abdi and Williams, 2010; Mohammed et al., 2016). In the PCA, we fitted our data to a correlation matrix, extracted the first two principal components, and ran these in our mixed-model ANOVA (loadings discussed in Results). In our ANOVA model, genotype and evolutionary watering environment were fixed effects and block nested within year as a random effect. Finally, to determine whether white flower color, pollen fertility, and chlorophyll fluorescence differed between genotypes (wild vs. hybrid) from different evolutionary watering environments, we ran a linear nested mixed-model ANOVA, with genotype and evolutionary water environment as fixed effects and block nested within year as a random effect. Due to varying sample sizes associated with white flower color, pollen fertility, and chlorophyll fluorescence, these traits were unable to be incorporated into our PCA.

To determine if rates of evolutionary divergence of four phenotypic traits (days to emergence, days to first flower, longest leaf length, and stem diameter) differed between genotypes (wild vs. hybrid) from different evolutionary watering environments (Low Rain, Control Unsheltered, and Double Rain), we performed a mixed-model, repeated-measures ANOVA. Similar to our mean trait analysis, evolutionary divergence rates of our traits were highly correlated (**Supplementary Table 2**) so, we ran a principal components analysis (PCA) fitted to a correlation matrix and extracted two principal components and ran these in our mixed-model ANOVA (loadings discussed in Results). Genotype, evolutionary watering environment, and their interaction were fixed between-subjects effects, and year was a random within-subjects effect. Finally, we ran a repeated-measures ANOVA for the frequency of white petalled plants with evolutionary watering environment as the main between-subjects effect and year as the random within-subjects effect. Due to smaller datasets for the traits chlorophyll fluorescence and pollen fertility (i.e., sample size within each experimental level within a treatment), we did not statistically compare these traits among genotypes or evolutionary watering environment.

Prior to running any PCA or mixed model ANOVA, response variables were transformed, if needed, to meet assumptions of normality. Furthermore, due to the non-orthogonality of these data, type III ANOVA results applied a Kenward-Roger's adjustment for computing the denominator degrees of freedom (Luke, 2017). Analyses were performed in R-Studio (Version 1.3.959; packages *stats* and *lme4*).

RESULTS

Trait Variation Between Crop-Wild Hybrid and Wild Populations From Varying Evolutionary Watering Environments

Hybrid seedlings, irrespective of the watering environment they evolved in, had significantly higher (∼6.0%) emergence frequency than wild seedlings. However, of the plants that emerged, survival to flowering (i.e., flowering frequency) did not differ between wild and hybrid populations (**Table 1**). Irrespective of genotype, seedling emergence frequency and flowering frequency did not significantly vary between plants from different evolutionary watering environment histories (**Table 1**). Finally, considering the genotype by evolutionary watering environment interaction, hybrid plants from double rain environments emerged significantly more frequently (29% more, **Table 1**), but did not differ significantly in their survival to flowering, than wild plants from the same environment (**Table 1**). However, seedling emergence frequency and flowering frequency of hybrid and wild populations from low rain, control unsheltered, and control sheltered environments did not significantly differ (**Table 1**).

Hybrid populations exhibited lower pollen fertility (∼9%, **Table 2**) and higher chlorophyll fluorescence (i.e., better

TABLE 1 | Mixed-model ANOVA for seedling emergence (n=1966) and flowering frequency (n=1413) fitted to a binomial distribution.

Parameter	df	Response	
		Seedling emergence frequency	Flowering frequency
		χ^2	χ^2
Genotype	1	**9.16**	0.01
Evolutionary Watering Environment	3	2.49	3.92
Genotype × Evolutionary Watering Environment	3	**9.81**	1.72

Bolded χ^2-statistics bolded indicate statistical significance below $P < 0.05$.

TABLE 2 | Mixed-model ANOVA of mean phenotypic trait values **(A)** principle component 1, **(B)** principle component 2, **(C)** pollen fertility, and **(D)** chlorophyll fluorescence (proxy of water-use-efficiency).

Response & Parameter	df (n,d)*	F**
(A) Principle Component 1 (n = 650; Days to Flower, Longest Leaf Length, Stem Diameter)		
Genotype (G)	1, 624	**92.12**
Evolutionary Watering Environment (EW)	3, 624	**3.22**
G × EW	3, 624	**16.62**
(B) Principle Component 2 (n = 650; Days to Seedling Emergence)		
G	1, 624	3.36^+
EW	3, 624	2.05
G × EW	3, 624	**3.38**
(C) Pollen fertility (n = 1,064)		
G	1, 1028	**34.65**
EW	3, 1026	0.85
G × EW	3, 1028	0.36
(D) Fluorescence (n = 413)		
G	1, 393	**8.12**
EW	3, 366	1.48
G × EW	3, 393	1.43
(E) White Flower Color (n = 343)		
EW	3,339	0.46

PC1 is a composite of trait variances of days to first flower, longest leaf length, and stem diameter and PC2 is represents the variance of days to emergence, exclusively.

**A Kenward-Roger's adjustment was applied for computing the denominator degrees of freedom.*

***Bolded F-statistics indicate statistical significance below $P < 0.05$ and values marked with $^+$ indicate marginal statistical differences.*

quantum use efficiency of PSII; **Table 2**) than wild populations, however, both traits did not differ between plants from different evolutionary watering environments and had no significant genotype by evolutionary watering environment interactions (**Table 2**; mean values presented in **Supplementary Table 3**). Finally, considering only hybrid populations, the frequency of white flowered plants did not significantly differ between populations from different evolutionary watering environments (**Table 2**; mean values presented in **Supplementary Table 3**).

Running our PCA, we found that two principal components were sufficient to cumulatively explain 83.5% of the trait variance in our four traits. The variance of principal component 1 (PC1) loaded heavily onto days to first flower (49.3%), leaf length (59.8%), and stem diameter (62.0%). Principal component 2 (PC2) loaded almost exclusively onto days to emergence (99.1 %). We, therefore, ran our ANOVA on our two proxy composite variables (PC1 and PC2). For traits that loaded onto PC1 (days to first flower, longest leaf length, and stem diameter), there was a significant genotype, evolutionary watering environment, and genotype by evolutionary watering environment effect. For the trait that loaded onto PC2 (days to emergence) there was a marginally significant genotype effect and a significant genotype by evolutionary watering environment interaction but no evolutionary watering environment effect (**Table 2**). Below, we present trends associated with PC2 (i.e., emergence time) followed by PC1 (flowering time, leaf length, and stem diameter) based on their model significances.

Hybrid plants tended to emerge slightly earlier (∼1 day), flower later (∼3 days), grow longer leaves (∼15%), and wider stems (∼35%) than wild plants. Irrespective of genotype, plants from double rain evolutionary environments flowered later (∼2 days), grow longer leaves (∼6%), and wider stems (∼8%) than plants from control sheltered evolutionary environments, with no differences between emergence times. Finally, days to emergence (PC2) did not differ between hybrid and wild plants from low rain, control unsheltered, control sheltered, and double rain environments (i.e., no genotype by evolutionary watering interaction; **Table 3**). However, hybrid plants from low rain, control unsheltered, and double rain environments took longer to flower, had longer leaves, and wider stem diameters then wild plants from the same environments (i.e., differed with respect to PC1; trait mean table presented in **Table 3**), with no difference between genotypes from control sheltered environments.

Evolutionary Divergence Rates of Crop-Wild Hybrid and Wild Populations From Extreme Watering Environments

Considering hybrid populations only, the watering environment in which hybrid population evolved did not significantly affect divergence rates of white-flower colored plants. In fact, the rate at which the proportion of white-flowered plants evolved in environments of low rain, control unsheltered, and double rain did not deviate from white-flowered plants of control sheltered environments (**Table 4**).

We found two principal components were sufficient to cumulatively explain 86.6% of the variation among divergence rates of our four traits. Principal component 1 (PC1) loaded, relatively evenly, onto the rate of evolutionary divergence of days to first flower (55.9%), leaf length (61.0%), and stem diameter (54.6%). Principle component 2 (PC2) loaded heavily onto rates of divergence of days to emergence (82.9 %) and days to first flower (50.2%). We, therefore, ran

TABLE 3 | Mean phenotype values* of four traits across evolutionary watering environments for wild and hybrid radish plants.

Phenotypic trait	Genotype	Evolutionary watering environment			
		Low rain ($n = 148$)	**Control unsheltered ($n = 158$)**	**Control sheltered ($n = 160$)**	**Double rain ($n = 184$)**
		Mean ($\bar{x}$ ± *SD*)			
Days to emergence	Wild	7.27 ± 2.76	7.04 ± 2.19	7.04 ± 3.91	7.25 ± 2.25
	Hybrid	6.21 ± 1.57	6.40 ± 1.77	7.34 ± 2.73	6.90 ± 2.62
Days to flower	Wild	34.70 ± 3.46	34.58 ± 3.52	35.08 ± 6.02	34.45 ± 4.17
	Hybrid	38.50 ± 7.57	38.25 ± 8.64	35.45 ± 6.42	40.29 ± 7.96
Longest leaf (cm)	Wild	20.38 ± 6.58	21.68 ± 6.32	21.90 ± 5.38	20.42 ± 6.25
	Hybrid	25.72 ± 9.26	24.07 ± 9.42	22.52 ± 10.34	26.36 ± 9.69
Stem diameter (mm)	Wild	7.31 ± 3.78	7.41 ± 2.71	8.65 ± 5.11	6.60 ± 2.46
	Hybrid	11.77 ± 6.24	11.09 ± 6.54	9.88 ± 7.45	13.17 ± 7.55

Mean trait values are presented as $\bar{x}$ ± SD.*

These are general trends associated with our traits of interest. Test statistics for these data are presented as part of the principle components analysis in **Table 2; a further description can be found in the methods and results.*

TABLE 4 | Mixed model ANOVA of mean evolutionary divergence rates of **(A)** principle component 1 (PC1), **(B)** principle component 2 (PC2), and **(C)** white flower color.

Response and parameter	df (n,d)*	F**
(A) Principle Component 1 ($n = 262$;Days to Flower, Longest Leaf Length, Stem Diameter)		
Genotype (G)	1, 48	**6.19***
Evolutionary Watering Environment (EW)	2, 48	0.81
G × EW	2, 48	1.28
(B) Principle Component 2 ($n = 262$;Days to Seedling Emergence, Days to Flower)		
G	1, 47	**26.61***
EW	2, 47	1.08
G × EW	2, 47	1.17
(C) White Flower Color ($n = 140$;only hybrid populations)		
EW	2, 24	0.29

PC1 is a composite of trait variances of days to first flower, longest leaf length, and stem diameter and PC2 is represents the variance of days to emergence and days to first flower.

**A Kenward-Roger's adjustment was applied for computing the denominator degrees of freedom.*

***Bolded F-statistics indicate statistical significance below $P < 0.05$.*

our ANOVA on our two proxy composite variables (PC1 and PC2). For the divergence rates of traits that loaded on to PC1 (days to first flower, longest leaf length, and stem diameter) and PC2 (days to emergence, days to first flowering) there was a significant genotype effect but no evolutionary watering environment or genotype by evolutionary watering environment interaction (**Table 4**; **Figures 2**, **3**). Below, we present trends of divergence rates associated with PC2 and then PC1.

After five generations of evolution, hybrid and wild populations evolved similar days to seedling emergence rates but in different in directions (PC2). Specifically, hybrid populations from extreme watering environments evolved earlier days to emergence relative to hybrid control sheltered populations (**Figure 2A**) whereas, wild populations from extreme watering environments evolved longer days to emergence relative to wild control sheltered populations (**Figure 2A**). The speed of evolution of days to first flower (PC1 & PC2) also differed, where hybrid populations from extreme watering environments evolved more slowly than wild populations from extreme watering environments relative to their control sheltered phenotypes, respectively (**Figure 2B**). Leaf morphology (PC1) evolved more slowly in hybrid populations and in different directions compared to wild populations. Specifically, hybrid populations from extreme watering environments evolved shorter leaves relative to hybrid control sheltered populations whereas, wild populations from extreme watering environments evolved longer leaves relative to wild control sheltered populations (**Figure 2C**). Lastly, after five generations, stem diameter morphology (PC1) evolved more slowly in hybrid populations compared to wild populations. Specifically, stem diameter phenotypes in hybrid population from extreme watering environments did not evolve away from hybrid control shelter phenotypes (**Figure 2D**); but wild populations from extreme watering environments evolved larger stem diameters relative to wild control sheltered population (**Figure 2D**).

DISCUSSION

After five generations of selection on wild and crop-wild hybrid populations across a soil-moisture gradient, and contrary to our expectations, wild populations were more phenotypically diverse and evolved selective traits faster than their hybrid relatives, even though white flower color (a crop derived trait) in hybrid populations remained at relatively

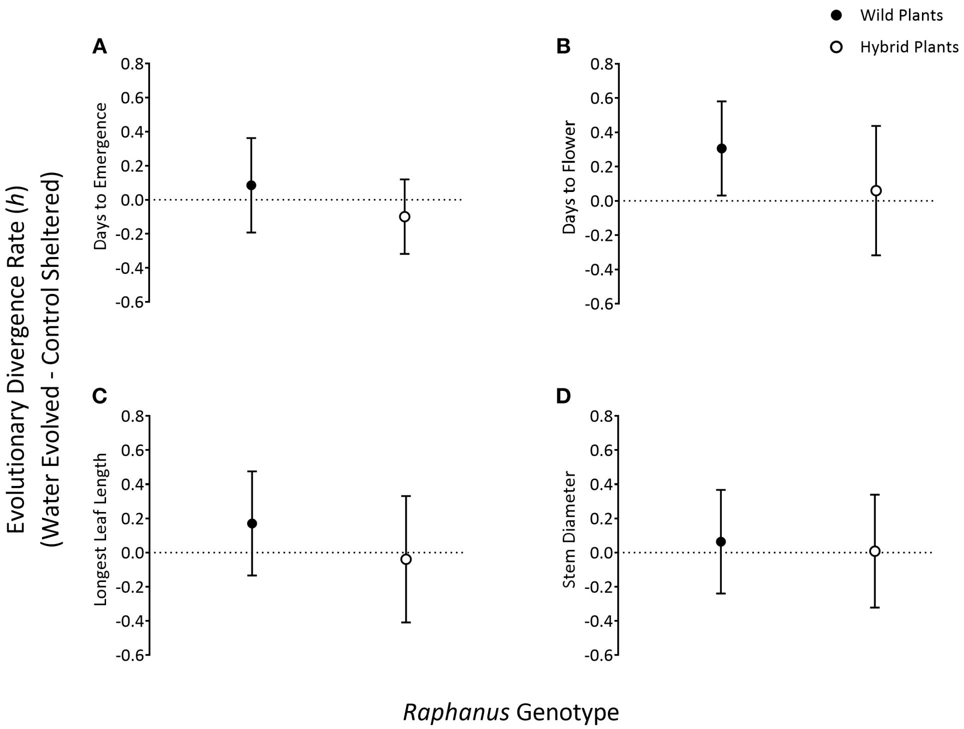

FIGURE 2 | Comparing evolutionary rate estimates [presented in haldanes (*h*) ± *SD*] of four traits—days to seedling emergence **(A)**, days to flowering **(B)**, longest leaf length **(C)**, and stem diameter **(D)**—between G_5 radish weed genotypes (wild—closed circles, hybrid—open circles). Among wild populations, values represent the change between wild, water-evolved populations and wild, control-sheltered (CS) populations. Similarly, among hybrid populations, values represent the change between hybrid, water-evolved, and control-sheltered populations.

high frequencies across watering environments. Further, the proportion of white flower color plants, after five generations, did not diverge away from control phenotypes in response to extreme watering conditions. This suggests that crop traits in our populations have introgressed and persisted across soil moisture environments (Strauss et al., 2004; Irwin and Strauss, 2005). Hybridization and evolutionary watering pressures, separately, promote increased emergence frequency, early emergence, later flowering, and larger morphology relative to wild populations. Furthermore, hybridization but not watering environment slowed rates of evolution across evolutionary watering environments. In contrast, wild populations across evolutionary watering environments grew to a smaller size, were quicker to flower and evolved relatively faster than crop-wild hybrid populations. Given the patterns in ROE we measured, we predict selection varied most in the control unsheltered treatment between years and plots whereas selection may have been more consistent in the sheltered plots (although still varying between years). Thus, based on the rate of evolution in wild versus hybrid populations measured here, wild populations may be more aggressive weeds than hybrid populations in Ontario if weediness is measured by rate of evolution (Bone and Farres, 2001; Whitney et al., 2006). We discuss the potentially adaptive weed strategies both wild and hybrid populations may be demonstrating and the implications of these strategies on weed management.

Hybridization and the Introduction of Canalized Traits as a "General Phenotype" Weed Strategy

Crop-wild hybrid populations emerged more frequently and earlier, flowered later, and were larger than wild populations. Among crop-wild hybrid populations, evolutionary rates across common gardens irrespective of their evolutionary watering selection history (**Table 4**; **Figures 2**, **3**). In contrast, wild populations exhibited faster evolutionary rate responses across evolutionary watering histories. This insensitivity of hybrid populations to diverse watering environments may be driven by crop trait inheritance patterns. Traits in crop populations are sometimes selected to have reduced environmental sensitivity (but see Sadras et al., 2009) and produce a specific phenotype in response to a particular environment or a consistent response in variable environments (Nicotra et al., 2010). Traits that display this environmental insensitivity are, more generally, referred to as canalized traits (Weinig, 2000; Valladares et al., 2007; Matesanz et al., 2010). To achieve these standards, breeders limit the phenotypic response by breeding for allelic homozygosity (either through dominant or recessive alleles), depending on the trait (e.g., seed size and flowering time) (Nicotra et al., 2010; Snow et al., 2010; Flint-Garcia, 2013); these traits, therefore, may share the same set of alleles that respond similarly across environments (i.e., genetically identical and correlated; Via and Lande, 1985). Introgression of these canalized crop traits into crop-wild hybrid

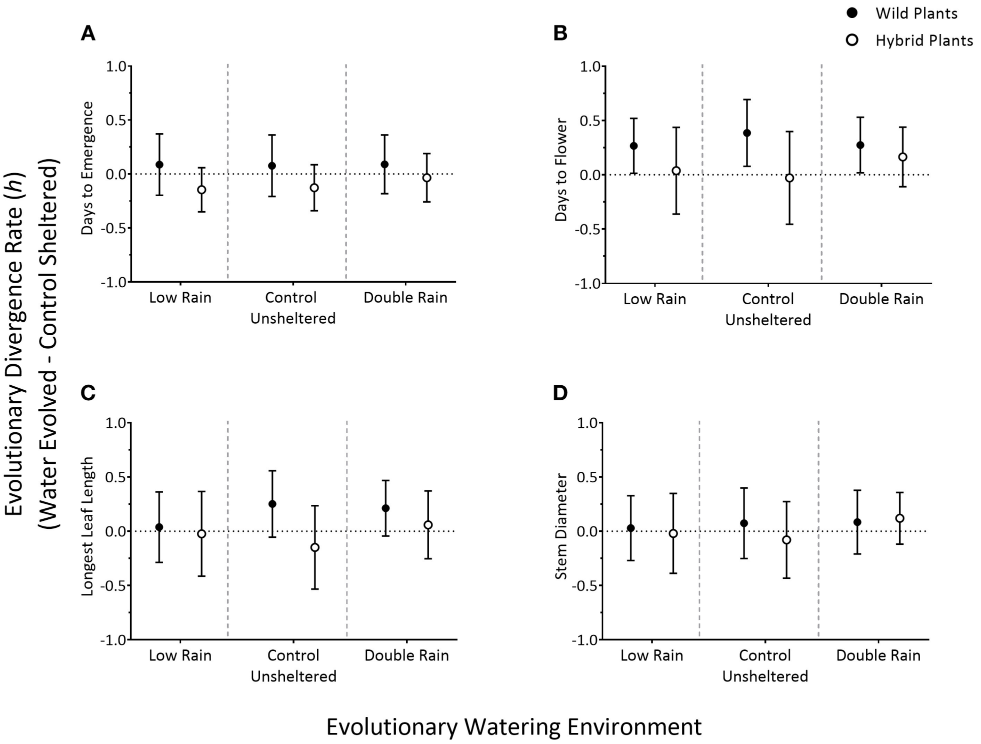

FIGURE 3 | Comparing evolutionary rate estimates [in haldanes (*h*) ± *SD*] of four traits—days to seedling emergence **(A)**, days to flowering **(B)**, longest leaf length **(C)**, and stem diameter **(D)**—of G_5 wild and hybrid radish plants (wild—closed circles, hybrid—open circles) from one of three environmental conditions (low-rain, control-unsheltered, double rain; separated by a gray dashed line) grown in common garden conditions. Values represent change between water-evolved plants and control sheltered plants for each respective treatment (i.e., change between Low Rain-evolved and Control Sheltered, Control Unsheltered-evolved and Control Sheltered, and Double Rain-evolved and Control Sheltered).

populations, may explain the lack of phenotypic plasticity and minimal differences in evolutionary rate estimates in our hybrid populations across environments.

Canalized trait responses, and therefore slow evolutionary rates, do not necessarily put crop-wild hybrids weeds at a disadvantage. Previous work conducted on related radish populations have found that hybrid radish populations have performed equally to, and at times better than, their wild radish counterparts across common gardens exposed to a variety of soil-moisture conditions (Shukla et al., unpublished data). Performing equally well, or slightly better, is characteristic of a generalist phenotype or "jack-of-all trades" adaptive strategy, where phenotypes display little plasticity and perform similarly across environments (Baker, 1965; Richards et al., 2006). This strategy has been seen before in other species (e.g., *Solanum hispidum* Pers., *Ageratum houstonianum* Mill., *Chloris virgata* Sw., and more, Hastwell and Panetta, 2005; *Corispermum macrocarpum* L. and *Salsola collina* Pall., Huang et al., 2009; Pichancourt and van Klinken, 2012; *Parkinsonia aculeata* L.). For example, fitness of a *Centura* species has similar expression across water flooding regimes (flooded vs. normal) (Richards et al., 2006). Similarly, two European lineages of *Taraxacum officinale* L. displayed a generalist strategy in response to water availability and shade (Oplaat and Verhoeven, 2015). Overall, our data suggest that hybridization and the introgression of crop traits, rather than extreme watering environments, may be a stronger factor influencing rates at which hybrid phenotypes evolve. The robust, and consistent, evolutionary rates displayed across extreme watering environments (i.e., lack of divergence from hybrid control phenotypes) suggest our crop-wild hybrid weeds are broadly tolerant to extreme watering regimes.

Water Availability Is Not a Strong Enough Disturbance to Drive Evolutionary Rates in Crop-Wild Hybrids

After five generations of growth under diverse and relatively extreme watering environments for Ontario, Canada, soil moisture alone does not appear to be a strong enough factor to drive rapid evolutionary divergence among crop-wild hybrid radish weeds. In response to water availability, hybrid weed populations may need longer than five generations for traits to evolve before they reach their "adaptive optimum" (Bock et al., 2015)—if they haven't already (Ord and Hundt, 2020). Alternatively, different abiotic and/or biotic factors (e.g., latitudinal clines, temperature, competition, herbivory, etc.) may elicit stronger and more rapid evolutionary responses and force hybrid weeds to "act," or to use their standing genetic variation

(Richards et al., 2006; Whitney and Gabler, 2008; Bock et al., 2015). For example, crop-wild hybrid sunflowers have rapidly (7–10 generations) evolved several morphological, phenological, and eco-physiological traits that increase survival (fitness-related trait) in response to varying herbicide, pesticide, and competition treatments (Mercer et al., 2007; Baack et al., 2008; Dlugosch and Parker, 2008; Presotto et al., 2016). Similarly, transgenic crops have contributed *Bt*, herbivory-resistance genes and glyphosate, herbicide-resistance genes from sunflower (*Helianthus* species) and kochia (*Kochia* species) plants, respectively, to sexually compatible, wild populations of related species. The resulting hybrid populations demonstrate strong selection over a few generations for these traits (Snow, 2002; Beckie et al., 2013). These selection pressures may drive rapid adaptation of weeds since pesticide/herbicide resistance and polyculture farming practices are important aspects in agroecology.

The relative success of crop-wild hybrid populations have varied across North America—from highly successful in California and Texas (Hegde et al., 2006; Hovick et al., 2012) to moderately successful in Michigan, USA and Ontario, Canada (Campbell et al., 2006; Campbell and Snow, 2007; Teitel et al., 2016a). Along with differences in soil moisture, one of the most apparent differences among these studies is temperature and length of growing season. Any one of these factors in conjunction with soil moisture could overcome canalized trait responses and increase the speed of contemporary evolution in hybrid radish weed populations. Furthermore, outside of an agronomic context, hybrid radish is a significant weed in natural and agricultural areas in California and Australia, as well as in its natural range in Eurasia (Ridley and Ellstrand, 2010). Our research suggests that soil moisture may impose variable strengths and directions of selection depending on where populations grow (cultivated vs. uncultivated areas) and may be reflected as diverse evolutionary rates; we encourage future research to compare rates of evolution in uncultivated areas. Finally, we recognize that the absence of phenotypic trait divergence in response to hybridization, water availability, and their interaction may be confounded with factors like abiotic and biotic pressures in their growth environment, phenotypic plasticity, and epigenetic effects of the maternal environment (Richards et al., 2006; Wolf and Wade, 2009; Germain et al., 2013; Campbell et al., 2015). With that in mind, future studies should consider taking the offspring of late generation plants (via a resurrection common garden approach; see Franks et al., 2018)—ensuring wild-wild and hybrid-hybrid mating—to measure evolutionary rates void of confounding environmental and epigenetic effects prior to dismissing the effect of soil moisture on evolutionary rates.

Water Availability as a Low-Disturbance Abiotic Factor Driving Evolutionary Rates in Wild Radish Populations

We found that wild radish populations were quicker to flower but had shorter leaves and thinner stem diameters than hybrid populations from the same evolutionary soil moisture environments. However, wild populations rapidly evolved later flowering phenologies and larger morphologies in response to extreme moisture environments (**Figure 2**) relative to wild control populations and did so faster than hybrid populations. Many weedy or wild relatives have evolved varying adaptive strategies to extremely low or high water availability over short time periods. For example, wild Mediterranean shrub *Fumana thymifolia* L. decreased seedling emergence during periods of drought (Jump et al., 2008) and wild weed *Lupinus luteus* L. delayed flowering in high rainfall environments (Berger et al., 2008; Berger and Ludwig, 2014). Delayed flowering and investment in growth can be an adaptive evolutionary approach to ensure survival in response to extreme—potentially unfavorable—environments (Grime, 1977; Berger and Ludwig, 2014). Though they may not flower as early, or for as long, investing in growth and defense against competitors increases opportunity for survival (Grime, 1977; Arendt, 1997).

Although our wild radish weed populations responded to watering environment, phenotypic changes and evolutionary rates were moderate compared to documented changes in response to other abiotic pressures. Wild or weedy plant species have displayed faster and stronger evolutionary rates in response to other abiotic environments (Bone and Farres, 2001; Berger and Ludwig, 2014), including but not limited to salinity (Kiang, 1982), herbicides (Powles et al., 1998; Mallory-Smith et al., 1999), and elevated soil pH (Snaydon and Davies, 1972; Davies and Snaydon, 1976). In each of these cases, the environment prompted the expression of novel phenotypes due to the traits' inherent plasticity and over a short period have been fixed due to their fitness-enhancing advantage (i.e., measured as reproductive success). Compared to our crop-wild hybrid populations, after five generations, our wild populations exhibited a more dramatic evolutionary response to watering environment but have been documented as having significant seed production more than or equally fecund to crop-wild hybrid populations (Shukla et al., unpublished data). Although wild radish have traditionally been known to grow and evolve relatively quickly in high disturbance environments (Snow and Campbell, 2005), our results suggest that wild radish populations can also evolve just as quickly in response to low-disturbance environments like soil moisture.

Implications for Weed Management and Future Directions

Weed management strategies within extreme watering environments will differ between wild and hybrid radishes. Hybrid seedlings may emerge more frequently, but these populations do not appear to evolve in response to soil moisture. Since hybrid populations responded to diverse watering environments in Ontario in similar ways ("generalist phenotype" strategy), weed management may be relatively consistent across moisture environments. Tilling repeatedly early in the season and delaying crop planting will support attempts to eradicate weedy crop-wild hybrid radish from agricultural environments under any moisture regime. Notably, hybrid radish appears to be easily controlled in agricultural environments worldwide but it is a significant weed in natural areas in California (Ridley and Ellstrand, 2010); suggesting that the environmental conditions that impose selection can vary depending on where populations are growing and may be reflected as different evolutionary rates. Therefore, eradication strategies of hybrid weeds in areas with different land-use histories may be more difficult to control and require new IWM strategies or strategies similar to those used for wild radish (see below); this, however, would need to be further investigated before any strategies can be implemented.

Evolutionary knowledge could be incorporated into managing plant population dynamics (and thus potentially weed management strategies) in a number of ways. For instance, reducing population size of a weed through altered cultural practices such as tilling or herbicides applications lowers the chance of population persistence via random genetic drift, assuming standing genetic variance is low (Goodman, 1987; Lande, 1993; Gomulkiewicz and Holt, 1995). However, if genetic variation is high (as in hybrid populations), crop-wild hybrid weed populations may still be able to respond to selection, overcome drift and reduce the degree to which hybrid populations are maladapted through rapid evolution (Husband and Campbell, 2004). This is where understanding rate of evolution can be a critical tool in weed management; if a weed population's ROE is slow, even moderate rates of genetic diversity will likely not be enough to support persistence, as population size may remain at a critically low level for too long (Gomulkiewicz and Holt, 1995). By understanding rate of evolution metrics and relative fitness of crop-wild hybrids, weed culling events (i.e., herbicide application or tilling) can be timed when the population is in a phase that is vulnerable to rapid demographic decline. Rapidly evolving populations will be slower to reach this vulnerable phase than slowly evolving populations, all else being equal; thus, it is important to know if weedy, crop-wild hybrid populations evolve at different rates from weedy progenitors. Knowing how these weed control methods act as selective pressures in promoting adaptive evolution is also vital in the execution of a successful IWM strategy. For example, extensive herbicide use can increase the rate of evolution of herbicide resistance. Therefore, mitigation techniques should diversify the herbicides used and reduce the number of application events to control weeds (Swanton and Weise, 1991; Bond and Grundy, 2001; Beckie, 2006). Although weed management strategies tend to vary between cultivars, understanding the effects of genetic diversity along with environmental context on the rate of adaptive evolution of weeds will be a key tool in an IWM plan. Here, we contribute to this work by starting to measure the rate of evolution in weed populations with elevated genetic diversity and diverse forms of selection imposed.

Wild radish weed mitigation strategies will differ from hybrid strategies, considering their ability to evolve relatively quickly in response to different watering environments. Wild radishes appear to pose a greater challenge in south-western Ontario agricultural environments because they are more likely to evade eradication based on traditional tilling schedules and herbicide applications (Monjardino et al., 2003; Warwick and Francis, 2005). For example, in Australia where wild radish is an aggressive weed, frequently rotating herbicide type and application time or altering crop harvest times (early vs. late) are strategies implemented to control wild radish weeds. Varying application strategies aim to prevent rapid evolution of herbicide resistance and/or phenology matching (between weed and crop), respectively, to ensure more successful weed removal and management (Ashworth et al., 2016). Knowing the rate at which new weeds are evolving, however, will help us implement a multi-faceted timed weed control strategy (i.e., varying tilling times and planting sites, varying water schedules, varying herbicide/pesticide treatments, etc.) that slows wild weed evolution. Furthermore, for existing weeds that have already employed a multi-faceted IWM strategy, periodically evaluating evolutionary rates of their weedy traits can support the efficacy of current IWM strategies and assess whether changes need to be made.

Research addressing the influence of the environment on the spread of crop-wild hybrid weeds is an important, preventative step in managing the risk of the evolution of crop-wild hybrid weeds. Our work evaluates the effect of watering on the rate of evolution of crop-wild hybrid weeds and, although our results suggest watering does not drive weedy trait evolution in crop-wild hybrids, it raises new questions on what facilitates the rate of evolution and success of hybrid radish documented in other environments (Campbell et al., 2006; Hegde et al., 2006; Campbell and Snow, 2007; Hovick et al., 2012; Teitel et al., 2016a). Although, research evaluating environmental variation on invasive potential can take time to properly test and evaluate, thorough research can be critical in creating and implementing effective IWM and policy. For example, to create IWM applicable across environments, assessing both wild and crop-wild hybrid genotypes in response to a range of abiotic and biotic selection forces and across a range of managed and unmanaged environments will be imperative. Alternatively, assessing multiple traits (particularly in connection to genetically modified crops) or non-canalized crop traits, if any, in these environments can make the difference in predicting and controlling invasive plant outbreaks and crop destruction. Finally, our work demonstrates only one of the many trajectories of crop-wild hybrid weed populations. Our research, more importantly, enacts the precautionary principle in environmental decision making, where precautionary measures should be taken even if results are not fully established or significant (Kriebel et al., 2001; Agriculture Agro-Food Canada, 2016).

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: figshare https://doi.org/10.6084/m9.figshare.12893927.v1.

AUTHOR CONTRIBUTIONS

LC designed the experiment. KS ran the data analysis with support from AL. KS and SS wrote the manuscript with support from LC and AL. KS, SS, and JB helped with experimental setup, data collection, and data analysis. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2020.600346/full#supplementary-material

REFERENCES

Abdi, H., and Williams, L. J. (2010). Principal component analysis. *WIREs Comp. Stat.* 2, 433–459. doi: 10.1002/wics.101

Agriculture and Agro-Food Canada (2016). *Reduced-Risk Strategy for Integrated Weed Management in Field Vegetables*.

Alexander, M. (1969). Differential staining of aborted and nonaborted pollen. *Stain Technol.* 44, 117–122. doi: 10.3109/10520296909063335

Anderson, E., and Stebbins, G. L. Jr. (1954). Hybridization as an evolutionary stimulus. *Evolution* 8, 378–388. doi: 10.1111/j.1558-5646.1954.tb01504.x

Antonovics, G., and Bradshaw, A. (1970). Evolution in closely adjacent plant populations VIII. Clinal patterns at a mine boundary. *Heredity* 25:349. doi: 10.1038/hdy.1970.36

Arendt, J. D. (1997). Adaptive intrinsic growth rates: an integration across taxa. *Q. Rev. Biol.* 72, 149–177. doi: 10.1086/419764

Arnold, M. L., and Martin, N. H. (2010). Hybrid fitness across time and habitats. *Trends Ecol. Evol.* 25, 530–536. doi: 10.1016/j.tree.2010.06.005

Ashworth, M. B., Walsh, M. J., Flower, K. C., Vila-Aiub, M. M., and Powles, S. B. (2016). Directional selection for flowering time leads to adaptive evolution in *Raphanus raphanistrum* (Wild radish). *Evol. Appl.* 9, 619–629. doi: 10.1111/eva.12350

Baack, E. J., Sapir, Y., Chapman, M. A., Burke, J. M., and Rieseberg, L. H. (2008). Selection on domestication traits and quantitative trait loci in crop–wild sunflower hybrids. *Mol. Ecol.* 17, 666–677. doi: 10.1111/j.1365-294X.2007.03596.x

Bagavathiannan, M. V., and Van Acker, R. C. (2008). Crop ferality: implications for novel trait confinement. *Agric. Ecosyst. Environ.* 127, 1–6. doi: 10.1016/j.agee.2008.03.009

Baker, H. G. (1965). *Characteristics and Modes of Origin of Weeds*. New York, NY: Academic Press Inc., 147–172.

Beckie, H. J. (2006). Herbicide-resistant weeds: management tactics and practices. *Weed Technol.* 20, 793–814. Available online at: https://www.jstor.org/stable/4495755?seq=1

Beckie, H. J., Blackshaw, R. E., Low, R., Hall, L. M., Sauder, C. A., Martin, S., et al. (2013). Glyphosate-and acetolactate synthase inhibitor–resistant kochia (*Kochia scoparia*) in western Canada. *Weed Sci.* 61, 310–318. doi: 10.1614/WS-D-12-00140.1

Berger, J. D., Adhikari, K. N., Wilkinson, D., Buirchell, B. J., and Sweetingham, M. W. (2008). Ecogeography of the Old World lupins. 1. Ecotypic variation in yellow lupin (*Lupinus luteus* L.). *Aust. J. Agric. Res.* 59, 691–701. doi: 10.1071/AR07384

Berger, J. D., and Ludwig, C. (2014). Contrasting adaptive strategies to terminal drought-stress gradients in Mediterranean legumes: phenology, productivity, and water relations in wild and domesticated *Lupinus luteus* L. *J. Exp. Bot.* 65, 6219–6229.doi :10.1093/jxb/eru006

Bock, D. G., Caseys, C., Cousens, R. D., Hahn, M. A., Heredia, S. M., Hübner, S., et al. (2015). What we still don't know about invasion genetics. *Mol. Eco.* 24, 2277–2297. doi: 10.1111/mec.13032

Bond, W., and Grundy, A. C. (2001). Non-chemical weed management in organic farming systems. *Weed Res.* 41, 383–405. doi: 10.1046/j.1365-3180.2001.00246.x

Bone, E., and Farres, A. (2001). Trends and rates of microevolution in plants. *Genetica* 112, 165–182. doi: 10.1023/A:1013378014069

Campbell, L. G., Parker, R. J., Blakelock, G., Pirimova, N., and Mercer, K. L. (2015). Maternal environment influences propagule pressure of an invasive plant, *Raphanus raphanistrum* (Brassicaceae). *Int. J. Plant Sci.* 176, 393–403. doi: 10.1086/680683

Campbell, L. G., Shukla, K., Sneck, M. E., Chaplin, C., and Mercer, K. L. (2016a). The effect of altered soil moisture on hybridization rate in a crop-wild system (*Raphanus* spp.). *PLoS ONE*. 11:166802. doi: 10.1371/journal.pone.0166802

Campbell, L. G., and Snow, A. A. (2007). Competition alters life history and increases the relative fecundity of crop-wild radish hybrids (*Raphanus* spp.). *New Phytol.* 173, 648–660. doi: 10.1111/j.1469-8137.2006.01941.x

Campbell, L. G., and Snow, A. A. (2009). Can feral weeds evolve from cultivated radish (*Raphanus sativus*, Brassicaceae)? *Am. J. Bot.* 96, 498–506. doi: 10.3732/ajb.0800054

Campbell, L. G., Snow, A. A., and Ridley, C. E. (2006). Weed evolution after crop gene introgression: Greater survival and fecundity of hybrids in a new environment. *Ecol. Lett.* 9, 1198–1209. doi: 10.1111/j.1461-0248.2006.00974.x

Campbell, L. G., Snow, A. A., and Sweeney, P. M. (2009b). When divergent life histories hybridize: Insights into adaptive life-history traits in an annual weed. *New Phytol.* 184, 806–818. doi: 10.1111/j.1469-8137.2009.03036.x

Campbell, L. G., Snow, A. A., Sweeney, P. M., and Ketner, J. M. (2009a). Rapid evolution in crop-weed hybrids under artificial selection for divergent life histories. *Evol. App.* 2, 172–186. doi: 10.1111/j.1752-4571.2008.00051.x

Campbell, L. G., Teitel, Z., and Miriti, M. N. (2016b). Contemporary evolution and the dynamics of invasion in crop-wild hybrids with heritable variation for two weedy life-histories. *Evol. Appl.* 9, 697–708. doi: 10.1111/eva.12366

Casper, B. B., and Jackson, R. B. (1997). Plant competition underground. *Annu. Rev. Ecol. Syst.* 28, 517–544. doi: 10.1146/annurev.ecolsys.28.1.545

Conner, J., and Via, S. (1993). Patterns of phenotypic and genetic correlations among morphological and life-history traits in wild radish, *Raphanus raphanistrum*. *Evolution*. 47, 704–711. doi: 10.2307/2410086

Davies, M. S., and Snaydon, R. W. (1976). Rapid population differentiation in a mosaic environment III. Measures of selection pressures. *Heredity* 36, 59–66. doi: 10.1038/hdy.1976.6

Dekker, J. (1997). Weed diversity and weed management. *Weed Sci.* 45, 357–363. doi: 10.1017/S0043174500092985

Délye, C., Menchari, Y., Michel, S., Cadet, É., and Le Corre, V. (2013). A new insight into arable weed adaptive evolution: mutations endowing herbicide resistance also affect germination dynamics and seedling emergence. *Ann. Bot.* 111, 681–691. doi: 10.1093/aob/mct018

Dlugosch, K. M., and Parker, I. M. (2008). Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. *Ecol. Lett.* 11, 701–709. doi: 10.1111/j.1461-0248.2008.01181.x

Ellstrand, N. C., and Hoffman, C. A. (1990). Hybridization as an avenue of escape for engineered genes. *Bioscience* 40, 438–442. doi: 10.2307/1311390

Ellstrand, N. C., and Marshall, D. L. (1985). The impact of domestication on distribution of allozyme variation within and among cultivars of radish, *Raphanus sativus* L. *Theor. Appl. Genet.* 69, 393–398. doi: 10.1007/BF00570908

Ellstrand, N. C., and Schierenbeck, K. A. (2006). Hybridization as a stimulus for the evolution of invasiveness in plants? *Euphytica* 97, 7043–7050. doi: 10.1073/pnas.97.13.7043

Flint-Garcia, S. A. (2013). Genetics and consequences of crop domestication. *J Agr. Food Chem.* 61, 8267–8276. doi: 10.1021/jf305511d

Franks, S. J., Hamann, E., and Weis, A. E. (2018). Using the resurrection approach to understand contemporary evolution in changing environments. *Evol. Appl.* 11, 17–28. doi: 10.1111/eva.12528

Gasser, C. S., and Fraley, R. T. (1989). Genetically engineering plants for crop improvement. *Science* 244, 1293–1299. doi: 10.1126/science.244.4910.1293

Germain, R. M., Caruso, C. M., and Maherali, H. (2013). Mechanisms and consequences of water stress–induced parental effects in an invasive annual grass. *Int. J. Plant Sci.* 174, 886–895. doi: 10.1086/670691

Gingerich, P. D. (1983). Rates of evolution: effects of time and temporal scaling. *Science* 222, 159–161. doi: 10.1126/science.222.4620.159

Gingerich, P. D. (1993). Quantification and comparison of evolutionary rates. *Am. J. Sci.* 293, 453–478. doi: 10.2475/ajs.293.A.453

Gingerich, P. D. (2001). Rates of evolution on the time scale of the evolutionary process. *Genetica* 8, 127–144. doi: 10.1023/A:1013311015886

Goldie, X., Gillman, L., Crisp, M., and Wright, S. (2010). Evolutionary speed limited by water in arid Australia. *in Proc. Roy. Soc. B.* 277, 2645–2653. doi: 10.1098/rspb.2010.0439

Gomulkiewicz, R., and Holt, R. D. (1995). When does evolution by natural selection prevent extinction? *Evolution* 49, 201–207. doi: 10.1111/j.1558-5646.1995.tb05971.x

Goodman, D. (1987). "The demography of chance extinction" in *Viable Populations for Conservation*, ed. M. E. Soulé (Cambridge: Cambridge University Press), 11–34. doi: 10.1017/CBO9780511623400.003

Government of Canada (2018). *Historical Climate Data - Buttonville A in E. a. N. Resources, ed*. Government of Canada.

Grime, J. P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *Am. Nat.* 111, 1169–1194. doi: 10.1086/283244

Haldane, J. B. S. (1949). Suggestions as to quantitative measurement of rates of evolution. *Evolution* 51–56. doi: 10.1111/j.1558-5646.1949.tb00004.x

Hartman, Y., Uwimana, B., Hooftman, D. A. P., Schranz, M. E., van de Wiel, C. C. M., Smulders, M. J. M., et al. (2013). Genomic and environmental selection patterns in two distinct lettuce crop-wild hybrid crosses. *Evol. Appl.* 6, 569–584. doi: 10.1111/eva.12043

Hastwell, G. T., and Panetta, F. D. (2005). Can differential responses to nutrients explain the success of environmental weeds? *J. Veg. Sci.* 16, 77–84. doi: 10.1111/j.1654-1103.2005.tb02340.x

Hegde, S. G., Nason, J. D., Clegg, J. M., and Ellstrand, N. C. (2006). The evolution of California's wild radish has resulted in the extinction of its progenitors. *Evolution* 60, 1187–1197. doi: 10.1554/05-634.1

Hendry, A. P., and Kinnison, M. T. (1999). The pace of modern life: measuring rates of micro-evolution. *Evolution* 53, 1637–1653. doi: 10.1111/j.1558-5646.1999.tb04550.x

Holm, L., Del, Y., Holm, E., and Herberger, T. (1997). *World Weeds: Natural Histories and Distribution*. New York, NY: John Wiley & Sons.

Hovick, S. M., Campbell, L. G., Snow, A. A., and Whitney, K. D. (2012). Hybridization alters early life-history traits and increases plant colonization success in a novel region. *Am. Nat.* 179, 192–203. doi: 10.1086/663684

Huang, Y., Zhao, X., Zhang, H., Huang, G., Luo, Y., and Japhet, W. (2009). A comparison of phenotypic plasticity between two species occupying different positions in a successional sequence. *Ecol. Res.* 24:1335. doi: 10.1007/s11284-009-0615-4

Husband, B. C. and L. G. Campbell (2004). *Population responses to novel environments: implications for ex situ plant conservation*. in Ex situ plant conservation: supporting species survival in the wild, eds. E. O. Guerrant, Jr, K. Havens, and M. Maunder (Washington DC: Island Press), p. 231–266.

Irwin, Rebecca, E., and Strauss, Sharon, Y. (2005). Flower color microevolution in wild radish: evolutionary response to pollinator-mediated election. *Am. Nat.* 165, 225–237. doi: 10.1086/426714

Jump, A. S., Peñuelas, J., Rico, L., Ramallo, E., Estiarte, M., Martínez-Izquierdo, J. A., et al. (2008). Simulated climate change provokes rapid genetic change in the Mediterranean shrub *Fumana thymifolia*. *Glob. Chang. Biol.* 13, 1605–1615. doi: 10.1111/j.1365-2486.2007.01521.x

Kay, Q. (1976). Preferential pollination of yellow-flowered morphs of *Raphanus raphanistrum* by *Pieris* and *Eristalis* spp. *Nature* 261:230. doi: 10.1038/261230a0

Kiang, Y. T. (1982). Local differentiations of *Anthoxanthum odoratum* L. population on roadsides. *Am. Midl. Nat.* 107, 340–350. doi: 10.2307/2425384

Kost, M. A., Alexander, H. M., Jason Emry, D., and Mercer, K. L. (2015). Life history traits and phenotypic selection among sunflower crop-wild hybrids and their wild counterpart: implications for crop allele introgression. *Evol. Appl.* 8, 560–572. doi: 10.1111/eva.12261

Kriebel, D., Tickner, J., Epstein, P., Lemons, J., Levins, R., Loechler, E. L., et al. (2001). The precautionary principle in environmental science. *Environ. Health Perspect.* 109, 871–876. doi: 10.1289/ehp.01109871

Lande, R. (1993). Risks of population extinction from demographic and environmental stochasticity and random catastrophes. *Am. Nat.* 142, 911–927.doi: 10.1086/285580

Langevin, S. A., Clay, K., and Grace, J. B. (1990). The incidence and effects of hybridization between cultivated rice and its related weed red rice (*Oryza sativa* L.). *Evolution* 4, 1000–1008. doi: 10.1111/j.1558-5646.1990.tb03820.x

Lavergne, S., and Molofsky, J. (2007). Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proc. Natl. Acad. Sci. U. S. A.* 104, 3883–3888. doi: 10.1073/pnas.0607324104

Luke, S. G. (2017). Evaluating significance in linear mixed-effects models in R. *Behav. Res. Methods.* 49, 1494–1502. doi: 10.3758/s13428-016-0809-y

Macnair, M. R., Smith, S. E., and Cumbes, Q. J. (1993). Heritability and distribution of variation in degree of copper tolerance in *Mimulus guttatus* at Copperopolis, California. *Heredity* 71, 445–455. doi: 10.1038/hdy.1993.162

Mallory-Smith, C., Hendrickson, P., and Mueller-Warrant, G. (1999). Cross-resistance of primisulfuron-resistant *Bromus tectorum* L. (downy brome) to sulfosulfuron. *Weed Sci.* 47, 256–257. doi: 10.1017/S0043174500091736

Matesanz, S., Gianoli, E., and Valladares, F. (2010). Global change and the evolution of phenotypic plasticity in plants. *Ann. NY Acad. Sci.* 1206, 35–55. doi: 10.1111/j.1749-6632.2010.05704.x

Maxwell, K., and Johnson, G. N. (2000). Chlorophyll fluorescence—a practical guide. *J. Exp. Bot*. 51:659–668. doi: 10.1093/jexbot/51.345.659

Mercer, K. L., Andow, D. A., Wyse, D. L., and Shaw, R. G. (2007). Stress and domestication traits increase the relative fitness of crop–wild hybrids in sunflower. *Ecol. Let.* 10, 383–393. doi: 10.1111/j.1461-0248.2007.01029.x

Mohammed, S. B., Khalid, A., Osman, S. E. F., and Helali, R. G. M. (2016). *Usage of Principal Component Analysis (PCA) in AI Applications*. Khartoum, Sudan. IJERT. 5.

Monjardino, M., Pannell, D. J., and Powles, S. B. (2003). Multispecies resistance and integrated management: a bioeconomic model for integrated management of rigid ryegrass (*Lolium rigidum*) and wild radish (*Raphanus raphanistrum*). *Weed Sci.* 51, 798–809. doi: 10.1614/P2002-118

Nason, J. D., and Ellstrand, N. C. (1995). Lifetime estimates of biparental inbreeding depression in the self-incompatible annual plant Raphanus sativus. *Evolution* 49, 307–316. doi: 10.1111/j.1558-5646.1995.tb02243.x

Nicotra, A. B., Atkin, O. K., Bonser, S. P., Davidson, A. M., Finnegan, E., Mathesius, U., et al. (2010). Plant phenotypic plasticity in a changing climate. *Trends Plant Sci.* 15, 684–692. doi: 10.1016/j.tplants.2010.09.008

Oplaat, C., and Verhoeven, K. J. F. (2015). Range expansion in asexual dandelions: selection for general-purpose genotypes? *J. Ecol.* 103, 261–268. doi: 10.1111/1365-2745.12347

Ord, T. J., and Hundt, P. J. (2020). Crossing extreme habitat boundaries: jack-of-all-trades facilitates invasion but is eroded by adaptation to a master-of-one. *Func. Ecol.* 34, 1404–1415. doi: 10.1111/1365-2435.13600

Panetsos, C. A., and Baker, H. G. (1967). The origin of variation in "wild" *Raphanus sativus* (Cruciferae) in California. *Genetica* 38, 243–274. doi: 10.1007/BF01507462

Pichancourt, J.-B., and van Klinken, R. D. (2012). Phenotypic plasticity influences the size, shape and dynamics of the geographic distribution of an invasive plant. *PLoS ONE* 7:e32323. doi: 10.1371/journal.pone.0032323

Powles, S. B., Lorraine-Colwill, D. F., Dellow, J. J., and Preston, C. (1998). Evolved resistance to glyphosate in rigid ryegrass (*Lolium rigidum*) in Australia. *Weed Sci.* 46, 604–607. doi: 10.1017/S0043174500091165

Presotto, A., Pandolfo, C., Poverene, M., and Cantamutto, M. (2016). Can achene selection in sunflower crop–wild hybrids by pre-dispersal seed predators hasten the return to phenotypically wild sunflowers? *Euphytica* 208, 453–462. doi: 10.1007/s10681-015-1579-9

Richards, C. L., Bossdorf, O., Muth, N. Z., Gurevitch, J., and Pigliucci, M. (2006). Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecol. Lett.* 9, 981–993. doi: 10.1111/j.1461-0248.2006.00950.x

Ridley, C. E., and Ellstrand, N. C. (2010). Rapid evolution of morphology and adaptive life history in the invasive California wild radish (*Raphanus sativus*) and the implications for management. *Evol. App.* 3, 64–76. doi: 10.1111/j.1752-4571.2009.00099.x

Rodgers, E. G. (1978). "Weeds and their control," in *Fundamentals of Plant-Pest Control*, ed. D.A. Roberts (San Francisco, CA: W. H. Freeman and Co.), 164–168.

Rohde, K. (1992). Latitudinal gradients in species diversity: The search for the primary cause. Oikos. 65, 514–527. doi: 10.2307/3545569

Sadras, V., Reynolds, M., De la Vega, A., Petrie, P., and Robinson, R. (2009). Phenotypic plasticity of yield and phenology in wheat, sunflower and grapevine. *Field Crop Res.* 110, 242–250. doi: 10.1016/j.fcr.2008.09.004

Sahli, H. F., Conner, J. K., Shaw, F. H., Howe, S., and Lale, A. (2008). Adaptive differentiation of quantitative traits in the globally distributed weed, wild radish (*Raphanus raphanistrum*). *Genetics* 180, 945–955. doi: 10.1534/genetics.107.085084

Schierenbeck, K. A., and Ellstrand, N. C. (2009). Hybridization and the evolution of invasiveness in plants and other organisms. *Biol. Invasions*. 11:1093. doi: 10.1007/s10530-008-9388-x

Snaydon, R. W., and Davies, M. S. (1972). Rapid population differentiation in a mosaic environment. II. Morphological variation in *Anthoxanthum odoratum*. *Evolution* 26, 390–405. doi: 10.1111/j.1558-5646.1972.tb01944.x

Sneck, M. (2012). *Evolutionary Responses to Global Change: An Experimental Test of the Effect of Altered Precipitation on Hybridization Rates in Sunflower (Helianthus)*. (Master Thesis). Rice University, Houston, TX, United States.

Snow, A. A. (2002). Transgenic crops—why gene flow matters. *Nat. Biotechnol.* 20, 542–542. doi: 10.1038/nbt0602-542

Snow, A. A., and Campbell, L. G. (2005). "Can feral radishes become weeds?" in *Crop Ferality and Volunteerism*, ed. J. B. Gressel (Florida, FL: CRC Press), 193-208.

Snow, A. A., Culley, T. M., Campbell, L. G., Sweeney, P. M., Hegde, S. G., and Ellstrand, N. C. (2010). Long-term persistence of crop alleles in weedy populations of wild radish (*Raphanus raphanistrum*). *New Phytol.* 186, 537–548. doi: 10.1111/j.1469-8137.2009.03172.x

Snow, A. A., Uthus, K. L., and Culley, T. M. (2001). Fitness of hybrids between weedy and cultivated radish: Implications for weed evolution. *Ecol. Appl.* 11, 999–1007. doi: 10.1890/1051-0761(2001)0110934:FOHBWA2.0.CO;2

Stanton, M. L., Snow, A. A., Handel, S. N., and Bereczky, J. (1989). The impact of a flower-color polymorphism on mating patterns in experimental populations of wild radish (*Raphanus raphanistrum* L.). *Evolution* 43, 335–346. doi: 10.1111/j.1558-5646.1989.tb04231.x

Strauss, S. Y., Irwin, R. E., and Lambrix, V. M. (2004). Optimal defence theory and flower petal colour predict variation in the secondary chemistry of wild radish. *J. Ecol.* 92, 132–141. doi: 10.1111/j.1365-2745.2004.00843.x

Swanton, C. J., and Weise, S. F. (1991). Integrated weed management: the rationale and approach. *Weed Technol.* 5, 657–663. doi: 10.1017/S0890037X00027512

Teitel, Z., Klimowski, A., and Campbell, L. G. (2016a). Assessing the effects of hybridization and precipitation on invasive weed demography using strength of selection on vital rates. *BMC Evol. Bio.* 16:266. doi: 10.1186/s12862-016-0833-7

Teitel, Z., Laursen, A. E., and Campbell, L. G. (2016b). Germination rates of weedy radish populations (*Raphanus* spp.) altered by crop-wild hybridisation, not human-mediated changes to soil moisture. *Weed Res.* 56, 149–158. doi: 10.1111/wre.12194

Valladares, F., Gianoli, E., and Gómez, J. M. (2007). Ecological limits to plant phenotypic plasticity. *New Phytol.* 176, 749–763. doi: 10.1111/j.1469-8137.2007.02275.x

Via, S., and Lande, R. (1985). Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* 39, 505–522. doi: 10.1111/j.1558-5646.1985.tb00391.x

Vigueira, C. C., Olsen, K. M., and Caicedo, A. L. (2013). The red queen in the corn: agricultural weeds as models of rapid adaptive evolution. *Heredity* 110, 303–311. doi: 10.1038/hdy.2012.104

Ward, J. K., Antonovics, J., Thomas, R. B., and Strain, B. R. (2000). Is atmospheric CO_2 a selective agent on model C3 annuals? *Oecologia* 123, 330–341. doi: 10.1007/s004420051019

Warwick, S. I., and Francis, A. (2005). The biology of Canadian weeds. 132. *Raphanus raphanistrum* L. *Can. J. Plant Sci.* 85, 709–733. doi: 10.4141/P04-120

Warwick, S. I., Phillips, D., and Andrews, C. (1986). Rhizome depth: the critical factor in winter survival of *Sorghum halepense* (L.) Pers. (Johnson grass). *Weed Res.* 26, 381–388. doi: 10.1111/j.1365-3180.1986.tb00721.x

Weinig, C. (2000). Plasticity versus canalization: population differences in the timing of shade-avoidance responses. *Evolution* 54, 441–451. doi: 10.1111/j.0014-3820.2000.tb00047.x

Whitney, K. D., Ahern, J. R., and Campbell, L. G. (2009). Hybridization-prone plant families do not generate more invasive species. *Biol. Invasions*. 11, 1205–1215. doi: 10.1007/s10530-008-9390-3

Whitney, K. D., and Gabler, C. A. (2008). Rapid evolution in introduced species, "invasive traits" and recipient communities: challenges for predicting invasive potential. *Divers Distrib.* 14, 569–580. doi: 10.1111/j.1472-4642.2008.00473.x

Whitney, K. D., Randell, R. A., and Rieseberg, L. H. (2006). Adaptive introgression of herbivore resistance traits in the weedv sunflower *Helianthus annuus*. *Am. Nat.* 167, 794–807 doi: 10.1086/504606

Wolf, J. B., and Wade, M. J. (2009). What are maternal effects (and what are they not)? *Phil. T. Roy. Soc. B* 364, 1107–1115. doi: 10.1098/rstb.2008.0238

Wright, S. (1968). *Evolution and the Genetics of Populations. Vol. 1. Genetic and Biométrie Foundations.* London and Chicago: University of Chicago Press.

Wright, S., Keeling, J., and Gillman, L. (2006). The road from Santa Rosalia: a faster tempo of evolution in tropical climates. *Proc. Natl. Acad. Sci. U. S. A.* 103, 7718–7722. doi: 10.1073/pnas.0510383103

Ye, C. Y., Tang, W., Wu, D., Jia, L., Qiu, J., Chen, M., et al. (2019). Genomic evidence of human selection on Vavilovian mimicry. *Nat. Ecol. Evol.* 3, 1474–1482. doi: 10.1038/s41559-019-0976-1

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Influence of Cereal Rye Management on Weed Control in Soybean

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Cereal rye as a cover crop is often used to improve soil health and as part of integrated weed management programs. Despite this, cereal rye biomass is often not managed for optimal weed suppression. This study evaluated the effects of managing cereal rye as part of an integrated weed management strategy in soybean. Factors consisted of levels of cereal rye management (no cereal rye, no nitrogen, or 20 kg/ha of nitrogen); cereal rye termination timing (20 or 10 d before soybean planting); and residual herbicide treatment applied at cereal rye termination (with or without). Winter annual weed control with cereal rye was generally greater compared to no cereal rye. Winter annual weed control was consistently better when cereal rye was terminated at 20 d before soybean planting compared to 10 d; while summer annual weed control was improved if termination was delayed. Effect of cereal rye management on summer annual weed control varied by weed species. In the absence of residual herbicides, Palmer amaranth control responded to the different levels of cereal rye management. However, morningglory spp. only responded to rye with supplemental N applications. Large crabgrass control was similar for treatments containing cereal rye, regardless of nitrogen input. Our results demonstrate the importance of cover crop management when incorporating cereal rye into an integrated weed management program for soybean.

Keywords: ***Amaranthus palmeri*, *Erigeron canadensis*, herbicide resistance, integrated weed management, *Ipomoea* spp. weed suppression, winter annual weeds**

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INTRODUCTION

Cover crops provide a number of ecological services, or benefits. These benefits include reducing erosion, preventing nutrient loss, providing pollinator habitat, fixing atmospheric nitrogen, and increasing soil microbial densities. In the Chesapeake Bay Watershed, adoption of cover crops for nutrient management has been very successful. Most cover crops are planted under cost-share programs that specify deadlines for fall planting dates, but allow termination in early spring. As a result, many cover crop fields receive a non-selective herbicide application in the spring prior to the rapid growth phase of most winter-hardy cover crops, which leads to very little cover crop residue present at planting.

Non-selective herbicide applications to terminate cover crops often provide excellent control of emerged weed seedlings. However, early spring termination often occurs before there is sufficient cover crop biomass to provide weed suppression of later-emerging weeds. Cover crops allowed to advance to the late-vegetative or early-reproductive stages produce significantly more biomass. In addition, the cover crop tissue contains a higher C:N ratio that resists decomposition and allows the dead biomass to persist longer (Wagger et al., 1998; Pittman et al., 2020).

Cover crops terminated later in the spring, provide weed suppression through direct plant-to-plant competition and/or as a physical barrier. Prior to cover crop termination, cover crops compete with weeds for resources required for growth (nutrients, moisture, sunlight) and typically cover crop species that gain a height advantage or rapidly shade the soil surface are the most competitive. These interactions occur between fall-seeded cover crops and winter annual weeds and weed species that germinate early spring before the cover crop is terminated.

After termination, desiccated cover crops tissue suppress weeds by altering light exposure at the soil surface, lowering night/day temperature fluctuations, lowering soil temperatures, and serving as a physical barrier that limits weed seedling growth (Teasdale and Mohler, 1993; Mirsky et al., 2011; DeVore et al., 2013). The higher the level of biomass the better weed suppression (Mohler and Teasdale, 1993).

Studies have documented lower weed densities and shorter weeds in the presence of cover crops (Montgomery et al., 2018; Wallace et al., 2019; DeSimini et al., in press). As a result, emerged weeds are susceptible to postemergence herbicides over a longer time period due to delayed emergence and slower growth.

Studies examining termination timing have shown that allowing an additional 2 wk of cover crop growth during the rapid stem elongation phase can result in a substantial increase in cover crop biomass (Mirsky et al., 2011; Cornelius and Bradley, 2017a; Whalen et al., 2020). However, there are few published studies examining late-spring termination on both winter annual and summer annual weeds.

Cover crops have been researched as an integrated weed management tool, particularly, to manage herbicide-resistant biotypes. Multiple-herbicide resistance has been documented in numerous weed species in the Mid-Atlantic region, including acetolactate synthase-inhibiting (ALS)- and glyphosate-resistant Palmer amaranth (*Amaranthus palmeri* [S. Wats.]), common ragweed (*Ambrosia artemisiifolia* [L.]), and horseweed (*Erigeron canadensis* L. [Cronq.]) (Heap, 2020). Although not widespread, common ragweed resistant to protoporphyrinogen oxidase inhibiting (PPO)-herbicides has also been documented in several Mid-Atlantic States (Heap, 2020). Preliminary studies have shown cover crops can play an important role in mitigating resistance and improving overall weed control. Cover crops, as a part of an integrated approach, requires additional research to better understand the contribution of the various tactics for overall weed control. This experiment was initiated to evaluate cereal rye (*Secale cereale* L.) management in combination with herbicides for integrated management of winter and summer annual weeds.

MATERIALS AND METHODS

This study was conducted over three soybean (*Glycine max* [L.] Merr.) growing seasons beginning in 2015 at the University of Delaware's Carvel Research and Education Center located near Georgetown, DE (38.64°N, 75.46°W). In 2015 and 2017, the soil was a Pepperbox loamy sand (loamy, mixed, mesic Aquic Arenic Paleudults) and in 2016 the soil was a Rosedale loamy sand (loamy, siliceous, mesic Arenic Hapludults). Soil texture was either loamy sand or sandy loam with 1.5–2.5% organic matter and pH ranging from 4.8 to 6.5 (**Table 1**).

The study included three factors, cereal rye management, timing of cereal rye termination, and use of residual herbicides. Cereal rye management was no rye, rye without additional N, or rye with 20 kg ha^{-1} of nitrogen applied before jointing. Rye was terminated 20 or 10 d prior to soybean planting. The residual herbicide treatment was included at the time of cover crop termination or no residual herbicide was used.

The entire site was drilled (rows 18 cm spacing) with 135 kg ha^{-1} of cereal rye in the fall (mid-October–mid-November). The no rye treatments were sprayed in the fall with glyphosate (2015) or clethodim (2016 and 2017).

There were two cereal rye termination timings, which were intended to be 20 or 10 days prior to soybean planting. Early-termination timings ranged from 18 to 29 days early preplant (EPP) while late terminations ranged from 10 to 16 days EPP (**Table 1**). Cereal rye was terminated with glyphosate (Roundup PowerMax®, Monsanto Company, St. Louis, MO) at 1.3 kg ae ha^{-1} plus 2,4-D at 0.5 kg ae ha^{-1} (Weedone® LV4, Nufarm, Inc., Alsip, IL). 2,4-D was included to control glyphosate-resistant weeds. Treatments with residual herbicides were treated with a pre-packaged mixture of chlorimuron plus thifensulfuron plus flumioxazin (19 + 61 + 6 g ai ha^{-1}, respectively) (Envive®, E.I. du Pont de Nemours and Company, Wilmington, DE) tankmixed with the glyphosate plus 2,4-D application.

Comparison treatments included no rye and no spring herbicide application (weedy check for winter annual weeds) and no rye but glyphosate applied early spring (non-treated check for summer annual weeds) to provide reference plots for visual ratings. Weed control ratings for the comparison treatments were not included in statistical analysis. The entire experimental site was treated 5 wk after soybean planting (WAP) with glyphosate plus fomesafen (Reflex®, Syngenta Crop Protection, Greensboro, NC) at 1.3 kg ae ha^{-1} plus 420 g ai ha^{-1}, respectively, plus non-ionic surfactant at 0.25 % v v^{-1} (Scanner®, Loveland Products, Greeley, CO).

TABLE 1 | Dates of field procedures and soil information.

Procedures/soils	2015	2016	2017
Early termination date	April 29	May 9	May 3
Late termination date	May 7	May 17	May 16
Planting date	May 20	May 27	June 1
Seeding rate (seeds ha^{-1})	60,700	72,800	72,800
Variety	4306R2/STS[a]	S43RY95[b]	S43RY95[b]
Postemergence application date	Jule 3	June 30	June 25
Soil texture (% sand)	Sandy loam (78)	Sandy loam (77)	Loamy sand (83)
Soil description (organic matter, pH)	2.5%, 4.8	1.5%, 6.5	1.5%, 5.4

[a] *Channel, St. Louis, MO, www.channel.com.*

[b] *Dyna-Gro Seed, Loveland, CO, www.dynagroseed.com.*

Soybeans were planted no-till in 38 cm rows at 60,700–72,800 seeds ha^{-1} between May 20 and June 1 (**Table 1**). Individual plots were 7.6 m long and 3 m wide (7 rows per plot). All herbicide applications were made with a tractor mounted sprayer, using compressed air, traveling 4.8 km h^{-1}. The spray volume was 187 L ha^{-1} and nozzles were 11,002 (Greenleaf AirMix®, Greenleaf Technologies, Covington, LA) with a pressure of 276 kPa. Treatments were replicated three times in 2015 and four times in 2016 and 2017. The plots were arranged in a randomized complete block design.

Cereal rye biomass was collected 1 wk before planting in 2015 and 2017, and 8 WAP in 2016. Rye biomass was collected in 1 m^{-2} quadrats in all plots without residual herbicide treatments to obtain a representative sample of biomass production. Rye biomass was dried to a constant weight and recorded.

Weed control was evaluated visually on a scale of 0–100, with 0 = no plant response and 100 = complete plant death. Winter annual weeds were rated at soybean planting and summer annual weeds were rated 1, 4, and 8 WAP. The middle five rows of each plot were harvested with a combine at physiological maturity. Yields were adjusted to 13% moisture content.

Statistical Analysis

Weed control data were arcsine square-root transformed prior to analysis to address variance in homogeneity and normality, and untransformed data are presented in the tables. Statistical analyses were conducted with PROC Mixed in SAS version 9.4 (SAS Institute Inc., Cary, NC), using year and replications within year as random effect. Fisher's protected LSD was used for mean separation at $P = 0.05$.

RESULTS

Cereal Rye Biomass

Differences in cereal rye biomass were achieved through supplemental spring nitrogen and termination timing each year. Early-spring nitrogen applications resulted in a 39% increase in biomass in 2015 and 2017 ($p = 0.026$), and a 124% increase in 2016 ($p = 0.0004$) (**Table 2**). In 2016, rye biomass was sampled 8 WAP, which allowed for a significant amount of biomass decomposition, but it is difficult to say how much decomposition had occurred (Poffenbarger et al., 2015; Sievers and Cook, 2018).

In 2015 and 2017, delaying rye termination resulted in a 60% increase in cereal rye biomass ($p = 0.0031$, **Table 2**). No differences were detected in 2016 when rye sampling was done 8 WAP. Early termination corresponded to stem elongation stage (Zadoks 35–37), while later termination occurred during boot stage (Zadoks 41–47). Levels of cereal rye biomass achieved in our trial were similar to other trials investigating weed suppression in the region (Mischler et al., 2010; Mirsky et al., 2011, 2017; Ryan et al., 2011a,b).

Winter Annual Weed Control

Glyphosate was used to remove cereal rye in the no rye treatment in 2015. This resulted in confounding between winter annual weed control and no rye treatments. So analysis did not include 2015 data. Winter annual weeds were rated within 3 d after planting and the main effects were significant but there were no interactions.

Horseweed and cutleaf eveningprimrose (*Oenothera laciniata* [Hill]) were the predominant species in 2016 and 2017, respectively. A multiple-resistant horseweed biotype (glyphosate- and ALS-resistant) was the dominant biotype at this site.

Horseweed control in 2016, rated at soybean planting was greatest for rye with N and least for rye without N ($p = 0.001$,

TABLE 2 | Infuence of cereal rye management and termination timing on cereal rye biomass production.

Main effects	Treatments	2015 and 2017[a]	2016[b]
		g m^{-2}	
Cereal rye management			
	No rye	–	–
	–N	538 b	89 b
	+N[c]	747 a	199 a
Termination timing[d]			
	10 EPP	792 a	148 ns
	20 EPP	493 b	140 ns

[a] *Cereal rye biomass collected 1 wk before soybean planting in 2015 and 2017.*
[b] *Cereal rye biomass collected 8 wk after soybean planting in 2016.*
[c] *Cereal rye received 20 kg ha^{-1} N before jointing.*
[d] *Intended termination timing 10 or 20 d early preplant (EPP), see **Table 1** for actual intervals between application and planting.*

TABLE 3 | Influence of cereal rye management, termination timing, and residual herbicide on horseweed in 2016, cutleaf eveninggprimrose in 2017, winter annual weed control in 2016 and 2017 when rated at soybean planting.

		ERICA[a]			
Main effects	Treatments	At planting	4 WAP	OEOLA	Other winter annual spp.
Cereal rye management		Control (%)			
	No rye	70 b	78 b	81 b	72 b
	–N	65 c	90 a	90 a	81 a
	+N[b]	75 a	82 b	94 a	81 a
Termination timing[c]					
	10 EPP	58 b	75 b	85 b	69 b
	20 EPP	82 a	91 a	92 a	87 a
Residual herbicide					
	None	69 b	83 ns	83 b	76 ns
	Yes[d]	71 a	83 ns	94 a	81 ns

[a] *ERICA, Erigeron canadensis, horseweed; EPP, early preplant; OEOLA, Oenothera lacinata, cutleaf eveningprimrose; W.A., winter annual; WAP, wk after planting.*
[b] *Cereal rye received 20 kg ha^{-1} N before jointing.*
[c] *Intended termination timing was 10 or 20 d early preplant (EPP), see **Table 1** for actual intervals between application and planting.*
[d] *Residual herbicide included a prepackaged mixture of chlorimuron plus thifensulfuron plus flumioxazin.*

Table 3). However, by 4 WAP, horseweed control was better for rye without N compared to rye with N or no rye ($p = 0.002$). Horseweed density was extremely high (over 100 plants m^{-2}) and intra-specific competition accounted for some inconsistency among rye management treatments. Cereal rye terminated 20 d EPP provided better horseweed control than cereal rye terminated 10 d EPP at both rating dates ($p = 0.0001$). At the time of early application, horseweed height was up to 13 cm and up to 23 cm at late termination. Residual herbicide did not have a significant influence on horseweed control since the biotype was predominately glyphosate- and ALS-resistant. 2,4-D was the herbicide active ingredient providing horseweed control and its effectiveness is reduced on larger horseweed plants.

Cutleaf eveningprimrose control was also greatest if cereal rye was present ($p = 0.0002$) and with earlier termination ($p = 0.004$) (**Table 3**) in 2017. In addition, control was better when chlorimuron plus thifensulfuron plus flumioxazin was included ($p = 0.0001$). Chlorimuron plus thifensulfuron plus flumioxazin is labeled for postemergence control of cutleaf eveningprimrose. In this trial, the rate of 2,4-D was not adequate to control cutleaf eveningprimrose, for instance glyphosate plus 2,4-D applied without cover crop or residual herbicide provided only 72% control.

Densities of the remaining winter annual weeds were low, so they were rated together. Remaining winter annual weeds included common chickweed (*Stellaria media* [L.] Vill.), henbit (*Lamium amplexicaule* L.), jagged chickweed (*Holosteum umbellatum* L.), knawel (*Scleranthus annuus* L.) and redstem filaree (*Erodium cicutarium* [L.] L'Her.). Cereal rye management ($p = 0.01$) and termination timing were significant ($p = 0.0001$) (**Table 3**). Remaining winter annual weed control was best when cereal rye was present, regardless of the amount, and when cereal rye was terminated 20 d EPP compared to applications closer to planting.

Summer Annual Weed Control

Palmer amaranth, morningglory species (predominantly *Ipomoea hederacea* Jacq. and secondarily *Ipomoea lacunosa* L.) and large crabgrass (*Digitaria sanguinalis* L. [Scop.]) were the dominant summer annual species and were present in each site year. Main effects were significant for all species rated. Cereal rye management by residual herbicide for Palmer amaranth control was the only interaction observed. Cereal rye terminated 10 d EPP provided better summer annual weed control than rye terminated several weeks before planting. At 1 WAP, cereal rye terminated 10 d EPP improved Palmer amaranth control by 6% compared to rye terminated 20 d EPP ($p = 0.017$, **Table 4**). Palmer amaranth control with residual herbicides was 67–71% regardless of cereal rye management, while treatments without a residual herbicide provided only 35–57% control ($p = 0.0151$). When residual herbicides were omitted, additional cereal rye management improved Palmer amaranth control. Rye with N improved Palmer amaranth control by 11% compared to rye without N and 22% greater than no rye. Loux et al. (2017) reported similar results, with benefits of cereal rye for *Amaranthus* spp. control only observed in the absence of preemergence herbicides.

TABLE 4 | Influence of cereal rye management, termination, timing, and residual herbicides on Palmer amaranth control.

			AMAPA[c] control		
Cereal rye management	**Residual herbicide[a]**	**Termination timing[b]**	**1 WAP**	**4 WAP**	**8 WAP**
			%		
+N[d]	Yes		70 a	95 a	96 a
+N	None		57 b	80 b	90 bc
–N	Yes		71 a	96 a	97 a
–N	None		46 c	72 c	85 c
No rye	Yes		67 a	92 a	93 ab
No rye	None		35 d	53 d	72 d
		10 EPP	61 a	85 a	91 ns
		20 EPP	55 b	78 b	87 ns

[a] *Residual herbicide included a prepackaged mixture of chlorimuron plus thifensulfuron plus flumioxazin.*

[b] *Intended termination timing was 10 or 20 d early preplant (EPP), see **Table 1** for actual intervals between application and planting.*

[c] *AMAPA, Amaranthus palmeri, Palmer amaranth; EPP, d early preplant; WAP, wk after planting.*

[d] *Cereal rye received 20 kg ha^{-1} N before jointing.*

At 4 WAP, trends were similar to those observed 1 WAP. Rye terminated 10 d EPP provided better Palmer amaranth control than rye terminated 20 d EPP ($p = 0.0016$, **Table 4**). Treatments that included residual herbicides provided at least 92% Palmer amaranth control, regardless of rye management ($p = 0.0001$). When residual herbicides were omitted, rye with N controlled Palmer amaranth 8% more than rye without N, and 27% greater than no rye.

The entire study was treated with glyphosate plus fomesafen 5 WAP to assess cereal rye management, termination timing, and residual herbicides for full-season weed control. At 8 WAP, data shows similar trends to what was observed prior to the glyphosate plus fomesafen applications (**Table 4**). Residual herbicides treatments provided at least 93% control of Palmer amaranth, regardless of rye management ($p = 0.0029$). When residual herbicides were omitted, cereal rye provided 85–90% Palmer amaranth control. Furthermore, rye with N alone provided similar control as the residual herbicide treatment with no rye. This is likely due to the increased rye biomass that suppressed growth of Palmer amaranth seedlings and thus were more susceptible to the glyphosate plus fomesafen treatment applied 5 WAP. Fomesafen is a contact herbicide that works best when weeds are small and in low densities. Cereal rye resulted in fewer, smaller amaranth plants, which improved control with the postemergence herbicide application.

At 1 WAP, morningglory spp. control was greater for rye with N compared to rye without N or no cereal rye $p = 0.003$, **Table 5**). Terminating cereal rye 10 d EPP provided 62% control compared to 54% when terminating 20 d EPP ($p = 0.002$). Including a residual herbicide provided 67% compared to 49% control with no residual herbicide (0.0001).

Trends for morningglory spp. control were similar at 4 WAP (**Table 5**). Control improved when N was applied to rye ($p = 0.005$), when rye was terminated 10 d EPP ($p =$

TABLE 5 | Influence of cereal rye management, termination timing, and residual herbicide on control of morningglory spp. and large crabgrass and soybean yield.

Main effects	Treatment	IPOSS[a] control			DIGSA control	Soybean yield
		1 WAP	4 WAP	8 WAP		
		%				kg ha^{-1}
Cereal rye management						
	No rye	54 b	59 b	89 b	68 b	2398 b
	–N	56 b	65 b	95 a	80 a	2877 a
	+N[b]	64 a	72 a	93 a	81 a	2871 a
Termination timing[c]						
	10 EPP	62 a	70 a	95 a	81 a	2805 ns
	20 EPP	54 b	61 b	89 b	72 b	2626 ns
Residual herbicide						
	None	49 b	53 b	91 ns	63 b	2560 b
	Yes[d]	67 a	77 a	93 ns	90 a	2870 a

[a] *DIGSA, Digitaria sanguinalis, large crabgrass; EPP, d early preplant; IPOSS, Ipomoea spp., morningglory species; WAP, wk after planting.*

[b] *Cereal rye received 20 kg ha^{-1} N before jointing.*

[c] *Intended termination timing was 20 d EPP and 10 d EPP, see **Table 1** for actual intervals between application and planting.*

[d] *Residual herbicide included a prepackaged mixture of chlorimuron plus thifensulfuron plus flumioxazin.*

0.0014), and when a residual herbicide was included ($p = 0.0001$). Morningglory spp. control improved with glyphosate plus fomesafen application when rated 8 WAP, but only the main effects of cereal rye management ($p = 0.002$) and termination timing ($p = 0.0001$) were significant (**Table 5**). The presence of cereal rye, regardless of management improved morningglory spp. control compared to no cereal rye (**Table 5**).

At 4 WAP, large crabgrass control was greater when cereal rye was present ($p = 0.0003$), when rye was terminated 10 d EPP ($p = 0.0008$), and when a residual herbicide was included ($p = 0.0001$) (**Table 5**). The greatest improvement in control was the result of including a residual herbicide at termination timing, improving control from 63 to 90%. At 8 WAP, all plots had >97% large crabgrass control due to the glyphosate plus fomesafen application.

Soybean Yield

Soybean yield was greater when cereal rye was present ($p = 0.0001$) and when a residual herbicide was included ($p = 0.001$) (**Table 5**). A yield increase of 20% was observed with cereal rye, regardless of management (**Table 5**). Including a residual herbicide resulted in a 12% increase in yield compared to no residual herbicide.

DISCUSSION

Altering cereal rye management resulted in different levels of biomass, with cereal rye responding to nitrogen applications. Our results show that while residual herbicide treatments were very effective for summer annual weed control, cereal rye with spring N was a consistently successful weed management tactic. While residual herbicides alone provided effective control in this trial, their effectiveness is dependent on environmental factors such as rainfall and soil type. For example, a lack of rainfall or too much rainfall can reduce the efficacy of residual herbicides resulting in lower levels of weed control. In addition, chlorimuron plus thifensulfuron plus flumioxazin will provide 3–4 wk of residual control. In our study, these residual herbicides were applied at least a week before soybean planting. As a result, additional tactics of cereal rye or residual applications close to planting were needed for the highest level of control. Since the Palmer amaranth biotype in this study was glyphosate- and ALS-resistant, there was only one active effective ingredient for residual control, which is not an effective resistance-management strategy (Norsworthy et al., 2012). Including cereal rye helped reduce the selection pressure on the herbicides used in this study.

Our research supports other studies that have shown cereal rye effectiveness in managing both winter (Hayden et al., 2012; Cornelius and Bradley, 2017b; Pittman et al., 2019; Sherman et al., 2020) and summer annual weeds (DeVore et al., 2013; Wiggins et al., 2015, 2016; Loux et al., 2017). Suppression of summer annual weeds is often positively correlated to higher cover crop biomass (Teasdale and Mohler, 2000; Ryan et al., 2011a,b). Cereal rye contains allelochemicals that can influence weed growth, although these effects often are reduced as rye reaches maturity and dissipate within a few weeks of rye termination (Reberg-Horton et al., 2005; Teasdale et al., 2012). Our study was not designed to separate the effects of allelopathy from plant to plant competition or the physical barrier from rye residues.

Additional N inputs or delaying cereal rye termination did improve weed control. These two approaches allowed cover crops to produce more biomass, resulting in additional mulch that hinders weed emergence and is more resistant to decay (Wagger, 1989; Mirsky et al., 2011). However, not all weed species responded consistently to the two levels of cereal rye management. No differences in winter annual weed control were observed among cereal rye treatments with and without N. Cereal rye affects winter annual weeds through inter-species competition, rather than physical suppression. The nitrogen applications were applied in mid-March and so there would have been no difference in ground cover or cereal rye biomass in the fall or early spring presumably when many of the winter annuals emerged. Furthermore, this study did not detect differences between the two levels of rye on the plant competition between winter annuals and cereal rye.

For summer annual weeds, we observed a rye management response only for Palmer amaranth control, and it occurred at all rating dates in the absence of residual herbicides. After the postemergence application, the rye with N, rye without N, and no rye biomass provided 90, 85, and 72% control, respectively. Morningglory spp. control was similar for rye without N and no rye. Only when rye received an application of N was there enough biomass production to improve morningglory spp. control at 1 and 4 WAP. Large crabgrass response was similar in the presence of rye, regardless of N application, and this control was greater than no rye. Our results imply that small-seeded species like Palmer amaranth and large crabgrass will respond to modest levels of rye biomass, but morningglory spp. need biomass levels $>538\ \mathrm{g\ m^{-2}}$ to influence seedling growth.

The effect of termination timing differed by weed life cycles. Winter annual weed control was better when cereal rye was terminated 20 d EPP, while summer annual weed control was improved when cereal rye was terminated 10 d EPP. In this trial, we did not explore why these differences were observed. However, this provides a challenge for farmers trying to manage winter and summer annual weeds efficiently. Additional research needs to focus on minimizing the number of herbicide applications, while maintaining high levels of weed control.

Increased soybean yield in the presence of cereal rye biomass cannot be attributed solely to improved weed control. Cereal rye mulch conserves soil moisture, which improved soybean yield.

Furthermore, this research is consistent with research showing that cereal rye does not negatively influence herbicide efficacy (Perkins et al., in press). While some research has demonstrated delayed cover crop termination can result in a reduction of herbicide reaching the soil (Whalen et al., 2020), this reduction in herbicide efficacy may be offset by increased cover crop biomass. Future research needs to investigate the relationship between cover crop biomass and soil-applied herbicide performance.

While cereal rye did not eliminate the need for soil-applied herbicides, it consistently improved weed control. This research contributes to our growing knowledge of weed control with cover crops and demonstrates the effects cereal rye management can have on ecological services.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

MV, BS, and QJ conceptualized, designed the study, and participated in its execution and data collection. MV and KV did the statistical analysis. KV wrote the first draft. MV, BS, and QJ edited the draft. All authors contributed to the article and approved the submitted version.

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REFERENCES

Cornelius, C. D., and Bradley, K. W. (2017a). Herbicide programs for the termination of various cover crop species. *Weed Tech.* 31, 514–522. doi: 10.1017/wet.2017.20

Cornelius, C. D., and Bradley, K. W. (2017b). Influence of various cover crop species on winter and summer annual weed emergence in soybean. *Weed Tech.* 31, 503–513. doi: 10.1017/wet.2017.23

DeSimini, S. A., Gibson, K. D., Armstrong, S. D., Zimmer, M., Maia, L. O. R., and Johnson, W. G. (in press). Effects of cereal rye and canola on winter and summer annual weed emergence in corn. *Weed Tech.* 1–7. doi: 10.1017/wet.2020.51

DeVore, J. D., Norsworthy, J. K., and Brye, K. R. (2013). Influence of deep tillage, a rye cover crop, and various soybean production systems on Palmer amaranth emergence in soybean. *Weed Tech.* 27, 263–270. doi: 10.1614/WT-D-12-00125.1

Hayden, Z. D., Brainard, D. C., Henshaw, B., and Ngoajio, M. (2012). Winter annual weed suppression in rye-vetch cover crop mixtures. *Weed Tech.* 26, 818–825. doi: 10.1614/WT-D-12-00084.1

Heap, I. (2020). *The International Herbicide-Resistant Weed Database*. Available online at: http://weedscience.org (accessed: August 19, 2020).

Loux, M. M., Dobbels, A. F., Bradley, K. W., Johnson, W. G., Young, B. G., Spaunhorst, D. J., et al. (2017). Influence of cover crops on management of amaranthus species in glyphosate- and glufosinate-resistant soybean. *Weed Tech.* 31, 487–495. doi: 10.1017/wet.2017.30

Mirsky, S. B., Curran, W. S., Mortensen, D. M., Ryan, M. R., and Shumway, D. L. (2011). Timing of cover-crop management effects on weed suppression in no-till planted soybean using a roller-crimper. *Weed Sci.* 59, 380–389. doi: 10.1614/WS-D-10-00101.1

Mirsky, S. B., Spargo, J. T., Curran, W. S., Reberg-Horton, S. C., Ryan, M. R., Schomberg, H. H., et al. (2017). Characterizing cereal rye biomass and allometric relationships across a range of fall available nitrogen rates in the eastern United States. *Agron. J.* 109, 1520–1531. doi: 10.2134/agronj2016.09.0557

Mischler, R. A., Curran, W. S., Duiker, S. W., and Hyde, J. A. (2010). Use of a rolled-rye cover crop for weed suppression in no-till soybeans. *Weed Tech.* 24, 253–261. doi: 10.1614/WT-D-09-00004.1

Mohler, C. L., and Teasdale, J. R. (1993). Response of weed emergence to rate of Vicia villosa Roth and *Secale cereale L.* residue. *Weed Res.* 33, 487–499.

Montgomery, G. B., McClure, A. T., Hayes, R. M., Walker, F. R., Senseman, S. A., and Steckel, L. E. (2018). Dicamba-tolerant soybean combined cover crop to control Palmer amaranth. *Weed Tech.* 32, 109–115. doi: 10.1017/wet.2017.96

Norsworthy, J. K., Ward, S. M., Shaw, D. R., Llewellyn, R. S., Nichols, R. L., Webster, T. M., et al. (2012). Reducing the risks of herbicide resistance: best management practices and recommendations. *Weed Sci.* 60, 31–62. doi: 10.1614/WS-D-11-00155.1

Perkins, C. M., Gage, K. A., Norsworthy, J. K., Young, B. D., Bradley, K. W., Bish, M. D., et al. (in press). Efficacy of residual herbicides influenced by cover crop residue for control of *Amaranthus* spp. in soybean. *Weed Tech.* 1–20. doi: 10.1017/wet.2020.77

Pittman, K., Barney, J., and Flessner, M. (2019). Horseweed (*Conyza canadensis*) suppression from cover crop mixtures and fall-applied residual herbicides. *Weed Tech.* 33, 303–311. doi: 10.1017/wet.2018.111

Pittman, K. B., Barney, J. N., and Flessner, M. L. (2020). Cover crop residue components and their effect on summer annual weed suppression in corn and soybean. *Weed Sci*. 68, 301–310. doi: 10.1017/wsc.2020.16

Poffenbarger, H. J., Mirsky, S. B., Weil, R. R., Kramer, M., Spargo, J. T., and Cavigelli, M. J. (2015). Legume proportion, poultry litter, and tillage effects on cover crop decomposition. *Agron*. J. 107, 2083–2096. doi: 10.2134/agronj15.0065

Reberg-Horton, S. C., Burton, J. D., Danehower, D. A., Ma, G., Monks, D. W., Murphy, J. P., et al. (2005). Changes over time in the allelochemical content of ten cultivars or rye (*Secale cereal* L.). *J. Chem Ecol*. 31, 179–193 doi: 10.1007/s10886-005-0983-3

Ryan, M. R., Curran, W. S., Grantham, A. M., Hunsberger, L. K., Mirsky, S. B., Mortensen, D. A., et al. (2011a). Effects of seeding rate and poultry litter on weed suppression from a rolled cereal rye cover crop. *Weed Sci*. 59, 438–444. doi: 10.1614/WS-D-10-00180

Ryan, M. R., Mirsky, S. B., Mortensen, D. A., Teasdale, J. R., and Curran, W. S. (2011b). Potential synergistic effects on cereal rye biomass and soybean planting density on weed suppression. *Weed Sci*. 59, 238–246. doi: 10.1614/WS-D-10-00110.1

Sherman, A. D., Haramoto, E. R., and Green, J. D. (2020). Integrating fall and spring herbicides with a cereal rye cover crop for horseweed (*Conyza canadensis*) management prior to soybean. *Weed Tech*. 34, 64–72. doi: 10.1017/wet.2019.116

Sievers, T., and Cook, R. L. (2018). Aboveground and root decomposition of cereal rye and hairy vetch cover crops. *Soil. Sci. Soc. Am. J*. 82, 14–155. doi: 10.2136/sssaj2017.05.0139

Teasdale, J. R., and Mohler, C. L. (1993). Light transmittance, soil temperature, and soil moisture under residue of hairy vetch and rye. *Agron. J*. 85, 673–680. doi: 10.2134/agronj1993.00021962008500030029x

Teasdale, J. R., and Mohler, C. L. (2000). The quantitative relationship between weed emergence and the physical properties of mulches. *Weed Sci*. 48, 385–392. doi: 10.1614/0043-1745(2000)048[0385:TQRBWE]2.0.CO;2

Teasdale, J. R., Rice, C. P., Cai, G., and Mangum, R. W. (2012). Expression of allelopathy in the soil environment: soil concetration and activity of benzoxazinoid compounds released by rye cover crop residue. *Plant. Ecol*. 213, 1893–1905 doi: 10.1007/s11258-012-0057-x

Wagger, M. G. (1989). Time of desiccation effects on plant composition and subsequent nitrogen release from several winter annual cover crops. *Agron. J*. 8, 236–241. doi: 10.2134/agronj1989.00021962008100020020x

Wagger, M. G., Cabrera,. M. L., and Ranells, N. N. (1998). Nitrogen and carbon cycling in relation to cover crop residue quality. *J. Soil Water Conserv*. 53, 214–218

Wallace, J., Curran, W., and Mortensen, D. (2019). Cover crop effects on horseweed (*Erigeron canadensis*) density and size inequality at the time of herbicide exposure. *Weed Sci*. 67, 327–328. doi: 10.1017/wsc.2019.3

Whalen, D. M., Shergill, L. S., Kinne, L. P., Bish, M. D., and Bradley, K. W. (2020). Integration of residual herbicides with cover crop termination in soybean. *Weed Tech*. 34, 11–18. doi: 10.1017/wet.2019.111

Wiggins, M. S., Hayes, R. M., and Steckel, L. E. (2016). Evaluating cover crops and herbicides for glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) control in cotton. *Weed Tech*. 30, 415–422. doi: 10.1614/WT-D-15-00113.1

Wiggins, M. S., McClure, M. A., Hayes, R. M., and Steckel, L. E. (2015). Integrating cover crops and POST herbicides for glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) control in corn. *Weed Tech*. 29, 412–418. doi: 10.1614/WT-D-14-00145.1

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SYSTEMATIC REVIEW
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The Pitfalls of Relating Weeds, Herbicide Use, and Crop Yield: Don't Fall Into the Trap! A Critical Review

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The growing recognition of the environmental and health issues associated to pesticide use requires to investigate how to manage weeds with less or no herbicides in arable farming while maintaining crop productivity. The questions of weed harmfulness, herbicide efficacy, the effects of herbicide use on crop yields, and the effect of reducing herbicides on crop production have been addressed over the years but results and interpretations often appear contradictory. In this paper, we critically analyze studies that have focused on the herbicide use, weeds and crop yield nexus. We identified many inconsistencies in the published results and demonstrate that these often stem from differences in the methodologies used and in the choice of the conceptual model that links the three items. Our main findings are: (1) although our review confirms that herbicide reduction increases weed infestation if not compensated by other cultural techniques, there are many shortcomings in the different methods used to assess the impact of weeds on crop production; (2) Reducing herbicide use rarely results in increased crop yield loss due to weeds if farmers compensate low herbicide use by other efficient cultural practices; (3) There is a need for comprehensive studies describing the effect of cropping systems on crop production that explicitly include weeds and disentangle the impact of herbicides from the effect of other practices on weeds and on crop production. We propose a framework that presents all the links and feed-backs that must be considered when analyzing the herbicide-weed-crop yield nexus. We then provide a number of methodological recommendations for future studies. We conclude that, since weeds are causing yield loss, reduced herbicide use and maintained crop productivity necessarily requires a redesign of cropping systems. These new systems should include both agronomic and biodiversity-based levers acting in concert to deliver sustainable weed management.

Keywords: weed-crop interference, cropping system, yield gap, crop loss, weeding, herbicide, trophic resource use, weed management

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INTRODUCTION

Since the onset of agriculture, a main objective of crop management has been the control of arable weeds, both by making the weed seed bank germinate at a time when the resulting plants would not hinder the crop and by eliminating weed plants at those times they would compete with the crop. At the beginning of the twentieth century, weed science books described losses per unit area of 20 to 50% without weed control, depending on the crop (Long, 1910; Fron, 1917). The increased availability of synthetic, highly effective herbicides in the middle of the twentieth century led to a decrease in weed species diversity and density (e.g., Andreasen et al., 1996; Andersson and Milberg, 1998; Robinson and Sutherland, 2002; Fried et al., 2009), and farmers largely lost interest in other weed management techniques. During that period, research studies focused on characterizing the harmfulness of particularly aggressive species, with most experimental studies conducted under controlled conditions and focusing on two-species situations (i.e., one crop or variety vs. one weed species) in order to determine harmfulness thresholds for triggering spraying operations (Caussanel et al., 1988; Clewis et al., 2001). Environmental and health issues (Stoate et al., 2009; Waggoner et al., 2013) have led to a recent legislation push for a reduction in pesticide use (Neumeister et al., 2007). Together with the expansion of herbicide resistance (Busi et al., 2013), this has triggered a shift from weed control exclusively based on systematic chemical herbicide applications to integrated weed management, where combinations of alternative preventive and curative techniques (which are only partially efficient) are used (Liebman and Gallandt, 1997). This shift raised the question of whether agricultural production will be impaired by weeds and how to move toward a weed management that relies little or not at all on herbicides.

Numerous studies thus investigated the effect of reducing pesticides on crop production (de Ponti et al., 2012; Seufert et al., 2012; Hossard et al., 2014; Lechenet et al., 2014, 2017a; Petit et al., 2015), the efficacy of herbicides to control weeds and to preserve crop production (Milberg and Hallgren, 2004; Fickett et al., 2013; Soltani et al., 2016), or the harmfulness of weeds for crop production (Milberg and Hallgren, 2004; Song et al., 2017). Their results and/or interpretations sometimes appear contradictory.

Consequently, this review paper critically analyses methods and results used in published studies that investigated weed harmfulness for crop production, herbicide impacts on weed flora and crop production as well as cropping-system impact on herbicide use. Our investigative framework discriminates the different conceptual models that have been used in the literature to explore the herbicide use-weed-crop yield nexus (**Figure 1**). Rather than writing a comprehensive review on the findings of the studies focusing on these relationships, we investigated the advantages and limits of each methodology as well as the implications of the methodological choices for interpreting results. The ultimate goal was to provide a methodological guide to answer two major questions, (1) when and how much do weeds affect crop production and (2) is it possible reconcile reduced herbicide use and yield preservation.

IMPACT OF WEEDS ON CROP PRODUCTION

There is a large literature on the effects of weeds on crop production (**Figure 1A**), revealing 1,532 articles published from 1956 to 2019 on the topic of "weed and yield loss" and available in the web of science database (see section Bibliometric Analysis of Literature and Weed-Borne Crop Yield Loss in **Supplementary Material** online). Oerke et al. (1994) published a book (later synthesized as a review paper (Oerke, 2006)) which remains so far the most complete report of the effect of weeds on crops around the world, revealing a high variability of yield loss due to weeds (see examples in section Range of Variation of Crop Yield Loss Due to Weeds Found in Literature online).

How and When Do Weeds Interfere With Crop Production?

Weeds interact directly with the crop through competition for water and mineral resources (Zimdahl, 2004), allelopathy (Kadioglu et al., 2005), and parasitism (Parker, 2009). Weeds can also host other organisms that can have either positive (DiTommaso et al., 2016) or negative (Mantle et al., 1977; Gutteridge et al., 2006) effects on the crop.

Most studies focused on competition between crops and non-parasitic weeds, experimentally assessing how co-habiting crop and weed plants take up resources like water (McGiffen et al., 1992), nitrogen (Teyker et al., 1991), and light (Rajcan and Swanton, 2001). Many studies aimed to identify the critical weed-free periods needed to avoid yield loss (Martin et al., 2001; Knezevic et al., 2002). Even if the impact of competition is often only visible late in the crop cycle (e.g., flowering), the weeds' harmfulness potential is determined very early (Kropff and Spitters, 1991; Hall et al., 1992; Fahad et al., 2015). For instance, if an oilseed rape (*Brassica napus* L.) crop remains weed-free until reaching 4–6 leaves, yield losses remain below 10%; conversely, weed control after 4 leaf stage targeting late-emerging weeds was not needed to limit yield loss (Martin et al., 2001). Because weeds compete with crops for resources, some studies advocate that increasing the resource pool diversity should alleviate crop:weed competition (Smith et al., 2010; Menalled et al., 2020). This is probably the case in conservation agriculture where the combination of no-till and cover crops modifies the resource pool diversity (Romdhane et al., 2019) in addition to filtering different weed communities compared to conventional farming (Chauhan et al., 2012; Trichard et al., 2013; Nichols et al., 2015; Cordeau et al., 2020).

How Crop: Weed Interference Is Quantified

The investigation of weed impacts on yield loss is overwrought with methodological difficulties (see the very detailed review of Swanton et al., 2015). There are roughly three types of methods (**Table 1**).

Herbicide trials (**Table 1A**) are annual standardized factorial experiments designed to assess the efficacy of modalities of herbicide use (single product, association or strategies) to control weed infestation (https://pp1.eppo.int/standards/PP1-152-4, https://pp1.eppo.int/standards/herbicides). Those trials

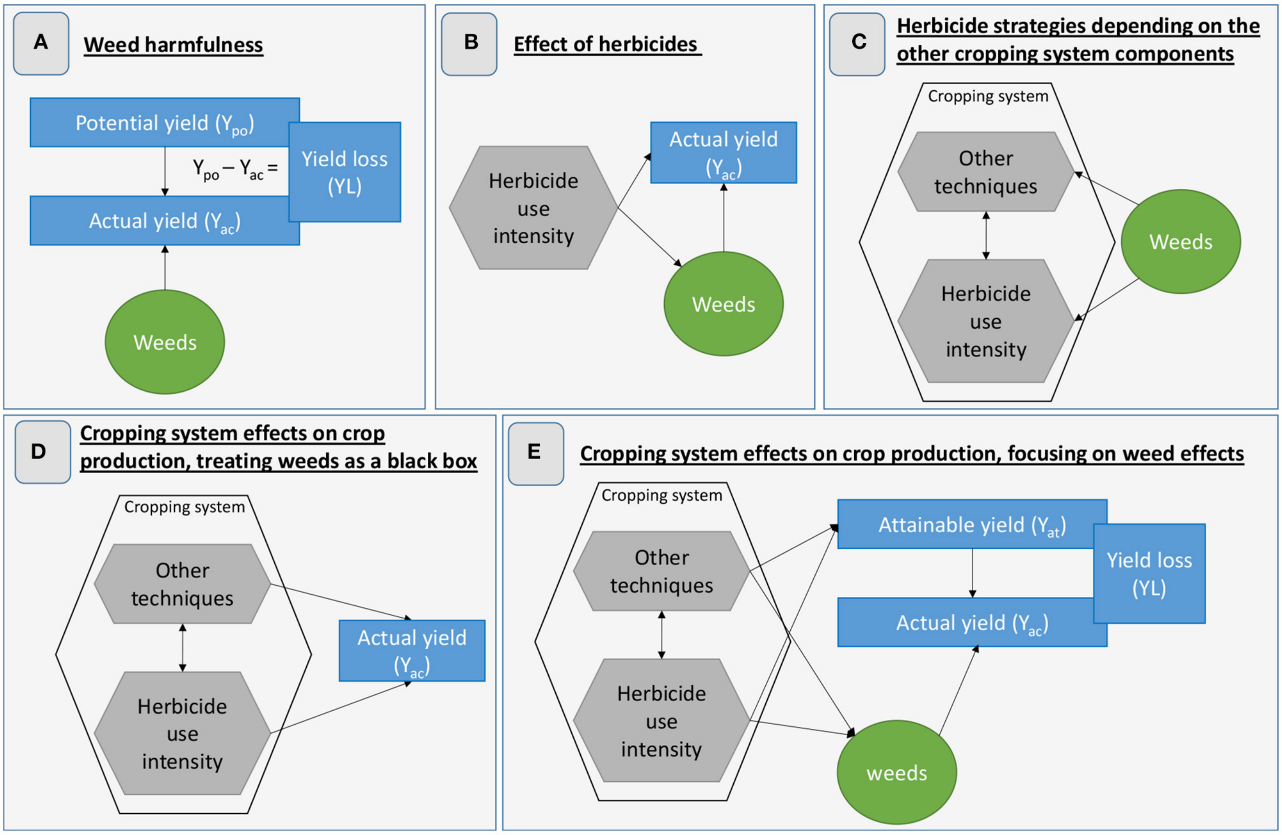

FIGURE 1 | Methodological framework used in the present study to determine the typology of studies investigating the relationships between farming practices including herbicides (gray octagons), weeds (green ovals), and crop yield (blue rectangles). **(A)** Weed harmfulness (section How Crop:Weed Interference Is Quantified), **(B)** Effect of herbicides (section Effect of Herbicides on Crop Production), **(C)** Herbicide strategies depending on the other cropping system components (section How Far Farmers Adapt Their Herbicide Strategies to the Other Farming Practices); **(D)** Cropping system effects on crop production, treating weeds as a black box (section The Comprehensive Effects of Cropping Systems on Crop Production). **(E)** Cropping system effects on crop production, focusing on weed effects (section The Comprehensive Effects of Cropping Systems on Crop Production).

that last beyond post-spraying weed assessment until crop harvest are sometimes used to assess yield loss by comparing yields between treated plots (as a proxy for weed-free control) and untreated plots (infested by weeds) (Florez et al., 1999; Milberg and Hallgren, 2004; Fickett et al., 2013) Some studies compared more complex situations, for example several modalities with an increasing use of herbicides in order to create a gradient of weed abundances (Dieleman et al., 1999; Boström and Fogelfors, 2002).

Other harmfulness studies identified zones inside a field or similar fields with a weed-density gradient but otherwise identical (**Table 1B**). Yield loss is then estimated as the difference of the yield in the different zones or fields relative to the maximum observed yield, and then linked the yield-loss estimation to a series of weed flora variables (e.g., plant densities, biomass). But simple weed metrics are not sufficient to explain yield loss as recent field studies reported that yield loss decreases with increasing diversity and richness in the weed community (Storkey and Neve, 2018; Adeux et al., 2019b).

Studies in greenhouse or garden plots create weed-density gradients, by transplanting weeds (usually a single species) at different dates and densities to mimic contrasting weed emergence flushes, and this at different crop stages (**Table 1C**). The biomass or grain production losses due to the presence of weeds are usually linked to weed variables and used to determine thresholds for weed management (Oliver, 1988). Frequently used variables were weed density (Cousens, 1985; McDonald and Riha, 1999) or, with better results, weed species specificity (Onofri and Tei, 1994), weed leaf area (Kropff and Spitters, 1991; Lotz et al., 1996; van Acker et al., 1997), or weed biomass (Milberg and Hallgren, 2004). All these approaches suffer from methodological drawbacks (**Table 1**), prominently among which the difficulty to estimate the potential yield in the absence of weeds obtained in the same pedoclimatic and cultural conditions.

Herbicide trials are primarily set up to assess the effect of crop protection (i.e., the difference between yield obtained with and without weeding) and not yield loss due to weeds. Treated plots are not necessary totally and constantly weed-free. Moreover, herbicides can be phytotoxic for the crop in certain weather

TABLE 1 | Critical summary of methods studying the effects of weeds on crop yield loss based literature analysis.

Method	Advantage	Limits	Consequences
A. Herbicide trials in fields			
Compare yield and weeds in sprayed and unsprayed fields/zones	- Many cropping systems and pedoclimates - Realistic multispecies weed flora	Weed-free control is rarely continuously and totally weed-free Possible phytotoxicity on crops	Underestimated yield loss
		Trials often set up in highly weed-infested areas	Overestimated yield loss, locally valid conclusions
		Annual studies	Disregards weed harmfulness for future crops
B. Other field trials			
Compare yield of zones/fields with a gradient of weed infestation to the highest observed yield, correlate yield loss to weed indicators	- Several cropping systems and pedoclimates - Realistic multispecies weed flora - Determines a critical weed-free period	The highest yield is lower than the potential (weed-free) yield	Underestimated yield loss
		Insufficient monitoring of processes, resources and flora	Confusing effects of weeds with those of environmental conditions determining the weed-infestation gradient
		Annual studies	Disregards weed harmfulness for future crops
C. Greenhouse, garden plots			
Transplant weeds at different densities and dates, correlate yield loss to weed indicators	- Weed-free control - Characterizing weed flora with indicators	Often a single crop-weed couple	Not applicable to multispecies weed communities observed in fields
		Insufficient monitoring of processes and resources	Local validity of harmfulness thresholds
		Indicators are too far from actual processes	
		Annual studies	Disregards weed harmfulness for future crops

conditions or at early crop stages (Cabanne et al., 1985; Carvalho et al., 2009). Both events can lead to underestimating potential yield. The best way to estimate yield losses at the annual scale consists in comparing the yield in weedy zones to that in weed-free controls without chemical or mechanical weeding (Adeux et al., 2019b). Indeed, mechanical weeding is also likely to affect crop growth, e.g., through modification in the nitrogen dynamics (Gilbert et al., 2009) or by uprooting crop plants (Rasmussen et al., 2009). But even the best of these approaches neglect long-term weed harmfulness even though this is the main reason why farmers relentlessly target weeds (Macé et al., 2007).

Last, these methods produce only locally valid conclusions, with a very high risk of confusing effects. Herbicide trials are usually set up preferentially in fields with an abundant flora or difficult-to-control species (e.g., *Alopecurus myosuroides* Huds., *Lolium multiflorum* Lam.). The results, therefore, mostly have a local validity, and any national estimation based on these data would probably overestimate weed-caused yield loss. Studies monitoring weed-infestation gradients risk confusing the effect of weed pressure with that of the local environmental conditions driving weed gradients. While experiments in controlled conditions (greenhouse, garden plots) do not suffer from this deficiency, they are disadvantaged by a highly artificialized weed flora.

Implications for Weed Management

Some field experiments attempted to provide indicators for deciding when to weed, depending on the crop and/weed stages. Trials such as those of **Table 1B** aimed to determine the critical weed-free periods needed to avoid yield loss (Martin et al., 2001; Knezevic and Datta, 2015). Methods to determine these critical periods have been largely criticized (Knezevic et al., 2002; Knezevic and Datta, 2015). Nutrient content in crops or resource availability in the field were rarely measured, even though the resources for which crops and weeds compete vary according to year, location and cropping system. Consequently, even for a given crop (e.g., maize, *Zea mays* L.), the critical weed control period varied considerably according to years, locations and authors, both in terms of onset (2 to 14 leaf) and end date (12 leaf to 1 week after flowering) (Hall et al., 1992; Hugo et al., 2014).

Other studies linked weeding decisions to a weed harmfulness threshold, based on empirical relationships correlating yield loss to weed indicators estimated in fields (**Table 1B**) or controlled conditions (**Table 1C**). The concept of harmfulness threshold is highly questionable (Oliver, 1988; O'Donovan, 1996; Swanton et al., 1999). In short, even the best of these thresholds usually disregard variability in water and nutrient resources, rarely quantify yield losses due to weed assemblages (Swinton et al., 1994), and only consider annual effects (McDonald and Riha, 1999; Munier-Jolain et al., 2002). In addition, the most pertinent weed indicators (i.e., those closest to processes implicated in crop-weed competition) such as relative leaf cover (Kropff and Spitters, 1991) are impractical for taking weed control decisions. Such practical limitations explain why weed densities are usually used to establish damage thresholds, i.e., the lowest weed density for which a decrease in crop yield is detected (Coble and

Mortensen, 1992). Last, the value of the threshold triggering weeding does not actually change the treatment frequency, and the sustainability of a cropping system relies on whether the decrease in herbicide use intensity is compensated by non-chemical cultural practices (Munier-Jolain et al., 2002).

EFFECT OF HERBICIDES ON CROP PRODUCTION

This section reports on studies that investigated the impact of herbicide use intensity on weed infestation or yield loss, but without analyzing the direct impact of weeds on crop yield (**Figure 1B**). To simplify we will disregard here the ever increasing problem of herbicide resistance (Busi et al., 2013) but discuss it in the conclusion section (section Synthesis and Conclusions).

Herbicide Trials

Herbicide trials (see definition in section How Crop:Weed Interference Is Quantified) have established that herbicides are efficient in controlling weeds but that their efficacy depends (unsurprisingly) on weed species (Jonathan et al., 1998), herbicide rates (Streibig, 1980), application dates (Stougaard et al., 1997), and spraying conditions (Blumhorst et al., 1990) (**Table 2A**). These effects have been summarized in handbooks (e.g., Mamarot and Rodriguez, 2003) and various decision-support systems (e.g., Kudsk, 2008). But when these trials attempt to determine to what extent the use of crop protection prevents yield losses, they encounter the same methodological setbacks to determine yield loss as the studies of section How Crop:Weed Interference Is Quantified (Cardina et al., 1997).

Most herbicide trials last for 1 year only, thus missing future yield losses due to the descendants of the weed seed production during the trial year. One rare exception (Boström and Fogelfors, 2002) assesses the effect of dosage and treatment frequency in a 10 year multi-site experiment. It showed no difference in crop yield of fields sprayed at 25 and 100% of a full dose, even though total weed densities increased by 43 to 67%. These and other results (Salonen, 1992; Blackshaw et al., 2006) remind us that herbicides, even though considered as the most efficient "hammer" against weeds, are not a 100% efficient tool to control the whole weed community and, more importantly, that there is no generic relationship between herbicides, weeds, and yield.

Farm-Field Surveys

Farm-field surveys monitor cultural practices and real-life weed floras in a large range of contrasting situations, at a regional (Petit et al., 2016; Yvoz et al., 2020) or even national scale (Rydberg and Milberg, 2000; Fried et al., 2008). They allow assessing the environmental, agronomic and ecological drivers of the in-field weed flora, and notably the relative contribution of agronomic and environmental factors (Schumacher, 1987; Fried et al., 2008; Seifert et al., 2015), landscape context and/or of farming systems (Gabriel et al., 2005; Geiger et al., 2010; Petit et al., 2016) on weed infestation levels and/or weed community composition. This approach has proved successful to detect long-term weed response to contrasted agricultural management strategies, for example, the generic signal of higher weed cover and/or weed seed bank abundance in organic vs. conventional systems (Hawes et al., 2010).

However, the suitability of this approach to assess the impacts of herbicide use on weed infestation in conventional fields is highly questionable (**Table 2B**). Annual surveys, particularly those disregarding past field history and initial weed pressure (e.g., Gaba et al., 2016), are meaningless to link herbicide use and post-weeding weed infestation or yield as farmers adapt herbicide use intensity to the initial weed infestation and to other cultural techniques (**Figure 2**). The absence of any correlation between herbicide use intensity and weed abundance sometimes reported in literature (Gabriel et al., 2005; Gaba et al., 2016; Petit et al., 2016) cannot be attributed to a lack of efficacy of herbicide use, as reported by some authors (Gaba et al., 2016) but either results from farmers' mental models (i.e., reduce herbicides to the benefit of non-chemical operations, trigger spraying based on observed weed communities; Kings, 2014) or from unsuitable protocols (i.e., assessing the effect of herbicide on weeds based on post-spraying weed surveys only, without any knowledge on pre-spraying floras; Gaba et al., 2016). To properly address the question of the impact of herbicide use on weed infestation, the weed flora should at the very least be surveyed twice a year, i.e., before and after chemical weeding (Milberg and Hallgren, 2004).

HOW FAR FARMERS ADAPT THEIR HERBICIDE STRATEGIES TO THE OTHER FARMING PRACTICES

This section deals with interaction between herbicide use and other farming practices (**Figure 1C**) Herbicide use intensity depends not only on initial weed infestation and the farmer's weeding strategy (**Figure 2**) but also on other practices (Beltran et al., 2013; Colbach and Cordeau, 2018), particularly in Integrated Weed Management (Swanton and Weise, 1991). The intensity decreases if fields are tilled, weeded mechanically and/or grown with diversified rotations (Yvoz et al., 2020), depending not only on the frequency but also the timing of non-chemical disturbances (**Table 3**).

These interactions results largely from the farmer's attitude and perceptions. Low herbicide use requires a long-term strategic management of weeds, aiming to prevent rather than to control weeds (Macé et al., 2007). Many farmers though focus on control rather than on prevention (Wilson et al., 2008), and the type of approach depends, among others, on the production situation. For instance, farmers with access to varieties tolerant to non-selective herbicides such as glyphosate frequently simplify rotations (Fausti et al., 2014) and tillage (Trigo and Cap, 2003; Cerdeira and Duke, 2006). They accept to plant into a weedy seedbed and rely on glyphosate applications on crop canopy to control weeds (Johnson et al., 2007). So, often farmers include integrated weed management options only when no other choice is available, for instance when weeds become resistant to herbicides (Llewellyn et al., 2004; Colas et al., 2020).

Risk aversion also influences weed management strategies, with farmers focusing on minimizing the risk of failure even

TABLE 2 | Critical summary of methods investigating the effects of herbicides on weeds and/or crop yield based literature analysis.

Method	Advantages	Limits	Consequences
A. Herbicide trials in fields			
See **Table 1A**			
B. Farm-field networks			
Correlate weeds or yield to herbicide use intensity	- Effect of herbicide strategies on weeds - Many cropping systems and production contexts - Identification of environmental, agronomical and ecological drivers	Herbicide use intensity depends on weed flora and cropping system	Confusing effects of herbicide use intensity with those of other practices and/or initial weed infestation (or its perception by farmers)
		Often only a single weed survey, in a single year	
		Bulk estimation of yield from harvest sale	Bad estimation of herbicide contribution to yield preservation

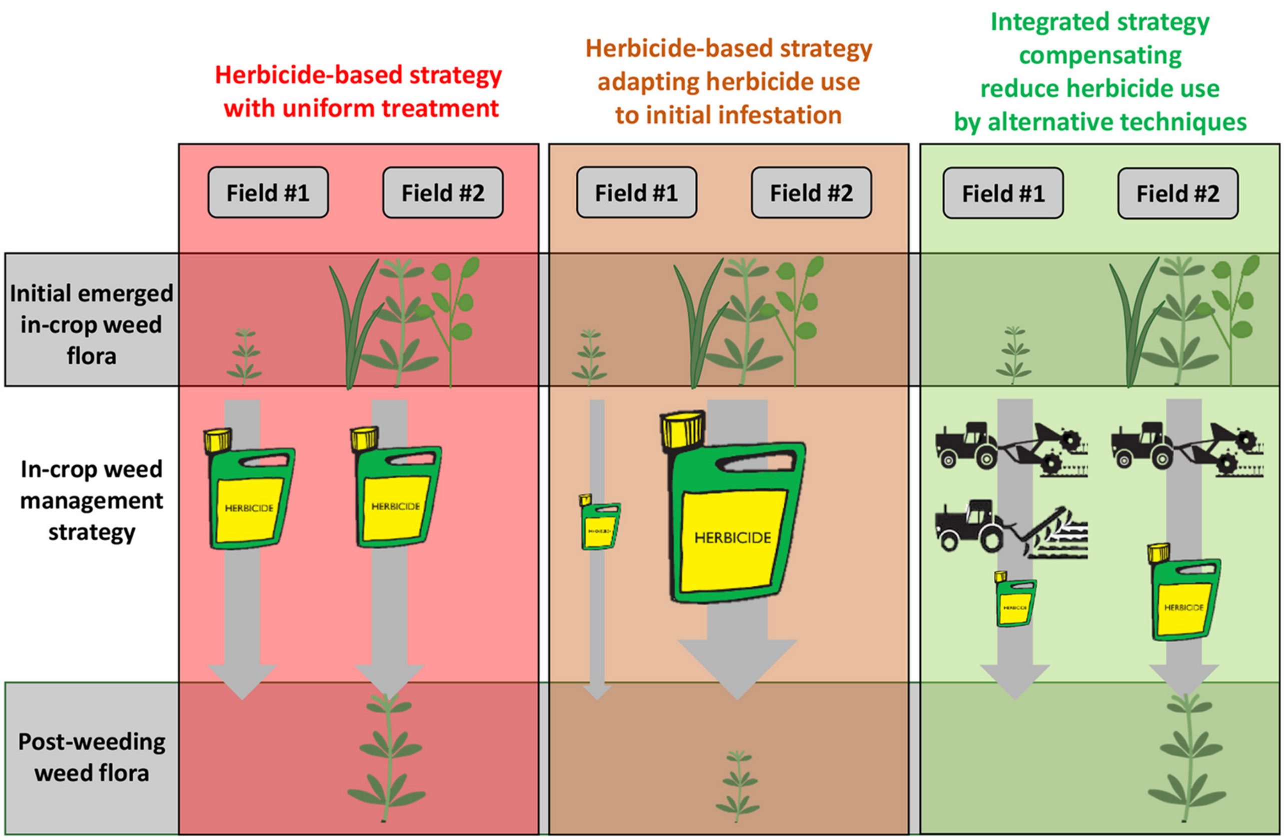

FIGURE 2 | Impact of initial weed infestation and of the farmer's herbicide strategy on herbicide use intensity and final weed flora.

at the cost of lowering their average economic performance (Wossink et al., 1997; Doohan et al., 2010; Ridier et al., 2013). This explains why herbicide use intensity tends to be higher in cropping systems taken from farm surveys and field monitoring networks than those tested in research stations (−3% averaged over rotation), proposed by advisors (−15%) or designed with simulations (−26%) (Colbach and Cordeau, 2018). Economic factors (e.g., herbicide prices, farm size) and labor requirements are also important determinants in the selection of a weed control technique by farmers (Wossink et al., 1997; Llewellyn et al., 2004; Hammond et al., 2006; Beltran et al., 2013; Jabbour et al., 2014b).

The way farmers perceive weeds is a major obstacle to reducing herbicide use (Rioux, 1994; Wossink et al., 1997), particularly if they attribute weeds to factors outside their control, such as weather events or uncontrolled weed growth in neighboring fields (Wilson et al., 2008; Doohan et al., 2010). Their preferences reflect a typical inverse relationship between perceived risk and benefit (Doohan et al., 2010), underestimating the risks resulting from overreliance on herbicides (Doohan et al.,

TABLE 3 | Main variations in herbicide use intensity (expressed by the treatment frequency index, TFI) as a function of other farming practices, identified in 272 cropping systems provided by farm surveys, agricultural statistics, and crop advisors.

Practice that allows reducing herbicide TFI	Instead of	Variation in herbicide TFI
Mechanical weeding ≥ 0.7 operations/year	<0.7 operations /year	−1.2
Frequency of superficial tillage Oct-March > 1 year/3	≤1 year/3	−0.8
Last tillage < 20 days prior to cash crop sowing	≥20 days	−0.7
Rotation with summer crops	Without	−0.6
Rotation with 50% spring/summer crops (or grassland) and 50% winter crops	Rotation dominated by either spring or winter crops	−0.6

Analysis based on classification and regression trees to identify splitting rules to discriminate farming practices and the resulting variation in TFI (according to Colbach and Cordeau, 2018).

2010) and overestimating the incidence of problematic weeds (Borger et al., 2012). Knowledge about weed biology and the effects of crop management practices is essential to overcome this deadlock. Indeed, farmers that exhibit great knowledge on these aspects and that critically discuss risks of weeds and benefits of management practices tend to have fields with a lower weed infestation (Jabbour et al., 2014a).

THE COMPREHENSIVE EFFECTS OF CROPPING SYSTEMS ON CROP PRODUCTION

This section reports on studies that investigated the effect of cropping systems on crop production, either without assessing weed floras or other pests ("black box" approach, **Figure 1D**), or by unraveling all components and effects of the general framework of **Figure 1E**, including weeds.

Cropping System Experiments

Cropping system experiments have been set up all over Europe aiming at a detailed, multiannual and multicriteria evaluation of novel cropping practices such as low-input or pesticide-free systems (Lechenet et al., 2017b). Most cropping system experiments were designed to test the feasibility of these systems with a wide range of objectives (e.g., reducing pesticide use or increasing crop diversity while maintain profitability, decreasing impact on soil structure, etc.) without looking at weeds (Deytieux et al., 2012; Giuliano et al., 2016), implemented in single sites or multi-site networks (Deytieux et al., 2016). Few experiments monitored weed floras (Chikowo et al., 2009; Debaeke et al., 2009; Adeux et al., 2017, 2019a; Jernigan et al., 2017) and even fewer assessed weed-driven yield loss, for instance by comparing yield in weedy and weed-free (manually weeded) zones (Teasdale and Cavigelli, 2010; Adeux et al., 2017). This lack of monitoring results in a high risk of confusing effects (**Table 4A**).

The holistic approach of cropping-system experiments compares coherent systems instead of factors, which can lead to seemingly contradictory results. For instance, a recent analysis of long-term experiments conducted in different regions concluded that yield would decrease by 5 to 13% compared to the yield obtained with current pesticide use if pesticide use was reduced by 50% (Hossard et al., 2014). This study was though unable to determine which pest was actually responsible for the yield decrease and, most importantly, how much of this yield decrease was due to a lower yield potential resulting from changes in management practices. For instance, delayed wheat sowing was reported to reduce emergence and survival of broad-leaved weed species (by about 20–30%) but in the absence of weeds decreased yield (by up to 30%), resulting from deteriorated weather or less productive cultivars (Christensen et al., 1994). If experiments specifically account for weeds, no correlation between herbicide use intensity, weed plant density and crop production was found, irrespective of the location (Eastern France, Chikowo et al., 2009; central France, Colbach et al., 2016; South-Western France, Adeux et al., 2017).

Consequently, most cropping-system trials and studies demonstrate local feasibility of reconciling reduced herbicide use and reduced crop yield loss but cannot explain causes and thus do not offer advice applicable elsewhere (Deytieux et al., 2016). Moreover, results collected in experimental stations (Deytieux, 2017) can differ from those collected from farms (Lechenet et al., 2017a) because experimenters do not farm as farmers do as they tend to explore extreme alternative strategies without having the economic survival of their farms depending on their success (Deytieux et al., 2012).

Farm Field Networks

To increase the number of investigated production situations and cropping systems, farm-field surveys (Seufert et al., 2012; Lechenet et al., 2014; Petit et al., 2016) and demonstration-farm networks like the French DEPHY network (Lechenet et al., 2017a) were set up but many methodological problems remain (**Table 2B**). Some of these could be alleviated by new statistical and survey methods. For instance, by including the production context in their analysis, Lechenet et al. (2017a) failed to detect any conflict between low herbicide use on one hand, and, on the other hand, high productivity at the cropping-system level (in 71% of the farms) or high profitability (in 79% of the farms). However, these authors did not monitor weeds or any other biotic or abiotic components, which hampers the identification of the causes of variability in farm-field networks (**Table 4B**).

Structural equation modeling (SEM) has been used to overcome these limits (Lamb et al., 2011; McLeod et al., 2015; Quinio et al., 2017). Among the few studies that include weed surveys, Quinio et al. (2017) thus discriminated the three pathways linking farming intensity (fallow management, sowing, chemical pest control and fertilization), crop yield, and weed

TABLE 4 | Critical synthesis of the methods investigating cropping system effects on weeds and crop yield based literature analysis.

Method	Advantages	Limits	Consequences
A. Cropping system experiments and experiment networks			
Multicriteria and multiannual evaluation of innovative cropping systems	- Actual fields - Demonstrating the feasibility of innovative systems - Measure state variables characterizing environment and crops	Weeds are rarely monitored Yield loss estimated but rarely measured Reduced herbicide use compensated by alternative practices	Confusing effects, e.g., confusing yield loss due to weeds with decease in potential yield due to changes in practices to compensate for reduced herbicide use
		Few systems in few pedoclimates	Results are difficult to extrapolate, even from networks
B. Farm-field networks			
Also see **Table 2B**			
Accounting for production context and cropping system	Many production contexts and cropping systems	No weed monitoring but using pesticide use intensity as proxy of pest incidence	Confusing yield loss due to weeds with decease in potential yield due to changes in practices to compensate for reduced herbicide use
PLS-PM and SEM[§] to disentangle relationships	As above + Network with weed monitoring + Less confusion of effects	Cropping system adapted to initial weed incidence	Confusion effects of weeds on farmers' decisions with those of practices on weeds
Agronomic diagnosis	- Measure state variables characterizing environment, crops and weeds - Yield components	Annual measurements	Neglects long-term effects of practices and weeds
		Difficult to monitor many contexts/systems	Conclusions sometimes difficult to extrapolate
C. Combine simulations with field measurements			
Compare measured yield to that simulated without weeds, from field history	- Many production contexts and/or cropping systems - Real-life farming practices - Identification of yield-limiting factors	Usually annual measurements	Neglects long-term effects of practices and weeds
		Weeds are rarely monitored	Confusing effects of weeds with those of other limiting factors
		Compare observed actual yield to simulated potential yield	Confusing effects of weeds with model bias
D. Simulate a virtual farm-field network			
Simulate many cropping systems from many regions with and without weeds, as well as with and without herbicides	Idem previous + Discriminate weed effects from other yield-limiting factors + Discriminate effects of herbicides from those of other practices	Simulation	Conclusions depend on model quality ("garbage in, garbage out")

[§] *PLS-PM partial least squares path modeling and SEM structural equation modeling.*

pressure (**Table 4B**). This analysis considerably reduced the risk of confusing effects, showing that crop yield increased with farming intensity and decreased with weed pressure (section Pathway Analysis of Field Survey Data online). Overall, the authors showed that farming intensity reduced weed pressure sufficiently to cancel any negative effects that weeds had on yield.

Diagnostic studies of farmers' fields go even further and investigate a large range of limiting factors of yield (Valantin-Morison and Meynard, 2008; Subedi and Ma, 2009). These studies measure state variables describing weeds and other pests (e.g., weed density and biomass at different stages), nutrition status (e.g., nitrogen absorption by both the crop and the weeds), resource availability in the soil, as well as crop yield components. Yield components are linked to potential limiting factors, often showing that weeds are the most important limiting factor (oilseed rape in France, Valantin-Morison and Meynard, 2008; maize in Eastern Canada, Subedi and Ma, 2009), identifying the weed variables that are the most linked to yield component variance (e.g., weed dry biomass and plant density accounting for nearly 40% of variance of oilseed rape grain number per m^2, Valantin-Morison and Meynard, 2008) or the main drivers of the weed floras (e.g., previous crop, tillage and oilseed rape sowing density, Valantin-Morison and Meynard, 2008). However, the cost of these measurements limits both the number of monitored situations and the duration of monitoring, thus disregarding any long-term effects of weeds and cultural practices.

Combining Simulation Models With Field Data

Simulation-based studies can go further, by using process-based crop models (with or without weeds) to estimate the potential yield (i.e., yield in the absence of weeds) which is so difficult to estimate in fields (**Table 4C**), the actual yield (in the presence of weeds) in many situations and cropping systems (**Table 4D**). Depending on which processes are included in the model, this approach allows identifying different yield-limiting factors to reduce the risk of confusing effects. The first approach compares the simulated potential yield to actual yield measured in fields (Affholder et al., 2003, 2013; van Ittersum et al., 2003; Silva et al., 2017). In addition to previously mentioned methodological drawbacks (**Table 4C**), this approach risks to confuse effects of agronomic and environmental conditions in the field with model bias and error.

In order to limit this risk, to cover more situations and to move beyond the annual scale, some teams went completely virtual (Colbach and Cordeau, 2018), which would not have been possible without the more recent development of mechanistic multispecies multiannual crop-weed dynamics models (see short description in section Weed Dynamics Models online). These authors simulated several hundred cropping systems provided by farm surveys, agricultural statistics or crop advisors over 30 years (to assess long-term effects) and with 10 weather scenarios. This approach allowed disentangling the effect of herbicide use intensity from that of other management practices by comparing the simulated weed floras and yields of the recorded cropping systems to those of these same systems minus herbicides (and without any other changes in practices). The relative effects of weeds and management practices on crop production were differentiated by comparing the yields of simulations run with and without weeds. As a result, this study could confirm and/or demonstrate the key conclusions of the present paper, and quantify them with values valid for a large range of production contexts and cropping systems. For instance, yield loss exceeds 50% when weed biomass exceeds crop biomass (further details in section Simulation Results Linking Yield Loss to Weed Biomass Online), or weed biomass during crop growth and yield loss increased by +116% and +62% (averaged over rotation), respectively, when herbicides were eliminated without redesigning the cropping system.

SYNTHESIS AND CONCLUSIONS

Impact of Weeds on Crop Production

Weed harmfulness for crop production is usually studied disregarding the complexity of the cultural practices, the multispecies nature of the weed flora (Massinga et al., 2001) and the processes underlying weed harmfulness, which limits the validity and the genericity of the results. Yield loss due to weeds tends to be underestimated because of the annual investigating scale and a poorly estimated potential yield. Despite existing methodological shortcomings, almost all studies conclude that weeds reduce yield if they are not controlled (Zimdahl, 2004), particularly if they emerge earlier or at crop emergence (Chikoye et al., 1995). The most accurate method to estimate yield loss at the annual scale consists in comparing the yield in weedy zones to that in weed-free controls without chemical or mechanical weeding. This approach allowed Adeux et al. (2019b) to conclude that weed diversity mitigates winter wheat yield loss and that not all weed communities are detrimental to crop productivity. Yield loss is more correlated to weed metrics closer to processes driving crop-weed competition than to weed density. Weed thresholds based on weed density are useless to forecast yield loss.

Effect of Herbicides on Crop Production

Because studies are usually annual and often limited to a single observation per field and per year, the contribution of herbicides to controlling weeds and yield loss can be underestimated. The lack of correlation between herbicide use intensity and weed abundance sometimes reported in literature (Gaba et al., 2016) cannot be attributed to a lack of efficacy of herbicide use, but rather to unsuitable survey protocols (i.e., assessing the effect of herbicide on weeds on post-spraying weed surveys only, without accounting for pre-spraying weed infestation). The weed flora must be assessed before and after weeding to evaluate its efficiency. And though herbicides are not always totally effective (even when weeds did not acquire resistance), they do reduce weed infestation and yield loss due to weeds.

How far Farmers Adapt Their Herbicide Strategies to the Other Farming Practices

The herbicide strategy used by a farmer in terms of commercial products, rates and timings of application depends on the other practices applied in the field as well as on the weed flora perceived by the farmer, his risk strategy and his production situation (Yvoz et al., 2020). Any attempt to assess the impact of herbicide use intensity on weed flora and yield loss must account for these interactions to avoid confusing effects (Quinio et al., 2017).

A Conceptual Framework Embedding the Herbicide-Weed-Yield Relationships

Based on the present review, we proposed a conceptual framework to synthesize the key variables and effects driving the relationships between herbicides, weeds and crop production (**Figure 3**), inspired by structural equation modeling using expert knowledge (Smith et al., 2014). This diagram not only illustrates that the effects of herbicides or weeds on crop productivity cannot be considered without accounting for the technical, and biophysical and socio-economic context. It also shows frequent feed-backs, e.g., herbicides indeed reduce weed densities but farmers increase herbicide use if they observed many emerging weeds. It is as yet difficult to quantify the individual links, particularly as the correlations are not necessarily linear, though some field and simulation studies cited above attempted to do this (Lamb et al., 2011; McLeod et al., 2015; Quinio et al., 2017).

Implications for Future Research and Weed Management

Our review demonstrates that understanding the herbicide use-weed-crop yield nexus requires to include all the components of the studied system (**Figure 1E**) and to deconstruct them in detail as illustrated in **Figure 3**. The apparent inconsistencies identified in the literature appear to have resulted from differences in methodological approaches and a few precautions are essential to avoid confusing effects (**Table 5**). The critical analysis of a large range of studies contrasting in terms of objectives and methodologies allowed us to answer the two questions addressed in this review, namely how harmful weeds are and whether herbicide use can be reduced without affecting yield. In summary, weeds are harmful for crop production but this harmfulness varies considerably and decreases when (1) weed biomass decreases, (2) weeds emerge later than the crop, (3) the

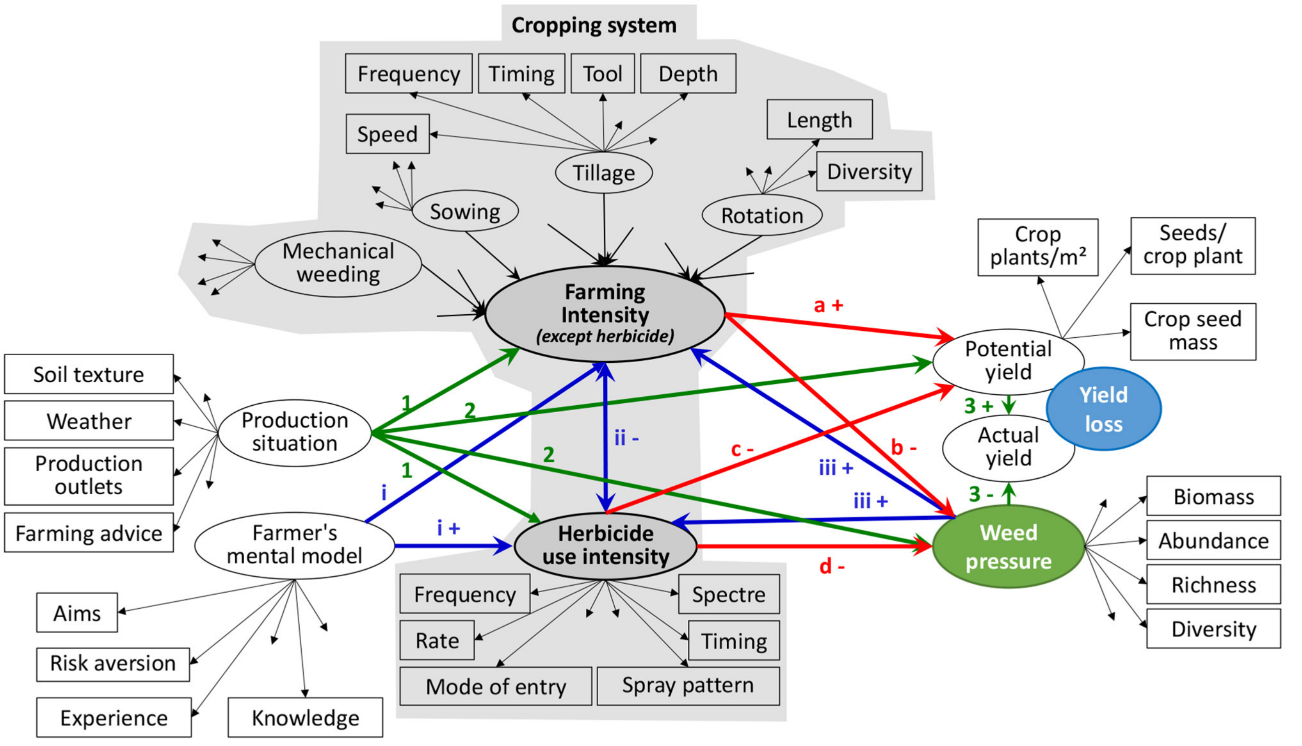

FIGURE 3 | Conceptual representation of cropping system effects on weeds and crop production in farmers' fields inspired by structural equation modeling to synthesize the relationships between herbicide use intensity, weed pressure, and yield loss based on the present literature analysis. Circles show latent variables (in bold the targets investigated in the present study), and rectangles manifest variables. The gray zone delimits the cropping system. Note that the arrows point from the latent variables to the manifest variables. Red arrows are effects of cropping system components (a. to create favorable conditions for crop growth, b. to create unfavorable conditions for weeds and to destroy them; c. phytotoxicity, d. weed destruction); green arrows show environmental effects (1. cropping choices depend on biophysical and socio-economical constraints; 2. crop growth and weed community depend on biophysical environment; 3: yield loss is the difference between attainable and actual yield due to weeds); blue arrows show farmers' decisions (i. farmers choose cropping techniques, including herbicides, based, among others, on their risk aversion; ii: the choice of the herbicide strategy depends on other techniques and vice-versa; iii: the choice of cropping techniques, particularly herbicides, depends on the past and present weed community). Positive (+) and negative (–) effects were shown for latent variables.

TABLE 5 | Major precautions needed to study the relationships between herbicides, weeds and yield to avoid methodological defects.

Precautions	In order to avoid to…	Suggestions
Take account of production contexts as well as farmers' objectives and perceptions	Generalize/extrapolate to situations outside the range of validity of the conclusions	- Survey farmers before (to identify their perceptions) or after the study to confront the results to their expertise - Collect variables describing the production context (pedoclimate, production outlets, use of irrigation…)
Consider all cropping system components in addition to herbicides	Confuse the effects of herbicides with those of practices introduced by farmers in order to compensate for reduced herbicide use	Document farming practices in detail, preferentially via interviews
Measure state variables describing weeds and resources (light, nitrogen, water…) before and after the studied practices	Confuse the effects of practices on weeds and the environment with those of practices introduced by farmers to adapt to weeds and environmental conditions	- Two surveys per year, before and after the studied practices - Measure resource availability
Measure variables close to the targeted processes	Missing the targeted effects	Measure weed and crop biomass and their ratio
Monitor over several years or measure indicators of future effects (e.g., weed seed production	Missing the effects of cropping systems and weeds on future crops	Sample/measure at the same locations over several years

weed community consists of many diverse species, (4) available resources increase (in highly fertilized/irrigated systems). Crop yield loss is highly correlated to weed variables closely linked to processes driving crop-weed competition but the best indicator variables (e.g., weed biomass at crop flowering) are useless for weed control decisions because they are measured too late.

These results advocate for a strategic long-term reasoning of weed management instead of tactical decisions based on current weed communities. Indeed, despite the undeniable weed harmfulness for crop production, well-reasoned integrated weed management can preserve crop production in cropping systems with reduced herbicide use even though herbicides remain to date the most efficient weed control technique, except in the case of herbicide-resistant weeds. However, in this particular case, many field studies show that integrated weed management can be highly efficient to control herbicide-resistant populations (e.g., Chauvel et al., 2001, 2009). These results have been synthesized already in reviews on strategies for managing herbicide-resistant populations (Beckie, 2006; Busi et al., 2013; Riar et al., 2013).

There is thus no unique single solution that is valid everywhere. Flexible solutions are required, considering the agronomical logic underlying cropping systems and the production context but also other potential levers that could be mobilized to enhance the biological regulation of weeds (Petit et al., 2018). These solutions might require to accept a certain level of weed presence in the field, and should also consider potential weed benefits for crop production, i.e., habitat provision for natural enemies (Dassou and Tixier, 2016; DiTommaso et al., 2016).

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

All the authors contributed to the analysis of the literature and writing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2020.615470/full#supplementary-material

REFERENCES

Adeux, G., Giuliano, S., Cordeau, S., Savoie, J.-M., and Alletto, L. (2017). Low-input maize-based cropping systems implementing IWM match conventional maize monoculture productivity and weed control. *Agriculture* 7:74. doi: 10.3390/agriculture7090074

Adeux, G., Munier-Jolain, N., Meunier, D., Farcy, P., Carlesi, S., Barberi, P., et al. (2019a). Diversified grain-based cropping systems provide long-term weed control while limiting herbicide use and yield losses. *Agron. Sustain. Dev.* 39:42. doi: 10.1007/s13593-019-0587-x

Adeux, G., Vieren, E., Carlesi, S., Bàrberi, P., Munier-Jolain, N., and Cordeau, S. (2019b). Mitigating crop yield losses through weed diversity. *Nat. Sustain.* 2, 1018–1026. doi: 10.1038/s41893-019-0415-y

Affholder, F., Poeydebat, C., Corbeels, M., Scopel, E., and Tittonell, P. (2013). The yield gap of major food crops in family agriculture in the tropics: assessment and analysis through field surveys and modelling. *Field Crops Res.* 143, 106–118. doi: 10.1016/j.fcr.2012.10.021

Affholder, F., Scopel, E., Madeira Neto, J., and Capillon, A. (2003). Diagnosis of the productivity gap using a crop model. Methodology and case study of small-scale maize production in central Brazil. *Agronomie* 23, 305–325. doi: 10.1051/agro:2003004

Andersson, T. N., and Milberg, P. (1998). Weed flora and the relative importance of site, crop, crop rotation, and nitrogen. *Weed Sci.* 46, 30–38. doi: 10.1017/S0043174500090135

Andreasen, C., Stryhn, H., and Streibig, J. (1996). Decline of the flora in Danish arable fields. *J. Appl. Ecol.* 33, 619–626. doi: 10.2307/2404990

Beckie, H. J. (2006). Herbicide-resistant weeds: management tactics and practices. *Weed Technol.* 20, 793–814. doi: 10.1614/WT-05-084R1.1

Beltran, J. C., White, B., Burton, M., Doole, G. J., and Pannell, D. J. (2013). Determinants of herbicide use in rice production in the Philippines. *Agric. Econ.* 44, 45–55. doi: 10.1111/j.1574-0862.2012.00631.x

Blackshaw, R. E., O'Donovan, J. T., Harker, K. N., Clayton, G. W., and Stougaard, R. N. (2006). Reduced herbicide doses in field crops: a review. *Weed Biol. Manag.* 6, 10–17. doi: 10.1111/j.1445-6664.2006.00190.x

Blumhorst, M. R., Weber, J. B., and Swain, L. R. (1990). Efficacy of selected herbicides as influenced by soil properties. *Weed Technol.* 4, 279–283. doi: 10.1017/S0890037X00025392

Borger, C., Michael, P., Mandel, R., Hashem, A., Bowran, D., and Renton, M. (2012). Linking field and farmer surveys to determine the most important changes to weed incidence. *Weed Res.* 52, 564–574. doi: 10.1111/j.1365-3180.2012.00950.x

Boström, U., and Fogelfors, H. (2002). Long-term effects of herbicide-application strategies on weeds and yield in spring-sown cereals. *Weed Sci.* 50, 196–203. doi: 10.1614/0043-1745(2002)050[0196:LTEOHA]2.0.CO;2

Busi, R., Vila-Aiub, M. M., Beckie, H. J., Gaines, T. A., Goggin, D. E., Kaundun, S. S., et al. (2013). Herbicide-resistant weeds: from research and knowledge to future needs. *Evol. Appl.* 6, 1218–1221. doi: 10.1111/eva.12098

Cabanne, F., Gaillardon, P., and Scalla, R. (1985). Phytotoxicity and metabolism of chlortoluron in two wheat varieties. *Pestic. Biochem. Physiol.* 23, 212–220. doi: 10.1016/0048-3575(85)90008-2

Cardina, J., Johnson, G. A., and Sparrow, D. H. (1997). The nature and consequence of weed spatial distribution. *Weed Sci.* 45, 364–373. doi: 10.1017/S0043174500092997

Carvalho, S. J. P. D., Nicolai, M., Ferreira, R. R., Figueira, A. V. D. O., and Christoffoleti, P. J. (2009). Herbicide selectivity by differential metabolism: considerations for reducing crop damages. *Sci. Agric.* 66, 136–142. doi: 10.1590/S0103-90162009000100020

Caussanel, J.-P., Kafiz, B., and Carteron, A. (1988). Analyse expérimentale des effets de concurrence d'une graminée adventice dans un blé de printemps en relation avec le désherbage. *Weed Res.* 28, 309–322. doi: 10.1111/j.1365-3180.1988.tb00809.x

Cerdeira, A. L., and Duke, S. O. (2006). The current status and environmental impacts of glyphosate-resistant crops: a review. *J. Environ. Q* 35, 1633–1658. doi: 10.2134/jeq2005.0378

Chauhan, B. S., Singh, R. G., and Mahajan, G. (2012). Ecology and management of weeds under conservation agriculture: a review. *Crop Protect.* 38, 57–65. doi: 10.1016/j.cropro.2012.03.010

Chauvel, B., Guillemin, J. P., and Colbach, N. (2009). Evolution of a herbicide-resistant population of *Alopecurus myosuroides* Huds. in a long-term cropping system experiment. *Crop Protect.* 28, 343–349. doi: 10.1016/j.cropro.2008.11.013

Chauvel, B., Guillemin, J. P., Colbach, N., and Gasquez, J. (2001). Evaluation of cropping systems for management of herbicide-resistant populations of blackgrass (*Alopecurus myosuroides* Huds.). *Crop Protect.* 19, 127–137. doi: 10.1016/S0261-2194(00)00065-X

Chikowo, R., Faloya, V., Petit, S., and Munier-Jolain, N. M. (2009). Integrated weed management systems allow reduced reliance on herbicides and long-term weed control. *Agric. Ecosyst. Environ.* 132, 237–242. doi: 10.1016/j.agee.2009.04.009

Chikoye, D., Weise, S. F., and Swanton, C. J. (1995). Influence of common ragweed (*Ambrosia artemisiifolia*) time of emergence and density on white bean (*Phaseolus vulgaris*). *Weed Sci.* 43, 375–380. doi: 10.1017/S0043174500081352

Christensen, S., Rasmussen, G., and Olesen, J. E. (1994). Differential weed suppression and weed control in winter wheat. *Aspects Appl. Biol.* 40, 335–342.

Clewis, S. B., Askew, S. D., and Wilcut, J. W. (2001). Common ragweed interference in peanut. *Weed Sci.* 49, 768–772. doi: 10.1614/0043-1745(2001)049[0768:CRIIP]2.0.CO;2

Coble, H. D., and Mortensen, D. A. (1992). The threshold concept and its application to weed science. *Weed Technol.* 6, 191–195. doi: 10.1017/S0890037X00034552

Colas, F., Cordeau, S., Granger, S., Jeuffroy, M.-H., Pointurier, O., Queyrel, W., et al. (2020). Co-development of a decision support system for integrated weed management: contribution from future users. *Eur. J. Agron.* 114:126010. doi: 10.1016/j.eja.2020.126010

Colbach, N., Bertrand, M., Busset, H., Colas, F., Dugué, F., Farcy, P., et al. (2016). Uncertainty analysis and evaluation of a complex, multi-specific weed dynamics model with diverse and incomplete data sets. *Environ. Model. Softw.* 86, 184–203. doi: 10.1016/j.envsoft.2016.09.020

Colbach, N., and Cordeau, S. (2018). Reduced herbicide use does not increase crop yield loss if it is compensated by alternative preventive and curative measures. *Eur. J. Agron.* 94, 67–78. doi: 10.1016/j.eja.2017.12.008

Cordeau, S., Baudron, A., and Adeux, G. (2020). Is tillage a suitable option for weed management in conservation agriculture? *Agronomy* 10:1746. doi: 10.3390/agronomy10111746

Cousens, R. (1985). A simple model relating yield loss to weed density. *Ann. Appl. Biol.* 107, 239–252. doi: 10.1111/j.1744-7348.1985.tb01567.x

Dassou, A. G., and Tixier, P. (2016). Response of pest control by generalist predators to local-scale plant diversity: a meta-analysis. *Ecol. Evol.* 6, 1143–1153. doi: 10.1002/ece3.1917

de Ponti, T., Rijk, B., and van Ittersum, M. K. (2012). The crop yield gap between organic and conventional agriculture. *Agric. Syst.* 108, 1–9. doi: 10.1016/j.agsy.2011.12.004

Debaeke, P., Munier-Jolain, N. M., Betrand, M., Guichard, L., Nolot, J. M., Faloya, V., et al. (2009). Iterative design and evaluation of rule-based cropping systems: methodology and case studies. A review. *Agron. Sustain. Dev.* 29, 73–86. doi: 10.1051/agro:2008050

Deytieux, V. (2017). *Performance et durabilité de systèmes de grande culture en production intégrée* (Ph.D. thesis). Université de Bourgogne Franche-Comté, Besançon, France.

Deytieux, V., Munier-Jolain, N., and Caneill, J. (2016). Assessing the sustainability of cropping systems in single- and multi-site studies. A review of methods. *Eur. J. Agron.* 72, 107–126. doi: 10.1016/j.eja.2015.10.005

Deytieux, V., Nemecek, T., Freiermuth Knuchel, R., Gaillard, G., and Munier-Jolain, N. M. (2012). Is integrated weed management efficient for reducing environmental impacts of cropping systems? A case study based on life cycle assessment. *Eur. J. Agron.* 36, 55–65. doi: 10.1016/j.eja.2011.08.004

Dieleman, J. A., Mortensen, D. A., and Martin, A. R. (1999). Influence of velvetleaf (*Abutilon theophrasti*) and common sunflower (*Helianthus annuus*) density variation on weed management outcomes. *Weed Sci.* 47, 81–89. doi: 10.1017/S004317450009069X

DiTommaso, A., Averill, K. M., Hoffmann, M. P., Fuchsberg, J. R., and Losey, J. E. (2016). Integrating insect, resistance, and floral resource management in weed control decision-making. *Weed Sci.* 64, 743–756. doi: 10.1614/WS-D-16-00052.1

Doohan, D., Wilson, R., Canales, E., and Parker, J. (2010). Investigating the human dimension of weed management: new tools of the trade. *Weed Sci.* 58, 503–510. doi: 10.1614/WS-D-09-00086.1

Fahad, S., Hussain, S., Chauhan, B. S., Saud, S., Wu, C., Hassan, S., et al. (2015). Weed growth and crop yield loss in wheat as influenced by row spacing and weed emergence times. *Crop Protect.* 71, 101–108. doi: 10.1016/j.cropro.2015.02.005

Fausti, S. W., Sluis, E. V. D., Qasmi, B. A., and Lundgren, J. (2014). "The effect of biotechnology and biofuels on U.S. corn belt cropping systems: updated version," *Economics Staff Paper - Department of Economics, South Dakota State University* (Brookings, SD), 23.

Fickett, N. D., Boerboom, C. M., and Stoltenberg, D. E. (2013). Soybean yield loss potential associated with early-season weed competition across 64 site-years. *Weed Sci.* 61, 500–507. doi: 10.1614/WS-D-12-00164.1

Florez, J. A., Fischer, A. J., Ramirez, H., and Duque, M. C. (1999). Predicting rice yield losses caused by multispecies weed competition. *Agron. J.* 91, 87–92. doi: 10.2134/agronj1999.00021962009100010014x

Fried, G., Norton, L. R., and Reboud, X. (2008). Environmental and management factors determining weed species composition and diversity in France. *Agric. Ecosyst. Environ.* 128, 68–76. doi: 10.1016/j.agee.2008.05.003

Fried, G., Petit, S., Dessaint, F., and Reboud, X. (2009). Arable weed decline in Northern France: crop edges as refugia for weed conservation? *Biol. Conserv.* 142, 238–243. doi: 10.1016/j.biocon.2008.09.029

Fron, G. (1917). *Plantes Nuisibles à L'agriculture. Encyclopédie agRicole.* Paris: J. B. Baillières & Fils.

Gaba, S., Gabriel, E., Chadœuf, J., Bonneu, F., and Bretagnolle, V. (2016). Herbicides do not ensure for higher wheat yield, but eliminate rare plant species. *Sci. Rep.* 6:30112. doi: 10.1038/srep30112

Gabriel, D., Thies, C., and Tscharntke, T. (2005). Local diversity of arable weeds increases with landscape complexity. *Perspect. Plant Ecol. Evol. Syst.* 7, 85–93. doi: 10.1016/j.ppees.2005.04.001

Geiger, F., Bengtsson, J., Berendse, F., Weisser, W. W., Emmerson, M., Morales, M. B., et al. (2010). Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. *Basic Appl. Ecol.* 11, 97–105. doi: 10.1016/j.baae.2009.12.001

Gilbert, P.-A., Vanasse, A., and Angers, D. A. (2009). Harrowing for weed control: impacts on mineral nitrogen dynamics, soil aggregation and wheat production. *Soil Tillage Res.* 103, 373–380. doi: 10.1016/j.still.2008.12.001

Giuliano, S., Ryan, M. R., Véricel, G., Rametti, G., Perdrieux, F., Justes, E., et al. (2016). Low-input cropping systems to reduce input dependency and environmental impacts in maize production: a multi-criteria assessment. *Eur. J. Agron.* 76, 160–175. doi: 10.1016/j.eja.2015.12.016

Gutteridge, R. J., Jenkyn, J. F., and Bateman, G. L. (2006). Effects of different cultivated or weed grasses, grown as pure stands or in combination with wheat, on take-all and its suppression in subsequent wheat crops. *Plant Pathol.* 55, 696–704. doi: 10.1111/j.1365-3059.2006.01405.x

Hall, M. R., Swanton, C. J., and Anderson, G. W. (1992). The critical period of weed control in grain corn (*Zea* mays). *Weed Sci.* 40, 441–447. doi: 10.1017/S0043174500051882

Hammond, C. M., Luschei, E. C., Boerboom, C. M., and Nowak, P. J. (2006). Adoption of integrated pest management tactics by Wisconsin farmers. *Weed Technol.* 20, 756–767. doi: 10.1614/WT-05-095R1.1

Hawes, C., Squire, G. R., Hallett, P. D., Watson, C. A., and Young, M. (2010). Arable plant communities as indicator of farming practice. *Agric. Ecosyst. Environ.* 138, 17–26. doi: 10.1016/j.agee.2010.03.010

Hossard, L., Philibert, A., Bertrand, M., Colnenne-David, C., Debaeke, P., Munier-Jolain, N., et al. (2014). Effects of halving pesticide use on wheat production. *Sci. Rep.* 4:4405. doi: 10.1038/srep04405

Hugo, E., Morey, L., Saayman-Du Toit, A. E., and Reinhardt, C. F. (2014). Critical periods of weed control for naked crabgrass (Digitaria nuda), a grass weed in corn in South Africa. *Weed Sci.* 62, 647–656. doi: 10.1614/WS-D-13-00152.1

Jabbour, R., Gallandt, E. R., Zwickle, S., Wilson, R. S., and Doohan, D. (2014a). Organic farmer knowledge and perceptions are associated with on-farm weed seedbank densities in Northern New England. *Weed Sci.* 62, 338–349. doi: 10.1614/WS-D-13-00098.1

Jabbour, R., Zwickle, S., Gallandt, E. R., Mcphee, K. E., Wilson, R. S., and Doohan, D. (2014b). Mental models of organic weed management: comparison of New England US farmer and expert models. *Renew. Agric. Food Syst.* 29, 319–333. doi: 10.1017/S1742170513000185

Jernigan, A. B., Caldwell, B. A., Cordeau, S., DiTommaso, A., Drinkwater, L. E., Mohler, C. L., et al. (2017). Weed abundance and community composition in a long-term organic vegetable cropping systems trial. *Weed Sci.* 65, 639–649. doi: 10.1017/wsc.2017.33

Johnson, W. G., Gibson, K. D., and Conley, S. P. (2007). Does weed size matter? An Indiana grower perspective about weed control timing. *Weed Technol.* 21, 542–546. doi: 10.1614/WT-06-094R.1

Jonathan, K. S., Michael, J. H., Peterson, D. E., Randy, W. L., and Boyer, J. E. (1998). Herbicide efficacy on four amaranthus species in Soybean (Glycine max). *Weed Technol.* 12, 315–321. doi: 10.1017/S0890037X00043876

Kadioglu, I., Yanar, Y., and Asav, U. (2005). Allelopathic effects of weeds extracts against seed germination of some plants. *J. Environ. Biol.* 26, 169–173.

Kings, D. (2014). Farmers' understandings of weeds and herbicide usage as environmental influences on agricultural sustainability. *J. Environ. Prot.* 5:923. doi: 10.4236/jep.2014.511094

Knezevic, S. Z., and Datta, A. (2015). The critical period for weed control: revisiting data analysis. *Weed Sci.* 63, 188–202. doi: 10.1614/WS-D-14-00035.1

Knezevic, S. Z., Evans, S. P., Blankenship, E. E., van Acker, R. C., and Lindquist, J. L. (2002). Critical period for weed control: the concept and data analysis. *Weed Sci.* 50, 773–786. doi: 10.1614/0043-1745(2002)050[0773:CPFWCT]2.0.CO;2

Kropff, M. J., and Spitters, C. J. T. (1991). A simple model of crop loss by weed competition from early observations on relative leaf area of the weeds. *Weed Res.* 31, 97–105. doi: 10.1111/j.1365-3180.1991.tb01748.x

Kudsk, P. (2008). Optimising herbicide dose: a straightforward approach to reduce the risk of side effects of herbicides. *Environmentalist* 28, 49–55. doi: 10.1007/s10669-007-9041-8

Lamb, E., Shirtliffe, S., and May, W. (2011). Structural equation modeling in the plant sciences: an example using yield components in oat. *Can. J. Plant Sci.* 91, 603–619. doi: 10.4141/cjps2010-035

Lechenet, M., Bretagnolle, V., Bockstaller, C., Boissinot, F., Petit, M.-S., Petit, S., et al. (2014). Reconciling pesticide reduction with economic and environmental sustainability in arable farming. *PLoS ONE* 9:e97922. doi: 10.1371/journal.pone.0097922

Lechenet, M., Dessaint, F., Py, G., Makowski, D., and Munier-Jolain, N. (2017a). Reducing pesticide use while preserving crop productivity and profitability on arable farms. *Nat. Plants* 3:17008. doi: 10.1038/nplants.2017.8

Lechenet, M., Deytieux, V., Antichi, D., Aubertot, J.-N., Bàrberi, P., Bertrand, M., et al. (2017b). Diversity of methodologies to experiment integrated pest management in arable cropping systems: analysis and reflections based on a European network. *Eur. J. Agron.* 83, 86–99. doi: 10.1016/j.eja.2016.09.012

Liebman, M., and Gallandt, E. R. (1997). "Many little hammers: ecological management of crop-weed interactions," in *Ecology in Agriculture,* ed L. E. Jackson (Davis, CA: Academic Press), 291–343.

Llewellyn, R. S., Lindner, R. K., Pannell, D. J., and Powles, S. B. (2004). Grain grower perceptions and use of integrated weed management. *Aust. J. Exp. Agric.* 44, 993–1001. doi: 10.1071/EA03115

Long, A. H. (1910). *Common Weeds of the Farm and Garden.* New York, NY: Frederick A. Stokes Company.

Lotz, L. A. P., Christensen, S., Cloutier, D., Fernandez Quintanilla, C., Legere, A., Lemieux, C., et al. (1996). Prediction of the competitive effects of weeds on crop yields bases on the relative leaf area of weeds. *Weed Res.* 36, 93–101. doi: 10.1111/j.1365-3180.1996.tb01805.x

Macé, K., Morlon, P., Munier-Jolain, N., and Quéré, L. (2007). Time scales as a factor in decision-making by French farmers on weed management in annual crops. *Agric. Syst.* 93, 115–142. doi: 10.1016/j.agsy.2006.04.007

Mamarot, J., and Rodriguez, A. (2003). *Sensibilité des Mauvaises Herbes aux Herbicides en Grandes Cultures*. Paris: ACTA.

Mantle, P., Shaw, S., and Doling, D. (1977). Role of weed grasses in the etiology of ergot disease in wheat. *Ann. Appl. Biol.* 86, 339–351. doi: 10.1111/j.1744-7348.1977.tb01848.x

Martin, S. G., van Acker, R. C., and Friesen, L. F. (2001). Critical period of weed control in spring canola. *Weed Sci.* 49, 326–333. doi: 10.1614/0043-1745(2001)049[0326:CPOWCI]2.0.CO;2

Massinga, R. A., Currie, R. S., Horak, M. J., and Boyer, J. (2001). Interference of Palmer amaranth in corn. *Weed Sci.* 49, 202–208. doi: 10.1614/0043-1745(2001)049[0202:IOPAIC]2.0.CO;2

McDonald, A., and Riha, S. (1999). Model of crop: weed competition applied to maize: *Abutilon theophrasti* interactions. I. Model description and evaluation. *Weed Res.* 39, 355–369. doi: 10.1046/j.1365-3180.1999.00151.x

McGiffen, M. E., Masiunas, J. B., and Huck, M. G. (1992). Tomato and nightshade (*Solanum nigrum* L. and *S.* ptycanthum Dun.) effects on soil water content. *J. Am. Soc. Horticult. Sci.* 117, 730–735. doi: 10.21273/JASHS.117.5.730

McLeod, E. M., Banerjee, S., Bork, E. W., Hall, L. M., and Hare, D. D. (2015). Structural equation modeling reveals complex relationships in mixed forage swards. *Crop Protect.* 78, 106–113. doi: 10.1016/j.cropro.2015.08.019

Menalled, U. D., Bybee-Finley, K., Smith, R. G., DiTommaso, A., Pethybridge, S. J., and Ryan, M. R. (2020). Soil-mediated effects on weed-crop competition: elucidating the role of annual and perennial intercrop diversity legacies. *Agronomy* 10:1373. doi: 10.3390/agronomy10091373

Milberg, P., and Hallgren, E. (2004). Yield loss due to weeds in cereals and its large-scale variability in Sweden. *Field Crops Res.* 86, 199–209. doi: 10.1016/j.fcr.2003.08.006

Munier-Jolain, N. M., Chauvel, B., and Gasquez, J. (2002). Long-term modelling of weed control strategies: analysis of threshold-based options for weed species with contrasted competitive abilities. *Weed Res.* 42, 107–122. doi: 10.1046/j.1365-3180.2002.00267.x

Neumeister, L., Williamson, S., Parente, S., and Cannell, E. (2007). *Pesticide uSe Reduction Strategies in Europe. Six Case Studies.* London: Pesticide Action Network Europe.

Nichols, V., Verhulst, N., Cox, R., and Govaerts, B. (2015). Weed dynamics and conservation agriculture principles: a review. *Field Crops Res.*183, 56–68. doi: 10.1016/j.fcr.2015.07.012

O'Donovan, J. T. (1996). Weed economic thresholds: useful agronomic tool or pipe dream? *Phytoprotection* 77, 13–28. doi: 10.7202/706097ar

Oerke, E. (2006). Crop losses to pests. *J. Agric. Sci.* 144, 31–43. doi: 10.1017/S0021859605005708

Oerke, E.-C., Dehne, H. W., Schonbeack, F., and Weber, A. (1994). *Crop Production and Crop Protection.* Amsterdam: Elsevier Science.

Oliver, L. R. (1988). Principles of weed threshold research. *Weed Technol.* 2, 398–403. doi: 10.1017/S0890037X00032152

Onofri, A., and Tei, F. (1994). Competitive ability and threshold levels of three broadleaf weed species in sunflower. *Weed Res.* 34, 471–479. doi: 10.1111/j.1365-3180.1994.tb02044.x

Parker, C. (2009). Observations on the current status of Orobanche and Striga problems worldwide. *Pest Manag. Sci.* 65, 453–459. doi: 10.1002/ps.1713

Petit, S., Cordeau, S., Chauvel, B., Bohan, D., Guillemin, J.-P., and Steinberg, C. (2018). Biodiversity-based options for arable weed management. A review. *Agron. Sustain. Dev.* 38:48. doi: 10.1007/s13593-018-0525-3

Petit, S., Gaba, S., Grison, A.-L., Meiss, H., Simmoneau, B., Munier-Jolain, N., et al. (2016). Landscape scale management affects weed richness but not weed abundance in winter wheat fields. *Agric. Ecosyst. Environ.* 223, 41–47. doi: 10.1016/j.agee.2016.02.031

Petit, S., Munier-Jolain, N., Bretagnolle, V., Bockstaller, C., Gaba, S., Cordeau, S., et al. (2015). Ecological intensification through pesticide reduction: weed control, weed biodiversity and sustainability in arable farming. *Environ. Manage* 56, 1078–1090. doi: 10.1007/s00267-015-0554-5

Quinio, M., De Waele, M., Dessaint, F., Biju-Duval, L., Buthiot, M., Cadet, E., et al. (2017). Separating the confounding effects of farming practices on weeds and winter wheat production using path modelling. *Eur. J. Agron.* 82, 134–143. doi: 10.1016/j.eja.2016.10.011

Rajcan, I., and Swanton, C. J. (2001). Understanding maize-weed competition: resource competition, light quality and the whole plant. *Field Crops Res.* 71, 139–150. doi: 10.1016/S0378-4290(01)00159-9

Rasmussen, J., Nielsen, H. H., and Gundersen, H. (2009). Tolerance and selectivity of cereal species and cultivars to postemergence weed harrowing. *Weed Sci*. 57, 338–345. doi: 10.1614/WS-08-109.1

Riar, D. S., Norsworthy, J. K., Steckel, L. E., Stephenson, D. O., Eubank, T. W., Bond, J., et al. (2013). Adoption of best management practices for herbicide-resistant weeds in midsouthern United States cotton, rice, and soybean. *Weed Technol.* 27, 788–797. doi: 10.1614/WT-D-13-00087.1

Ridier, A., Ghali, M. B. E., Nguyen, G., and Kephaliacos, C. (2013). The role of risk aversion and labor constraints in the adoption of low input practices supported by the CAP green payments in cash crop farms. *Rev. Étud. Agric Environ.* 94, 195–219. doi: 10.4074/S1966960713012034

Rioux, R. (1994). Influence de la fumure azotée sur la compétition entre le chiendent et l'orge. *Phytoprotection* 65, 61–64.

Robinson, R. A., and Sutherland, W. J. (2002). Post-war changes in arable farming and biodiversity in Great Britain. *J. Appl. Ecol.* 39, 157–176. doi: 10.1046/j.1365-2664.2002.00695.x

Romdhane, S., Spor, A., Busset, H., Falchetto, L., Martin, J., Bizouard, F., et al. (2019). Cover crop management practices rather than the composition of cover crop mixtures affect microbial communities in no-till agroecosystems. *Front. Microbiol.* 10:1618. doi: 10.3389/fmicb.2019.01618

Rydberg, N. T., and Milberg, P. (2000). A survey of weeds in organic farming in Sweden. *Biol. Agric. Horticult.* 18, 175–185. doi: 10.1080/01448765.2000.9754878

Salonen, J. (1992). Efficacy of reduced herbicide doses in spring cereals of different competitive ability. *Weed Res.* 32, 483–491. doi: 10.1111/j.1365-3180.1992.tb01909.x

Schumacher, W. (1987). Measures taken to preserve arable weeds and their associated communities in central Europe. *Monogr. Br. Crop Protect. Council* 109–112.

Seifert, C., Leuschner, C., and Culmsee, H. (2015). Arable plant diversity on conventional cropland—the role of crop species, management and environment. *Agric. Ecosyst. Environ.* 213, 151–163. doi: 10.1016/j.agee.2015.07.017

Seufert, V., Ramankutty, N., and Foley, J. A. (2012). Comparing the yields of organic and conventional agriculture. *Nature* 485, 229–U113. doi: 10.1038/nature11069

Silva, J. V., Reidsma, P., Laborte, A. G., and van Ittersum, M. K. (2017). Explaining rice yields and yield gaps in Central Luzon, Philippines: an application of stochastic frontier analysis and crop modelling. *Eur. J. Agron.* 82, 223–241. doi: 10.1016/j.eja.2016.06.017

Smith, R. G., Davis, A. S., Jordan, N. R., Atwood, L. W., Daly, A. B., Grandy, A. S., et al. (2014). Structural equation modeling facilitates transdisciplinary research on agriculture and climate change. *Crop Sci.* 54, 475–483. doi: 10.2135/cropsci2013.07.0474

Smith, R. G., Mortensen, D. A., and Ryan, M. R. (2010). A new hypothesis for the functional role of diversity in mediating resource pools and weed–crop competition in agroecosystems. *Weed Res.* 50, 37–48. doi: 10.1111/j.1365-3180.2009.00745.x

Soltani, N., Dille, J. A., Burke, I. C., Everman, W. J., Vangessel, M. J., Davis, V. M., et al. (2016). Potential corn yield losses from weeds in North America. *Weed Technol.* 30, 979–984. doi: 10.1614/WT-D-16-00046.1

Song, J.-S., Kim, J.-W., Im, J.-H., Lee, K.-J., Lee, B.-W., and Kim, D.-S. (2017). The effects of single- and multiple-weed interference on soybean yield in the far-Eastern region of Russia. *Weed Sci.* 65, 371–380. doi: 10.1017/wsc.2016.25

Stoate, C., Baldi, A., Beja, P., Boatman, N. D., Herzon, I., van Doorn, A., et al. (2009). Ecological impacts of early 21st century agricultural change in Europe - a review. *J. Environ. Manage* 91, 22–46. doi: 10.1016/j.jenvman.2009.07.005

Storkey, J., and Neve, P. (2018). What good is weed diversity? *Weed Res.* 58, 239–243. doi: 10.1111/wre.12310

Stougaard, R. N., Maxwell, B. D., and Harris, J. D. (1997). Influence of application timing on the efficacy of reduced rate postemergence herbicides for wild oat (*Avena fatua*) control in spring barley (*Hordeum vulgare*). *Weed Technol.* 11, 283–289. doi: 10.1017/S0890037X00042962

Streibig, J. (1980). Models for curve-fitting herbicide dose response data. *Acta Agric. Scand.* 30, 59–64. doi: 10.1080/00015128009435696

Subedi, K. D., and Ma, B. L. (2009). Assessment of some major yield-limiting factors on maize production in a humid temperate environment. *Field Crops Res.* 110, 21–26. doi: 10.1016/j.fcr.2008.06.013

Swanton, C. J., Nkoa, R., and Blackshaw, R. E. (2015). Experimental methods for crop–weed competition studies. *Weed Sci*. 63, 2–11. doi: 10.1614/WS-D-13-00062.1

Swanton, C. J., Weaver, S., Cowan, P., Acker, R. V., Deen, W., and Shrestha, A. (1999). Weed thresholds: theory and applicability. *J. Crop Product.* 2, 9–29. doi: 10.1300/J144v02n01_02

Swanton, C. J., and Weise, S. F. (1991). Integrated weed management - the rationale and approach. *Weed Technol.* 5, 657–663. doi: 10.1017/S0890037X00027512

Swinton, S. M., Buhler, D. D., Forcella, F., Gunsolus, J. L., and King, R. P. (1994). Estimation of crop yield loss due to interference by multiple weed species. *Weed Sci.* 42, 103–109. doi: 10.1017/S0043174500084241

Teasdale, J. R., and Cavigelli, M. A. (2010). Subplots facilitate assessment of corn yield losses from weed competition in a long-term systems experiment. *Agron. Sustain. Dev.* 30, 445–453. doi: 10.1051/agro/2009048

Teyker, R., Hoelzer, H., and Liebl, R. (1991). Maize and pigweed response to nitrogen supply and form. *Plant Soil* 135, 287–292. doi: 10.1007/BF00010918

Trichard, A., Alignier, A., Chauvel, B., and Petit, S. (2013). Identification of weed community traits response to conservation agriculture. *Agric. Ecosyst. Environ.* 179, 179–186. doi: 10.1016/j.agee.2013.08.012

Trigo, E. J., and Cap, E. J. (2003). The impact of the introduction of transgenic crops in Argentinean agriculture. *AgBioForum* 6, 87–94.

Valantin-Morison, M., and Meynard, J. M. (2008). Diagnosis of limiting factors of organic oilseed rape yield. A survey of farmers' fields. *Agron. Sustain. Dev.* 8, 527–539. doi: 10.1051/agro:2008026

van Acker, R. C., Lutman, P. J. W., and Froud-Williams, R. J. (1997). Predicting yield loss due to interference from two weed species using early observations of relative weed leaf area. *Weed Res.* 37, 287–299. doi: 10.1046/j.1365-3180.1997.d01-52.x

van Ittersum, M. K., Leffelaar, P. A., van Keulen, H., Kropff, M. J., Bastiaans, L., and Goudriaan, J. (2003). On approaches and applications of the Wageningen crop models. *Eur. J. Agron.* 18, 201–234. doi: 10.1016/S1161-0301(02)00106-5

Waggoner, J., Henneberger, P., Kullman, G., Umbach, D., Kamel, F., Beane Freeman, L., et al. (2013). Pesticide use and fatal injury among farmers in the agricultural health study. *Int. Arch. Occup. Environ. Health* 86, 177–187. doi: 10.1007/s00420-012-0752-x

Wilson, R. S., Tucker, M. A., Hooker, N. H., Lejeune, J. T., and Doohan, D. (2008). Perceptions and beliefs about weed management: perspectives of Ohio grain and produce farmers. *Weed Technol.* 22, 339–350. doi: 10.1614/WT-07-143.1

Wossink, G., de Buck, A., van Niejenhuis, J., and Haverkamp, H. (1997). Farmer perceptions of weed control techniques in sugarbeet. *Agric. Syst.* 55, 409–423. doi: 10.1016/S0308-521X(96)00097-2

Yvoz, S., Petit, S., Biju-Duval, L., and Cordeau, S. (2020). A framework to type crop management strategies within a production situation to improve the comprehension of weed communities. *Eur. J. Agron.* 115:126009. doi: 10.1016/j.eja.2020.126009

Zimdahl, R. L. (2004). *Weed-Crop Competition - A Review*. Oxford: Balckwell Publishing.

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Low Energy Laser Treatments Control Annual Ryegrass (*Lolium rigidum*)

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Increasing concern for the ongoing availability and efficacy of herbicides is driving interest in the development of alternative physical and thermal weed control methods. Fortunately, improvements in weed detection through advancements in computing hardware and deep learning algorithms are creating an opportunity to use novel weed control tools, such as lasers, in large-scale cropping systems. For alternative control options, there are two key weed control timing opportunities, early and late post-crop emergence. Weed density for the early timing is typically higher, with a shorter window for control. Conversely, late post-emergent treatment of surviving and late-emerging weeds would occur in lower densities of larger and more variably sized weeds, given a prior weed control effort, but with a longer available weed control period. Research in laser weeding to date has primarily focused on early growth stage weeds and the ability of this approach to control larger weeds remains unknown. This study used a 25 W, 975 nm fiber-coupled diode laser to evaluate the opportunity for control of annual ryegrass (*Lolium rigidum* Gaudin) and the influence of four different growth stages (three-leaf, seven-leaf, mid-tillering, and late-tillering). Annual ryegrass plants at each growth stage were treated using a laser-focused to a 5 mm diameter with five different irradiation durations developing energy densities of 1.3, 2.5, 6.4, 19.1, and 76.4 J mm^{-2}. At the three-leaf stage, all plants were controlled at 76.4 J mm^{-2} and 93.3% controlled at 19.1 J mm^{-2}. Complete control of seven-leaf plants was only achieved at 76.4 J mm^{-2}. Although laser treatments did not control mid-tillering stage plants, 76.4 J mm^{-2} reduced biomass by 60.2%. No similar reductions in biomass were recorded for the largest plants. This initial research assists in the development of novel weed control options in the context of large-scale conservation cropping systems. Future research should investigate the influence of laser treatments on additional weed species and the impact of increased laser power on larger weeds.

Keywords: laser weeding, site-specific weed control, annual ryegrass, fiber laser, growth stage

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INTRODUCTION

The widespread adoption of conservation agriculture in large-scale cropping systems has resulted in improved productivity outcomes by focusing on minimal soil disturbance, increased soil cover, and diverse cropping species. These outcomes are based on enhanced soil health due to improved soil structure, better nutrient cycling and increased organic matter (Kassam et al., 2012). Without the opportunity for using tillage-based weed control in these production systems, weed management is heavily reliant on herbicides. The only alternatives suitable for routine use in cropping systems are agronomic approaches that increase crop competition, including higher plant densities (Lemerle et al., 2001, 2004), narrow row spacing and strategic fertilizer placement (Kristensen et al., 2008; Bajwa et al., 2015). Recently in Australian cropping systems, harvest weed seed control has been adopted as a standard approach for targeting weed seeds collected during crop harvest (Walsh et al., 2013). There are no currently available physical or thermal weed control methods that provide equivalent levels of efficacy and cost-effectiveness as herbicides in large-scale conservation cropping systems (Coleman et al., 2019; Bauer et al., 2020). However, the selection pressure from repeated use of herbicides with similar modes of action has resulted in the widespread evolution of herbicide resistance (Heap, 2020). The problem of resistance for weed control is compounded by a lack of availability of new herbicide modes of action, negative public perception of herbicide use and increasing regulation that is restricting herbicide development (Duke, 2012). Reduced herbicide options are a significant threat to conservation agriculture in large-scale production systems driving the need for research and development of feasible alternatives.

Site-specific application technologies enable the use of non-selective thermal weed control methods in large-scale cropping systems. While research and development has continued for non-chemical control options, these approaches have not typically progressed to commercialization, owing to high or unknown control costs and a lack of in-crop selectivity. Fortunately, site-specific weed control (SSWC) offers an opportunity for selective application of non-selective methods, improving their relevance in large-scale production systems (Coleman et al., 2019). Until recently, the bottleneck in SSWC has been the accurate, precise and reliable detection of weeds. However, advancements in deep learning, now being translated into the agricultural domain, have seen in-crop weed detection become increasingly realistic (López-Granados, 2011; Wang et al., 2019). Greater computational speed, miniaturization, and reduced costs are creating the opportunity for detection and fine-grained identification to be feasible in large-scale cropping systems and at a level of specificity that enables alternative control options. At the highest spatial resolution for weed detection, recent assessments of instance segmentation for common weeds demonstrate the opportunity for exact targeting of plant center and stem locations in both grass and broadleaf weeds (Champ et al., 2020; Lottes et al., 2020). These developments would enable the use of high precision weed control options such as laser and electrical weeding. However, challenges remain in the reliability of detection, generalization to other weed species and the ability to handle diverse environments.

Laser treatments offer one of the highest levels of precision for targeted weed control and can be applied as either pyrolytic spot or stem cutting treatments. A laser is a highly directional beam of electromagnetic energy that results in the heating of the targeted area. Since the 1970s, there have been intermittent research efforts on the use of lasers for the control of seedling and early growth stage monocot and dicot weeds, which have highlighted the opportunity for this approach. Couch and Gangstad (1974) found treatment with a CO_2 laser (10,600 nm) reduced biomass of water hyacinth (*Eichhornia crassipes* [Mart.] Solms). Other glasshouse studies report significant biomass reductions in volunteer rye (*Secale cereale* L.) and wild oat (*Avena fatua* L.) by CO_2 laser-based stem clipping. However, complete control was not observed with the regrowth of tillers post-clipping (Bayramian et al., 1992).

Further research on the use of laser cutting for weed control found up to 2.3 J per mm of stem thickness was required to cut stems of charlock mustard (*Sinapis arvensis* L.) with a 50 W CO_2 laser and 0.6 mm^2 beam area (Heisel et al., 2001). If the cut was performed below the apical meristem, there was a 90% reduction in biomass for the dicot weeds. A significant reduction in biomass was observed when two-leaf stage perennial ryegrass (*Lolium perenne* L.) plants were cut 2 cm above the soil surface with the laser, compared to the scissors, suggesting a heating effect of the laser treatment on growth. Differences were also found between 10,600 and 355 nm wavelength lasers for cutting efficiency of charlock mustard and winter wheat (*Triticum aestivum* L.) based on the increased absorption and hence explosive heating of water by the longer wavelength laser (Schou et al., 2002).

Rather than cutting of plant tissue, laser-based cellular ablation, and pyrolysis seek to disrupt the cellular function of the apical meristem through exposure to high temperatures. Evidence of the effectiveness of laser pyrolysis with diode and CO_2 lasers has been observed for the control of cultivated tobacco (*Nicotiana tabacum* L.) (Wöltjen et al., 2008), barnyard grass (*Echinochloa crus-galli* [L.] P. Beauv.) (Wöltjen et al., 2008; Marx et al., 2012) and redroot pigweed (*Amaranthus retroflexus* L.) at the seedling, two-leaf and four-leaf growth stages. Marx et al. (2012) found that the lowest lethal energy dose was 54 and 25 J for barnyard grass and redroot pigweed, respectively. The research to date has focused on early growth stage weeds (seedling to four-leaf) with no known studies on laser efficacy on larger/older weeds (seven-leaf to late-tillering). Broadly, two key in-crop weed control timing opportunities exist in large scale cropping systems, early and late post-crop emergence. Weed densities at the early timing are typically larger, with densities in Australian production systems of approximately five plants m^{-2} (Llewellyn et al., 2016). During this critical crop growth stage, weed control timing within a 2- to 3-week period is vital in minimizing the impact of weeds on yield potential.

In comparison, the control of late post-emergent weeds, which have escaped treatment, is more focused on minimizing seed set for future seasons. Surviving and late-emerging weeds at this stage may be present in lower densities of larger weeds and may be more variable in weed size given the prior weed control

TABLE 1 | Sowing dates and treatment dates for the four growth stages across three separate trials in 2019 and 2020.

Growth stage	Planting dates		
	Trial 1 (T1)	Trial 2 (T2)	Trial 3 (T3)
Late-tillering	11/04/2019	31/07/2019	18/05/2020
Mid-tillering	28/04/2019	17/08/2019	28/05/2020
Seven-leaf	12/05/2019	30/08/2019	9/06/2020
Three-leaf	25/05/2019	14/09/2019	22/06/2020
Laser treatment	18/06/2019	8/10/2019	30/07/2020
Plant harvest	12/07/2019	25/10/2019	21/08/2020

effort. However, there is a longer available weed control period. Hence, developing control options that target larger growth stage weeds for complementary use with initial herbicide treatments is important in understanding the use-case for novel tools.

Annual ryegrass (*Lolium rigidum* Gaudin) is the dominant weed of Australian grain production systems. There are no studies that have investigated the efficacy of laser treatments for the control of annual ryegrass. Addressing these gaps in growth stage and species, this research aimed to evaluate (i) the irradiation energy requirement (as determined by treatment duration) of spot laser treatments to control annual ryegrass and (ii) the influence of growth stage on energy requirement for annual ryegrass control.

MATERIALS AND METHODS

Pot trials evaluating the effects of spot laser treatments on annual ryegrass growth and survival were conducted in 2019 and 2020, located in an outdoor growth facility at the I.A. Watson International Grains Research Center in Narrabri, NSW, Australia (−30.276790, 149.810460). Plants were established by planting at least five seeds from a commercial annual ryegrass seed lot at a depth of 10 mm in 100 or 200 mm diameter black plastic pots filled with a commercial potting mixture, Ultragrow Platinum potting mix (Centenary Landscaping, Queensland, Australia). The plantings were conducted four times at 2-week intervals, with five replicates per treatment at each planting time, establishing four growth stages for laser treatment. Pots were routinely watered to maintain soil near field capacity and were fertilized as required with a complete liquid fertilizer. Plants were thinned to one plant per pot at the one to two-leaf stage. The resulting annual ryegrass growth stages at the time of laser treatment were three-leaf, seven-leaf, mid-tillering, and late-tillering (**Table 1** and **Figure 1**).

Laser Treatment

Pyrolytic laser treatments were applied using a continuous wave (CW) 25 W fiber-coupled laser diode (OptLaser, Piaseczno, Poland). The laser produces a 975 ± 10 nm beam delivered to a fixed focal length lens of 36.3 mm with an optical fiber cable. Power control was provided by adjusting input driver voltage to the laser diode controller between 0 and 5 V through a custom Python 3 (Van Rossum and Drake, 2009) command-line interface software and Arduino driver.

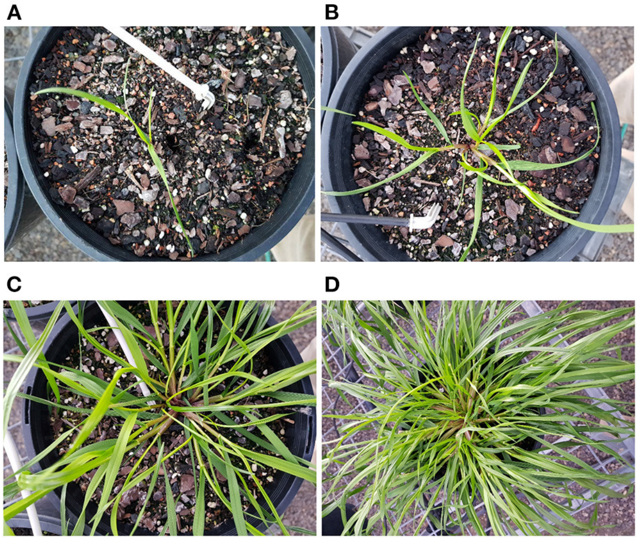

FIGURE 1 | Indicative plant sizes for the four annual ryegrass growth stages treated **(A)** three-leaf, **(B)** seven-leaf, **(C)** mid-tillering, and **(D)** late-tillering before laser treatment.

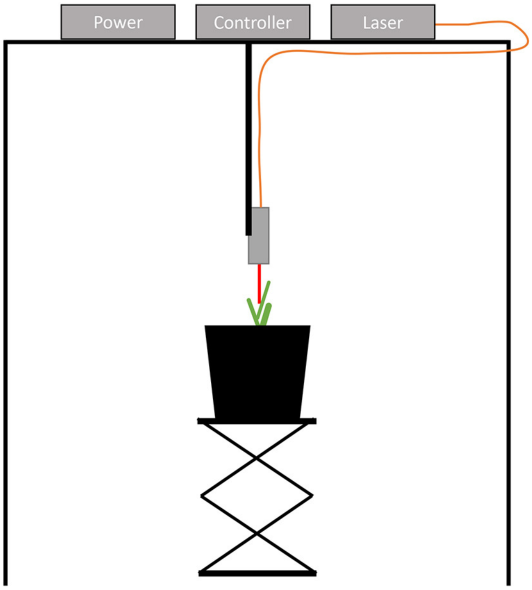

FIGURE 2 | Diagram of laser interlock and manual targeting method for the CW 25 W 975 nm fiber-coupled diode laser.

The laser was mounted in a custom-built double-interlock system (**Figure 2**) enabling precise targeting of weeds and safe operation of the laser, which was contained within an additional interlocked housing. Individual pots were positioned manually.

TABLE 2 | Summary of laser exposure and energy parameters for the CW 25 W, 975 nm fiber-coupled diode laser.

Laser	Beam diameter (mm)	Exposure time (s)	Total energy (J)	Energy density (J mm^{-2})
CW 975 ± 10 nm fiber-coupled diode laser	5	0	0	0
		1	25	1.3
		2	50	2.5
		5	125	6.4
		15	375	19.1
		60	1,500	76.4

Energy density is calculated as the total incident laser energy (based on a constant 25 W expressed as J s^{-1}, multiplied by exposure time) divided by the total area of the laser spot, with 5 mm width.

FIGURE 3 | Annual ryegrass three-leaf stage plants at 3 weeks post-laser treatment illustrating plants considered controlled **(Left)** and uncontrolled **(Right)** with new growth emerging from the uncontrolled growing point.

Targeting was achieved using a 3% of total power setting and a Thorlabs NIR Detector Card (ThorLabs Newton New Jersey, USA). Plants were treated with a 5 mm beam width developed at 42 mm from lens surface for 1, 2, 5, 15, and 60 s (**Table 2**). The selection of laser beam diameter and hence laser beam area (spot size) through laser optics has been shown to impact the total energy required (Wöltjen et al., 2008; Marx et al., 2012; Kaierle et al., 2013), with larger spot sizes improving ease of targeting, whilst smaller diameters reducing potential energy wastage. Earlier studies suggested that the larger spot sizes used provided improved performance, though the results were inconclusive (Mathiassen et al., 2006). A key benefit of larger spot sizes is the reduced requirement for exact positioning. Very narrow beams may miss treating the growing point from small positioning errors. The 5 mm beam diameter used in this study was based on findings from Marx et al. (2012) and Kaierle et al. (2013), where weeds up to the four-leaf stage with laser beam widths up to 6 mm were tested. As plants grow, the growing point also increases in size, such that larger beam sizes may be needed to target the larger growing points. The 5 mm beam width was selected to accommodate both seedling stage annual ryegrass and more mature plants. A fan inside the interlock was used to remove smoke condensing on the lens, thus preventing damage and reduced performance. The laser was positioned directly above the plant, targeting the base of the leaves or center of tillers depending on plant growth stage.

Following treatment, plants were returned to the outdoor growth facility. Laser treatment effects were assessed through mortality counts and dry weight cuts at 25, 29, and 22 days after treatment for Trial 1 (T1), Trial 2 (T2), and Trial 3 (T3), respectively. Plants were deemed to be dead if no new growth was present (**Table 1** and **Figure 3**). Surviving plants were harvested by cutting at ground level and placing in an envelope or small paper bag, depending on plant size. Plants were then oven-dried at 70°C for 3 days before weighing. Plant biomass data were converted to a percentage of untreated control for analysis and presentation.

Statistical Analysis

Treatment durations were converted to energy density using:

$$\rho_{energy} = \frac{P \times t}{\pi \, r_{beam}{}^{2}}$$

Where ρ_{energy} is the energy density in J mm^{-2}, P is laser power in Watts, t is treatment duration in seconds and r_{beam} is the radius of the laser beam in millimeters. Energy density allows more straightforward comparison of different beam widths, by standardizing incident energy by spot area. The percentage of untreated control values were analyzed using the Dose-Response Curve (DRC) package (Ritz et al., 2015) implemented in RStudio (RStudio Team, 2015; R Core Team, 2019) statistical software. Dose-response analyses were plotted with ggplot2 (Wickham, 2016).

Dry weight with a standard error of the mean ($n = 15$) and survival counts as a percentage of total replicates are presented in **Table 3**, with all three trial results grouped. An analysis of variance was conducted in RStudio with no significant difference at the trial level. The response of plants as a percentage of treated control biomass response to increasing laser energy treatment curves was fitted with the DRC package using a three-parameter log-logistic function:

$$y = d/(1 + exp(b(log(x) - log(e))))$$

Where d is the upper limit, b is the slope of the curve, and e is the inflection point or effective dose for 50% control. The choice of model was determined as the optimum fit through comparison with other functions based on the minimization of the Akaike information criterion. Lack of fit testing was performed for the resulting curves, with no significance found ($P < 0.05$) indicating an appropriate model was chosen. Effective doses (ED) at the 50% (ED_{50}) and 90% (ED_{90}) dry weight reduction levels were determined with the DRC package on the modeled dose-response curves and compared for significance from zero and each other.

TABLE 3 | Annual ryegrass dry weight in response to increasing laser energy density treatments when applied to four different growth stages based on three trials.

Growth stage	Energy density ($J\ mm^{-2}$)	Dry weight (g)	Percent control (%)
Three-leaf	0	0.58 ± 0.09	0
	1.3	0.47 ± 0.09	6.7
	2.5	0.31 ± 0.09	6.7
	6.4	0.08 ± 0.04	53.3
	19.1	0.01 ± 0.01	93.3
	76.4	0	100
Seven-leaf	0	3.22 ± 0.36	0
	1.3	3.10 ± 0.27	0
	2.5	3.23 ± 0.37	0
	6.4	1.54 ± 0.31	6.7
	19.1	0.44 ± 0.13	26.7
	76.4	0	100
Mid-tillering	0	10.77 ± 0.47	0
	1.3	11.07 ± 0.59	0
	2.5	10.16 ± 0.61	0
	6.4	11.23 ± 0.75	0
	19.1	9.79 ± 0.51	0
	76.4	4.28 ± 0.87	13.3
Late-tillering	0	22.17 ± 1.38	0
	1.3	21.34 ± 1.79	0
	2.5	22.15 ± 1.80	0
	6.4	21.50 ± 2.01	0
	19.1	21.45 ± 1.85	0
	76.4	19.85 ± 0.95	0

Observation error (±) is the standard error of the mean ($n = 15$). Mean dry weights presented by treatment and growth stage, with controlled plants considered as zero mass. Percent control is calculated from controlled weeds as a percentage of total replicates ($n = 15$).

RESULTS

The laser energy levels used in this study were, in general, too low given that the highest treatments tested were only effective on three-leaf and seven-leaf stage annual ryegrass, with no control of older growth stages. Three-leaf annual ryegrass was controlled with 76.4 J mm^{-2}, with only one survivor at 19.1 J mm^{-2} laser energy treatments. The highest laser energy treatment of 76.4 J mm^{-2} controlled all seven-leaf plants (**Table 3**), with no consistent control observed at other treatment energy levels.

Laser treatments consistently reduced annual ryegrass biomass at the earliest growth stages but had little or no effect on tillering plants (**Table 3**). Biomass reductions of 46.6 and 85.8% were observed for three-leaf stage plants following treatment with the 2.5 and 6.4 J mm^{-2}, respectively. At the seven-leaf stage, there were reductions of 52.1 and 86.2% observed for the 6.4 and 19.1 J mm^{-2} treatments, respectively. At the mid-tillering stage, annual ryegrass growth was only reduced at the highest energy treatment, which resulted in a 60.2% reduction in biomass. At the late-tillering growth stage, there was a minor decrease in plant biomass of 10.4% observed at the highest laser energy treatment.

Laser Energy Dose-Response

The laser energy density required to reduce the growth of older and larger annual ryegrass plants is substantially greater than the density required to affect the growth of seedlings similarly. Dose-response curves were developed to show the relationship between increasing laser energy doses and annual ryegrass biomass reductions (**Figure 4**). The growth of annual ryegrass was restricted in a log-logistic relationship with increasing energy treatments. The log-logistic curve failed to fit on the late-tillering growth stage, given the lack of impact on biomass. As highlighted by the ED_{50} values, there was a 2.4-fold higher energy requirement ($P < 0.05$) to achieve a 50% reduction in the biomass of seven-leaf annual ryegrass plants (6.13 ± 0.37 J mm^{-2}) compared with three-leaf plants (2.60 ± 0.37 J mm^{-2}) (**Figure 4**). The energy requirements for ED_{50} of mid-tillering plants were 10.1 and 23.8-fold greater ($P < 0.05$) than the seven and three-leaf growth stages. There were no differences ($P > 0.05$) between the ED_{90} values of 8.32 ± 2.33 J mm^{-2} and 13.87 ± 5.27 J mm^{-2} for the three and seven-leaf growth stage. ED_{90} values were not found within the range of energy levels tested for either mid-tillering or late-tillering growth stages. The comparatively flat dose-response curves highlight that there was little or no effect of the laser treatments on tillering plants.

DISCUSSION

The use of a laser beam to deliver targeted energy for pyrolytic, thermal plant damage demonstrated the potential for control of three- and seven-leaf annual ryegrass plants, with no control observed for later growth stages. Complete control of three- and seven-leaf plants at 76.4 J mm^{-2} and 93.3% control of three-leaf at 19.1 J mm^{-2} illustrate the potential of lasers as an effective SSWC tool for seedling annual ryegrass plants. The lack of control of larger plants even at the highest energy dose indicates that substantially higher laser energy treatments beyond those tested would be required to control, or at least significantly impact the growth of these older annual ryegrass growth stages. Nevertheless, control of three- and seven-leaf weeds is encouraging for the deployment of lasers in large-scale cropping systems, indicating laser capability in weed control, though not necessarily specific energy doses required given the exploratory nature of the energy treatments used.

The lowest dose at which high levels of control of three-leaf stage annual ryegrass was observed was 19.1 J mm^{-2}, considerably higher than results recorded for barnyard-grass. Attempts at controlling four-leaf stage barnyard grass (Marx et al., 2012) were not successful. Control at the three-leaf stage occurred with ~3 J mm^{-2}, though there was substantial variability in the energy required (Wöltjen et al., 2008). Previous studies evaluating lasers for pyrolysis have not attempted to control seven-leaf or larger weeds, with most focusing on dicotyledonous weeds at early growth stages (Mathiassen et al., 2006; Wöltjen et al., 2008; Marx et al., 2012; Kaierle et al., 2013; Xiong et al., 2017). The finding of complete control of seven-leaf annual ryegrass at the highest energy treatment of 76.4 J mm^{-2} is a significant preliminary finding, however, at the next highest

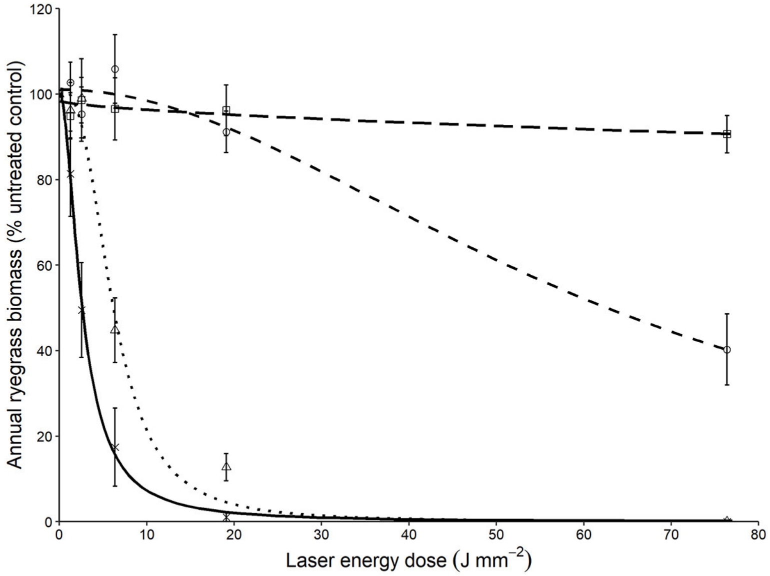

FIGURE 4 | Influence of increasing laser energy density doses on the biomass of annual ryegrass plants when applied at four plant growth stages expressed as the percentage of the untreated control. Growth stages treated included three-leaf, seven-leaf, mid-tillering, and late-tillering. Error bars are provided as standard errors of the mean (*n* = 15).

treatment, 19.1 J mm^{-2}, only 23.3% control was achieved. These results suggest that smaller increments in laser energy between the chosen levels are required to determine the point of adequate control more precisely.

Our CW 25 W fiber-coupled diode laser delivered inadequate energy after 60 s for control of tillering annual ryegrass. This result highlighted the inadequate power and hence low rate of energy delivery of the laser in addition to the low maximum treatments used for large weeds. In large-scale crop production environments, even low weed densities of 5 plants m^{-2} (Llewellyn et al., 2016) dictate that per-weed treatment times must be significantly lower than the 15 and 60 s durations that were found to control three-leaf stage annual ryegrass. These results and this requirement for large-scale systems suggest that (1) more energy is required for control of larger weeds and (2) if lasers are to be relevant in large-scale production systems, laser power must be sufficient to deliver the energy required in a short duration. The present study used energy treatments that were too low for the large weeds tested, with future research seeking to increase total energy delivered. Concerning laser power, it appears that the lethal temperature of thermal control options is inversely related to the exposure time (Sutcliffe, 1977; Ascard et al., 2007), where the rate of delivery does not seem to impact the efficacy of laser weeding. For example, Wöltjen et al. (2008) observed no difference in energy requirement for control of barnyard grass between a 500 W CO_2 laser and a 250 W diode laser. It should be noted that previous results have suggested that laser wavelength is an important factor (Kaierle et al., 2013) in the efficiency of control of seedling weeds whereby this comparison used two distinct laser wavelengths. Thus, it remains unclear as to whether a rapid energy delivery rate from a high-power laser would result in adequate heat movement through the larger weeds or simply pyrolyze surface tissue, with char acting as an insulator protecting the plant from further damage. Similar insulating effects of char are used for fire safety practices in buildings (White and Dietenberger, 2010).

The spot size of 5 mm chosen for the present study was based on findings in Marx et al. (2012) and Kaierle et al. (2013). Studies incorporating larger spot sizes and larger ranges of energy densities would be necessary for future research in determining the importance of spot size in controlling large weeds. Further, incorporating adaptive optics techniques that could change the spot size in the field depending on target weed size and environment could help enable smarter targeting of weeds by size.

The present study represents the first investigation of annual ryegrass, and to the best of our knowledge is the first evaluation of lasers for control of grass weeds larger than the four-leaf growth stage. Annual ryegrass was controlled at the highest energy levels at both the three- and seven-leaf growth stages, with larger plants less sensitive to the energy treatments indicating a strong growth stage and plant size effect on efficacy. The variability in efficacy was found to increase with lower doses, indicating consistent levels of weed control requires delivery of larger quantities of energy. A more detailed analysis of temperature changes and heat movement in both the plant and soil at each energy dose may provide some answers on variability and targeting. Further investigation is required to determine the practicality and energy requirements needed for consistent control of tillering and mature annual ryegrass. The use of more powerful lasers would increase the practicality of energy delivery by reducing the required treatment times. The targeted nature of lasers, coupled with advancements in the precision of weed recognition, are offering new opportunities for weed control tools.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

GC and CB: conceptualization, methodology, analysis, and writing. CS: conceptualization, methodology, and reviewing. SL-S: conceptualization, reviewing, and editing. MW: conceptualization, methodology, reviewing, and editing. All authors contributed to the article and approved the submitted version.

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REFERENCES

Ascard, J., Hatcher, P. E., Melander, B., and Upadhyaya, M. K. (2007). "Thermal weed control," in *Non-Chemical Weed Management: Principles, Concepts and Technology*, eds M. K. Upadhyaya and R. E. Blackshaw (Wallingford: CABI), 155–175. doi: 10.1079/9781845932909.0155

Bajwa, A. A., Mahajan, G., and Chauhan, B. S. (2015). Nonconventional weed management strategies for modern agriculture. *Weed Sci.* 63, 723–747. doi: 10.1614/WS-D-15-00064.1

Bauer, M. V., Marx, C., Bauer, F. V., Flury, D. M., Ripken, T., and Streit, B. (2020). Thermal weed control technologies for conservation agriculture—a review. *Weed Res.* 60, 241–250. doi: 10.1111/wre.12418

Bayramian, A., Fay, P. K., and Dyer, W. E. (1992). "Weed control using carbon dioxide lasers," in *Proceedings of the Western Society of Weed Science*, ed R. G. Lym (Salt Lake City: WSWS), 55–56.

Champ, J., Mora-Fallas, A., Goëau, H., Mata-Montero, E., Bonnet, P., and Joly, A. (2020). Instance segmentation for the fine detection of crop and weed plants by precision agricultural robots. *Appl. Plant Sci.* 8:e11373. doi: 10.1002/aps3.11373

Coleman, G. R. Y., Stead, A., Rigter, M. P., Xu, Z., Johnson, D., Brooker, G. M., et al. (2019). Using energy requirements to compare the suitability of alternative methods for broadcast and site-specific weed control. *Weed Technol.* 33, 633–650. doi: 10.1017/wet.2019.32

Couch, R., and Gangstad, E. (1974). Response of waterhyacinth to laser radiation. *Weed Sci.* 22, 450–453. doi: 10.1017/S0043174500037991

Duke, S. O. (2012). Why have no new herbicide modes of action appeared in recent years? *Pest Manag. Sci.* 68, 505–512. doi: 10.1002/ps.2333

Heap, I. M. (2020). *The International Survey of Herbicide Resistant Weeds*. Available online at: http://www.weedscience.com (accessed November 12, 2020).

Heisel, T., Schou, J., Christensen, S., and Andreasen, C. (2001). Cutting weeds with a CO_2 laser. *Weed Res.* 41, 19–29. doi: 10.1046/j.1365-3180.2001.00212.x

Kaierle, S., Marx, C., Rath, T., and Hustedt, M. (2013). Find and irradiate - lasers used for weed control. *Laser Tech. J.* 10, 44–47. doi: 10.1002/latj.201390038

Kassam, A., Friedrich, T., Derpsch, R., Lahmar, R., Mrabet, R., Basch, G., et al. (2012). Conservation agriculture in the dry mediterranean climate. *F. Crop. Res.* 132, 7–17. doi: 10.1016/j.fcr.2012.02.023

Kristensen, L., Olsen, J., and Weiner, J. (2008). Crop density, sowing pattern, and nitrogen fertilization effects on weed suppression and yield in spring wheat. *Weed Sci.* 56, 97–102. doi: 10.1614/WS-07-065.1

Lemerle, D., Cousens, R. D., Gill, G. S., Peltzer, S. J., Moerkerk, M., Murphy, C. E., et al. (2004). Reliability of higher seeding rates of wheat for increased competitiveness with weeds in low rainfall environments. *J. Agric. Sci.* 142, 395–409. doi: 10.1017/S002185960400454X

Lemerle, D., Verbeek, B., and Orchard, B. (2001). Ranking the ability of wheat varieties to compete with *Lolium rigidum*. *Weed Res.* 41, 197–209. doi: 10.1046/j.1365-3180.2001.00232.x

Llewellyn, R., Ronning, D., Ouzman, J., Walker, S., Mayfield, A., and Clarke, M. (2016). *Impact of Weeds in Australian Grain Production: The Cost of Weeds to Australian Grain Growers and the Adoption of Weed Management and Tillage Practices*. Canberra: GRDC.

López-Granados, F. (2011). Weed detection for site-specific weed management: mapping and real-time approaches. *Weed Res.* 51, 1–11. doi: 10.1111/j.1365-3180.2010.00829.x

Lottes, P., Behley, J., Milioto, A., Stachniss, C., Chebrolu, N., Milioto, A., et al. (2020). Robust joint stem detection and crop - weed classification using image sequences for plant - specific treatment in precision farming. *J. F. Robot.* 37, 20–34. doi: 10.1002/rob.21901

Marx, C., Barcikowski, S., Hustedt, M., Hustedt, S., Haferkamp, H., Rath, T., et al. (2012). Design and application of a weed damage model for laser-based weed control. *Biosyst. Eng.* 113, 148–157. doi: 10.1016/j.biosystemseng.2012.07.002

Mathiassen, S. K., Bak, T., Christensen, S., and Kudsk, P. (2006). The effect of laser treatment as a weed control method. *Biosyst. Eng.* 95, 497–505. doi: 10.1016/j.biosystemseng.2006.08.010

R Core Team (2019). *R: A Language and Environment for Statistical Computing (version 3.6.1)*. Available online at: https://www.r-project.org/

Ritz, C., Baty, F., Streibig, J. C., and Gerhard, D. (2015). Dose-response analysis using R. *PLoS ONE* 10:e0146021. doi: 10.1371/journal.pone.0146021

RStudio Team (2015). *RStudio: Integrated Development Environment for R (Version 1.1.456)*. Available online at: http://www.rstudio.com/

Schou, J., Heisel, T., Nordskov, A., Christensen, S., Jensen, P. S., Thestrup, B., et al. (2002). "Quantitative laser cutting of plants," in *International Symposium on High-Power Laser Ablation 2002*, ed C. R. Phipps (Taos, NM: SPIE), 9. doi: 10.1117/12.482143

Sutcliffe, J. (1977). *Plants and Temperature.* London: Edward Arnold (Publishers) Ltd.

Van Rossum, G., and Drake, F. L. (2009). *Python 3 Reference Manual*. Scotts Valley, CA: CreateSpace.

Walsh, M., Newman, P., and Powles, S. (2013). Targeting weed seeds in-crop: a new weed control paradigm for global agriculture. *Weed Technol.* 27, 431–436. doi: 10.1614/WT-D-12-00181.1

Wang, A., Zhang, W., and Wei, X. (2019). A review on weed detection using ground-based machine vision and image processing techniques. *Comput. Electron. Agric.* 158, 226–240. doi: 10.1016/j.compag.2019.02.005

White, R. H., and Dietenberger, M. A. (2010). "Chapter 18 - fire safety of wood construction," in *Wood Handbook - Wood as an Engineering Material*, ed. J. R. Ross (Madison, WI: Forest Products Laboratory), 18-1–18-22.

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis (Version 3.3.0)*. New York, NY: Springer-Verlag. Available online at: https://ggplot2.tidyverse.org

Wöltjen, C., Haferkamp, H., Rath, T., and Herzog, D. (2008). Plant growth depression by selective irradiation of the meristem with CO_2 and diode lasers. *Biosyst. Eng.* 101, 316–324. doi: 10.1016/j.biosystemseng.2008.08.006

Xiong, Y., Ge, Y., Liang, Y., and Blackmore, S. (2017). Development of a prototype robot and fast path-planning algorithm for static laser weeding. *Comput. Electron. Agric.* 142, 494–503. doi: 10.1016/j.compag.2017.11.023

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Distribution and Control of Herbicide-Resistant Italian Ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] in Winter Wheat (*Triticum aestivum* L.) in North Carolina

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Italian ryegrass is consistently ranked as one of the most problematic weeds of winter wheat in the Southeastern United States. To determine the distribution of resistant Italian ryegrass biotypes, seed was collected from locations throughout North Carolina and screened with diclofop, pinoxaden, mesosulfuron, and pyroxsulam. Results identified evidence of resistance to diclofop at all locations sampled throughout the state. Resistance to mesosulfuron, pyroxsulam, and pinoxaden were confirmed in 11, 19, and five percent of sampled locations, respectively. Additionally, Italian ryegrass biotypes resistant to multiple and all herbicides tested were identified, eliminating POST herbicide application as an option for control. Adjusting tillage practices may be an option for sustainable weed management to maintain effective control and maximize crop yield. Companion studies were established in the Coastal Plain and Piedmont regions of North Carolina in 2013 and 2014 to evaluate the effect of tillage on Italian ryegrass efficacy with herbicides. Herbicide treatments consisted of pyroxasulfone PRE only, mesosulfuron, or pinoxaden and POST only applications of mesosulfuron plus pyroxasulfone or pinoxaden plus pyroxasulfone. Tillage treatments included no-till and conservation tillage. Treatments containing pinoxaden provided the greatest Italian ryegrass control, regardless of tillage system. The use of pyroxasulfone PRE controlled a higher percentage of Italian ryegrass in the Piedmont when compared to the Coastal Plain, which is believed to be due to multiple flushes during the growing season in the Coastal Plain. Herbicide treatment was still a significant factor in Italian ryegrass control, but Italian ryegrass seed head density was consistently lower in the no-till system. Tillage may be stimulating germination, allowing greater control with PRE herbicides. An integrated system of herbicides and tillage may allow for greater yield and reduce selection pressure on POST herbicides.

Keywords: Italian ryegrass, tillage, *Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot, herbicide resistance, mechanical management

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is a staple crop grown worldwide (Gupta et al., 2008). Growers around the globe produce over 26 billion bushels of wheat per year (United States Department of Agriculture, 2014). The United States produces ∼2 billion bushels per year, of which 44 million are produced in North Carolina (United States Department of Agriculture, 2014). Winter wheat accounts for 75% of wheat production in the United States (Agriculture Marketing Resource Center, 2015). Winter wheat is planted in mid- to late- November and harvested around June in North Carolina (Weisz, 2013). As in many agronomic cropping systems, an effective weed management program plays an important role in a successful wheat growing season. Weed interference can cause significant yield reductions in winter wheat (Liebl and Worsham, 1987; Wilson and Wright, 1990).

Weedy *Lolium* spp. are a ubiquitous problem in wheat production worldwide (Llewellynn and Powles, 2001; Barros et al., 2005; Trusler et al., 2007). Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot], a common problem weed in Southeastern United States winter wheat production (Liebl and Worsham, 1987; Grey and Bridges, 2003; Grey et al., 2012). Studies conducted in Oregon showed yield losses of up to 60% that were attributable to increased densities of Italian ryegrass in winter wheat over the course of 2 years. These studies also investigated the effect of Italian ryegrass competition on wheat yield reduction by variety, however decreased yields were observed as Italian ryegrass populations increased, regardless of wheat variety (Appleby et al., 1976). Liebl and Worsham (1987) quantified yield losses associated with interference from Italian ryegrass reporting for every 10 Italian ryegrass plants m^{-2}, wheat yield was decreased by an average of 4.2%. In subsequent studies, Italian ryegrass has been shown to reduce wheat yield by >30% with as little as nine ryegrass plants m^{-2} present (Hashem et al., 1998; Scursoni et al., 2012).

Effective chemical control of Italian ryegrass is limited as this species has evolved resistance to all herbicides labeled in winter wheat (Grey and Bridges, 2003; Hoskins et al., 2005; Grey et al., 2012; Heap, 2020). The preemergence (PRE) herbicides applied for Italian ryegrass control in winter wheat include acetolactate synthase (ALS) (herbicide group [HG] 2)-, microtubule synthesis (HG 3)-, photosystem II (PSII) (HG 5)-, and very-long-chain fatty acid (VLCFA) (HG 15)-inhibiting herbicides. The postemergence (POST) herbicides applied for Italian ryegrass in winter wheat include acetyl CoA carboxylase (ACCase) (HG 1)- and ALS-inhibiting herbicides. North Carolina Italian ryegrass populations evolved resistance to ACCase- and ALS- inhibiting herbicides in 1990 and 2007, respectively (Heap, 2020). Multiple herbicide-resistant (HG 1 and 2) North Carolina Italian ryegrass populations were confirmed in 2007 as well (Heap, 2020). Resistance to the VLCFA-inhibiting herbicides remain unevolved in North Carolina (Everman, personal communication). Thus, North Carolina farmers growing winter wheat cannot solely rely on herbicides to sustainably control Italian ryegrass.

Mechanical control (i.e., tillage) is efficacious on Italian ryegrass in winter wheat (Oveson and Appleby, 1971; Moyer et al., 1994; Bond et al., 2014). Tillage provides weed control by burying the seed deep within the soil profile resulting in seedlings never reaching the soil surface or seeds remaining dormant (Pollard and Cussans, 1981; Ball, 1992). However, much of the tillage efficacy research has focused on deep tillage (i.e., moldboard plow) which is not as commonly used anymore with the trend of adopting reduced- or no till agriculture. Previous research has also demonstrated implementing tillage can influence Italian ryegrass germination (Bueno et al., 2007; Trusler et al., 2007; Ichihara et al., 2009). Thus, tillage may impart enough of a stimulus to induce Italian ryegrass germination which allows for better control when a preemergence herbicide is applied (Stougaard et al., 1984; Shaw, 1996; Rasmussen, 2003). The combination of implementing tillage in addition to applying herbicides could increase the control of Italian ryegrass in North Carolina wheat.

Currently, winter wheat is planted into no-till fields in the Piedmont regions, while planted into conservation tilled (>30% of residue left on soil surface) fields in the Coastal Plains regions of North Carolina and both regions are inhabited by herbicide-resistant Italian ryegrass populations. Thus, it is of interest to determine the efficacy of tillage and effective herbicides on North Carolina Italian ryegrass populations. The objectives of this research were to determine the distribution and the efficacy of conservation tillage practices of herbicide-resistant Italian ryegrass in North Carolina. The hypotheses of the research were that herbicide-resistant Italian ryegrass is pervasive across North Carolina and that tillage impacts Italian ryegrass control and density.

MATERIALS AND METHODS

Distribution of Herbicide-Resistant Italian Ryegrass in North Carolina

Italian ryegrass seeds were collected from wheat fields in North Carolina in the spring of 2012 and 2013. Sample locations were chosen on a longitudinal spacing of every 13 degree min, and a latitude spacing of every 10 degree min, resulting in a total of 239 locations selected for sampling. Sites were only sampled if an agricultural area was present within 3 km of the central grid point. When Italian ryegrass was found, seed heads from that location were collected and marked with the GPS coordinate of the location. Italian ryegrass seed was collected from 155 of the 239 locations (**Figure 1**). One hundred and thirty-six of these locations were sampled in 2012, with the other 20 being sampled in 2013. Collected seeds were sown into Fafard 2B potting mix in 9 by 13 cm flats in a greenhouse, with each flat containing one sampled Italian ryegrass population. After emergence, approximately three to four seedlings were transferred into 10 cm square pots filled with Fafard 2B potting mix. Overhead irrigation was supplied and light was supplemented by 1,000 watt metal halide bulbs for 12 h day^{-1}. Average day/night temperatures in the greenhouse were 25/15 C.

Once the plants reached three- to five-leaf stage, herbicide treatments were applied. Herbicide treatments consisted of: a non-treated check, two ACCase- (diclofop-methyl [1,077 g

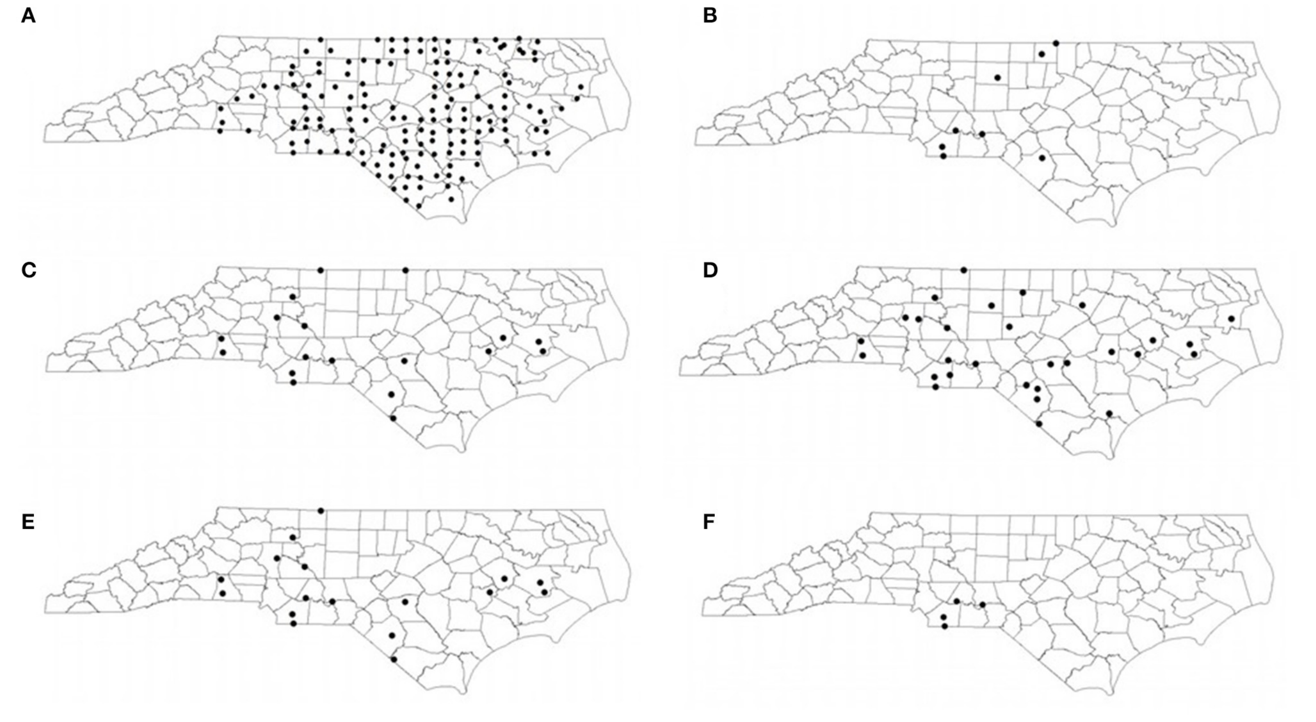

FIGURE 1 | **(A)** Distribution of the 155 sites sampled in North Carolina for putative herbicide-resistant Italian ryegrass in 2012 and 2013. All sampled Italian ryegrass populations have evolved resistance to diclofop applied at the maximum-labeled rate (acetyl CoA carboxylase-inhibiting herbicide). **(B)** Distribution of the eight North Carolina Italian ryegrass samples that have evolved resistance to pinoxaden applied at the maximum-labeled (acetyl CoA carboxylase-inhibiting herbicide). **(C)** Distribution of the 18 North Carolina Italian ryegrass populations that have evolved resistance to mesosulfuron applied at the maximum-labeled rate (acetolactate synthase-inhibiting herbicide). **(D)** Distribution of the 29 North Carolina Italian ryegrass populations that have evolved resistance to pyroxsulam applied at the maximum-labeled rate (acetolactate synthase-inhibiting herbicide). **(E)** Distribution of the 17 North Carolina Italian ryegrass populations that have evolved resistance to both acetolactate synthase-inhibiting herbicides applied at the maximum-labeled rate (mesosulfron and pyroxsulam). **(F)** Distribution of the four North Carolina Italian ryegrass samples that have evolved resistance to all tested acetyl CoA carboxylase (diclofop and pinoxaden)- and acetolactate synthase (mesosulfron and pyroxsulam)-inhibiting herbicides applied at the maximum-labeled rate.

ai ha^{-1}]; pinoxaden [61 g ai ha^{-1}]), and two ALS-inhibiting herbicides (pyroxsulam [18 g ai ha^{-1} plus non-ionic surfactant at 0.5% v v^{-1}]; mesosulfuron [15 g ai ha^{-1} plus methylated seed oil at 1% v v^{-1}]). Herbicide and adjuvant rates are based on the maximum-labeled rates of the respective treatment. One lethal rate of each herbicide was included as resistance to the selected herbicides has already evolved in North Carolina Italian ryegrass populations. Treatments were applied in a spray chamber calibrated to deliver 140 L ha^{-1} of solution at 207 kPa 46 cm above the plant height with TeeJet TT8002 EVS nozzles (TeeJet® nozzles; Spraying Systems Co., Wheaton, IL). Treatments were arranged in a randomized complete block design with four replications and repeated once in time. Visual control ratings were taken at 14, 21, and 28 days after herbicide treatment. Control was estimated as a sum of total chlorosis, necrosis, and stunting on a rating scale ranging from 0 to 100%; where 0% equaled no control and 100% equaled complete control.

Tillage Impact on Italian Ryegrass Control, Density, and Wheat Yield

The study locations during the 2013–2014 growing season were at a private farm near Hertford, North Carolina (36.18 N, −76.38 W) and at the Piedmont Research Station in Salisbury, North Carolina (35.70 N, −80.62 W). These two locations represent the Coastal Plains (Hertford) and the Piedmont (Salisbury) regions of North Carolina. The Hertford location is tilled prior to wheat planting, while the Salisbury had been in continuous no till for ∼30 years before research was initiated. The soil of the field location near Hertford, North Carolina is a Roanoke silt loam (fine, mixed, semiactive, thermic Typic Endoaquult). The soil of the field at the Piedmont Research Station is a Lloyd clay loam (fine, kaolinitic, thermic Rhodic Kanhapludult). Planting at all locations was done in the fall of the respective year with a 3 m wide grain drill. Winter wheat was seeded at a rate of 72 seeds meter^{-1}, with a 19 cm row spacing. Locations were planted twice with one study being conservatively-tilled and the other study being no-tilled prior to sowing. No tillage was further implemented on the tillage studies. Tillage was conducted with a chisel plow in Hertford, while tillage was conducted with a coulter-blade plow with a rolling-spike harrow attachment in Salisbury. Each study was tilled and planted on the same day. The experimental design was a randomized complete block with four replications. Plots were 3 m wide by 10 m long for all studies. Five herbicide programs

TABLE 1 | Herbicide treatment, application timing, and rates applied in no-till and conventional tillage systems for Italian ryegrass control.

Herbicide program	Timing	Rate (g ai ha^{-1})[a]
Non-treated		
Pyroxasulfone	PRE	74.4
Pyroxasulfone fb mesosulfuron[a]	PRE fb POST	74.4 fb 15
Pyroxasulfone fb pinoxaden	PRE fb POST	74.4 fb 60.5
Mesosulfuron[a] + pyroxasulfone	POST	15 + 74.4
Pinoxaden + pyroxasulfone	POST	60.5 + 74.4

[a] *Included MSO at a rate of 0.25% v v^{-1}.*

MSO, methylated seed oil; PRE, pre-emergence; fb, followed by; POST, post-emergence.

were included in the experiment (**Table 1**). Treatments were applied with a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} of solution at 207 kPa 46 cm above the plant height with TeeJet XR11002 nozzles (TeeJet® nozzles; Spraying Systems Co., Wheaton, IL). Italian ryegrass control was visually assessed at the postemergence herbicide timing and prior to wheat harvest. Italian ryegrass control was visually assessed on a rating scale ranging from 0 to 100%, where 0% equaled no control and 100% equaled complete control. Italian ryegrass densities were estimated prior to winter wheat harvest by averaging the frequency of seed heads recorded in three one-meter^2 areas within a plot.

Statistical Analysis

All statistical analyses were performed using Statistical Analysis Software, SAS 9.3 (SAS Institute, Inc., NC, USA).

Distribution of Herbicide-Resistant Italian Ryegrass in North Carolina

A herbicide-susceptible Italian ryegrass population was identified based on control ratings and was defined as a population from a location sample where there was complete control observed across all replications and in both runs. The pinoxaden+mesosulfuron- and pyroxsulam-susceptible Italian ryegrass populations were collected from Stokes and Carteret County, North Carolina, respectively. No diclofop-susceptible Italian ryegrass populations were collected from the 155 sampled. Once a herbicide-susceptible Italian ryegrass population was determined, Italian ryegrass control data were subjected to ANOVA using PROC GLIMMIX and treatment means were separated using Dunnett's Procedure ($P < 0.05$) to separate Italian ryegrass populations that exhibited lower control than the selected susceptible populations. Moreover, Italian ryegrass populations were concluded to be herbicide-resistant if control was <50% as the discriminating rates of the applied herbicides should result in complete control. Outliers were not removed as they represent the variability of resistance within sampled locations most likely due to segregation (Poirier et al., 2014). Variability between runs was not significant, therefore runs were combined for analysis.

Tillage Efficacy on Italian Ryegrass

Italian ryegrass control and seed head density data were subjected to ANOVA using PROC GLM and treatment means were separated using Fisher's Protected LSD ($P < 0.05$). PROC Corr was also conducted to determine if Italian ryegrass seed density, control prior to winter wheat harvest, and winter wheat yield data were correlated.

RESULTS AND DISCUSSION

Distribution of Herbicide-Resistant Italian Ryegrass in North Carolina

It is important to note that this survey method is predisposed to select for herbicide resistance. When a field was visited, samples were collected only when Italian ryegrass was visible above the crop canopy, meaning it is likely that these plants were escapes from previous herbicide applications. The four herbicides screened can be broken down into their mode of action group, either ACCase or ALS-inhibiting herbicides.

Acetyl CoA Carboxylase-Inhibiting Herbicides

No single Italian ryegrass population could be identified as susceptible to diclofop (**Figure 1**). This result was not unexpected as diclofop has been used extensively and recurrently in North Carolina winter wheat production (Heap, 2020; Everman, personal communication; Kuk and Burgos, 2007). Resistance to pinoxaden was not confirmed in North Carolina until 2007 (Heap, 2020). While pinoxaden has the same mode of action as diclofop, pinoxaden can control diclofop-resistant Italian ryegrass populations, and resistance has not been reported to be as common in similar surveys (Kuk and Burgos, 2007; Salas et al., 2013; Bararpour et al., 2018). Out of the 155 sampled populations in North Carolina, eight Italian ryegrass populations exhibited controls levels below those of the herbicide-susceptible population when treated with pinoxaden, elucidating the evolution of resistance in the select populations (**Figure 1**). The distribution of pinoxaden-resistant Italian ryegrass populations are isolated in Southwestern North Carolina. Since all population showed signs of resistance to diclofop, these four populations are cross-resistant to the tested ACCase-inhibiting herbicides.

Acetolactate Synthase-Inhibiting Herbicides

Eighteen Italian ryegrass populations exhibited controls levels below those of the herbicide-susceptible population when treated with mesosulfuron, elucidating the evolution of resistance in the select populations (**Figure 1**). Resistance to pyroxsulam was more common than resistance to mesosulfuron in the sampled Italian ryegrass populations. Twenty-nine Italian ryegrass populations exhibited controls levels below those of the herbicide-susceptible population when treated with pyroxsulam, elucidating the evolution of resistance and that resistance is widespread throughout the state (**Figure 1**). Out of the 155 sampled populations in North Carolina, 17 Italian ryegrass populations were cross resistant to both ALS-inhibiting herbicides (**Figure 1**).

Four Italian ryegrass populations were found to have evolved resistance to all four of the tested herbicides (**Figure 1**). The four multiple-herbicide-resistant Italian ryegrass populations are isolated in Southwest North Carolina. These four locations were identified in Richmond, Stanly, and Union counties. Chemical control of Italian Ryegrass in winter wheat would likely be limited or impossible exclusively with POST herbicides. Findings from this survey can be a valuable resource to growers when making decisions concerning Italian ryegrass control in winter wheat. While resistance may not be present on every farm near one of the sample locations, the possibility should be taken into account when designing a herbicide program.

While the herbicide resistance survey was conducted 6–7 years ago, the distribution of the herbicide-resistant Italian ryegrass populations would likely be similar today. Wheat production in North Carolina has declined (2015: 600,000 ha; 2020: ∼100,000 ha) since this work was conducted and minimal (if any) herbicides are applied to the wheat planted, depending on grain price (United States Department of Agriculture, 2014, 2019). While pyroxasulfone (not included in the original survey) has been applied to the wheat grown within North Carolina since the research was conducted, no control failures have been reported with this herbicide. While that does not suggest that pyroxasulfone-resistant Italian ryegrass populations have not evolved within North Carolina, the lack of control failure complaints suggest that pyroxasulfone remains efficacious on North Carolina Italian ryegrass populations.

Tillage Impact on Italian Ryegrass Control, Density, and Wheat Yield

Tillage was evaluated in separate companion trials placed adjacent to each other in each location and year, so results cannot be directly compared; however, trends can be observed between tillage types. No differences in control were observed at the POST application for the three treatments containing pyroxasulfone PRE in either tillage system with control ranging from 76 to 80% in the no-till and 81–88% in the tilled system (**Table 2**).

TABLE 2 | Italian ryegrass control at the postemergence timing in winter wheat averaged across locations (Hertford and Salisbury, North Carolina) and years (2013 and 2014); experiments were separated by tillage environment.

		No-Till		Till	
Herbicide program	**Timing**	%			
Non-treated		0	B	0	b
Pyroxasulfone	PRE	79	A	81	a
Pyroxasulfone fb mesosulfuron	PRE fb POST	80	A	86	a
Pyroxasulfone fb pinoxaden	PRE fb POST	76	A	88	a
Mesosulfuron + pyroxasulfone	POST	0	B	0	b
Pinoxaden + pyroxasulfone	POST	0	B	0	b

Columns that share the same letters are not statistically different based on Fisher's LSD ($P < 0.05$).
PRE, pre-emergence; fb, followed by; POST, post-emergence.

TABLE 3 | Italian ryegrass control prior to winter wheat harvest across both experiment locations (Hertford and Salisbury, North Carolina) and years (2013 and 2014); experiments were separated by tillage environment.

		No-Till		Till	
Herbicide program	**Timing**	%			
Non-treated		0	D	0	d
Pyroxasulfone	PRE	52	C	69	c
Pyroxasulfone fb mesosulfuron	PRE fb POST	54	C	77	bc
Pyroxasulfone fb pinoxaden	PRE fb POST	95	A	97	a
Mesosulfuron + pyroxasulfone	POST	70	B	79	b
Pinoxaden + pyroxasulfone	POST	87	A	93	a

Columns that share the same letters are not statistically different based on Fisher's LSD ($P < 0.05$).
PRE, pre-emergence; fb, followed by; POST, post-emergence.

Late season Italian ryegrass control, prior to harvest, varied greatly across herbicide programs and locations, but was not impacted by year. The greatest Italian ryegrass control was observed when pinoxaden was applied POST (**Table 3**). Reduced efficacy of mesosulfuron in both tillage systems when compared to pinoxaden indicates an established or emerging issue with resistance to the ALS-inhibiting herbicides (Kuk and Burgos, 2007). Although ALS-resistant Italian ryegrass plants may be present, treatments of mesosulfuron plus pyroxasulfone applied total POST resulted in greater control than a single PRE application of pyroxasulfone when averaged over locations and years. Two-pass herbicide programs provided higher levels of control for all treatments, however only one two-pass program was significantly greater than its total POST counterpart (**Table 4**). A significant interaction of herbicide program and location averaged over years was also observed for late season Italian ryegrass control. Trends were similar to the herbicide program analysis, but differences in Italian ryegrass response in Hertford and Salisbury due to ALS-resistant biotypes are more apparent (**Table 4**). The greatest Italian ryegrass control was observed where pyroxasulfone was followed by an application of pinoxaden regardless of location or tillage system. In the tilled system, the total POST pinoxaden program provided similar levels of control, however control in the no-till system was lower at both locations. Pyroxasulfone applied PRE provided 35 and 69% control at Hertford and Salisbury, respectively, in the no-till system, and 57 and 84% control at Hertford and Salisbury, respectively, in the tilled system. Applying mesosulfuron POST or mesosulfuron plus pyroxasulfone POST did not significantly improve Italian ryegrass control at either location for no-till or tilled systems (**Table 4**).

Italian ryegrass seed head density was affected by a significant year, location, and herbicide program interaction. The highest Italian ryegrass seed head density was observed at Hertford in 2013 with 336 and 476 seed heads m^{-2} in the no-till and tilled systems, respectively (**Table 5**). Within each year, seed head density was greatest in Hertford compared to Salisbury within each tillage system (**Table 5**). This high density of Italian ryegrass in the non-treated at Hertford is likely due to a heterogeneous distribution of ALS-resistant Italian ryegrass plants, and the impacts of such high densities are apparent in all efficacy evaluations taken in this study. The large range of densities within a tillage system (4–336 and 0–476 seed heads m^{-2} in no-till

TABLE 4 | Italian ryegrass control in conservation and no tillage systems prior to winter wheat harvest as influenced by herbicide program and location averaged over years.

Herbicide program	Timing	No-Till				Till			
		Hertford		Salisbury		Hertford		Salisbury	
		%							
non-treated		0	C	0	C	0	c	0	c
pyroxasulfone	PRE	35	C	69	B	57	b	84	ab
pyroxasulfone fb mesosulfuron	PRE fb POST	69	BC	73	B	78	ab	80	b
pyroxasulfone fb pinoxaden	PRE fb POST	93	A	99	A	96	a	99	a
mesosulfuron + pyroxasulfone	POST	40	C	67	B	68	b	86	ab
pinoxaden + pyroxasulfone	POST	87	A	87	B	88	a	97	a

Columns that share the same letters are not statistically different based on Fisher's LSD ($P < 0.05$).
PRE, preemergence; fb, followed by; POST, postemergence.

TABLE 5 | Italian ryegrass seed head density counts prior to winter wheat harvest across separated by experiment locations (Hertford and Salisbury, North Carolina) and years (2013 and 2014); experiments were separated by tillage environment.

Herbicide program	Timing	No-Till								Till							
		2013				2014				2013				2014			
		Hertford		Salisbury		Hertford		Salisbury		Hertford		Salisbury		Hertford		Salisbury	
		Plants m^{-2}															
Non-treated		299	AB	94	DE	167	CD	78	EFG	476	a	118	bc	128	bc	59	cd
Pyroxasulfone	PRE	250	BC	43	EFG	71	EFG	25	EFG	152	b	23	d	61	cd	20	d
Pyroxasulfone fb mesosulfuron	PRE fb POST	169	CD	22	EFG	20	EFG	38	EFG	54	cd	13	d	45	cd	11	d
Pyroxasulfone fb pinoxaden	PRE fb POST	4	EFG	9	EFG	15	EFG	7	G	0	d	0	d	3	d	2	d
Mesosulfuron + pyroxasulfone	POST	336	A	59	EFG	93	DEF	48	EFG	413	a	17	d	72	bcd	24	d
Pinoxaden + pyroxasulfone	POST	28	EFG	43	EFG	18	EFG	15	EFG	9	d	1	d	3	d	2	d

Columns that share the same letters are not statistically different based on Fisher's LSD ($P < 0.05$).
PRE, pre-emergence; fb, followed by; POST, post-emergence.

TABLE 6 | Winter wheat yield averaged across experiment locations (Hertford and Salisbury, North Carolina) and years (2013 and 2014); experiments were separated by tillage environment.

Herbicide program		No-Till Timing		Till tons ha^{-1}	
Non-treated		3.3	C	3.2	c
Pyroxasulfone	PRE	3.8	BC	4.3	a
Pyroxasulfone fb mesosulfuron	PRE fb POST	4.0	B	4.3	a
Pyroxasulfone fb pinoxaden	PRE fb POST	4.6	A	4.9	a
Mesosulfuron + pyroxasulfone	POST	3.2	C	3.6	b
Pinoxaden + pyroxasulfone	POST	4.3	AB	4.9	a

Columns that share the same letters are not statistically different based on Fisher's LSD ($P < 0.05$).
PRE, pre-emergence; fb, followed by; POST, post-emergence.

and tilled, respectively) makes statistical treatment separation difficult. However, clear trends can be observed. Similar to late season control ratings, treatments with pinoxaden POST resulted in the lowest Italian ryegrass seed head densities, regardless of tillage system. In 6 out of 8 instances, mesosulfuron plus pyroxasulfone POST was not significantly different from the non-treated, however there was only one instance of treatments being significantly different where the same herbicides were applied sequentially (**Table 5**). Although the seed head densities were not significantly different due to the high degree of variability, the lower densities where a PRE was followed by a POST compared to the total POST combination emphasizes the value of a PRE herbicide to control Italian ryegrass. Correlation analysis results detected a relatively high negative correlation between Italian ryegrass control prior to harvest and Italian ryegrass seed head density in the no-till ($R = -0.63$; $P < 0.0001$) and tilled systems ($R = -0.50$; $P < 0.0001$).

Winter wheat yield was significantly affected by year and herbicide program for both tillage systems. There was a significant location effect for the no-till system, and a significant year by location interaction for the tilled system. When averaged over herbicide program in the no-till system, wheat yield was greater in 2014 (4.2 tons ha^{-1}) compared to 2013 (3.6 tons ha^{-1}) and at Salisbury (4.8 tons ha^{-1}) compared to Hertford (3.0 tons ha^{-1}). In the tilled system, the yield was also greater in Salisbury (5.4 tons ha^{-1}) than Hertford (2.8 tons ha^{-1}) when averaged over location and herbicide program. The year by location interaction for wheat yield in the tilled system showed a similar trend, however greater yields in Hertford were observed in 2014 and in Salisbury in 2013 (data not shown). The effects of environment on crop yield are well-documented and come as no surprise (Bassett et al., 1989; Laidig et al., 2017). Although not part of the analysis, of interest was that higher yields were observed, in general, in the tilled system, regardless of year and location (data not shown) (Šíp et al., 2013).

Winter wheat yield was affected by herbicide program, therefore results are averaged over years and locations within each tillage system. Treatments containing pinoxaden gave the greatest level of Italian ryegrass control, and subsequently resulted in the greatest winter wheat yield in both no-till and tilled systems (**Table 6**). Following the trends in Italian ryegrass seed head density, the lowest yields were observed in the non-treated and where mesosulfuron was applied as part of a total POST program. Pyroxasulfone followed by mesosulfuron applied to winter wheat did not yield significantly different from the highest yielding in the tilled system. Correlation analysis identified a relatively high negative correlation between Italian ryegrass seed head density and winter wheat yield in the no-till and tilled systems ($R = -0.69$; $P < 0.0001$ and $R = -0.61$; $P < 0.0001$, respectively).

The results of the tillage system study gives clear conclusions on the importance of herbicide program to control Italian ryegrass. Effective POST herbicides are critical to maximize control and reduce Italian ryegrass seed head. The negative correlation between seed head density and winter wheat yield reveals the importance of reducing populations, not just improving control. In addition, the use of an effective PRE herbicide preserved winter wheat yield potential even where herbicide-resistant biotypes occur (Bond et al., 2014; Liu et al., 2016). Looking closely at Italian ryegrass seed head density and control data in the different tillage systems, trends emerge which suggest greater germination in the tilled system. The use of a PRE in the tilled system resulted in a lower percentage of Italian ryegrass when compared to the no-till system. The higher density may be due to stimulated germination due to tillage at planting (Forcella and Lindstrom, 1988; Chauhan et al., 2006; Bueno et al., 2007). This may have enabled the PRE treatment to control more Italian ryegrass, leaving less for the POST treatment to control, explaining the differences in late season Italian ryegrass control, seed head density, and yield of two-pass and one-pass herbicide programs containing mesosulfuron.

To further investigate the impact of tillage on Italian ryegrass, future studies should investigate the role of light and tillage on germination, as well as the occurrence of multiple germination flushes throughout the growing season. The distribution of herbicide-resistant Italian ryegrass populations in North Carolina should be sampled again in the future with the inclusion of screening pyroxasulfone. Since pyroxasulfone is one of the only effective herbicides to control Italian ryegrass, North Carolina wheat farmers have likely recurrently and extensively applied the herbicide. Over reliance of pinoxaden and pyroxasulfone since the herbicide screen was first conducted could have selected for resistant Italian ryegrass populations within the state (Kaundun, 2013; Busi et al., 2018; Heap, 2020).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

EJ conducted analysis and writing. ZT conducted research and writing. WE supervised research, analysis, and writing. All authors contributed to the article and approved the submitted version.

REFERENCES

Agriculture Marketing Resource Center (2015). *Wheat overview and wheat production*. Available online at: https://www.agmrc.org/commodities-products/grains-oilseeds/wheat

Appleby, A. P., Colbert, D. R., and Olson, P. D. (1976). Winter wheat yield reduction from interference by Italian ryegrass. *Agronomy J*. 34, 63–466. doi: 10.2134/agronj1976.00021962006800030007x

Ball, D. A. (1992). Weed seedbank response to tillage, herbicides, and crop rotation sequence. *Weed Sci*. 40, 654–659. doi: 10.1017/S0043174500058264

Bararpour, T., Korres, N. E., Nilda, R., Burgos, N. R., Hale, R. R., and Tseng, T. P. (2018). Performance of pinoxaden on the control of diclofop-resistant Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum*) in winter wheat. *Agriculture* 8:14. doi: 10.3390/agriculture8070114

Barros, J. F. C., Basch, G., and de Carvalho, M. (2005). Effect of reduced doses of a post-emergence graminicide mixture to control *Lolium rigidum* G. in winter wheat under direct drilling in Mediterranean environment. *Crop Prot*. 24, 880–887. doi: 10.1016/j.cropro.2005.01.020

Bassett, L. M., Allan, R. E., and Rubenthaler, G. L. (1989). Genotype X environment interactions on soft white winter wheat quality. *Agron. J*. 81, 955–960. doi: 10.2134/agronj1989.00021962008100060022x

Bond, J. A., Eubank, T. W., Bond, R. C., Golden, B. R., and Edwards, H. M. (2014). Glyphosate-resistant Italian ryegrass (*Lolium perenne* ssp. multiflorum) control with fall-applied residual herbicides. *Weed Technol*. 28, 361–370. doi: 10.1614/WT-D-13-00149.1

Bueno, J., Amiama, C., and Hernanz, J. L. (2007). No-tillage drilling of Italian ryegrass (*Lolium multiflorum* L.): crop residue effects, yields and economic benefits. *Soil Till. Res*. 95, 61–68. doi: 10.1016/j.still.2006.11.002

Busi, R., Porri, A., Gaines, T. A., and Powles, S. B. (2018). Pyroxasulfone resistance in *Lolium rigidum* is metabolism-based. *Pestic Biochem. Phys*. 148, 74–80. doi: 10.1016/j.pestbp.2018.03.017

Chauhan, B. S., Gill, G. S., and Preston, C. (2006). Tillage system effects on weed ecology, herbicide activity and persistence: a review. *Aust. J. Exp. Agric*. 46, 1557–1570. doi: 10.1071/EA05291

Forcella, F., and Lindstrom, M. F. (1988). Weed seed populations in ridge and conventional tillage. *Weed Sci*. 36, 500–503. doi: 10.1017/S0043174500075263

Grey, T. L., and Bridges, D. C. (2003). Alternatives to diclofop for the control of Italian ryegrass (*Lolium multiflorum*) in winter wheat (*Triticum aestivum*). *Weed Technol*. 17, 219–223. doi: 10.1614/0890-037X(2003)017[0219:ATDFTC]2.0.CO;2

Grey, T. L., Cutts, G. S., Sosnoskie, L., and Culpepper, A. S. (2012). Italian ryegrass (*Lolium perenne*) control and winter wheat response to POST herbicides. *Weed Technol*. 26, 644–648. doi: 10.1614/WT-D-12-00046.1

Gupta, P. K., Mir, R. R., Mohan, A., and Kumar, J. (2008). Wheat genomics: present status and future prospects. *Int. J. Plant Genom*. 2008:896451. doi: 10.1155/2008/896451

Hashem, A., Radosevich, S. R., and Roush, M. L. (1998). Effect of proximity factors on competition between winter wheat (*Triticum aestivum*) and Italian ryegrass (*Lolium multiflorum*). *Weed Sci*. 46, 181–190. doi: 10.1017/S0043174500090391

Heap, I. (2020). *International survey of herbicide resistant weeds*. Available online at: www.weedscience.org/in.asp (accessed August 15, 2020).

Hoskins, A., Young, B. G., Krausz, R. F., and Russin, J. S. (2005). Control of Italian ryegrass (*Lolium multiflorum*) in winter wheat. *Weed Technol*. 19, 261–265. doi: 10.1614/WT-03-118R3

Ichihara, M., Yamashita, M., Sawada, H., Kida, Y., and Asai, M. (2009). Influence of after-ripening environments on the germination characteristics and seed fate of Italian ryegrass (*Lolium multiflorum*). *Weed Biol. Manag*. 9, 217–224. doi: 10.1111/j.1445-6664.2009.00342.x

Kaundun, S. S. (2013). Resistance to acetyl-CoA carboxylase-inhibiting herbicides. *Pest Manag. Sci*. 70, 1405–1417. doi: 10.1002/ps.3790

Kuk, Y. I., and Burgos, N. R. (2007). Cross-resistance profile of mesosulfuron-methyl-resistant Italian ryegrass in the southern United States. *Pest Manag. Sci*. 63, 349–357. doi: 10.1002/ps.1338

Laidig, F., Pieph, H. P., Rentel, D., Drobek, T., Meyer, U., and Huesken, A. (2017). Breeding progress, environmental variation and correlation of winter wheat yield and quality traits in German official variety trials and on-farm during 1983–2014. *Theor. Appl. Genet*. 130, 223–245. doi: 10.1007/s00122-016-2810-3

Liebl, R., and Worsham, A. D. (1987). Interference of Italian ryegrass (*Lolium multiflorum*) in wheat (*Triticum aestivum*). *Weed Sci*. 35, 819–823. doi: 10.1017/S0043174500079406

Liu, M., Hulting, A. G., and Mallory-Smith, C. (2016). Characterization of multiple herbicide-resistant Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) populations from winter wheat fields in Oregon. *Weed Sci*. 64, 331–338. doi: 10.1614/WS-D-15-00147.1

Llewellynn, R. S., and Powles, S. B. (2001). High levels of herbicide resistance in rigid ryegrass (*Lolium rigidum*) in the wheat belt of Western Australia. *Weed Technol*. 15, 242–248. doi: 10.1614/0890-037X(2001)015[0242:HLOHRI]2.0.CO;2

Moyer, J. R., Roman, E. S., Lindwall, C. W., and Blackshaw, R. E. (1994). Review: Weed management in conservation tillage systems for wheat production in North and South America. *Crop Prot*. 13, 244–259. doi: 10.1016/0261-2194(94)90012-4

Oveson, M. M., and Appleby, A. P. (1971). Influence of tillage management in a stubble mulch fallow-winter wheat rotation with herbicide weed control. *Agron. J*. 63, 19–20. doi: 10.2134/agronj1971.00021962006300010008x

Poirier, A. H., York, A. C., Jordan, D. L., Chandi, A., Everman, W. J., and Whitaker, J. R. (2014). Distribution of glyphosate- and thifensulfuron-resistant palmer amaranth (*Amaranthus palmeri*) in North Carolina. *Int J Agron*. 2014:747810. doi: 10.1155/2014/747810

Pollard, F., and Cussans, G. W. (1981). The influence of tillage on the weed flora in a succession of winter cereal crops on a sandy loam soil. *Weed Res*. 21, 185–190. doi: 10.1111/j.1365-3180.1981.tb00115.x

Rasmussen, I. A. (2003). The effect of sowing date, stale seedbed, row width and mechanical weed control on weeds and yields of organic winter wheat. *Weed Res*. 44, 12–20. doi: 10.1046/j.1365-3180.2003.00367.x

Salas, R. A., Burgos, N. R., Mauromoustakos, A., Lassiter, R. B., Scott, R. C., and Alcober, E. A. (2013). Resistance to ACCase and ALS inhibitors in *Lolium perenne* ssp. *multiflorum* in the United States. *J. Crop Weed* 9, 168–183.

Scursoni, J. A., Palmano, M., De Notta, A., and Delfino, D. (2012). Italian ryegrass (*Lolium multiflorum* Lam.) density and N fertilization on wheat (*Triticum aestivum* L.) yield in Argentina. *Crop Prot*. 32, 36–40. doi: 10.1016/j.cropro.2011.11.002

Shaw, D. R. (1996). Development of stale seedbed weed control programs for southern row crops. *Weed Sci*. 44, 413–416. doi: 10.1017/S0043174500094108

Šíp, V., Vavera R, Chrpová, J., Kusá, H., and RuŽek, P. (2013). Winter wheat yield and quality related to tillage practice, input leveland environmental conditions. *Soil Tillage Res*. 132, 77–85. doi: 10.1016/j.still.2013.05.002

Stougaard, R. N. G., Kapusta, G., and Roskamp, G. (1984). Early preplant herbicide applications for no-till soybean (*Glycine max*) weed control. *Weed Sci*. 32, 293–298. doi: 10.1017/S0043174500059014

Trusler, C. S., Peeper, T. F., and Stone, A. E. (2007). Italian ryegrass (*Lolium multiflorum*) management options in winter wheat in Oklahoma. *Weed Technol*. 21, 151–158. doi: 10.1614/WT-06-038.1

United States Department of Agriculture (2014). *Wheat: Planted acreage, harvest acreage, production, yield and farm price*. Available online at: https://www.ers.usda.gov/topics/crops/wheat/

United States Department of Agriculture (2019). *State agricultural overview: North Carolina*. Available online at: https://quickstats.nass.usda.gov/results/8F40D33F-FDC6-329D-B18B-8716E781DABE

Weisz, R. (2013). "Small grain growth and development," in *Small Grain Production Guide*, ed D. L. Wright (Raleigh, NC: North Carolina State University), 1–4.

Wilson, B. J., and Wright, K. J. (1990). Predicting the growth and competitive effects of annual weeds in wheat. *Weed Res*. 30, 201–211. doi: 10.1111/j.1365-3180.1990.tb01704.x

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Integrated Weed Management Systems to Control Common Ragweed (*Ambrosia artemisiifolia* L.) in Soybean

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As resistance to herbicides limits growers' weed management options, integrated weed management (IWM) systems that combine non-chemical tactics with herbicides are becoming critical. A 2 year integrated weed management (IWM) study was conducted at three locations in VA, USA. The factorial study evaluated: (1) soybean planting date (early or late planted) (2) with or without winter cover (cereal rye/wheat or no cover), and (3) with or without harvest weed seed control (HWSC). Prior to soybean planting in the first year, winter cover resulted in a 22% reduction in common ragweed density compared to no cover. At soybean harvest in the first year, the lowest common ragweed densities were in the late planted plots following winter wheat, and common ragweed aboveground biomass was reduced by 46 and 22% at two locations in late planted compared to early planted soybean. To evaluate the impact of the first year's treatments and HWSC, full season soybeans were planted across the trial in the second year. Prior to soybean planting in the second year, late planting in the first year common ragweed density was reduced by 83% at one location, but significant reductions were not observed elsewhere. When comparing winter cover to no cover, common ragweed densities were reduced by 31 and 49% at two locations and densities were similar at the third location. Harvest weed seed control reduced common ragweed density by 43% at one location compared to the conventional harvest plots but no significant reductions were observed at the other locations or at other rating timings. However, there was a significant location by planting date by winter cover interaction and the overall lowest common ragweed densities (4.1 to 10.3 plants m^{-2}) were in the late planted plots with winter cover. This research indicated that winter cover, late planting, and HWSC can reduce common ragweed populations with late planting being the most influential. Therefore, double-cropping soybean after wheat is likely the most viable means to better control common ragweed using IWM as it combines both winter cover and late planting date.

Keywords: cover crops, harvest weed seed control, planting date, weed density, seed retention

INTRODUCTION

As herbicide resistance in weed species continues to develop (Heap, 2020) there is a need to develop new integrated weed management (IWM) strategies for weed control systems that rely a multi-tactic approach to control weeds (Swanton and Weise, 1991; Thill et al., 1991). A multi-tactic approach is crucial, as weeds can quickly adapt to high selection pressures from a single tactic approach (Thill et al., 1991; Norsworthy et al., 2012). To combat the growing problem of herbicide resistance in the USA, farmers need to rapidly adopt IWM strategies (Redlick et al., 2017). Weed control tactics that can be components of an IWM system include using cover crops, tillage, cultural practices, harvest weed seed control (HWSC), and herbicide programs, among others (Swanton and Weise, 1991).

Common ragweed is a major problem in the Mid-Atlantic soybean production region (Scruggs et al., 2019) due to resistance to four different sites of action (SOA) including groups 2, 5, 9, and 14 (Heap, 2020). There are also biotypes reported to be multiple resistant to groups 2 and 9, 2, and 14, and 2, 9, and 14 in several states around the USA (Heap, 2020). Coupled with the fact that common ragweed presents significant allergenic risk, common ragweed is listed as the ninth most common and troublesome weed in all broadleaf crops, and the number seventh most troublesome weed in soybeans by the Weed Science Society of America (WSSA) (Van Wychen, 2016). A common ragweed density as low as 4 plants 10 m^{-1} row reduced soybean yield up to 132 kg ha^{-1} and when left uncontrolled all-season, soybean yield can be reduced up to 62%, with densities of up to 160 plants m^{-2} (Coble et al., 1981). An IWM approach is needed to limit the potential impact common ragweed can have on soybean production.

Weed scientists in the USA are adapting HWSC strategies developed in Australia as part of an IWM approach (Norsworthy et al., 2016; Beam et al., 2019; Shergill et al., 2020). HWSC removes or kills seed that are retained on the mother plant with harvest operations (Walsh et al., 2013). There are several HWSC systems including narrow windrow burning, direct bale, chaff removal, chaff lining, and seed impact mills (i.e., the integrated Harrington Seed Destructor and Seed Terminator) (Walsh et al., 2013). All of these systems are being used commercially in Australia (Walsh et al., 2017a) where they are similarly effective on rigid ryegrass (*Lolium rigidum* Gaud.) (Walsh et al., 2017b), and some are being used on an experimental basis by early adopters in the USA (Schwartz-Lazaro et al., 2017; Tidemann et al., 2017; Walsh et al., 2018; Soni et al., 2019; Shergill et al., 2020). If weed seed has already shattered and is on the ground, HWSC is not effective (Walsh and Powles, 2014). Common ragweed seed retention was generally 80% or more at soybean harvest in the mid-Atlantic region (Schwartz-Lazaro et al., 2020). Beam et al. (2019) reported 22 to 26% reductions in common ragweed density in the growing season following HWSC. Both reports indicate potential for HWSC to manage common ragweed.

Agronomic cultural practices can also be deployed to further impact summer annual weeds like common ragweed including crop rotation, planting date, and row spacing. To give crops a competitive advantage over weeds, well-adapted genetics and agronomic practices need to be used. Delaying crop planting can result in lower weed densities due to asynchrony with weed emergence periodicity. Common ragweed emerges earlier than most summer annual weeds in the mid-Atlantic and has a shorter emergence periodicity. The majority of common ragweed emergence (>90%) occurs by mid-May in many regions of the USA (Myers et al., 2014; Werle et al., 2014; Barnes et al., 2017), prior to when double crop soybean is typically planted. Due to this early germination window, double-cropping soybean after wheat harvest is a tool that may be utilized to manage common ragweed in the mid-Atlantic region. Reducing soybean row width from 72 to 38 cm also increases crop competitiveness against weeds (Norsworthy et al., 2012) and can even lower weed fecundity (Chandler et al., 2001).

Cover crops are another tactic as part of an IWM program that can impact weeds. Cover crops suppress weeds physically (light, temperature, and impedance), nutritively (nitrogen immobilization) and chemically via allelochemicals (Moore et al., 1984; Barnes and Putnam, 1987; Teasdale and Mohler, 1993, 2000; Teasdale et al., 2012; Mirsky et al., 2013; Wells et al., 2017; Williams et al., 2018). Cereal rye has been shown to consistently produce the most biomass of fall-planted grass cover crops (Finney et al., 2009; Mirsky et al., 2013). While there is compelling evidence in the literature that cover crops can suppress weeds, suppression of summer annual weeds is variable driven by cover crop biomass levels, weed species, and management (Teasdale and Mirsky, 2015; Wallace et al., 2018).

There is also only limited research on the interaction between cover crops and other weed control tactics (Teasdale et al., 2005; Ryan et al., 2011a,b; Nord et al., 2012; Norsworthy et al., 2016; Hay et al., 2019), specifically cover crops with HWSC, despite numerous calls for such research (Swanton and Weise, 1991; Thill et al., 1991; Swanton et al., 2008; Harker and O'Donovan, 2013). It is likely that integration of management techniques that target various common ragweed life stages will provide better control than tactics used individually. The objective of this research was to evaluate integrated common ragweed management strategies in soybean including planting time, use of a cover crop, and HWSC.

MATERIALS AND METHODS

Site Description

A 2 year study to evaluate integrated approaches to common ragweed management in soybean was conducted at three locations in Virginia, USA. Locations included Kentland Farm in Blacksburg initiated in 2016, a grower's field in Lawrenceville initiated in 2016, and the Southern Piedmont Agricultural Research and Extension Center in Blackstone initiated in 2017. Soil types were a Ross loam (Fine-loamy, mixed, superactive, mesic Cumulic Hapludolls) with a pH of 6.6 and 3.4% organic matter. At Blacksburg (37°11'40.2 "N 80°34'16.6" W; 510 m), an Emporia sandy loam (Fine-loamy, siliceous, subactive, thermic Typic Haludults) with a pH of 5.48 and 0.9% organic matter at Lawrenceville (36°39'01.2 "N 77°49'34.2" W; 101 m), and an Appling sandy loam (Fine, kaolinitic, thermic, Typic

Kanhapludults) with a pH of 6.42 and 3% organic matter at Blackstone (37°04’59.6 “N 77°58’19.5” W; 125 m). Blacksburg and Blackstone sites did not have a naturalized common ragweed population, so these sites were over seeded with common ragweed in late fall prior to the initiation of the experiment. These seeds were harvested from the Lawrenceville location in an area outside but adjacent to the study. This population of common ragweed was approximately 30% glyphosate resistant, based on glyphosate response in replicated research trials adjacent to this study. Common ragweed seed were spread using a rotary spreader at ∼11.6 million seed ha^{-1} on November 11, 2016 and November 15, 2017 at Blacksburg and Blackstone, respectively. This rate of common ragweed seed was used to ensure a dense uniform stand and account for potential low germination of ripe dormant seed (Willemsen, 1975; Baskin and Baskin, 1977).

Experiments were a factorial design with 3 factors, each with 2 levels and 5 replications and arranged as a randomized complete block. Factors included (1) soybean planting date, (2) ± winter cover, and (3) ± HWSC. Soybean planting dates were in mid- to late May, to represent early planted soybean or early July to represent late planted soybean (**Table 1**). Winter cover was either cereal rye, planted in the fall prior to early planted soybean, or wheat planted in the fall and harvested prior to late planted soybean. Plots without winter cover were left fallow over the winter. HWSC was implemented at the end of the soybean growing season as described by Beam et al. (2019). All crop residues and weed seeds contained therein were removed from the plot. Using similar methods, Matthews et al. (1996) found that of the rigid ryegrass seeds that enter the combine between 75 and 85% were collected in the chaff cart and up to 94% of wild mustard (*Sinapis arvensis* L.) seeds were captured. Plots without HWSC had crop residues evenly distributed back across the plot as with a standard harvest operation. Fertility, herbicide programs, planting dates, row spacing, crop varieties, and other practices were selected to mimic standard production practices for the region and are described below and in **Table 1**. While these differences between treatments certainly impacted results, it makes the results directly applicable to farmers by putting results into the management context of production agriculture. All herbicide applications were made using a 6-nozzle boom with 45.7 cm nozzle spacing equipped with XR11002 nozzles calibrated to apply 140 L ha^{-1} of spray solution. All plots measured 4.57 by 7.62 m.

First Year of the Study

Early Planted Soybean

Cereal rye, variety not stated (Southern States Cooperative, Richmond VA, USA), was drilled on 16.5 cm spacing at 134 kg ha^{-1}. Cereal rye planting date, along with other termination, planting, and harvesting dates are located in **Table 1**. Cereal rye was terminated 2 wk before soybean planting using a roller crimper and glyphosate (Roundup Powermax, Monsanto Co, St. Louis, MO, USA) at 1,126 g ae ha^{-1} plus 2,4-D (Shredder Amine 4, WinField Solutions LLC, St. Paul, MN, USA) at 532 g ae ha^{-1} plus flumioxazin (Valor SX, Valent USA Corp., Walnut Creek, CA, USA) at 89.25 g ai ha^{-1}. Early planted soybean plots that had no winter cover received the same herbicide application as the plots with cereal rye. Soybeans were planted into the early planted plots in rows on 76 cm centers, at 407,550 seed ha^{-1} (AG48X7 in 2017 and AG56X8 in 2018, Monsanto Co., St. Louis MO, USA) with 6 rows per plot. At planting, glufosinate (Liberty 280 SL, Bayer CropScience LP, Research Triangle Park, NC, USA) was applied at 59.38 g ai ha^{-1} plus ammonium sulfate at 1.68 kg ha^{-1} (Spray Grade Ammonium Sulfate, DSM Chemicals North America, Inc., Augusta, GA, USA) and crop oil concentrate (Crop Oil Concentrate, Southern States Cooperative, Richmond, VA, USA) at 1% v v^{-1}. Early planted plots both with and without winter cover residue were fertilized at soybean planting with

TABLE 1 | Dates for cover crop and soybean planting, cover crop termination, POST herbicide application, and soybean harvest for all locations and years of the experiment.

Field operation	Blacksburg		Lawrenceville		Blackstone	
	2016–2017	2018	2016–2017	2018	2017–2018	2019
Cereal rye planted	October 10	–	November 8	–	November 16	–
Winter wheat planted	October 19	–	November 8	–	November 16	–
Winter wheat nitrogen application	March 13	–	March 8	–	February 28	–
Winter wheat POST herbicide application	February 20	–	March 8	–	February 28	–
Cereal rye terminated/early planted burndown and residual herbicide application	May 3	–	May 9	–	May 9	–
Early soybean planting[a]	May 18	May 28	May 26	May 22	May 23	May 22
Wheat harvested	June 27	–	June 22	–	June 18	–
Late planting burndown and residual herbicide application	June 27	–	June 22	–	June 18	–
Late soybean planting[a]	July 6	–	June 28	–	July 3	–
Early planted POST herbicide application	June 29	July 9	July 7	July 3	July 3	June 19
Late planted POST herbicide application	August 18	–	August 6	–	August 13	–
Soybean harvest	November 14	October 29	November 7	October 25	October 24	

[a] *Additional burndown applied at planting.*

56 kg ha^{-1} of P_2O_5 and 56 kg ha^{-1} of K_2O. When common ragweed average height reached 30 cm tall in the no cover plots, a POST application of glyphosate plus fomesafen (Flexstar GT 3.5, Syngenta Crop Protection LLC, Greensboro, NC, USA) at 1,107 g ae plus 274 g ai ha^{-1} plus non-ionic surfactant (Scanner, Loveland Products, Greeley, CO, USA) at 0.25% v v^{-1} was made. The POST herbicide application timing was late by design, ensuring that not all common ragweed plants were controlled by the herbicide program and allowing all treatment effects to be measured, but still realistically similar to what often occurs in grower fields. Early planted soybean harvest occurred and HWSC treatments implemented in the fall of the year. Yield, however, was not measured due to poor soybean stand from drought and deer herbivory at all locations.

Late Planted Soybean

Winter wheat was drilled (SS8340 in 2017, Southern States Cooperative, Richmond, VA, USA and Hilliard in 2018, Featherstone Seed, Amelia, VA, USA) at 134 kg ha^{-1}, on 16.5 cm spacing (**Table 1**). Plots with a wheat cover crop had 56 kg ha^{-1} of N and thifensulfuron (Harmony SG, Corteva, Indianapolis, IN, USA) applied at 26.25 g ai ha^{-1} plus non-ionic surfactant at 0.25% v v^{-1} in late winter. Wheat was harvested in June of each year (**Table 1**). Late planted plots that had a wheat cover or that had been left fallow had glufosinate applied at 65.52 g ai ha^{-1} plus flumioxazin at 89.25 g ai ha^{-1} plus crop oil concentrate at 1% v v^{-1} immediately after wheat harvest. Late planted soybean (AG48X7 in 2017 and AG56X8 in 2018) were drilled at 494,000 seed ha^{-1} in rows on 33 cm centers with 15 rows per plot. Glufosinate was applied again at 65.52 g ai ha^{-1} plus crop oil concentrate at 1% v v^{-1} following drilling soybean. Late planted plots, both with and without winter wheat, were fertilized at soybean planting with 56 kg ha^{-1} of P_2O_5 and 56 kg ha^{-1} of K_2O. A postemergence application of glyphosate plus fomesafen plus non-ionic surfactant at 0.25% v v^{-1} was made when common ragweed average height was 30 cm tall in the no cover plots, for reasons previously described.

Second Year of the Study

The second year of the study was used to evaluate the effect of different IWM tactics (soybean planting time, winter cover, and HWSC) on common ragweed populations in the following growing season. Following soybean harvest in the first year of the study, the site was left fallow over the winter. In year 2, the entire study at each site was planted full season soybean (early planted) using the same herbicide program, fertility, planting rate, and row spacing as previously described, with the exceptions of soybean variety (AG56X8 and AG41X8 in 2018 and 2019, respectively).

Site Specific Management

Blacksburg contained large crabgrass (*Digitaria sanguinalis* (L.) Scop), giant foxtail (*Setaria faberi* Herrm.), and johnsongrass (*Sorghum halepense* (L.) Pers.) that was controlled with sethoxydim (Poast, BASF Corp., Research Triangle Park, NC, USA) at 315 g ai ha^{-1} plus crop oil concentrate at 1% v v^{-1} on June 17, 2017 in the full season soybean plots and August 16, 2017 in the late planted soybean plots. The Lawrenceville site was previously in tobacco and the soil pH was low, slowing the growth of both the cereal rye and winter wheat. To help correct this problem and get sufficient biomass for weed control, the field was fertilized with 50.4 kg of N, 16.8 kg of P_2O_5, and 67.2 kg of K_2O ha^{-1} plus 560 kg ha^{-1} of lime (as per soil test recommendation) in mid-February 2017. The Blackstone site contained large crabgrass that was controlled with sethoxydim at 315 g ai ha^{-1} plus crop oil concentrate at 1% v v^{-1} on June 12, 2018.

Data Collection and Analyses

Common ragweed density measurements were conducted at preplant herbicide application, at POST herbicide application and harvest in two random 0.25 m^2 quadrats per plot. Height data were collected by measuring 10 random common ragweed plants per plot at cereal rye termination or wheat harvest for both the winter cover and no cover plots for each planting timing and again just prior to the POST herbicide application. At soybean harvest, common ragweed density measurements were taken in two random 0.25 m^2 quadrats per plot and four representative common ragweed plants were hand harvested and air dried. The samples were weighed and then threshed to determine total seed remaining on the plant at the time of soybean harvest. Using the density at soybean harvest and the average number of seeds per plant the total number of seeds that could be impacted by HWSC was calculated. Data collected in the second year of the experiment included common ragweed density and height, as described for year 1.

All data were analyzed in JMP Pro 14 (SAS Institute Inc., Cary, NC, USA) with a model that included main effects of location, planting date, winter cover, HWSC, block, and interactions with all main effects, excluding block. All model effects were considered to be fixed effects. The models were reduced using stepwise model selection to remove non-significant interactions. Main model terms were never removed. Means were separated using Fisher's Protected LSD ($P = 0.05$).

RESULTS AND DISCUSSION

First Year of the Study

Common Ragweed Density

Cereal rye biomass, assessed just prior to termination, was 5,940, 2,205, and 1,508 kg ha^{-1} at Blacksburg, Lawrenceville, and Blackstone, respectively, due to difference in inherent soil fertility and growing conditions. These biomass levels are below the 8,000 kg ha^{-1} threshold that has been reported for summer annual weed suppression (Teasdale and Mohler, 1993). At wheat harvest, most of the wheat residue was removed from the plots with the harvest operation. The remaining wheat residue was ∼15 to 20 cm in height.

For initial common ragweed density, there was a significant location by planting time interaction (**Table 2**). Common ragweed density at Blacksburg in the late planted timing was 0 plants m^{-2} compared to all other locations and planting timings, which had similar common ragweed densities of 92.4 to 116.8 plants m^{-2} (data not shown). Winter cover as a main effect alone was significant for common ragweed density prior to soybean

TABLE 2 | Effects table for common ragweed density across all locations for years 1 and 2 of field experiments at Blacksburg and Lawrenceville, VA, USA in 2017–2018 and Blackstone, VA, USA in 2018–2019.

Model effects[a]	**Year 1**			**Year 2**		
	At soybean planting	**At POST**	**At harvest**	**At soybean planting**	**At POST**	**At harvest**
	———————————	————————	——————— ***P*****-values** ———	———————	————————	———
Block	0.321	**0.032**	**0.002**	0.798	**<0.001**	0.446
Location	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.809	**<0.001**
Planting time	**0.003**	**<0.001**	0.797	**<0.001**	**<0.001**	**0.005**
Cover	**0.031**	**<0.001**	**<0.001**	**<0.001**	**0.028**	0.601
HWSC	–	–	–	**<0.001**	**0.070**	0.424
Location by planting time	**<0.001**	**0.013**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Location by cover	.	**<0.001**	**<0.001**	**<0.001**	0.106	.
Location by HWSC	–	–	–	**<0.001**	.	.
Planting time by cover	.	0.705	**<0.001**	.	**0.016**	.
Planting time by HWSC	–	–	–	.	.	.
Cover by HWSC	–	–	–	.	.	.
Location by planting time by cover	.	**<0.001**	**0.001**	.	**0.006**	.
Location by planting time by HWSC	–	–	–	.	.	.
Location by cover by HWSC	–	–	–	.	.	.
Planting time by cover by HWSC	–	–	–	.	.	
Location by planting time by cover by HWSC	–	–	–	.	.	.
Global ANOVA	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

[a] *-, effect not included in the model;., effect removed from the model using stepwise selection. Bold values denotes significant $p < 0.05$.*

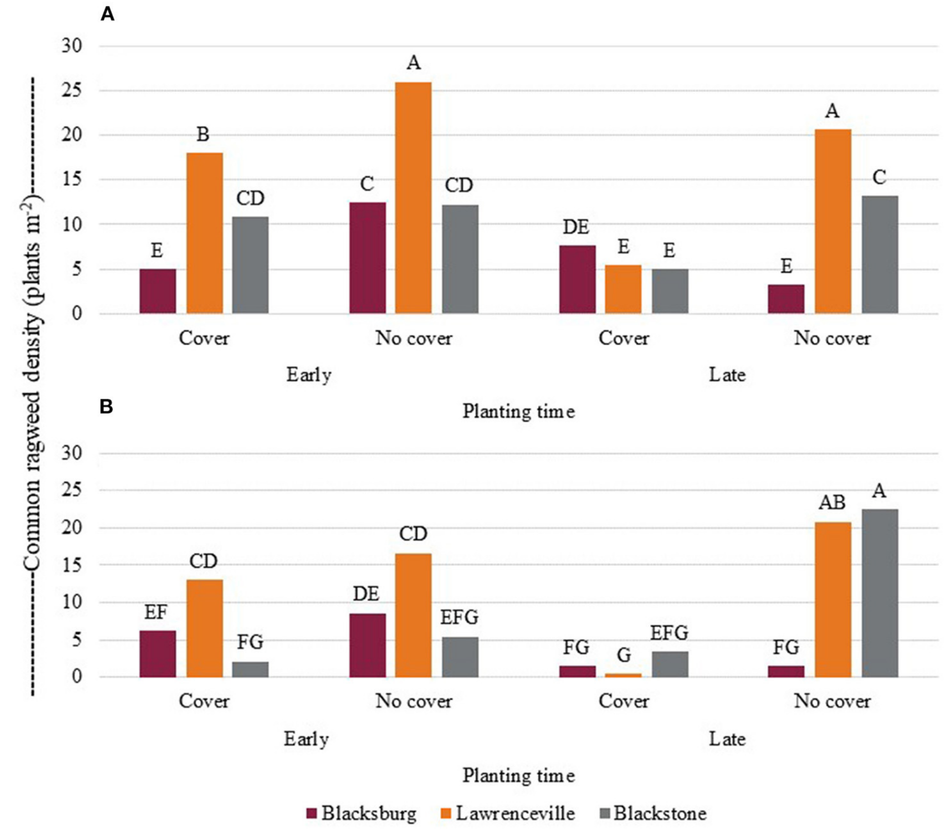

FIGURE 1 | Common ragweed density **(A)** at POST herbicide application (6 wk after planting) and **(B)** at soybean harvest by location, planting time, and ± winter cover in the first year of the field experiment. Means are considered statistically different when they do not share a letter according to Fisher's Protected LSD $_{(P=0.05)}$.

planting. In the plots that had a winter cover, either cereal rye or winter wheat, common ragweed density was lower at 77.2 plants m^{-2} compared to the no cover plots, which had a density of 99 plants m^{-2} (data not shown).

At POST herbicide application (6 WAP), there was a significant location by planting time by winter cover interaction for common ragweed density (**Table 2**). Overall, the Lawrenceville location had greater common ragweed densities than either Blacksburg or Blackstone. Both the Blacksburg and Blackstone locations had similar common ragweed densities for each treatment. Across all three locations, the late planted plots with winter cover had the least common ragweed with 7.6, 5.4, and 5 plants m^{-2} at Blacksburg, Lawrenceville, and Blackstone, respectively (**Figure 1A**). The greatest common ragweed densities were both at the Lawrenceville location in both treatments without winter cover with 26 and 20.6 plants m^{-2} (**Figure 1A**) in the early planted without cover and the late planted without cover treatments, respectively. In most instances, plots with winter cover had lower common ragweed densities compared to the no cover plots for both soybean planting times.

At harvest, a significant location by planting time by winter cover interaction was observed for common ragweed density (**Table 2**). Common ragweed densities at harvest were again overall greater at the Lawrenceville location and densities were similar at both the Blacksburg and Blackstone locations. The treatments with the greatest overall density were the late planted no cover treatments with 20.8 and 22.5 plants m^{-2} (**Figure 1B**) at Lawrenceville and Blackstone, respectively. Comparing the winter cover and no cover treatments within the late planting timing, there was a 98% reduction in common ragweed densities at Lawrenceville and 85% at Blackstone. At the Blacksburg location, regardless of winter cover, the common ragweed densities in the late planted treatments were the same at 1.5 plants m^{-2} (**Figure 1B**). Common ragweed density at soybean harvest is mostly the result of emergence after postemergence herbicide application. The Blacksburg site was the northernmost and highest elevation site, likely providing from better germination conditions for common ragweed compared to other sites.

It has been reported that common ragweed has a short germination window in the spring compared to many summer annual weeds. Barnes et al. (2017) reported that 90% of common ragweed emergence occurs around the first to middle of May in Nebraska. In Pennsylvania, Delaware, and New Jersey, it has been reported that 95% of common ragweed emergence for the growing season occurs around mid-April to the first of May (Myers et al., 2004). Werle et al. (2014) reported that 90% of cumulative common ragweed emergence occurs around mid-May in Iowa. Common ragweed emergence patterns are similar across a wide area of the United States. These dates of common ragweed emergence are similar to the current study, which saw little common ragweed emergence occurring after preplant herbicide application in the treatments that were late planted either behind wheat (what is known as a double-crop soybean)

TABLE 3 | Effects table for initial common ragweed height and at POST herbicide application across all locations for year 1 of the field experiment at Blacksburg and Lawrenceville, VA, USA in 2017 and Blackstone, VA, USA in 2018.

Model effects[a]	**Year 1**		**Year 2**	
	Initial	**At POST**	**Initial**	**At POST**
	Late planted			
	P-values			
Block	**<0.001**	0.801	0.323	**<0.001**
Location	**<0.001**	**<0.001**	**0.026**	**<0.001**
Planting time	–	**<0.001**	**0.001**	**0.042**
Cover	**<0.001**	**<0.001**	**<0.001**	0.791
HWSC	–	**–**	0.910	**<0.001**
Location by planting time	–	**<0.001**	**<0.001**	**<0.001**
Location by cover	**<0.001**	**<0.001**	**<0.001**	.
Location by HWSC	–	–	.	**0.018**
Planting time by cover	–	**<0.001**	**<0.001**	.
Planting time by HWSC	–	–	.	0.193
Cover by HWSC	–	–	**0.002**	**0.014**
Location by planting time by cover	–	**<0.001**	**<0.001**	.
Location by planting time by HWSC	–	–	.	**0.004**
Location by cover by HWSC	–	–	.	.
Planting time by cover by HWSC	–	–	.	.
Location by planting time by cover by HWSC	–	–	.	.
Global ANOVA	**<0.001**	**<0.001**	**<0.001**	**<0.001**

[a] *-, effect not included in the model;., effect removed from the model using stepwise selection. Bold values denotes significant $p < 0.05$.*

or had been left fallow until late soybean planting timing and planted in late June to early July. Amuri et al. (2010) reported that overall weed densities in double-crop soybean following winter wheat were lower when residues were left on the soil surface instead of burning. This trend of lower weed densities in late planted with soybean with winter cover is similar to what was observed in the current study.

Common Ragweed Height

Initial common ragweed height was measured prior to wheat harvest in the late planted soybean treatments. In the late planted treatments, a significant location by winter cover interaction was observed (**Table 3**). At all locations, common ragweed height was reduced in the late planted treatments with winter cover (60, 50, and 29% at Blacksburg, Lawrenceville, and Blackstone, respectively) compared to the no cover treatments (**Figure 2A**). The wheat growing in competition with common ragweed resulted in shorter plants at the time of wheat harvest than where left fallow.

Common ragweed heights at POST herbicide applications showed a significant location by planting time by winter cover interaction (**Table 3**), similar to the density data. Common ragweed height in early planted treatments was similar regardless of whether there was winter cover or not at the Lawrenceville and Blackstone locations, with heights ranging from 31.7 to 38.0 cm (**Figure 2B**). At the Blacksburg location, the common ragweed plants in the early planted, winter cover treatments were shorter than the common ragweed plants in the early planted, no cover

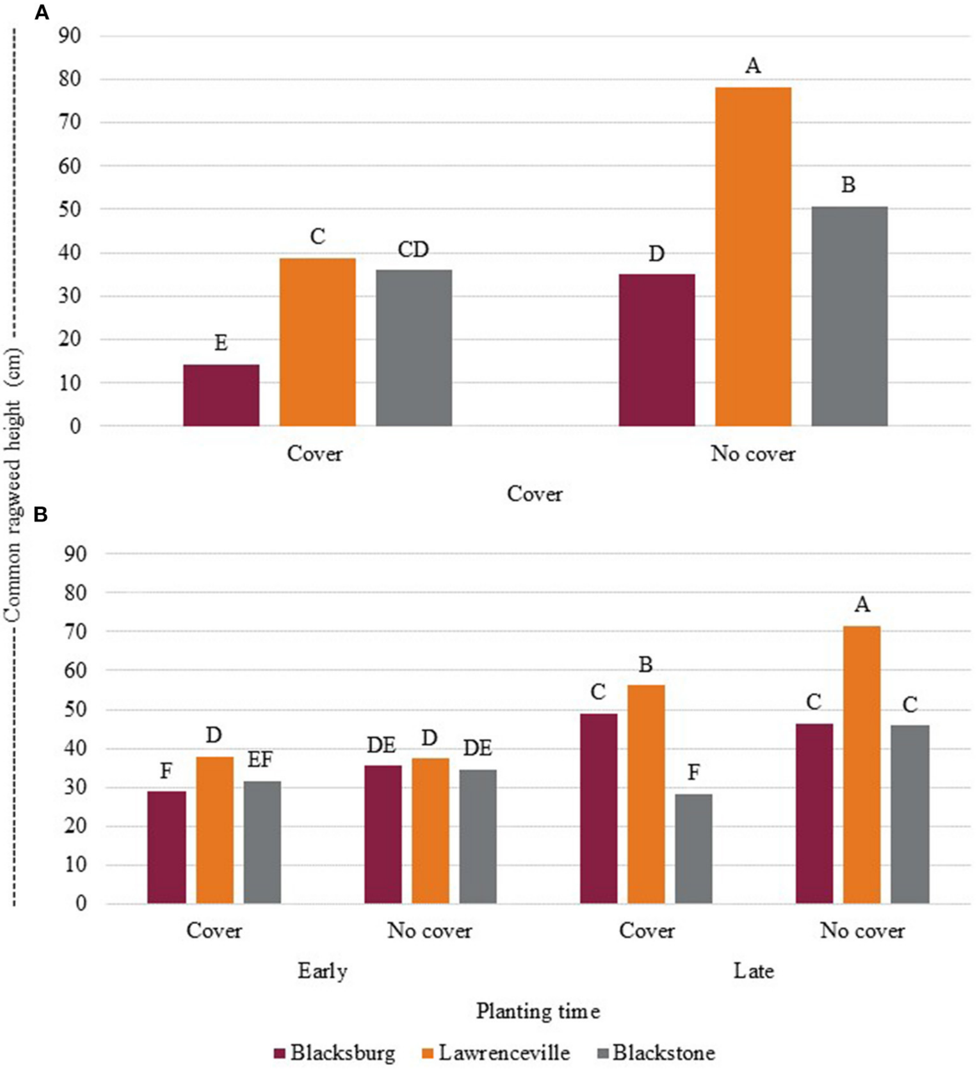

FIGURE 2 | Common ragweed height **(A)** at late planting across all locations in year 1 of the field experiment and **(B)** at POST herbicide application (6 wk after planting) by location, planting time, and ± winter cover (cereal rye for early planting and winter wheat for late planting) in year 1 of the field experiment. Means are considered statistically different when they do not share a letter according to Fisher's Protected LSD $_{(P=0.05)}$.

treatments with heights of 28.9 and 35.5 cm, respectively, a 19% reduction. The difference in location was likely the result of greater cereal rye biomass in Blacksburg location compared to the other locations. In most instances, common ragweed was shorter in the early planted treatments when compared to late planted treatments regardless of winter cover. At the Blacksburg location, there was no difference in common ragweed height in either late planted treatment. At Lawrenceville, common ragweed was 21% shorter in the late planted, winter cover treatment (56.3 cm) compared to the late planted, no cover treatment (71.6 cm). At Blackstone, a similar trend was seen with plants in the late planted, winter cover treatment (28.4 cm) being shorter than the late planted no cover treatment (45.9 cm), by 38% (**Figure 2B**). Competition from a cover crop has been shown to reduce the height of Palmer amaranth. Hay et al. (2019) reported a 26 to 40% reduction in Palmer amaranth height when grown in competition with winter wheat compared to no cover crop. This finding is similar to the current study where a 19 to 38% reduction in common ragweed height was observed with winter cover compared to no cover crop.

Common Ragweed Biomass and Seed Retention at Harvest

Common ragweed aboveground biomass and seed retention data were collected just prior to soybean harvest. A significant location by planting time interaction was observed with common ragweed aboveground biomass (**Table 4**). At the Blacksburg location, the common ragweed biomass was less in the late planted treatments (22.6 g plant^{-1}) compared to the early planted treatments (42.3 g plant^{-1}), a 46% reduction (**Figure 3A**). At the Lawrenceville and Blackstone locations, biomasses were similar across both soybean planting timings with biomasses ranging from 26.6 to 35.4 g plant^{-1}.

A significant location by planting time by winter cover interaction was observed for common ragweed seed retention at soybean harvest (**Table 4**). Common ragweed seed retention was variable across the three locations, ranging from 836 to 3,611 seed plant^{-1}. Although not always significantly different from all treatments, the numerically greatest seed retention was in the Lawrenceville early planted no cover treatment and the Blackstone late planted no cover treatment with 3,609 and 3,611 seed plant^{-1}, respectively (**Figure 3B**). Common ragweed that emerges later in the growing season and grown in competition with a soybean crop are smaller and produce less aboveground biomass and seed (Dickerson and Sweet, 1971; Simard and Benoit, 2012). Simard and Benoit (2012) reported that common ragweed produced 3,694 seed plant^{-1} when grown in competition with soybean. This finding is similar to what was observed in the current study, however, in the current study only seed retained at harvest were recorded.

Second Year of the Study

Common Ragweed Density

Common ragweed density in the second year of the experiment was collected prior to preplant herbicide application in the spring and again at POST herbicide application when common ragweed reached 30 cm in height. There were multiple significant interactions observed for common ragweed density at the

TABLE 4 | Effects table for common ragweed biomass and seed retention at soybean harvest across all locations for year 1 of the field experiment at Blacksburg and Lawrenceville, VA, USA in 2017 and Blackstone, VA, USA in 2018.

Model effects[a]	**Year 1**		**Year 2**	
	Biomass (g plant $^{-1}$)	**Retention (seed plant^{-1})**	**Biomass (g plant^{-1})**	**Retention (seed plant^{-1})**[b]
	————————————————	***P*-values**	————————————————	
Block	**0.035**	**0.037**	0.251	0.380
Location	0.563	0.157	**<0.001**	**<0.001**
Planting time	**0.011**	0.729	0.355	0.720
Cover	**0.002**	0.183	0.808	0.144
HWSC	–	–	0.417	0.908
Location by planting time	**0.009**	0.215	.	**0.021**
Location by cover	–	0.912	.	.
Location by HWSC	–	–	.	.
Planting time by cover	–	0.977	.	.
Planting time by HWSC	–	–	.	.
Cover by HWSC	–	–	.	.
Location by planting time by cover	–	**0.025**	.	.
Location by planting time by HWSC	–	–	.	.
Location by cover by HWSC	–	–	.	.
Planting time by cover by HWSC	–	–	.	.
Location by planting time by cover by HWSC	–	–	.	.
Global ANOVA	**<0.001**	**0.040**	**<0.001**	**<0.001**

[a] *-, effect not included in the model.;., removed from the model using stepwise selection.*

[b] *Only the Blacksburg and Lawrenceville locations were included in this analysis.*

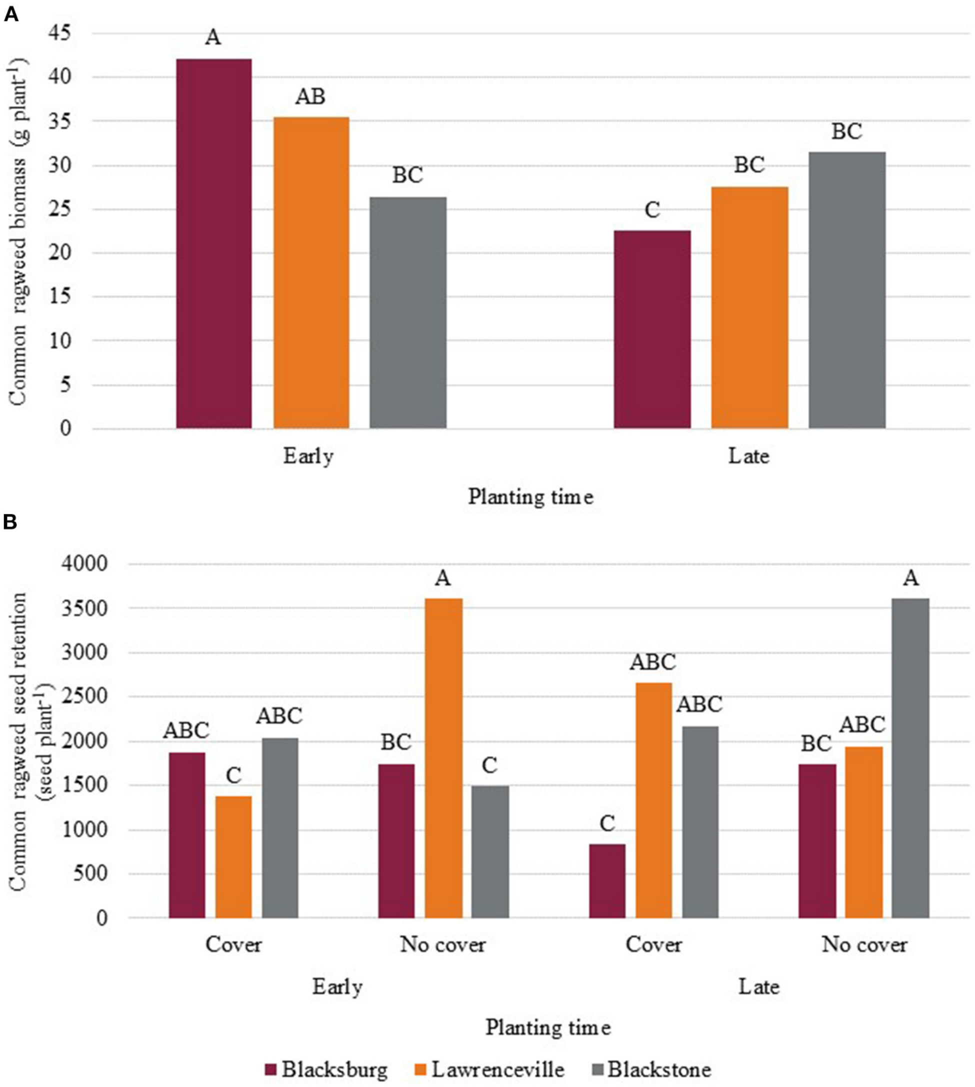

FIGURE 3 | (A) common ragweed biomass at soybean harvest by location and planting time for the first year of the field experiment and **(B)** common ragweed seed retention at soybean harvest by location, planting time, and cover (cereal rye for early planting and winter wheat for late planting) for the first year of the field experiment. Means are considered statistically different when they do not share a letter according to Fisher's Protected LSD $_{(P=0.05)}$.

start of the second year of the experiment. These interactions include location by planting time, location by winter cover, and location by HWSC (**Table 2**). Since all these interactions included location, these interactions are likely influenced by differences among locations observed in the first year of the study.

At the Blacksburg location, common ragweed densities were 83% lower in the late planted treatments from year 1 compared to the early planted treatments (**Figure 4A**). At the Lawrenceville and Blackstone locations, densities were similar between the early and late planted treatments with densities of 83.0 and 81.2 and 52.8 and 46.8 plants m^{-2}, respectively.

When comparing treatments across location based on winter cover, common ragweed densities at the Blacksburg location were similar between the winter cover and no cover treatments the prior year with densities of 41.2 and 44.6 plants m^{-2}, respectively (**Figure 4B**). At the Lawrenceville location, common ragweed density in the winter cover treatments (55.1 plants m^{-2}) were 50% lower than in the no cover treatments (109.1 plants m^{-2}). At the Blackstone location, similar to the Lawrenceville location, the common ragweed density in the winter cover treatments (40.7 plants m^{-2}) were 31% lower than in the no cover treatments (58.9 plants m^{-2}).

The effect of HWSC on common ragweed density was only different at Lawrenceville where HWSC significantly reduced common ragweed density compared to the conventional harvest treatments with densities of 59.7 and 104.5 plants m^{-2}, respectively, a 43% reduction (**Figure 4C**). Prior research on HWSC has demonstrated that it can be variable on a species

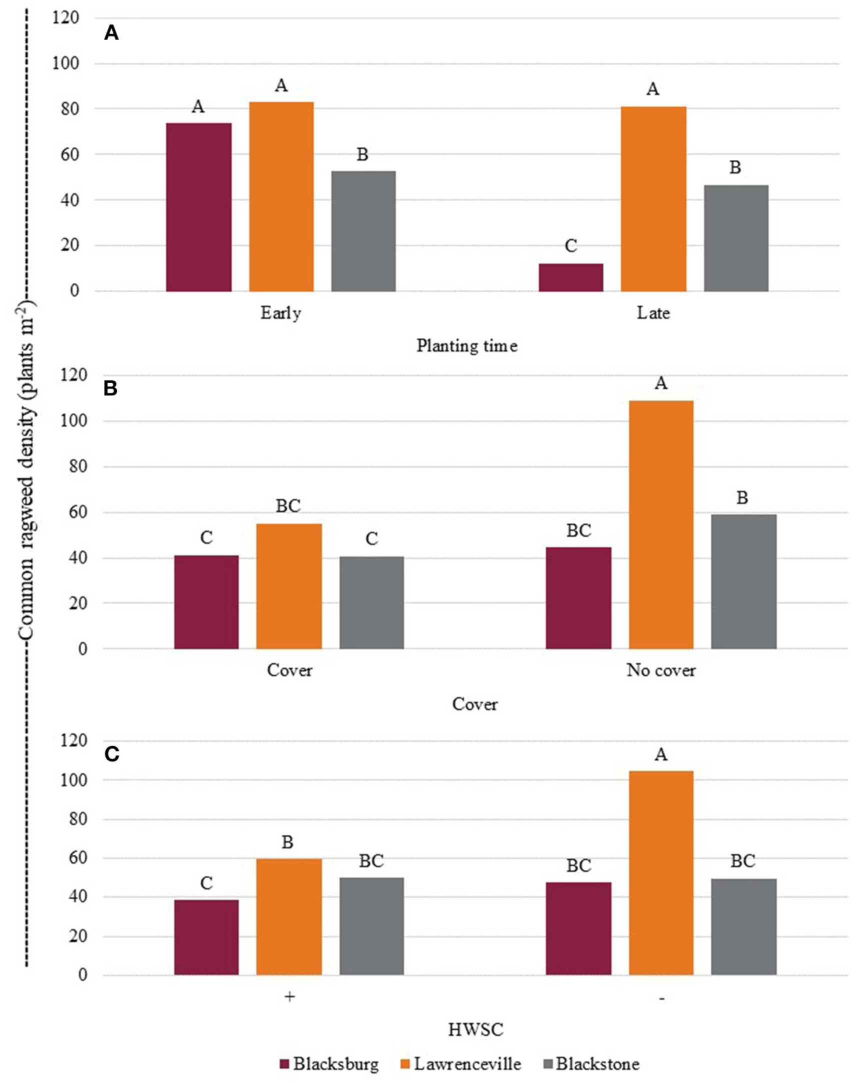

FIGURE 4 | Common ragweed density at preplant herbicide application in year 2 of the experiment **(A)** by location and planting time **(B)** by location and ± winter cover and **(C)** by location and ± harvest weed seed control (HWSC) prior to preplant herbicide application. Means are considered statistically different when they do not share a letter according to Fisher's Protected LSD $_{(P=0.05)}$.

such as common ragweed. Beam et al. (2019) demonstrated that a one-time implementation of HWSC can reduce common ragweed density by 22% in the spring of the following year prior to preplant herbicide application, similar to the Lawrenceville location in the current study. Norsworthy et al. (2016) reported variability in the effect of HWSC at reducing weed density when using field residue removal with Palmer amaranth (*Amaranthus palmeri* S. Wats.). The efficacy of HWSC can be influenced by weed seed retention and the size of soil seedbank in a given field (Walsh et al., 2017b). The Lawrenceville location had a naturalized common ragweed infestation where as other sites had introduced seed at the initiation of the experiment, potentially accounting for this difference.

At POST herbicide application in the second year of the study, a significant location by planting time by winter cover interaction was observed (**Table 2**). This interaction includes two of the three tactics evaluated (planting time and winter cover), indicating that these tactics work better together than separately, but this varied by location. Similar to in the first year of the study, the late planted, winter cover treatments had less common ragweed compared to the other treatments across all locations with 4.7, 6.7, and 10.3 plants m^{-2} at Blacksburg, Lawrenceville, and Blackstone, respectively (**Figure 5A**). While not significantly greater than some treatments, the greatest common ragweed densities were in the Blacksburg early planted, no cover treatment and the Blackstone late planted, no cover treatment with 29.2 and 30.1 plants m^{-2}, respectively. There was no significant effect of HWSC on common ragweed density at POST herbicide application (**Table 2**). The effect of HWSC on weed populations can be variable depending on the quantity of seed shattered

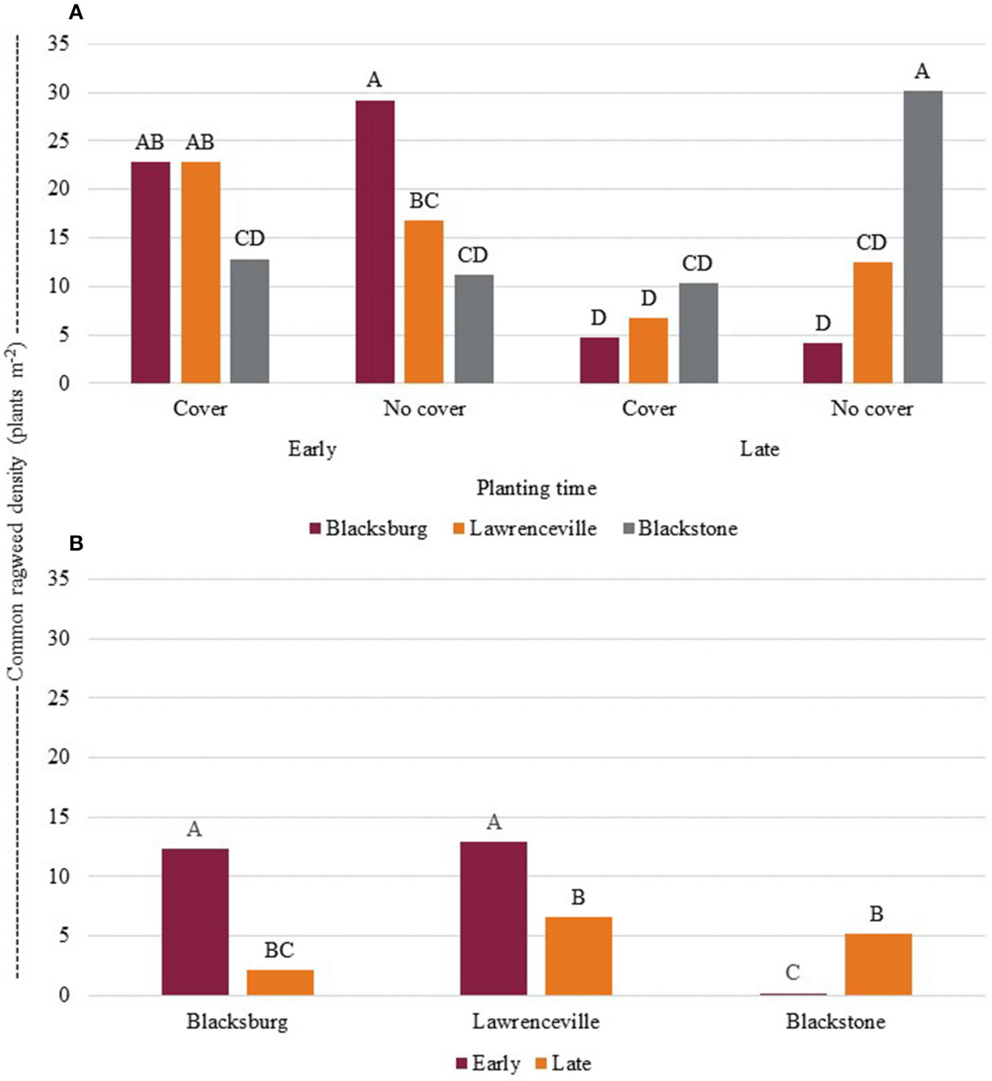

FIGURE 5 | Common ragweed density **(A)** at POST herbicide application by location, planting time, and ± winter cover (cereal rye for early planting and winter wheat for late planting) in year 2 of the experiment. POST herbicide applications were made when common ragweed reached 30 cm in height and **(B)** at soybean harvest by location and planting time in year 2 of the experiment. Means are considered statistically different when they do not share a letter according to Fisher's Protected LSD (P=0.05).

prior to HWSC implementation, efficacy of subsequent herbicide applications, and the size of the soil seedbank (Norsworthy et al., 2016; Tidemann et al., 2016; Walsh et al., 2017b; Beam et al., 2019).

At soybean harvest, in the second year of the study a significant location by planting time interaction was observed (**Table 2**). Similar to the first year of the study the treatments that had been late planted had significantly lower common ragweed densities compared to the treatments that had been planted early in year 1 at Blacksburg and Lawrenceville locations. Common ragweed densities were 2.1 and 6.6 plants m^{-2} in the late planted treatments at Blacksburg and Lawrenceville, respectively, compared to 12.3 and 12.9 plants m^{-2} in the early planted treatments (**Figure 5B**). Conversely at the Blackstone location, common ragweed densities were lower in the early planted treatments compared to the late planted treatments with 0.18 and 5.2 plants m^{-2}. The reason for this difference among locations not clear, but may be due to differences in weather in 2019 (the second year of the study at this site) compared to 2018 for the other locations.

Common Ragweed Height

Common ragweed height, at the beginning of the second year of the study a significant location by planting time by winter cover interaction was observed similar to the first year of the study (**Table 3**). Common ragweed height ranged from 3.1 to 7.8 cm across all locations and treatments (**Figure 6A**). The shortest common ragweed plants were observed in the early planted with

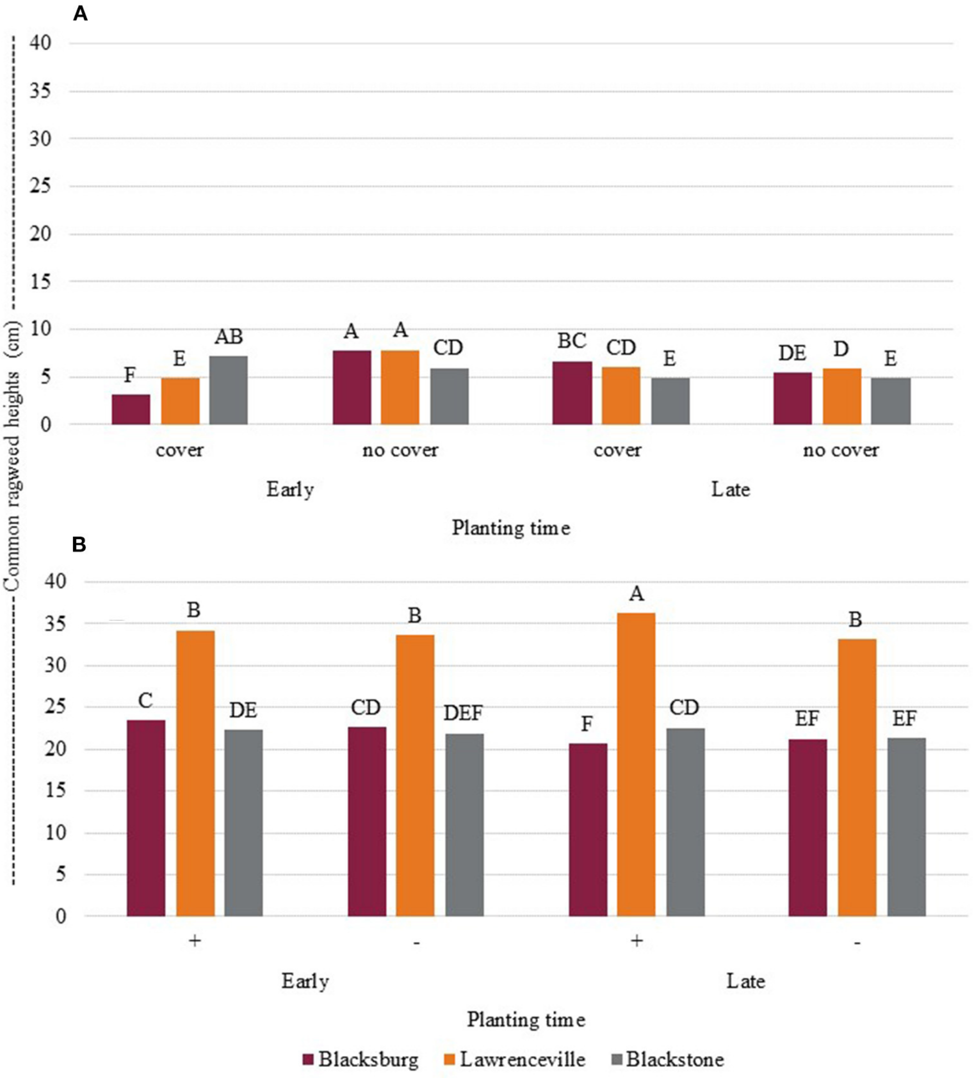

FIGURE 6 | Common ragweed heights **(A)** at preplant herbicide application in year 2 of the experiment by location, planting time, and location and **(B)** at POST application in the second year of the experiment by location, planting time, and HWSC. Means are considered statistically different when they do not share a letter according to Fisher's Protected LSD $_{(P=0.05)}$.

winter cover plots in Blacksburg. The tallest common ragweed plants were observed in the early planted with no winter cover plots. Common ragweed height at POST applications, in the second year of the study a significant location by planting time by HWSC interaction was observed. The Lawrenceville location had common ragweed that was significantly taller (33.1 to 36.3 cm) compared to the other locations (21.2 to 23.5 cm) (**Figure 6B**).

Common Ragweed Biomass and Seed Retention at Harvest

Common ragweed aboveground biomass in the second year of the study was only significant by location (**Table 4**). Common ragweed aboveground biomass was 47.81, 16.95, and 27.13 g plant^{-1} at Blacksburg, Lawrenceville, and Blackstone, respectively. Common ragweed seed retention data for the second year of the study only included the Blackstone and Lawrenceville locations (**Table 4**). At the Blackstone location, common ragweed failed to set seed by soybean harvest due to severe drought conditions. There was a significant location by planting time interaction observed for common ragweed seed retention. At Blacksburg, common ragweed seed retention was 886 and 681 seed plant^{-1} in the early and late planted treatments, respectively. At Lawrenceville, common ragweed seed retention was 375 and 525 seed plant^{-1} in the early and late planted treatments, respectively (data not shown).

Research Implications

Herbicide resistance is an increasing problem and diversifying weed control strategies into an IWM system is a necessity. The current study demonstrates that using multiple methods including planting timing, winter cover, and HWSC can help reduce common ragweed populations in 1 year, but effects varied by location. It appears that the planting timing (late planting or double-cropping soybean after winter wheat compared to early planted) reduces common ragweed populations more than other treatments tested in this study. Therefore, double-cropping soybeans after wheat is a recommended strategy for integrated common ragweed management, where feasible. This system should be used in conjunction with crop rotation and other weed management techniques to keep common ragweed densities at manageable levels. Including a winter cover (wheat or cereal rye) resulted in similar or reduced common ragweed density and reduced common ragweed heights across locations at soybean planting. The effectiveness of HWSC was variable but reduced common ragweed densities at one of three locations. Variability in HWSC has been demonstrated in other research (Norsworthy et al., 2016; Walsh et al., 2017b; Beam et al., 2019) and highlights the need for additional research. The impact of planting timing and HWSC and its effect on soil seedbanks should be evaluated further.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

SB and MF performed the research and analyzed the data. SB, MF, and SM wrote the paper. CC, DCH, and DLH edited the paper. All authors designed the research.

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REFERENCES

Amuri, N., Brye, K. R., Gbur, E. E., Oliver, D., and Kelley, J. (2010). Weed populations as affected by residue management practices in a wheat-soybean double-crop production system. *Weed Sci.* 58, 234–243. doi: 10.1614/WS-09-088.1

Barnes, E. R., Werle, R., Sandell, L. D., Lindquist, J. L., Knezevic, S. Z., Sikkema, P. H., et al. (2017). Influence of tillage on common ragweed (*Ambrosia artemisiifolia*) emergence pattern in Nebraska. *Weed Technol.* 31, 623–631. doi: 10.1017/wet.2017.38

Barnes, J., and Putnam, A. (1987). Role of benzoxazinones in allelopathy by rye (*Secale cereale* L.). *J. Chem. Ecol.* 13, 889–896. doi: 10.1007/BF01020168

Baskin, J. M., and Baskin, C. C. (1977). Role of temperature in the germination ecology of three summer annual weeds. *Oecologia* 30, 377–382. doi: 10.1007/BF00399768

Beam, S. C., Mirsky, S., Cahoon, C., Haak, D., and Flessner, M. (2019). Harvest weed seed control of Italian ryegrass [*Lolium perenne* ssp *multiflorum* (Lam.) Husnot], common ragweed (*Ambrosia artemisiifolia* L.), and Palmer amaranth (*Amaranthus palmeri* S. Watson). *Weed Technol.* 33, 627–632. doi: 10.1017/wet.2019.46

Chandler, K., Shrestha, A., and Swanton, C. J. (2001). Weed seed return as influenced by the critical weed-free period and row spacing of no-till glyphosate-resistant soybean. *Can. J. Plant Sci.* 81, 977–880. doi: 10.4141/P01-049

Coble, H. D., Williams, F. M., and Ritter, R. L. (1981). Common ragweed (*Ambrosia artemisiifolia*) interference in soybeans (*Glycine max*). *Weed Sci.* 29, 339–342. doi: 10.1017/S0043174500062081

Dickerson, C. T., and Sweet, R. D. (1971). Common ragweed ecotypes. *Weed Sci.* 19, 64–66. doi: 10.1017/S0043174500048281

Finney, D. M., Creamer, N. G., Schultheis, J. R., Wagger, M. G., and Brownie, C. (2009). Sorghum sudangrass as a summer cover and hay crop for organic fall cabbage production. *Renew. Agric. Food Syst.* 24, 225–233. doi: 10.1017/S174217050999007X

Harker, K. N., and O'Donovan, J. T. (2013). Recent weed control, weed management, and integrated weed management. *Weed Technol.* 27, 1–11. doi: 10.1614/WT-D-12-00109.1

Hay, M. M., Dille, J. A., and Peterson, D. E. (2019). Integrated pigweed (*Amaranthus* spp.) management in glufosinate-resistant soybean with a cover crop, narrow row widths, row-crop cultivation, and herbicide program. *Weed Technol.* 33, 710–719. doi: 10.1017/wet.2019.62

Heap, I. (2020). *The International Survey of Herbicide Resistant Weeds*. Available online at: www.weedscience.org (accessed December 28, 2020).

Matthews, J. M., Llewellyn, P. S., Reeves, T., and Jaeschke, R. (1996). "Seed catching; a method to control annual weeds," in *8th Australian Agronomy Conference, University of Southern Queensland*. Toowoomba, QLD: Australian Society of Agronomy, 684–685.

Mirsky, S. B., Ryan, M. R., Teasdale, J. R., Curran, W. S., Reberg-Horton, C. S., Spargo, J. T., et al. (2013). Overcoming weed management challenges in cover crop-based organic rotational no-till soybean production in the eastern United States. *Weed Technol.* 27, 193–203. doi: 10.1614/WT-D-12-00078.1

Moore, R. E., Niemczura, W. P., Kwok, O. C. H., and Patil, S. S. (1984). Inhibitors of ornithine carbamoyltransferase from *Pseudomonas syringae* pv. *Phaseaolicola. Tetrahedron Lett.* 25, 3931–3934. doi: 10.1016/0040-4039(84)80033-7

Myers, M. W., Curran, W. S., VanGessel, M. J., Calvin, D. D., Mortensen, D. A., Majek, B. A., et al. (2004). Predicting weed emergence for eight annual weed species in the northeastern United States. *Weed Sci.* 52, 913–919. doi: 10.1614/WS-04-025R

Nord, E. A., Ryan, M. R., Curran, S., Mortensen, D. A., and Mirsky, S. B. (2012). Effects of management type and timing on weed suppression in soybean no-till planted into rolled-crimped cereal rye. *Weed Sci.* 60, 624–633. doi: 10.1614/WS-D-12-00024.1

Norsworthy, J. K., Korres, N. E., Walsh, M. J., and Powles, S. B. (2016). Integrating herbicide programs with harvest weed seed control and other fall management practices for the control of glyphosate-resistant Palmer amaranth

(*Amaranthus palmeri*). *Weed Sci.* 64, 540–550. doi: 10.1614/WS-D-15-00210.1

Norsworthy, J. K., Ward, S. M., Shaw, D. R., Llewellyn, R. S., Nichols, R. L., Webster, T. M., et al. (2012). Reducing the risks of herbicide resistance: best management practices and recommendations. *Weed Sci.* 60, 31–62. doi: 10.1614/WS-D-11-00155.1

Redlick, C., Syrovy, L. D., Duddu, H. S. N., Benaragama, D., Johnson, E. N., Willenburg, C. J., et al. (2017). Developing an integrated weed management system for herbicide-resistant weeds using lentil (*Lens culinaris*) as a model crop. *Weed Sci.* 65, 778–786. doi: 10.1017/wsc.2017.47

Ryan, M. R., Curran, W. S., Grantham, A. M., Hunsberger, L. K., Mirsky, S. B., Mortensen, D. A., et al. (2011a). Effects of seeding rate and poultry litter on weed suppression from a rolled cereal rye cover crop. *Weed Sci.* 59, 438–444. doi: 10.1614/WS-D-10-00180.1

Ryan, M. R., Mirsky, S. B., Mortensen, D. A., Teasdale, J. R., and Curran, W. S. (2011b). Potential synergistic effects of cereal rye biomass and soybean planting density on weed suppression. *Weed Sci.* 59, 238–246. doi: 10.1614/WS-D-10-00110.1

Schwartz-Lazaro, L. M., Norsworthy, J. K., Walsh, M. J., and Bagavathiannan, M. V. (2017). Efficacy of the integrated Harrington seed destructor on weeds of soybean and rice production systems in the southern United States. *Crop Sci.* 57, 2812–2818. doi: 10.2135/cropsci2017.03.0210

Schwartz-Lazaro, L. M., Shergill, L. S., Evans, J. A., Bagavathiannan, M. V., Beam, S. C., Bish, M. D., et al. (2020). Seed shattering phenology at soybean harvest of economically important weeds in multiple regions of the United States. Part 1: broadleaf species. *Weed Sci*. 1–29. doi: 10.1017/wsc.2020.80

Scruggs, E., Beam, S., and Flessner, M. (2019). *Common Ragweed (Ambrosia artemisiifolia) Control in Soybeans*. Blacksburg, VA: Virginia Cooperative Extension SPES143NP.

Shergill, L. S., Bejleri, K., Davis, A., and Mirsky, S. B. (2020). Fate of weed seeds after impact mill processing in midwestersn and mid-Atlantic United States. *Weed Sci.* 68, 92–97. doi: 10.1017/wsc.2019.66

Simard, M. J., and Benoit, D. L. (2012). Potential pollen and seed production from early- and late-emerging common ragweed in corn and soybean. *Weed Technol.* 26, 510–516. doi: 10.1614/WT-D-11-00178.1

Soni, N., Nissen, S. J., Westra, P., and Norsworthy, J. K. (2019). Seed retention of winter annual grass weeds at winter wheat harvest maturity shows potential for harvest weed seed control. *Weed Technol.* 34, 266–271. doi: 10.1017/wet.2019.108

Swanton, C. J., Mahoney, K. J., Chandler, K., and Gulden, R. H. (2008). Integrated weed management knowledge-based weed management systems. *Weed Sci.* 56, 168–172. doi: 10.1614/WS-07-126.1

Swanton, C. J., and Weise, S. F. (1991). Integrated weed management: the rationale and approach. *Weed Technol.* 5, 657–663. doi: 10.1017/S0890037X00027512

Teasdale, J. R., and Mirsky, S. B. (2015). Tillage and planting date effects on weed dormancy, emergence, and early growth in organic cotton. *Weed Sci.* 63, 477–490. doi: 10.1614/WS-D-14-00112.1

Teasdale, J. R., and Mohler, C. L. (1993). Light transmittance, soil-temperature, and soil-moisture under residue of hairy vetch and rye. *Agron. J.* 85, 673–680. doi: 10.2134/agronj1993.00021962008500030029x

Teasdale, J. R., and Mohler, C. L. (2000). The quantitative relationship between weed emergence and the physical properties of mulches. *Weed Sci.* 48, 385–392. doi: 10.1614/0043-1745(2000)048[0385:TQRBWE]2.0.CO;2

Teasdale, J. R., Pillai, P., and Collins, R. T. (2005). Synergism between cover crop residue and herbicide activity on emergence and early growth of weeds. *Weed Sci.* 53, 521–527. doi: 10.1614/WS-04-212R

Teasdale, J. R., Rice, C., Cai, G., and Mangum, R. A. (2012). Expression of allelopathy in the soil environment: soil concentration and activity of benzoxazinoid compounds released by rye cover crop residue. *Plant Ecol.* 213, 1893–1905. doi: 10.1007/s11258-012-0057-x

Thill, D. C., Lish, J. M., Callihan, R. H., and Bechinski, E. J. (1991). Integrated weed management-a component of integrated pest management: a critical review. *Weed Technol.* 5, 648–656. doi: 10.1017/S0890037X00027500

Tidemann, B. D., Hall, L. M., Harker, K. N., and Alexander, B. C. S. (2016). Identifying critical control points in the wild oat (*Avena fatua*) life cycle and the potential effects of harvest weed-seed control. *Weed Sci.* 64, 463–473. doi: 10.1614/WS-D-15-00200.1

Tidemann, B. D., Hall, L. M., Harker, K. N., and Beckie, H. J. (2017). Factors affecting weed seed devitalization with the Harrington seed destructor. *Weed Sci.* 65, 650–658. doi: 10.1017/wsc.2017.23

Van Wychen, L. (2016). *2016 Survey of the Most Common and Troublesome Weeds in Broadleaf Crops, Fruits and Vegetables in the United States and Canada*. Weed Science Society of America National Weed Survey Dataset. Available online at: http://wssa.net/wp-content/uploads/2016-Weed-Survey_Broadleaf-crops.xlsx (accessed August 19, 2019).

Wallace, J. M., Keene, C. L., Curran, W., Mirsky, S. S., Ryan, M. R., and VanGessel, M. J. (2018). Integrated weed management strategies in cover crop-based, organic rotational no-till corn and soybean in the mid-Atlantic region. *Weed Sci.* 66, 94–108. doi: 10.1017/wsc.2017.53

Walsh, M., Newman, P., and Powles, S. (2013). Targeting weed seeds in-crop: a new weed control paradigm for global agriculture. *Weed Technol.* 27, 431–436. doi: 10.1614/WT-D-12-00181.1

Walsh, M., Ouzman, M., Newman, P., Powles, S., and Llewellyn, R. (2017a). High levels of adoption indicate that harvest weed seed control is now an established weed control practice in Australian cropping. *Weed Technol.* 31, 341–347. doi: 10.1017/wet.2017.9

Walsh, M. J., Aves, C., and Powles, S. B. (2017b). Harvest weed seed control systems are similarly effective on rigid ryegrass. *Weed Technol.* 31, 178–183. doi: 10.1017/wet.2017.6

Walsh, M. J., Broster, J. C., Schwaartz-Lazaro, L. M., Norsworthy, J. K., Davis, A. S., Tidemann, B. D., et al. (2018). Opportunities and challenges for harvest weed seed control in global cropping systems. *Pest Mgmt Sci.* 74, 2235–2245. doi: 10.1002/ps.4802

Walsh, M. J., and Powles, S. B. (2014). High seed retention at maturity of annual weeds infesting crop fields highlights the potential for harvest weed seed control. *Weed Technol.* 28, 486–493. doi: 10.1614/WT-D-13-00183.1

Wells, M. S., Reberg-Horton, S. C., Mirsky, S. B., Maul, J. E., and Hu, S. (2017). *In situ* validation of fungal N translocation to cereal rye mulches under no-till soybean production. *Plant Soil* 410, 153–165. doi: 10.1007/s11104-016-2989-8

Werle, R., Sandell, L. D., Buhler, D. D., Hartzler, R. G., and Lindquist, J. L. (2014). Predicting emergence of 23 summer annual weed species. *Weed Sci.* 62, 267–279. doi: 10.1614/WS-D-13-00116.1

Willemsen, R. W. (1975). Effect of stratification temperature and germination temperature on germination and the induction of secondary dormancy in common ragweed seeds. *Am. J. Bot.* 62, 1–5. doi: 10.1002/j.1537-2197.1975.tb12333.x

Williams, A., Wells, M. S., Dickey, D. A., Hu, S., Maul, J., Raskin, D. T., et al. (2018). Establishing the relationship of soil nitrogen immobilization to cereal rye residues in a mulched system. *Plant Soil* 426, 95–107. doi: 10.1007/s11104-018-3566-0

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

NOMENCLATURE

Common ragweed, *Ambrosia artemisiifolia* L. AMBEL; cereal rye, *Secale cereale* L.; soybean *Glycine max* (L.) Merr.; winter wheat, *Tritcum aestivum* L.

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Fecundity and Seed Dormancy Variation Within and Among *Echinochloa* Species

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The *Echinochloa* genus contains two of the world's top five worst weeds. The persistence and weediness of *Echinochloa* spp. are partly due to its seed longevity and variable seed dormancy. In the USA, specifically in Arkansas, multiple species infest the same field in many cases. Persistence could vary across species and dormancy affects infestation level. Studies were conducted to evaluate the seed production potential and dormancy of *Echinochloa* species in the State. Ninety-four accessions were characterized in a common garden in Fayetteville, Arkansas, USA. The species were *Echinochloa colona* (L.) Link, *E. crus-galli* (L.) P. Beauv*, E. muricata* P. Beauv and *E. walteri* (Pursh). Only one accession was identified as *E. walteri* and was excluded from data analysis. Seeds were after-ripened for 6 months at room temperature and germinated at 32°C day/23°C night with 12-h daylength. Germination was monitored for 14 d. The *Echinochloa* species in Arkansas were predominantly *E. colona* (78%). *E. colona* had the highest seed production and the lowest seed dormancy among species. Dormancy within each species varied greatly, especially for *E. colona*, with a germination capacity (GC) of 41–99%. Only 2.7% of 73 *E. colona* accessions were dormant. *E. crus-galli* had 56–79% GC; 33% of the accessions were dormant. *E. muricata* had 2–39% germination, with all accessions considered dormant. *E. colona* had the highest fecundity, with 72,973 seeds per plant. This was 2.3- and 2.6x higher than that of *E. crus-galli* and *E. muricata*, respectively. High seed production and high germination capacity must have contributed to the dominance of *E. colona* among other *Echinochloa* species. *E. muricata* is expected to persist longer in the soil seedbank compared to *E. colona* and *E. crus-galli*. Seed production, dormancy and longevity will affect interspecies population dynamics in response to management tactics.

Keywords: dormancy-breaking treatment, *Echinochloa* species, *E. crus-galli*, *E. walteri*, *E. colona*, *E. muricata*, seedbank, seed dormancy

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INTRODUCTION

Echinochloa species are the most problematic weed in rice fields, with *E. crus-galli* and *E. colona* recorded among the ten most common weeds in vegetable farms, rice (*Oryza sativa* L.), and upland row crops in Arkansas including cotton (*Gossypium hirsutum* L.), sugarcane (*Saccharum offcinarum* L.), sorghum (*Sorghum bicolor* L.), peanut (*Arachis hypogea* L.) and cassava (*Manihot esculenta* Crantz) (Holm et al., 1991, Dowler, 1995; Ciocarlan, 2000; Norsworthy et al., 2013). These species are major global weeds in rice. It is important agronomically and economically to study the characteristics of these species under similar growing conditions since weedy *Echinochloa* can invade and dominate a crop field after only one season and cause significant yield losses (Ruiz-Santaella et al., 2006). *Echinochloa* species are prolific seed producers. *E. crus-galli* can produce up to 1,000,000 seeds plant^{-1} contingent upon environmental conditions. Seed size and weight vary across species and these traits are somewhat correlated with seed dormancy (Maun and Barrett, 1986). Studies on dormancy of *Echinochloa* species in the United States have been chiefly on *E. crus-galli*. Few studies have been conducted on the germination behavior of *E. colona* (Chun and Moody, 1987; Lin and Kuo, 1996; Kovach et al., 2010); one study was conducted on *E. walteri*; and none on *E. muricata*. Understanding the abundance and distribution of *E. colona*, *E. muricata*, and *E. crus-galli*, requires good comprehension of the dormancy behavior of these species (Bryson and Reddy, 2012). This knowledge informs long-term weed management strategies.

Echinochloa species have innate dormancy at seed maturity, as many plant species are, which is broken when the seed is separated from the mother plant. Notwithstanding, a proportion of the seeds will stay dormant for 3–7 months after maturity (Honek and Martinkova, 1996; ShengGan et al., 2007). In general, seed dormancy can be broken after prolonged after-ripening at room temperature (Finch-Savage and Leubner-Metzger, 2006). Different proportions of seeds of numerous weedy species do not germinate despite sufficient after-ripening period, showing some level of secondary dormancy. In the field, numerous non-dormant seeds are driven into secondary dormancy by unfavorable conditions. Seed dormancy in the field can be overcome by modulating soil temperature and light using synthetic or natural ground cover (Rahn, 1968; Benech-Arnold et al., 2000; Probert, 2000). Tillage can also encourage germination of weed seeds and allow for control measures of emerged seedlings prior to planting the crop.

Most research on seed dormancy focused on treatments or techniques to break dormancy. Little is known about interspecies variation in dormancy within the same genus (Finch-Savage and Leubner-Metzger, 2006). Furthermore, variation in seed germination behavior among ecotypes of the same species could be high and could impact the efficacy of weed management tactics. For example, the weedy relative of rice has a wide range of dormancy, with 84–100% germination capacity (GC) at 35°C and a much wider range at lower temperatures (Tseng et al., 2013). It is common to have more than one species in a field, which presents a problem in weed management because of variability in germination behavior (Grundy, 2003; Donohue, 2005; Finch-Savage and Leubner-Metzger, 2006).

Temperature and light are two major environmental factors that control seed dormancy. Light requirement for germination differs across species (Kovach et al., 2010), but *E. colona*, *E. crus-galli*, and *E. walteri* require light for germination (Maun and Barrett, 1986; Chauhan and Johnson, 2009; Kovach et al., 2010) and complete darkness induces secondary dormancy (Kovach et al., 2010). Thus, *Echinochloa* seeds buried deep in the soil profile where the seed can no longer perceive light will go into deep dormancy, helping build a persistent soil seedbank. Extreme soil temperature fluctuation is common in temperate regions, which also promotes secondary dormancy. Warm temperature in the summer breaks seed dormancy and allows seed emergence. The effectiveness of cultural practices, such as stale seedbed, for weed management requires knowledge of weed emergence patterns, which is a manifestation of the dormancy status and germination requirements of the seeds. Information on the inter-species variation in dormancy and fecundity would be useful for reducing soil seedbank and enhancing weed control (Vleeshouwers and Kropff, 2000; Fischer et al., 2009). Specifically, differences in emergence patterns, if known, will allow growers to adjust tillage operation and crop planting ahead of the anticipated early cohort of the weed. Growers would also know the critical weed-free period and conduct cultivation and herbicide applications at the proper time, in anticipation of further weed emergence. Knowledge of dormancy trait will inform growers or crop managers about roughly how many cropping seasons it would take to reduce the soil seedbank to a minimum. The objective of this study was to evaluate the fecundity and dormancy characteristics of *Echinochloa* species occurring in Arkansas, in the US Mid-south. The goal is to provide data that could improve the robustness of crop management decision models and better prediction models on how crop management practices affect the population dynamics of major weed species.

MATERIALS AND METHODS

Seed Production Potential

Ninety-four (94) *Echinochloa* accessions were collected from rice and soybean fields in Arkansas, USA (**Figure 1**). Seedlings were raised in the greenhouse and transplanted at the 4-leaf stage in a common garden at 1.5 × 1.5 m spacing at the Milo Shult Agricultural Research and Extension Center, Fayetteville, AR. Transplanting occurred on July 5, 2012 and June 28, 2013. In 2012, the whole field was sprayed with a mixture of glyphosate (1.12 kg ae ha^{-1}) and *S*-metolachlor (1.79 kg ai ha^{-1}) 7 d before transplanting to control other weeds. The field was irrigated when needed and kept weed-free by hoeing other weeds. In 2013, a mixture of pendimethalin (1.0 kg ai ha^{-1}) and *S*-metolachlor (1.0 kg ai ha^{-1}) was sprayed 7 d after transplanting. Nitrogen fertilizer (60 kg N ha^{-1}) was applied on the day of transplanting by manually incorporating the fertilizer around the base of each plant. Insecticide (imidacloprid, 0.22% granule) was applied at 0.20 kg ai ha^{-1} to control wireworms. Each accession had four biological replications. Mature panicles were harvested by hand and air-dried for 7 d. A subset of 10 panicles per plant were threshed and cleaned to remove sterile florets. The seeds from these 10 panicles were weighed and the weight of 500 seeds was recorded. The remaining panicles were also threshed and the weight of the seeds was recorded. The total number of seeds plant^{-1} was estimated based on the total seed weight of the representative panicles harvested, multiplied by the total panicles plant^{-1}.

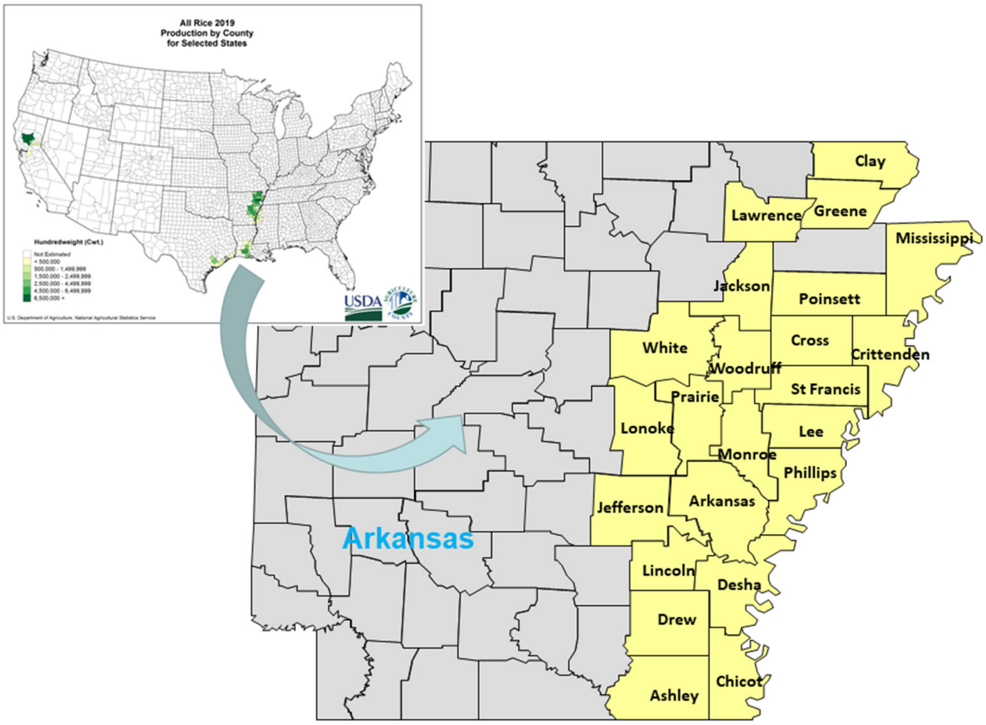

FIGURE 1 | Counties from where *Echinochloa* samples were collected in Arkansas, USA. Rice-producing states in the US (https://www.nass.usda.gov/Charts_and_Maps/Crops_County/index.php#ar).

Pre-germination Temperature Treatments to Break Seed Dormancy of *Echinochloa colona*

Three accessions, identified morphologically as *E. colona*, were selected randomly out of 94 accessions to represent different seed sizes. Seeds were stored at room temperature (about 25°C) in the dark for 180 d. Batches of 50 seeds were counted and placed in Petri plates lined with filter paper. Seeds were incubated for 7 d in four conditions: (1) 50°C; (2) 4°C; (3) −20°C; and 4) ambient temperature (25 °C) with four replications per condition. At the end of incubation period, the Petri dishes were arranged, completely randomized, in a tray and were placed in a growth chamber at 32°C day/23°C night temperature with 12-h photoperiod. Seed germination was recorded at 7, 14, and 21 d after incubation. At each germination evaluation period, the germinated seeds were removed from the Petri dish after counting.

GC (%) was calculated using the formula:

$$GC = \frac{\text{total number of seeds germinated}}{\text{total number of viable seeds}} \times 100 \qquad (1)$$

Dormancy Evaluation of *Echinochloa* Species

Ten (10) representative panicles harvested from each field-grown plant were air-dried in the greenhouse (36°C) for 7 d, threshed and stored at room temperature (25–28°C) for 180 d in the dark. Ninety-two accessions comprised of 72 *E. colona*, 9 *E. crus-galli*, and 10 *E. muricate* were used in this study. The same method for seed germination was used as in Section Pre-germination Temperature Treatments to Break Seed Dormancy of *Echinochloa colona*, except that for this study, the seeds were stored only at room temperature prior to germination. The germination condition was similar to the ideal germination conditions for *E. colona* at 30°C day/20°C night temperature cycle (Chauhan and Johnson, 2009). Seed germination was evaluated at 7 and 14 d of incubation for accessions with high GC. The observation period was extended to 21 d for large-seeded accessions (generally *E. muricata*) with low GC. The GC was calculated using Equation (1).

Statistical Analysis

Data collected were analyzed using JMP for Windows software Version 11.0 (SAS, 2011). To determine the effect of temperature on seed dormancy, an analysis of variance (ANOVA) was conducted on the GC data. The GC of all accessions studied in 2012 and 2013 were pooled in the absence of year effect. Cluster analysis was done on the average GC of each accession to determine statistically supported grouping of accessions based on germination.

Cluster Analysis

The accessions were grouped using the cubic clustering criterion in SAS-JMP (12.1) (**Figure 2**). The number of statistically distinct clusters was the point where the cubic clustering criterion reached a maximum, beyond which the curve declines with each additional number of clusters.

RESULTS

Seed Production Potential of *Echinochloa* Species in a Common Garden

E. colona produced the highest amount of seed (539 seeds panicle^{-1}) and *E. muricata* produced the lowest (259 seeds panicle^{-1}) (**Table 1**). Accounting for the total number of panicles, the estimated total seed production per plant was significantly different among species. *E. colona* produced the highest estimated number of seeds (72,973 seeds plant^{-1}), *E. crus-galli* had 31,911 seeds plant^{-1}, and *E. muricata* produced an estimated 27,589 seeds plant^{-1}. The lone *E. walteri* accession produced 25,392 seeds plant^{-1}, which was within the range of *E. muricata* seed production.

Dormancy-Breaking Temperature Treatment for *E. colona*

In this study, seed storage at different pre-germination temperatures, from freezing (−20°C) to hot (50°C), did not have a big effect on GC (**Table 2**). All pre-germination incubation temperature treatments resulted in 65–77% germination at 32/23°C day/night temperature with 12-h photoperiod. Seven days of incubation at 25°C (room temperature) resulted in the highest GC, which was significantly higher only than the GC at 4°C. Incubation at 4°C for 7 d resulted in 65% germination.

FIGURE 2 | Cubic clustering criterion. The point where the cubic clustering criterion reached a maximum, beyond which the curve declines with each additional number of clusters.

Species Differences in Seed Dormancy

Among the three species tested, *E. colona* had the highest average GC (77%) with a range of 41–99% (**Table 3**). Of the 74 *E. colona* accessions tested, 73% had a maximum GC of 80–100% and 47% of accessions had an average GC of at least 80% (**Supplementary Table 1**). Therefore, this specie is not highly dormant. The temperature used was within the optimum germination temperature for *E. colona* and *E. crus-galli* (Chauhan and Johnson, 2009). *E. crus-galli* had the second highest average GC (53%) with a range of 11–79% across accessions. This was at least twice as high as the GC reported previously for *E. crus-galli* (25.7 ± 24.9%) and inferior to var. *oryzicola* after 270 d of after-ripening (92.9 ± 7.3%), germinated at 30°C with 12 h photoperiod (Barrett and Wilson, 1983). While it is not possible to make direct comparisons to other studies on different species of *Echinochloa*, it is useful to list other germination reports to provide an overview of the germination behavior of *Echinochloa* in a wide range of environments. Brod (1968) reported 79–86% germination of *E. crus-galli* after 5 mo of storage at room temperature (Brod, 1968). All previous studies determined that 30°C is the optimum temperature for germination of *E. crus-galli* (Kasahara and Kinoshita, 1952;

TABLE 2 | Germination capacity of *Echinochloa colona* from Arkansas, averaged across accessions, after storage at various temperatures for 7 d, Altheimer Laboratory, Milo Shult Agricultural Research and Extension Center, University of Arkansas, Fayetteville, USA.

Storage temperature[a]	Mean	Std Dev	Min	Max	LSD[b]
	-------------------------	%	--------------------		
−20°C	69	12	47	84	9
4°C	65	13	42	82	
25°C	77	10	64	90	
50°C	72	10	58	91	

[a] *Three E. colona accessions were selected randomly from accessions collected in 2010–2011. Seeds were incubated for 7 d. Fifty seeds were then germinated, in three replications, at 32/23°C light/dark cycle with 12-h photoperiod.*

[b] *Means were compared using Fisher's least significant difference (LSD) test (α = 0.05).*

TABLE 1 | Seed production of *Echinochloa* species in a common garden, averaged across accessions and years, Milo Shult Agricultural Research and Extension Center, University of Arkansas, Fayetteville, AR, USA.

Species[a]	Panicles plant^{-1}			Total seeds panicle^{-1}			Total seeds plant^{-1}		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
E. colona	140	63	258	539	234	1,739	72,973	9,098	217,217
E. crus-galli	56	40	65	345	249	396	31,911	7,186	71,494
E. muricata	63	20	111	259	107	565	27,589	13,189	37,589
E. walteri[c]	50			510			25,392		
LSD[b]	12			156			25,220		

[a] *Data were obtained from 94 accessions with four biological replicates per accession, across 2 years.*

[b] *Means were compared using Fisher's LSD test (P = 0.05).*

[c] *E. walteri was excluded from the analysis because only one accession represented this specie.*

TABLE 3 | Germination capacity of *Echinochloa* species from Arkansas, USA, grown in a common garden at the Milo Shult Agricultural Research and Extension Center, Fayetteville, AR, averaged by species across 2 years (2012 and 2013).

Species[a]	Number of Accessions	Germination capacity[b]			
		Mean	Std Dev	Min	Max
		----------------%	-----------------		
E. colona	74	77	13	41	99
E. crus-galli	9	53	25	11	79
E. muricata	10	18	23	2	73
LSD[c]		11			

[a] *E. walteri was excluded from this analysis because only one accession was collected.*
[b] *Panicles were air-dried in the greenhouse (36°C) for 7 d, threshed and placed in paper bags, and stored at 25–28°C in the dark for 180 d. Fifty seeds were then germinated, in three replications, at 32/23°C light/dark cycle with 12-h photoperiod.*
[c] *Means were compared using Fisher's least significant difference test (α = 0.05).*

TABLE 4 | Germination capacity of *Echinochloa* species *E. colona, E. crus-galli*, and *E. muricata* from Arkansas, USA, averaged across accessions in a cluster.

Accession grouping	No. of accessions[a]	Germination capacity[b]			
		Mean	Median	Min	Max
		-------------------	%	------------------	
Cluster 1	65	81	82	66	99
Cluster 2	18	52	55	27	64
Cluster 3	9	9	6	2	21

[a] *Four plants per accession were grown in a common garden at the Milo Shult Agricultural Research and Extension Center, Fayetteville, Arkansas, USA in 2012 and 2013.*
[b] *E. walteri, the fourth species encountered in sampled fields, was not included in the analysis because only one accession was collected. Echinochloa panicles were air-dried in the greenhouse (36°C) for 7 d, threshed and placed in paper bags, and stored at 25–28°C in the dark for 180 d. Fifty seeds were then germinated, in three replications, at 32/23°C light/dark cycle with 12 h of light.*

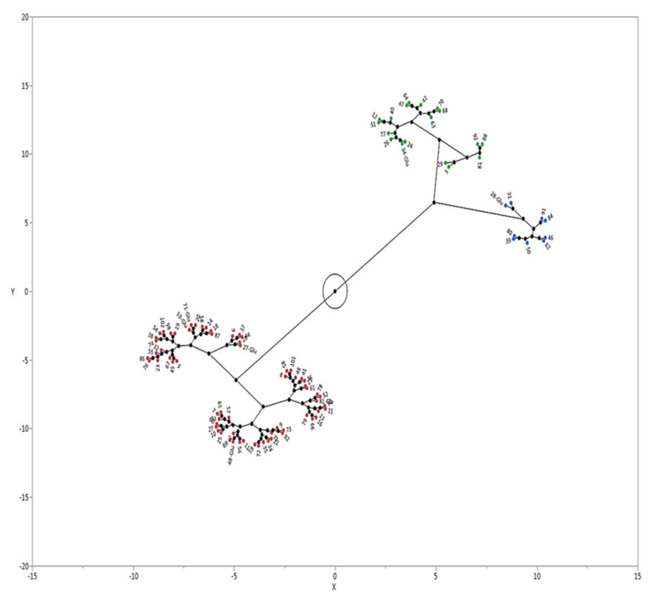

FIGURE 3 | Castellation plot showing three clusters of *Echinochloa* species from Arkansas, USA based on germination capacity. The accessions were grown in a common garden in Fayetteville, Arkansas. Seeds were after-ripened for 180 d at 25°C and germinated in a growth chamber at 32°C day/23°C night temperature with 12-h photoperiod. Cluster 1 = *E. colona* (red); Cluster 2 = *E. crus-galli* (green); cluster 3 = *E. muricata* (blue).

Arai and Miyahara, 1963,Brod, 1968). In the present study, *E. muricata* had the lowest GC (18%) with a range of 2–73% across accessions (**Table 3**).

Echinochloa accessions separated into three clusters based on GC (**Figure 3**). Cluster 1 was the largest group with 65 accessions (i. 61 *E. colona*; ii. 3 *E. crus-galli*; iii. 1 *E. muricata*). The average GC of each accession in this cluster ranged from 66 to 99 % (**Table 4**). Cluster 1 was the least dormant group of accessions. The majority (84%) of the *E. colona* accessions, 33% of *E. crus-galli*, and 10% of *E. muricata* accessions were in this group. The second cluster had 18 accessions (12 *E. colona*, 4 *E. crus-galli* and 2 *E. muricata*) with an average GC of 52%. A marginally larger category (44%) of *E. crus-galli* accessions grouped in this intermediate cluster relative to Cluster 1. The third cluster had nine accessions, mainly *E. muricata*, with only two *E. crus-galli* accessions. This cluster consisted of the most dormant accessions, with an average GC of 9%. Most of the *E. muricata* (70%) were among the most dormant accessions.

Intra-Species Variation in Seed Dormancy

Cluster Analysis of *E. colona* Germination Capacity

As indicated in the previous section, there was a large variation in the GC of *E. colona*, while the vast majority of accessions were in the low dormancy group. When analyzed with other species, the *E. colona* accessions were divided into four clusters (**Figure 4**). Cluster 1 (blue) had 46 accessions, with GC ranging from 74 to 92% and an average of 83% (**Table 5**). Cluster 2 (red) consisted of 20 accessions with a GC range of 51–71% and an average of 63%. Cluster 3 (brown) consisted of four non-dormant accessions with the highest average GC of 97%. This focused study showed that the majority (63%) of *E. colona* accessions had low dormancy level, with a few non-dormant accessions. This supports the overall classification of *E. colona* as the least dormant species in the previous section. Cluster 4 (green) consisted of the two most dormant *E. colona* accessions with a GC of 42%. Compared to other species, the most dormant *E. colona* were less dormant than most *E. muricata* accessions and the most dormant *E. crus-galli* accessions (see Section Species Differences in Seed Dormancy).

Cluster Analysis of *E. crus-galli* Germination Capacity

The nine *E. crus-galli* accessions were split into two groups (**Figure 5**). Two-thirds of these grouped in cluster 1, with GC ranging from 56 to 79% and an average of 68% (**Table 6**). One-third of the accessions were more dormant, showing an average GC of 22%, with the maximum GC of only 34%. Overall, the highest GC of *E. crus-galli* was 79% and the lowest was 11% when germinated at 30/20°C with 12-h photoperiod.

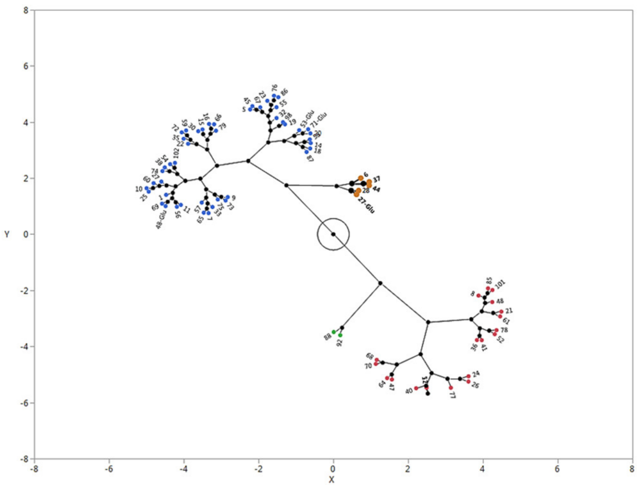

FIGURE 4 | Castellation plot showing four clusters of *E. colona* from Arkansas, USA based on germination capacity. The accessions were grown in a common garden in Fayetteville, Arkansas. Seeds were after-ripened for 180 d at 25°C and germinated in a growth chamber at 32°C day/23°C night temperature with 12-h photoperiod. Cluster 1 = blue; Cluster 2 = red; cluster 3 = brown, Cluster 4 = green.

TABLE 5 | Germination capacity of *Echinochloa colona* from Arkansas, USA, averaged across accessions in a cluster.

Accession grouping	No. of accessions[a]	Germination capacity[b]			
		Mean	Median	Min	Max
		------------------%------------------			
Cluster 1	46	83	82	74	92
Cluster 2	20	63	64	51	71
Cluster 3	4	97	97	95	99
Cluster 4	2	42	42	41	42

[a] *Four plants per accession were grown in a common garden at the Agricultural Research and Extension Center, Fayetteville, Arkansas, USA in 2012 and 2013.*
[b] *E. colona panicles were air-dried in the greenhouse (36°C) for 7 d, threshed and placed in paper bags, and stored at 25–28°C in the dark for 180 d. Fifty seeds were then germinated, in three replications, at 32/23°C light/dark cycle with 12 h of light.*

Cluster Analysis of Germination Capacity of *E. muricata*

The *E. muricata* accessions separated into two clusters on the basis of germination (**Figure 6**). Cluster 1 had seven accessions, with a GC between 2 and 15% and an average of 6% (**Table 7**). The three accessions in cluster 2 had lower dormancy, with an average GC of 46%, and the highest germination being 73%. This one accession in Cluster 2 had the highest GC among *E. muricata* accessions. The bulk of *E. muricata* accessions were highly dormant. Given that this specie also has the largest seeds, *E. muricata* will most likely persist for the longest time in the soil compared to *E. colona* and *E. crus-galli*.

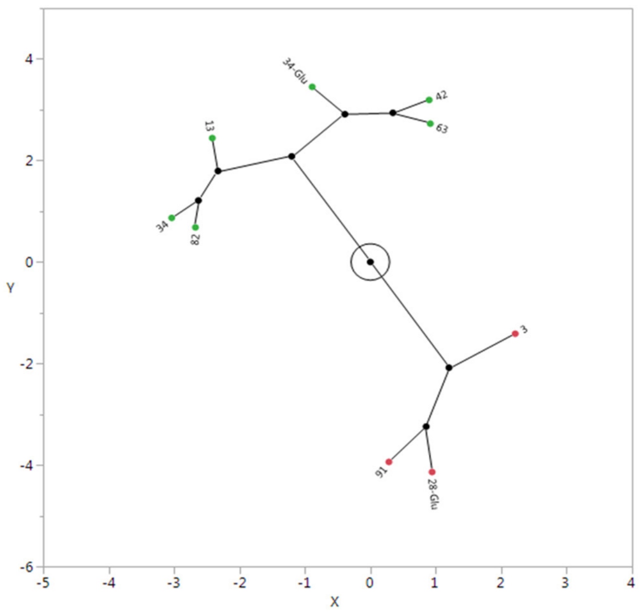

FIGURE 5 | Castellation plot showing two clusters of *E. crus-galli* from Arkansas, USA based on germination capacity. The accessions were grown in a common garden in Fayetteville, Arkansas. Seeds were after-ripened for 180 d at 25°C and germinated in a growth chamber at 32°C day/23°C night temperature with 12-h photoperiod. Cluster 1 = green; Cluster 2 = red.

TABLE 6 | Germination capacity of *Echinochloa crus-galli* in Arkansas, USA, averaged across accessions in a cluster.

Accession grouping	No. of accessions[a]	Germination capacity[b]			
		Mean	Median	Min	Max
		------------------%------------------			
Cluster 1	6	68	69	56	79
Cluster 2	3	22	21	11	34

[a] *Four plants per accession were grown in a common garden at the Agricultural Research and Extension Center, Fayetteville, Arkansas, USA in 2012 and 2013.*
[b] *E. crus-galli panicles were air-dried in the greenhouse (36°C) for 7 d, threshed and placed in paper bags, and stored at 25–28°C in the dark for 180 d. Fifty seeds were then germinated, in three replications, at 32/23°C light/dark cycle with 12 h of light.*

DISCUSSION

Seed Production Potential

Assessment of the relative contribution of seed production to weed population dynamics, persistence, or weediness of species entails growing such plants in the same environment at the same time without competition. For example, weed-weed and weed-crop associations, the duration of interference, and season of the year alter weed seed production tremendously (Bagavathiannan et al., 2012). The seed production potential of *E. crus-galli* in the common garden (without competition) ranged from about 7,000 to about 72,000 per plant across 2 years. The main factors affecting this large variation are high intraspecies diversity in plant type and climate-related variability across years. The year

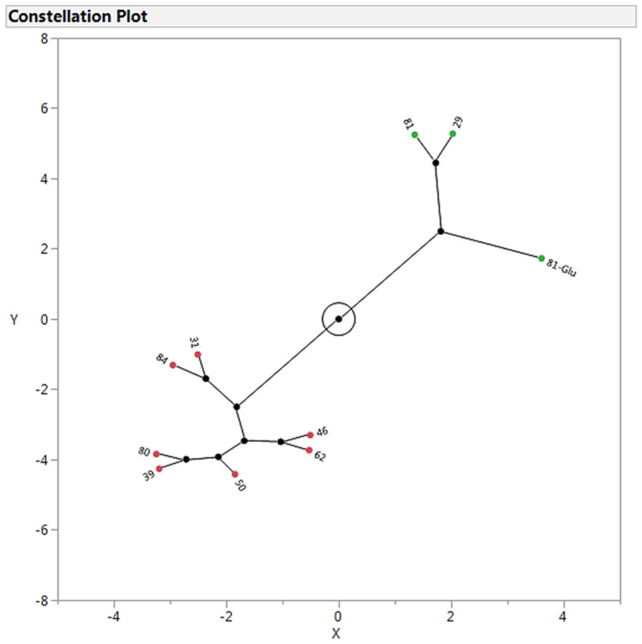

FIGURE 6 | Castellation plot showing two clusters of *E. muricata* from Arkansas, USA based on germination capacity. The accessions were grown in a common garden in Fayetteville, Arkansas. Seeds were after-ripened for 180 d at 25°C and germinated in a growth chamber at 32°C day/23°C night temperature with 12-h photoperiod. Cluster 1 = Red; Cluster 2 = green.

TABLE 7 | Germination capacity of *Echinochloa muricata* from Arkansas, USA, averaged across accessions in a cluster.

Accession grouping	No. of accessions[a]	Germination capacity[b]			
		Mean	Median	Min	Max
		----------------%	-------------------		
Cluster 1	7	6	5	2	15
Cluster 2	3	46	39	27	73

[a] Four plants per accession were grown in a common garden at the Agricultural Research and Extension Center, Fayetteville, Arkansas, USA in 2012 and 2013.

[b] *E. muricata panicles were air-dried in the greenhouse (36°C) for 7 d, threshed and placed in paper bags, and stored at 25–28°C in the dark for 180 d. Fifty seeds were then germinated, in three replications, at 32/23°C light/dark cycle with 12 h of light.*

effect cannot be overstated. *E. crus-galli* growing with cotton full-season, can produce 35,500 seeds plant^{-1} in year 1 and about half that in year 2 (Bagavathiannan et al., 2012). The crop effect may not be as large as that of year or climatic effect. In the same study, *E. crus-galli* growing with rice full season produced about 39,000 seeds plant^{-1} in year 1, very similar to that with cotton. The same principle applies to seed production of other species. Seed production data from other regions or locations generated at different times can only be examined in conjunction with information on associated plant and environmental factors. A large dataset across space and time would allow one to detect species-specific behavioral patterns, if any, and would be highly informative in formulating location-specific and broadscale management strategies.

Dormancy-Breaking Treatments for *E. colona*

Conducting successful research on weedy species requires the capability to obtain sufficient germination to grow enough plants for an indoor test, or establish a large enough population for a field test. This need is most acute in evaluating germination requirements, germination behavior, seed longevity, plant growth traits, or response to treatments. Sufficient after-ripening time is a primary requirement for good germination of *Echinochloa* (Chauhan and Johnson, 2009) as it is for the majority of plant species. Research in the Philippines revealed that optimum GC occurred after 60 d of after-ripening. Extending the after-ripening duration to 90 d did not increase the germination further (Chauhan and Johnson, 2009). Given sufficient after-ripening period, and sufficient moisture in the soil, temperature becomes the primary determinant for germination. The temperature to which the seeds are exposed prior to germination, and the duration of such exposure, largely determine whether the seed will germinate or stay dormant. Hence, we tested various seed storage temperatures to break dormancy. We learned that storage at room temperature is the best condition for *E. colona*. Research by Chauhan and Johnson (2009) also showed the same with incubation at room temperature (25°C) resulting in the highest germination (76%) of *E. colona,* which is almost identical to our findings about *E. colona* from Arkansas. Exposure to a wide range of pregermination temperatures has a small effect on *E. colona* GC, as long as the seeds are germinated under optimum conditions at some point. Extended exposure to extreme temperatures could drive the seeds into deep dormancy, while fluctuating temperatures could help break dormancy (Benech-Arnold et al., 1990; Martínez-Ghersa et al., 1997) as this weakens the hard seed coat. Storing dry seed at a constant temperature promotes secondary dormancy (Martínez-Ghersa et al., 1997), but soil temperature fluctuates diurnally and seasonally in the field. When briefly exposed to extremely high temperature, i.e., 120°C for 5 min, more than 70% of the seeds germinated; germination decreased at temperatures higher than this and the seeds were killed at 180°C (Chauhan and Johnson, 2009). That means, at least for *E. colona*, brief exposure to high temperatures will not reduce germination. Conditions that break dormancy can vary across species. Thus, it would be beneficial to determine such condition for each specie that contributes to the weed community composition.

Dormancy Across and Within Species

The four *Echinochloa* species tested vary widely in GC. *E. colona* was the least dormant of the species tested. About 16% of *E. colona* accessions had an intermediate level of dormancy (**Table 4**). The separation of *E. colona* accessions into four clusters indicates a significant dormancy level grouping. The range of GC within *E. colona* cluster 1 was also especially large, showing differences among accessions within the cluster. Such intra-cluster variation may not be statistically significant, but in terms of weed management to reduce the soil seed bank, a 33% difference in germination means a substantial difference in population size and the amount of possible new seed deposits in cases where the population is not managed effectively. At the species level, this large variation in seed dormancy entails long-term weed management goals.

E. crus-galli looks generally like *E. colona*, but with higher dormancy. *E. crus-galli* normally has 5–50% non-dormant seed that can germinate soon after maturation; the rest will stay dormant and remain in the soil seedbank for a long time (Honek and Martinkova, 1996). Only 39% of freshly deposited *E. crus-galli* seeds germinate in the field at one time; the rest germinate at various times thereafter (Kon et al., 2007; Chauhan and Johnson, 2009). The extended germination period presents a challenge to farmers in achieving effective season-long weed control. The *E. crus-galli* accessions from Arkansas separated into three germination categories, with almost equal proportions in Clusters 1 and 2, showing the greatest intra-species variability in dormancy among the species tested. In that sense, *E. crus-galli* may be the most unpredictable in terms of population response to management tactics. Barrett and Wilson (1983) reported similar findings. They tested *E. crus-galli* var. *crus-galli* accessions that have been post-ripened for 9 mo and germinated at 30°C with 12-h photoperiod for 14 d. Eleven accessions had GCs between 0 and 25%, four had 26–50%, two had 51–75%, and one had >75% (Barrett and Wilson, 1983).

E. muricata was the most dormant among the Arkansas species tested and the most heavily biased toward the opposite end of the spectrum compared to *E. colona*. Here it is tempting to argue that seed size is a contributing factor to seed dormancy (Barrett and Wilson, 1983). However, this hypothesis needs to be tested empirically and is best illustrated with different seed sizes of the same species. It may be comforting to a rice farmer to know that *E. muricata* is rarely found in the rice field; rather, it thrives in ditches and field edges (N. Roma-Burgos, observation during sample collection). It is expected to persist the longest, based on its large seed size and deep dormancy.

Of the accessions collected, only one was identified as *E. walteri* based on morphological traits (Hussain, 2016). Our data on this specie is, therefore, not conclusive. Nevertheless, the GC of *E. walteri* (49%) was consistent with what Kovach et al. (2010) and Buhler and Hoffman (1999) reported under similar germination conditions as the current experiment. They also reported that *E. walteri* requires alternating light and dark germination conditions, and does not germinate in total darkness. Light is therefore a crucial trigger for *E. walteri* germination. This means that *E. walteri* seed, when buried deep in the soil profile where the seed can no longer sense light, will go into deep dormancy. Light requirement, however, is species-specific; both *E. colona* and *E. crus-galli* germinate in darkness, but light increases germination (Kovach et al., 2010). In earlier studies, Buhler and Hoffman (1999) reported high dormancy of *Echinochloa* species and interspecies variability in GC.

Significance of Findings

Weedy *Echinochloa* species, specifically *E. colona* and *E. crus-galli* are among the world's worst weeds and are the primary weeds in rice production. These two species are the most common in Arkansas (U.S. Mid-south). Little is known about what drives species dominance, among which could be seed production and dormancy. The fact that *E. colona* produces the highest seed number (up to about 217,000 plant^{-1}) and has the lowest dormancy implies that it would be predominant in any growing season, as was captured in the collection of samples. The difference in seed dormancy between and within species of *Echinochloa* is high, which means that there will be consistently high level of infestation and a persistent soil seed bank to maintain species dominance. This data set partly explains the relative species abundance in the US Mid-south. Exposure of *E. colona* seeds to freezing (−20°C) or high (50°C) temperature does not reduce its germination capacity. This implies that extreme winters or superhot summers are not going to reduce infestation levels. In temperate regions, we could not rely on winter kill to reduce weed population size. *E. muricata* is deeply dormant and thrives more in ditches and field edges rather than in the crop field, but has been observed to invade rice fields in a few cases. Growers need to be vigilant in stopping any encroachment because this specie will have a very large, persistent seedbank that can plague the crop indefinitely.

DATA AVAILABILITY STATEMENT

The dataset presented in this article can be made available upon request to the corresponding author Nilda Roma-Burgos via email: nburgos@uark.edu.

AUTHOR CONTRIBUTIONS

NR-B conceptualized and designed the experiment, supervised the implementation of the experiment, wrote the manuscript with HT, revised the manuscript, and secured research funding. HT conducted the experiment, collected and analyzed data, prepared figures and tables, and wrote the first draft of the manuscript with guidance from NR-B. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2021.623425/full#supplementary-material

REFERENCES

Arai, M., and Miyahara, M. (1963). Physiological and ecological studies on Barnyard Grass (*Echinochloa crus-galli* Beauv. var. oryzicola Ohwi): V. On the germination of the seed. *Jap. J. Crop Sci*. 31, 362–366. doi: 10.1626/jcs.31.362

Bagavathiannan, M. V., Norsworthy, J. K., Smith, K. L., and Neve, P. (2012). Seed production of barnyardgrass (*Echinochloa crus-galli*) in response to time of emergence in cotton and rice. *J. Agric. Sci*. 150, 717–714. doi: 10.1017/S0021859611000876

Barrett, S. C. H., and Wilson, B. F. (1983). Colonizing ability in the *E. crus-galli* complex (barnyardgrass). II. Seed biology. *Can. J. Bot*. 61, 556–562. doi: 10.1139/b83-063

Benech-Arnold, R. L., Ghersa, C. M., Sanchez, R. A., and Insausti, P. (1990). Temperature effects on dormancy release and germination rate in *Sorghum halepense* (L.) Pers. seeds: a quantitative analysis. *Weed Res*. 30, 81–89. doi: 10.1111/j.1365-3180.1990.tb01690.x

Benech-Arnold, R. L., Sanchez, R. A., Forcella, F., Kruk, B. C., and Ghersa, C. M. (2000). Environmental control of dormancy in weed seedbanks in soil. *Field Crops Res*. 67, 105–122. doi: 10.1016/S0378-4290(00)00087-3

Brod, G. (1968). Unter Suchengen zur Biologie und Okologie der Huhner-hirse *E. crus-galli* (L.) Beauv. *Weed Res*. 8,115–127. doi: 10.1111/j.1365-3180.1968.tb01409.x

Bryson, C. T., and Reddy, K. N. (2012). "Diversity of *Echinochloa* in the US Mid-South," in *Abstract retrieved from Proc. 2012 Weed Science Society of America Annual Meeting* (Waikoloa, HI).

Buhler, D. D., and Hoffman, M. L. (1999). *Echinochloa, in Andersen's Guide to Practical Methods of Propagating Weeds and Other Plants*. Lawrence, KS: Weed Science Society of America.

Chauhan, B. S., and Johnson, D. E. (2009). Seed germination ecology of junglerice (*Echinochloa colona*): a major weed of rice. *Weed Sci*. 57, 235–240. doi: 10.1614/WS-08-141.1

Chun, J. C., and Moody, K. (1987). "Ecotypic variation in *Echinochloa colona*," in *Abstract extracted from Proc. 11th Asian Pacific Weed Science Society Conference* (Taipei: Asian Pacific Weed Science Society), 3–27.

Ciocarlan, V. (2000). "The genus Echinochloa Beauv," in *Flora ilustrată a României: Pteridophyta et Spermatophyta* (Bucureşti: Ceres), 1141.

Donohue, K. (2005). Seeds and seasons: interpreting germination timing in the field. *Seed Sci. Res*. 15, 175–187. doi: 10.1079/SSR2005208

Dowler, C. C. (1995). "Weed Survey-Southern States," in *Proc. 1995* (Memphis, TN: Southern Weed Science Society), 290–325.

Finch-Savage, W. E., and Leubner-Metzger, G. (2006). Seed dormancy and the control of germination. *New Phytol*. 171, 501–523. doi: 10.1111/j.1469-8137.2006.01787.x

Fischer, A. J., Linquist, B., Moechnig, M., Mutters, R., Hill, J. E., Greer, C., et al. (2009). "Alternative rice stand establishment systems to manage herbicide-resistant weeds," in *Abstract extracted from Proc. Weed Science Society of America Annual Meeting* (Orlando, FL).

Grundy, A. C. (2003). Predicting weed emergence: a review of approaches and future challenges. *Weed Res*. 43, 1–11. doi: 10.1046/j.1365-3180.2003.00317.x

Holm, L. G., Plucknett, D. L., Pancho, J. V., and Herberger, J. P. (1991). *The World's Worst Weeds*. Malabar, FL: Krieger.

Honek, A., and Martinkova, Z. (1996). Geographic variation in seed dormancy among populations of *E. crus-galli. Oecologia* 108, 419–423. doi: 10.1007/BF00333716

Hussain, T. (2016). *Characterization of Echinochloa spp. in Arkansas* (Master's thesis). Fayetteville, AR: University of Arkansas.

Kasahara, Y., and Kinoshita, O. (1952). Studies on the control of barnyardgrass in the paddy field. *Proc. Crop Sci. Soc. Jpn*. 21, 319–320. doi: 10.1626/jcs.21.319

Kon, K. F., Follas, G. B., and James, D. E. (2007). Seed dormancy and germination phenology of grass weeds and implications for their control in cereals. *NZ Plant Prot*. 60, 174–182. doi: 10.30843/nzpp.2007.60.4597

Kovach, D. A., Widrlechner, M. P., and Brenner, D. M. (2010). Variation in seed dormancy in *Echinochloa* and the development of a standard protocol for germination testing. *Seed Sci. Technol*. 38, 559–571. doi: 10.15258/sst.2010.38.3.04

Lin, R. J., and Kuo, W. H. J. (1996). Seasonal changes in the germinability of buried seeds of *Echinochloa colonum* (L.) Link. and *Alopecurus aequalis* Sobol. var. *amurensis*. *Mem. Coll. Agric*. 36, 233–244.

Martínez-Ghersa, M. A., Satorre, E. H., and Ghersa, C. M. (1997). Effect of soil water content and temperature on dormancy breaking and germination of three weeds. *Weed Sci*. 45, 791–797. doi: 10.1017/S0043174500088986

Maun, M. A., and Barrett, S. C. H. (1986). The biology of Canadian weeds. 77. *E. crus-galli* (L.) Beauv. *Can. J. Plant Sci*. 66, 739–759. doi: 10.4141/cjps86-093

Norsworthy, J. K., Bond, J., and Scott, R. C. (2013). Weed management practices and needs in Arkansas and Mississippi Rice. *Weed Technol*. 27, 623–630. doi: 10.1614/WT-D-12-00172.1

Probert, R. J. (2000). "The role of temperature in the regulation of seed dormancy and germination," in *Seeds: The Ecology of Regeneration in Plant Communities*, ed. M. Fenner (Wallingford: CAB International), 261–292.

Rahn, E. M. (1968). Life history studies as related to weed control in the Northeast. 5. Barnyardgrass. *Agric. Exp. Stat. Univ. Delaware Bull*. 368, 1–46.

Ruiz-Santaella, J. P., De Prado, R., Wagner, J., Fischer, A. J., and Gerhards, R. (2006). Resistance mechanisms to cyhalofop-butyl in a biotype of *Echinochloa phyllopogon* (Stapf) Koss. from California. *J. Plant Dis. Prot*. 20, 95–100.

SAS (2011). *JMP®, Version 11*. Cary, NC: SAS Institute Inc.

ShengGan, W., Qiang, W., XuePing, Z., ChangXing, W., LiPing, C., and JinLiang, S. (2007). Dormancy and dormancy breaking in barnyardgrass *E. crus-galli*. *Acta Agric. Univ. Zhejiangensis* 19, 225–228.

Tseng, T. M., Burgos, N. R., Shivrain, V. K., Alcober, E. A., and Mauromoustakos, A. (2013). Inter- and intra-population variation in dormancy of *Oryza sativa* (weedy red rice) and allelic variation in dormancy-linked loci. *Weed Res*. 53, 440–451. doi: 10.1111/wre.12044

Vleeshouwers, L. M., and Kropff, M. J. (2000). Modeling field emergence patterns in arable weeds. *New Phytol*. 148, 445–457. doi: 10.1046/j.1469-8137.2000.00773.x

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IPSIM-Cirsium, a Qualitative Expert-Based Model to Predict Infestations of *Cirsium arvense*

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Throughout Europe, *Cirsium arvense* is the most problematic perennial weed in arable crops, whether managed under organic or conventional agriculture. Non-chemical control methods are limited with partial efficacy. Knowledge is missing on their effect across a wide gradient of cropping systems and pedoclimates. To achieve effective *Cirsium arvense* management ensuring crop productivity while limiting the reliance of cropping systems on herbicide, expert-based models are needed to gather knowledge on the effect of individual levers and their interactions in order to (i) design and assess finely tuned combinations of farming practices in different pedoclimates and (ii) support decisions for *Cirsium arvense* control. Based on expert-knowledge and literature, we developed IPSIM-*Cirsium*, a hierarchical qualitative model which evaluates the infestation of *Cirsium arvense* as a function of farming practices, climate conditions, soil descriptors and their interactions. IPSIM-*Cirsium* is a multi-attribute model considering all possibilities of interactions between factors, it estimates the infestation rate of the field graded according to a four-level scale. The model outputs were confronted to independent field observations collected across 6 fields, over a 16-year period in 3 sites. IPSIM-*Cirsium* showed a satisfactory predictive quality (accuracy of 78.2%). IPSIM-Cirsium can be used as a tool for crop advisors and researchers to assist the design of systems less reliant on herbicides, for farmers and advisers to assess *ex-ante* prototypes of cropping systems, and for teachers as an educational tool to share agroecological weed management knowledge.

Keywords: integrated weed management, *Canada thistle*, qualitative modeling, injury profile simulator, cropping practices, soil, climate

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INTRODUCTION

Weed management is essential to limit their harmfulness against crops such as yield loss, decline of crop harvest quality and harvest difficulties (Colbach et al., 2021). Nowadays, weed management relies on herbicides, and its intensive use raises concern on public health, soil-water-air contamination, biodiversity maintenance (Stoate et al., 2009), and development of herbicide resistance (Powles and Yu, 2010). Reducing the reliance of cropping systems on pesticides is promoted throughout Europe (e.g., EU legislation and the French ECOPHYTO National Action

Plan). Authorities strengthened the criteria to deliver marketing authorizations for pesticides, leading to a dynamic of withdrawal of herbicide (e.g., Carbamate herbicides such as Butylat or Chlorobufam) over the past decades (Chauvel et al., 2012). In addition, public policies aim at decreasing the use of widely used authorized herbicides, such as Glyphosate. Decreasing herbicide use while ensuring crop productivity and economic profitability of farming systems requires a deep redesign of cropping systems implementing 'many little hammers' to curtail weed population increase (Liebman et al., 1997). However, the management of perennial weeds remain of high concern in integrated cropping systems (Favrelière et al., 2020). While annual weeds rely on their seed to maintain their population over the years, perennial weeds base their survival on their vegetative reproduction. *Cirsium arvense* (L) Scop. is the most problematic perennial weed in Europe. A density of 15 and 30 shoots/m² can reduce cereal yield by 35% and more than 50%, respectively (Hodgson, 1968; Favrelière, 2019). Seed production by *C. arvense* is sometimes reported to be sizable (Gruber and Claupein, 2009), but Donald (1990) observed that it can be restricted, limiting harvest pollution with weed seeds. Restraining *C. arvense* infestation in a particular location and avoiding seed production is crucial to its establishment in new locations within a given landscape since, as many Asteraceae species, *C. arvense* seeds are transported by the wind (Tiley, 2010), implying a management at the landscape scale. Weedy green biomass at harvest timings located above the cutting bar of the combine harvest may increase harvest difficulties (Mézière et al., 2015), but this was not precisely quantified. The prickly mature foliage deters livestock from grazing (Schreiber, 1967). Non-chemical control methods are limited and with partial effects (Melander et al., 2013; Davis et al., 2018). Most herbicide-free weed management levers rely on intensive tillage, high diversity of crop in the crop sequence and increased competitiveness with subsidiary crops (Lukashyk et al., 2008; Brandsæter et al., 2012; Melander et al., 2012; Miller, 2016). Despite existing knowledge on particular levers and their effect with long-term perspectives, information on long-term combination of multiple levers in various production contexts remains scarce because the effect of interactions between cropping practices and pedoclimate remain only partially known.

Expert knowledge is needed to elucidate the significance of different integrated weed management tactics in various pedoclimates and production situations, evaluate the emphasis of each practice, their interactions and synthesize this knowledge as a model to assess designed strategies and forecast future weed dynamics. This expert approach, associated with literature, is the aim of the IPSIM framework (Injury Profile SIMulator) developed by Aubertot and Robin (2013), using wheat-eyespot as a case study to present a proof of concept. Models were developed to understand the impact of *C. arvense* on the yield in cereal fields (Donald and Khan, 1996; Rasmussen and Nielsen, 2020), without considering neither cropping practices, nor pedoclimate and field environment. In the literature, some models simulate the long-term effect of cropping systems on multiple weed species and quantify the impact of weeds through multiple criteria including yield loss (Colbach et al., 2021), but most of these models do not include perennial species. Models and/or decision support tools dedicated to perennial weeds are scarce. They focus only on non-chemical cropping practices (Favrelière et al., 2016), and on chemical efficiency (Liu et al., 2019), but do not consider the interaction of cropping practices with pedoclimate and field environment. In addition, they are not designed to assess cropping systems or to be used as an educational tool to design innovative cropping systems. Our objectives are: (i) to identify the most significant cropping practices and pedoclimate variables, and their combinations impacting the growth of *C. arvense*, (ii) to better understand their efficacy to replace chemical-only control methods, (iii) to determine interactions between cropping practices and pedoclimate to tackle the complexity of a limited part of agroecosystems, and finally (iv) to develop an evaluation tool for farmers and advisers through a consensual model, simple to use.

MATERIALS AND METHODS

IPSIM Method Using the DEXi Software

The conception of the *C. arvense* model relies on the IPSIM platform. IPSIM, i.e., Injury Profile SIMulator, was first designed by (Aubertot and Robin, 2013). IPSIM is a generic modeling method which aims at apprehending cropping practices, pedoclimate and environmental factors to explain injuries caused by a single or several pests, on a specific or a set of crops. Cropping practices refer to all the cultivation techniques used in the process of crop production (e.g., tillage, harvest, sowing, etc.), pedoclimate refer to the soil and weather components impacting the development of the considered pest (e.g., soil texture, rainfall, temperature, etc.), and field environment to the abiotic or biotic factors encountered in the field surroundings (e.g., field margins, host plants, etc.). All these components are selected according to their significance in the explanation of the injury profile of the considered pests.

This platform requires the organization of its hierarchy according to a specific plan, implemented with the DEXi software (Bohanec, 2020). The DEX method, implemented by the software DEXi, supports qualitative hierarchical attribute aggregation. Originally, this method was designed as a decision modeling method based on the subdivision of a complex problem into smaller and less complex subproblems. These subproblems are represented by hierarchically structured attributes, i.e., variables that characterize the complex problem. Terminal attributes of the hierarchy represent inputs (or input indicators), while the root represents the main output of the model. Any number of aggregated attributes (internal nodes in the hierarchy) can be placed between inputs and outputs; they correspond to subproblems and represent intermediate or partial outputs of evaluation. A DEX model is used so that the input attributes are filled in by the user of the model, providing a description of the problem at hand. Then, the values of aggregated attributes are determined with the aggregation of the corresponding input attributes or underlying attributes. The aggregation takes place in accordance with aggregating tables, previously formulated by domain experts. Aggregating tables consist of elementary "if-then" rules that describe output values for all combinations of input values. Each aggregated attribute in the model has an associated aggregating table. During and after their construction, all tables are verified by DEXi for completeness and consistency. Attributes used in the model are qualitative variables, either ordinal or nominal. The use of quantitative variables is not possible directly through the DEXi software, however upstream converters can be designed to discretize quantitative variables before they are used in the model, or to convert nominal variables into ordinal ones (e.g., the name of a cultivar can be converted into a qualitative level of resistance to a disease).

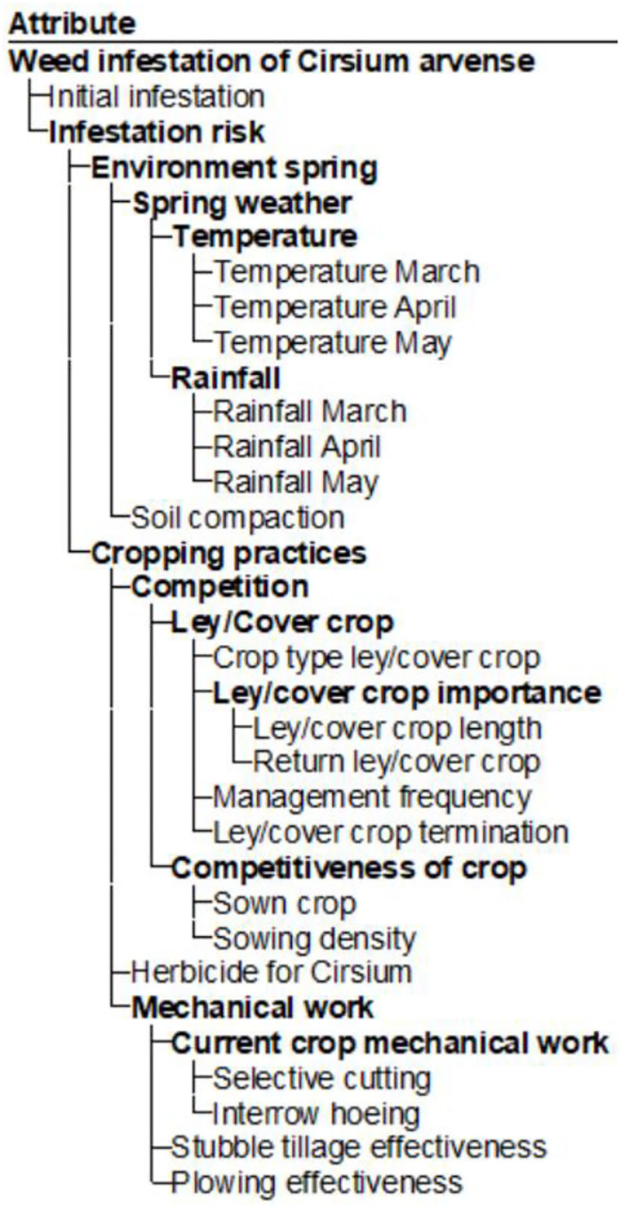
The building process of an IPSIM model requires three steps: (i) identifying and structuring the attributes, (ii) defining attribute scales, and (iii) defining the aggregating tables (Aubertot and Robin, 2013).

Definition of Attributes

The IPSIM method aims at apprehending a wide variety of factors or indicators to model an injury profile. A generic pattern of IPSIM main attributes is to consider any factor that might harm or benefit to the single or multiple modeled organisms, directly (e.g., control method) or indirectly (e.g., type of soil). These factors are considered either punctual or on a larger scale to be considered for several years. IPSIM-*Cirsium* is a static deterministic model. IPSIM-*Cirsium* aims at representing only the infestation of *C. arvense* in an identified field. Therefore, the output of the model is defined to express a weed infestation rate, represented as a qualitative variable. This qualitative output variable can be translated into quantitative variables as density (number of shoots/m²), biomass above ground (g/m²), or percentage of covering of the field.

Factors were chosen first according to the literature with keywords involving general and generic growing factors (e.g., Temperature, Rainfall, Soil, Relative Humidity, Photoperiod) and control methods (e.g., Competitive crops, Cover crops, Tillage, Cropping practices, etc.) related to *C. arvense.* This literature analysis was made using commercial databases (EconLit, Food Science Source, Web of Science, MEDLINE®, Saga Web, Scopus, TAIR) and free databases (Google Scholar, Agricola, ProdINRA, PubMed). The list of beneficial and detrimental factors was then confronted to experts during workshops to co-design the model. The experts were chosen nationally from research institutes (INRAE), technical institutes (Arvalis, Terres Inovia, Acta), and Chambers of Agriculture according to their participation on *Cirsium arvense* control programs or expertise. These co-design workshops aimed at validating the input attribute choices, structure, and interactions and to identify new attributes that could have been omitted.

The aggregated attributes of IPSIM-*Cirsium* as main factors were defined according to (i) the weed environment and (ii) the weed management methods. The weed environment is spatially limited to the considered field and temporally to the current year of evaluation of weed infestation rate. Control methods were chosen to also focus on the spatial field environment and the transfer of individuals of *Cirsium arvense* between fields due to skipped cleaning of tool was omitted here in the sake of simplicity. However, control methods were considered in the 4 years preceding the evaluation of the weed infestation. This wide time window for control methods is explained by the perennial character of *Cirsium arvense*. Input attributes are then chosen as indicators of the risk of infestation linked to the environment of the field and control methods efficacy that impact the growth of *C. arvense*. The structure of attributes of IPSIM-*Cirsium* is presented in **Figure 1**.



Attribute

- **Weed infestation of Cirsium arvense**
  - Initial infestation
  - **Infestation risk**
    - **Environment spring**
      - **Spring weather**
        - **Temperature**
          - Temperature March
          - Temperature April
          - Temperature May
        - **Rainfall**
          - Rainfall March
          - Rainfall April
          - Rainfall May
      - Soil compaction
    - **Cropping practices**
      - **Competition**
        - **Ley/Cover crop**
          - Crop type ley/cover crop
          - **Ley/cover crop importance**
            - Ley/cover crop length
            - Return ley/cover crop
          - Management frequency
          - Ley/cover crop termination
        - **Competitiveness of crop**
          - Sown crop
          - Sowing density
      - Herbicide for Cirsium
      - **Mechanical work**
        - **Current crop mechanical work**
          - Selective cutting
          - Interrow hoeing
        - Stubble tillage effectiveness
        - Plowing effectiveness

FIGURE 1 | Hierarchical structure of IPSIM-Cirsium. Bold and not in bold terms represent aggregated and basic attributes, respectively. (20 basic attributes and 13 aggregated attributes) (screenshot of the DEXi software).

Attribute Scales

The following step is to set scale values to each attribute. Aggregated and input attributes of IPSIM-*Cirsium* have either two or three levels of scale (e.g., *Unfavorable, Moderately* *favorable,* and *Favorable*). They are represented by words and can be either ordinal or nominal. *Unfavorable* means that this attribute is detrimental to the user and therefore detrimental to the control of *Cirsium arvense* (**Figure 2**). This scale order is designed directly under DEXi software and will be prevalent for the establishment of aggregating tables.

Scale values are sometimes a result of a conversion of quantitative or qualitative variables. For example, the amount of rain per month is categorized into three levels: *Favorable to Cirsium, Moderately Favorable to Cirsium,* and *Unfavorable to Cirsium*. The levels of this attribute were defined by a converter using two thresholds. Some attributes are purely descriptive and need to be converted prior being used in the model. A converter is then used to qualify this information into a qualitative value that can be used by the model (e.g., Tools used for the cover crop destruction must be categorized as *Favorable* or *Unfavorable* to the development of *Cirsium arvense*). The corresponding converters are defined by considering international literature and expertise, and need to be adapted for each considered region, especially for the sowing rate of the crop.

In a few instances, attributes are described by a two-level scale (e.g., for *Return of the ley* attribute, the user only must specify if he had ley in the three preceding years or not). Attributes generally have a three-level scale (e.g., *Sown crop* can generate a closed, moderately closed, or open canopy). The output attribute of the model IPSIM-*Cirsium* has a four-level scale (i.e., *Very low infestation, Low infestation, Intermediate infestation, and High infestation*). We chose to define four levels of infestation in order to describe the evolution of infestation throughout several years.

Aggregating Tables

The last step to build an IPSIM model is the definition of aggregative tables for each aggregated attribute and the output of the model. During the aggregation of underlying attributes in the attribute tree, decision rules must be edited to characterize any aggregation possibilities. Collectively, these rules were initially called "Utility functions." These aggregative rules are simple "*if-then*" functions that enable the model to provide a specific answer to any situation it is confronted to. Aggregative tables are represented in a tabular form in the DEXi software and aim at considering scale orders of the underlying attributes (**Figure 3B**).

To consider each aggregation possibility, we consider all the combinations of scale levels of the underlying attributes. For example, *Competitiveness of crop* is composed of the aggregation of *Sown crop* and *Sowing density*. *Sown crop* and *Sowing density* are both three-level-scaled attributes. Therefore, nine aggregation possibilities need to be explored for the aggregated attribute *Competitiveness of crop*. Each possibility needs to be filled row by row. This process enables a high level of flexibility for each situation encountered. Aggregating tables are defined using literature and expert knowledge, as summarized in **Table 1**. However, some situations lack scientific consensus in the literature, especially in the combination of several cropping practices. This problem was fixed with expert knowledge. Yet, some possible decisions are sometimes marred with subjectivity of the experts during the process of filling in the aggregative rules.

Calculation of Weights

Weights are widely used in model analysis to describe the importance of each attribute. Weights are defined by the aggregative tables defined at each aggregation of attributes. Originally mainly used on quantitative models, the DEX method managed to adapt weight calculation to qualitative models, too. Weights are obtained by constructing a hyperplane that approximates the points (decision rules) of an aggregative table, to minimize the least squares criterion. Relative weights are then calculated from the slope of this hyperplane: the higher the slope in the direction of an attribute, the higher the weight of this attribute (Bohanec, 2020).

There are four types of weights: *local* and *global* weights, *normalized* or not. Normalized weights consider the number of values per scale (analysis of the weight of IPSIM-*Cirsium* will rely on normalized weights only); they are calculated by normalizing all scales to the unit interval, thus ruling out the effect of scales having different numbers of values. Local weights are described for each aggregate attribute and the corresponding aggregative table, regardless of attributes and functions elsewhere in the model. Consequently, the sum of the local weights of attributes underlying each aggregated attribute equals to 100%. In contrast, global weights represent the importance of attributes in the context of the whole model. For each attribute, they are calculated by multiplying the local weight of that attribute with the global weight of its parent attribute. The global weight of the root attribute is assumed to be 100%. In this way, the sum of all the input attributes' global weights in the model is 100%, too. For example: if we consider the global normalized weight of *Competitiveness of crop* (2%) and the local normalized weight of *Sown crop* (50%), the global normalized weight of *Sown crop* is 1% (2% × 50%), as shown in **Table 3**.

These weights enable an approximate overview of the importance of each attribute, input, or aggregated ones. It is an equivalent of sensitivity analysis for quantitative models (Aubertot and Robin, 2013). Weights can also be used to define the aggregative tables, in a reverted strategy of modeling with DEXi software. This strategy was left out in favor of the description of each situation row by row, taking into account the literature and expert knowledge available.

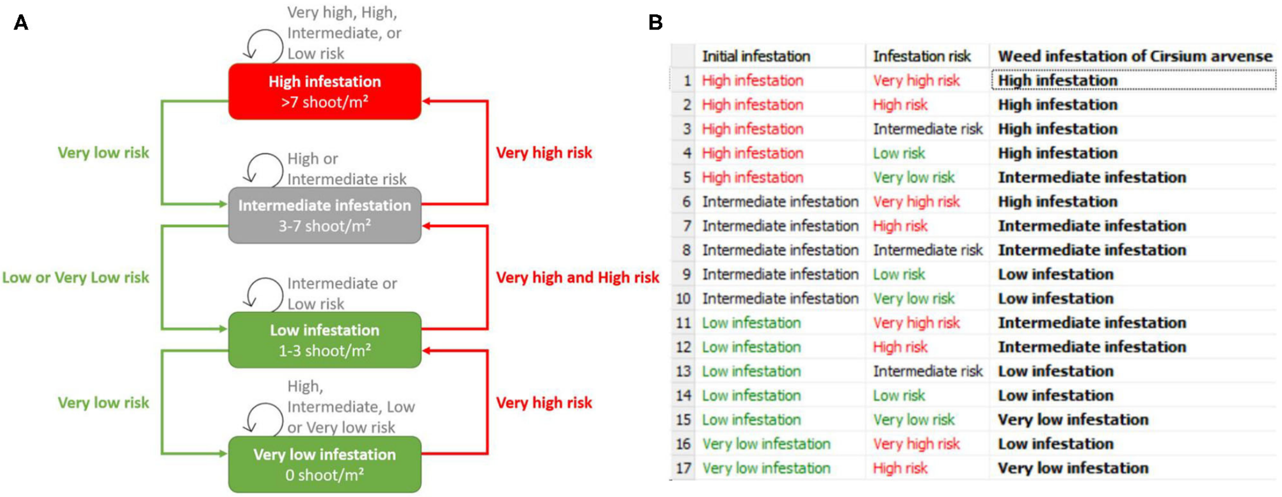
Assessment of the Predictive Quality of IPSIM-*Cirsium*

Data Collection

Several datasets (D) were used in the evaluation of the predictive quality of IPSIM-*Cirsium*, summarized in **Table 2**. D1 was collected at the INRAE experimental farm in Bretenière (47°14' 11.2" N, 5°05'56.1" E), 15 km southeast of Dijon, France. The complete description of the long-term cropping system experiment (crop sequence and associated management, including intensity of tillage herbicide, use herbicide types, mechanical weeding, etc.) implemented from 2000 to 2017 was synthesized by Adeux et al. (2019). The reference cropping system (CS) called S1 was characterized by a 3-year oilseed rape—winter wheat—winter barley rotation, systematic moldboard plowing in summer-autumn and herbicides as sole curative weed

Attribute	Scale
Weed infestation of Cirsium arvense	**High infestation**; Intermediate infestation; ***Low infestation***; ***Very low infestation***
Initial infestation	**High infestation**; Intermediate infestation; ***Low infestation***; ***Very low infestation***
Infestation risk	**Very high risk**; **High risk**; Intermediate risk; ***Low risk***; ***Very low risk***
Environment spring	**Favorable environment for Cirsium**; Moderately favorable environment for Cirsium; ***Unfavorable environment for Cirsium***
Spring weather	**Favorable weather for Cirsium**; Moderately favorable weather for Cirsium; ***Unfavorable weather for Cirsium***
Temperature	**High temperature**; Average temperature; ***Low Temperature***
Temperature March	**Unfavorable**; Moderately favorable; ***Favorable***
Temperature April	**Unfavorable**; Moderately favorable; ***Favorable***
Temperature May	**Unfavorable**; Moderately favorable; ***Favorable***
Rainfall	**High amount of rain**; Average amount of rain; ***Low amount of rain***
Rainfall March	**Unfavorable**; Moderately favorable; ***Favorable***
Rainfall April	**Unfavorable**; Moderately favorable; ***Favorable***
Rainfall May	**Unfavorable**; Moderately favorable; ***Favorable***
Soil compaction	**High compaction**; Moderate compaction; ***Low compaction***
Cropping practices	**Ineffective practices**; Moderately effective practices; ***Effective practices***
Competition	**Ineffective competition**; Moderately effective competition; ***Effective competition***
Ley/Cover crop	**Low effectiveness of ley/cover crop**; Moderate effectiveness of ley/cover crop; ***High effectiveness of ley/cover crop***
Crop type ley/cover crop	**Open canopy**; Moderately closed canopy; ***Closed canopy***
Ley/cover crop importance	**Low importance**; Medium importance; High importance
Ley/cover crop length	**Unfavorable length**; Moderately favorable length; ***Favorable length***
Return ley/cover crop	**Long ley return**; ***Short ley return***
Management frequency	**Low management**; Moderate management; ***High management***
Ley/cover crop termination	**Unfavorable termination**; Moderately favorable termination; ***Favorable termination***
Competitiveness of crop	**Low competitiveness**; Moderate competitiveness; ***High competitiveness***
Sown crop	**Open canopy**; Moderately closed canopy; ***Closed canopy***
Sowing density	**Unfavorable rate**; Moderately favorable rate; ***Favorable rate***
Herbicide for Cirsium	**No herbicide**; Moderately effective herbicide; ***Highly effective herbicide***
Mechanical work	**Ineffective mechanical work**; Moderately effective mechanical work; ***Effective mechanical work***
Current crop mechanical work	**Ineffective current crop practices**; Moderately effective current crop practices; ***Effective current crop practices***
Selective cutting	**Unsatisfying num of repetition**; Neutral; ***Satisfying num of repetition***
Interrow hoeing	**Unsatisfying num of repetition**; Neutral; ***Satisfying num of repetition***
Stubble tillage effectiveness	**Ineffective stubble tillage**; Moderately effective stubble tillage; ***Effective stubble tillage***
Plowing effectiveness	**Ineffective plowing**; Moderately effective plowing; ***Effective plowing***

FIGURE 2 | Attribute scales of IPSIM-Cirsium. All of the scales are ordered from values favorable to *Cirsium arvense* (i.e., detrimental to the user) on the left-hand side (Red color) to values unfavorable to *Cirsium arvense* (i.e., beneficial to the user) on the right-hand side (Green color). (screenshot of the DEXi software).



	Initial infestation	Infestation risk	Weed infestation of Cirsium arvense
1	High infestation	Very high risk	**High infestation**
2	High infestation	High risk	**High infestation**
3	High infestation	Intermediate risk	**High infestation**
4	High infestation	Low risk	**High infestation**
5	High infestation	Very low risk	**Intermediate infestation**
6	Intermediate infestation	Very high risk	**High infestation**
7	Intermediate infestation	High risk	**Intermediate infestation**
8	Intermediate infestation	Intermediate risk	**Intermediate infestation**
9	Intermediate infestation	Low risk	**Low infestation**
10	Intermediate infestation	Very low risk	**Low infestation**
11	Low infestation	Very high risk	**Intermediate infestation**
12	Low infestation	High risk	**Intermediate infestation**
13	Low infestation	Intermediate risk	**Low infestation**
14	Low infestation	Low risk	**Low infestation**
15	Low infestation	Very low risk	**Very low infestation**
16	Very low infestation	Very high risk	**Low infestation**
17	Very low infestation	High risk	**Very low infestation**

FIGURE 3 | Decision rules for final level of infestation ("Weed infestation of *Cirsium arvense*"). Rules are designed according to the Initial level of infestation, represented in **(A)** by square labeled here as four-level scale : *High infestation*, *Intermediate infestation*, *Low infestation*, and *Very low infestation* (Green cells are beneficial for the field, Gray cell is neutral for the field and Red cell is detrimental for the field); and the Risk of infestation, represented by arrows, enabling to lower the infestation of *C. arvense* (Green downward arrows), increase the infestation (Red upward arrows), or maintain the same level of infestation (Gray circular arrows). **(B)** Represents the "Weed infestation of *Cirsium arvense*" decision rules translated in the DEXi software (screenshot of the DEXi software) for the 20 possible combinations (four level of Initial infestation, five level of Infestation risk).

management tool. All alternative cropping systems (S2, S3, S4, and S5) were designed to mimic farmers aiming at reducing herbicide reliance through contrasted agronomical pathways and resulted in more complex 6-year rotations. S2 was a transition from reduced tillage (i.e., no inversion tillage, 2001–2010) to no-till conservation agriculture (2010–2017). S3, S4, and S5 implemented moldboard plowing every 2 years on average over the 2001–2017 period. However, weed management relies

TABLE 1 | Literature on the effects of climate, soil and cropping practices on the growth of *Cirsium arvense*.

Factors	Direction	Intensity	Impact on *C. arvense*	References
Temperature	–	+	Temperature increases the germination and growth of shoots of *C. arvense*	Bostock, 1978; Wilson, 1979; Sciegienka et al., 2011
Rainfall	–	+	Probability of emergence and biomass production of *C. arvense* increase when water regime increases	Hamdoun, 1972; Wilson, 1979; Liew et al., 2012
Soil compaction	–	+	Compaction due to tractor weight do not impact the growth of *C. arvense*, it even gives *C. arvense* a small advantage over other plants and weeds	Hausman et al., 2010; Brandsæter et al., 2011; Hochstrasse et al., 2012
Ley	+	+ + +	Alfalfa, hemp, rye, grass or leguminous meadow on a 3-year period help reduce *C. arvense*	Edwards et al., 2000; Hochstrasse et al., 2012; Weill, 2015; Favrelière, 2019
Cover crop	+	+	Cover crop can be used to smother the weed and help reduce the weed infestation	Lukashyk et al., 2008; Thomsen et al., 2015
Competitive crop	+	+	Some crop such as long straw cereals maintain a high level of competition against *C. arvense*	Rasmussen, 2011; Melander et al., 2012; Taramarcaz, 2019
Herbicide	+	+ + +	Herbicides are a curative way to control *C. arvense*. Used at early stage and during several years, they efficiently control the weed.	Hume, 1982; Verwijst et al., 2017; Tavaziva et al., 2019
Selective cutting	+	+	Selective cutting is an efficient control operation for *C. arvense,* it reduces number of shoots over years	Hansen, 1918; Lukashyk et al., 2008; Verwijst et al., 2017; Tavaziva et al., 2019
Interrow hoeing	+	++	Repeated interrow hoeings enable a great control of aerial shoots of *C. arvense*	Graglia et al., 2006; Campiglia et al., 2012
Stubble tillage	+	++	Efficient mechanical control against *C. arvense* lowers the regrowth capacity, and increasing the depth exhausts the weed. If followed by dry weather, uprooting the weed helps the decay of it, especially before the carbohydrate mobilization by the root system	Lukashyk et al., 2008; Armengot et al., 2015; Thomsen et al., 2015; Brandsæter et al., 2017; Taramarcaz, 2019
Plowing	+	++	Plowing enables a destruction of the root system of *C. arvense*, added to tillage it helps the destruction of the weed	Pekrun and Claupein, 2004; Brandsæter et al., 2011; Hochstrasse et al., 2012; Thomsen et al., 2015; Weill, 2015

The factor can be beneficial (+) or detrimental (–) to control C. arvense. Intensity of the effect is represented with 3 levels: low (+), moderate (++), and high (+ + +).

uniquely on herbicide in S3, on mechanical tools and herbicide in S4 and only on mechanical tools in S5 (Adeux et al., 2019). Cover crop was sown since 2007 in each of the summer fallow period of a preceding spring or summer crop. Alfalfa was implemented for 1–3 years in S5. These four alternative CS also implemented a wide array of preventive and cropping weed management tools such as false seedbed techniques, delayed sowing of winter cereals, and higher seeding rates. The set of decision rules characterizing each of the five cropping systems was replicated on two blocks (in a 1.7 ha field). All individual farming operations were recorded from 1999 to 2017 in the 10 fields. The abundance of *Cirsium arvense* was assessed every year from 2002 to 2017, at crop flowering, after all weeding operations, by counting the density of shoots in 8 fixed zones per field with four and one 0.36 m^2 quadrats in 2001–2013 and 2014–2017, respectively. Since the zones were fixed over the 2001–2017 period, the maximal density recorded in the four quadrats per zone over the 2001–2013 period was selected to be representative of the zone level, to assess the evolution of *Cirsium arvense* with 1280 surveys (i.e., eight zones by five cropping systems by two blocks × 16 years). Maximal density was chosen here to represent the Potential of Infestation described in Adeux et al. (2017).

D2 was conducted in Sours (48°24′38.16″N, 1°35′53.16″ E), France. Three systems were surveyed from 2011 to 2020: Autonomous system, Dr. Durupt system and Productor system. These three systems were all conducted in organic conditions with different intensity of tillage, ley implement and rotation as *Cirsium arvense* control methods. Autonomous and Dr. Durupt systems were conducted in CAPABLE project (CASDAR AAP IP 2017) in a system experiment. Alfalfa was implemented for 3 years, with three management per year (e.g., chopping, mowing). No cover crop was implemented in these experiments. The compaction of soil was characterized as moderate. In Dr. Durupt, Autonomous and Productor systems, intensity of stubble tillage

TABLE 2 | Summary of the datasets used in the evaluation of the predictive quality.

Dataset	Number of cropping systems	Years of observation	Number of observations	Assessment methods	Location
D1	5	2001–2017	1,280	Density of shoot: 32 random quadrats from 2001 to 2013, 8 random quadrats from 2014 to 2017	Bretenière, France
D2	3	2011–2020	117	Density of shoot: 16 fixed quadrats Chicouène method assessment	Sours, France
D3	5	2015–2017	120	Density of shoot: 16 fixed quadrats	Salvagnac, France

was low, high, and average, respectively. Dr. Durupt system was plowed every 2 years, Autonomous system every year, and Productor system only once every 4 years. Selective cutting and interrow hoeing intensity were low in the three systems. The abundance of *Cirsium arvense* was assessed every year in June with two different methods. Autonomous and Productor systems were surveyed according to the Chicouène method (Chicouene and Arbiotech, 2000) and Dr. Durupt and Autonomous systems were surveyed with the use of 16 0.5 m²-fixed-quadrats from 2018 to 2020. Only the Chicouène method was performed from 2011 to 2020, resulting in a single abundance value per system per year.

D3 was conducted in Salvagnac (43°54′23.0″N, 1°41′19.0″ E), South of France. Five cropping systems were evaluated from 2015 to 2017: Progressive Tillage Control, Progressive Sustainable Tillage Control, Cover crop, Shallow Tillage Control, and Shallow Sustainable Tillage Control. These different factors were all tested on the same field, on bare soil except for the cover crop (Sorghum) in the Cover crop treatment. Progressive Tillage Control consisted of an increase of 5 cm depth for each stubble cultivation performed each month. Progressive Sustainable Tillage Control consisted of an increase of 5 cm depth for each stubble cultivation performed whenever *Cirsium arvense* reached five-leaf stage. Cover crop consisted of the use of sorghum (*Sorghum sudanense*), chopped during summer to control *Cirsium arvense*, sown every year in May. Shallow Tillage Control consisted of repetitions of stubble cultivation at 8–10 cm depth every month, while Shallow Sustainable Tillage Control was performed at 8–10 cm whenever *Cirsium arvense* reached five-leaf stage. All the cropping systems are conducted in organic conditions, without the use of any herbicide (organic or not). No selective cutting nor interrow hoeing were performed. Plowing was performed once every four years. Each cropping system was repeated in three blocks. The number of shoots of *Cirsium arvense* was assessed every year in four fixed plots in each cropping system, composed of four quadrats of 0.25 m². The four quadrats of each plot were then summed. D3 assessed 60 values of *C. arvense* density per year, resulting in 120 values for 2016 and 2017.

These survey values were then translated into four levels of infestation according to the scale of the output attribute of IPSIM-*Cirsium*: *Very low*, *Low*, *Intermediate*, and *High* corresponding to 0 thistle/m², 0.01–2.99 thistle/m², 3.00–6.99 thistle/m², and ≥7thistle/m², respectively. This scale was developed according to co-design workshops.

Statistical Analysis

The evaluation of the predictive quality of IPSIM-*Cirsium* was performed by comparing calculated values (outputs of the model) and observed values (in the field experiment), described earlier. Values were calculated for June of each year, therefore calculated values were compared to values observed in June. The comparison of values led to the construction of a confusion matrix. The confusion matrix is a table that shows the performance of an ordinal or nominal model where rows represent observed values and columns represent calculated values. To summarize confusion matrix, several metrics were computed to evaluate the predictive quality of IPSIM-Cirsium: accuracy, quadratic weighted Cohen's kappa, precision, recall, and F1-Score. The accuracy is the number of correctly calculated values (i.e., calculated value is equal to observed value) among all the calculated values (Nguwi and Cho, 2010), defined as:

$$Accuracy = \frac{A}{N}$$

where A is the number of correctly assigned calculated values and N the number of calculated values. On the other hand, Cohen's kappa is expressing a score of agreement level between two annotators: observed and calculated value (Cohen, 1960), described as:

$$\kappa \equiv \frac{p_o - p_e}{1 - p_e}$$

where κ is the agreement among observed and calculated annotators (p_o, the relative observed agreement; p_e, the expected agreement when both annotators are randomly chosen). κ rates in between -1 and $+1$ and can be interpreted as the proportion of variability explained by the model (Fleiss and Cohen, 1973). Values of κ describe the agreement between observed and calculated annotators: <0; 0.01–0.20; 0.21–0.40; 0.41–0.60; 0.61–0.80; and 0.81–1 values qualify the agreement as Poor; Slight; Fair; Moderate; Substantial; and Almost perfect, respectively (McHugh, 2012). F1 score is calculated from the precision p (number of correctly calculated values divided by the number of observed values for each class) and the recall r (number of correctly calculated results divided by the total of calculated values for each class), expressing the harmonic mean between

TABLE 3 | Normalized weights of IPSIM-Cirsium.

Attributes defining the final weed infestation	Local level						Global level					
	1	2	3	4	5	6	1	2	3	4	5	6
. Initial infestation	**66**						**66**					
. Infestation risk	**34**						**34**					
. . Environment spring		**33**						**11**				
. . . Spring weather			**57**						**7**			
. . . . Temperature				**50**						**3**		
. . . . . Temperature March					33						1	
. . . . . Temperature April					33						1	
. . . . . Temperature May					33						1	
. . . . Rainfall				**50**						**3**		
. . . . . Rainfall March					33						1	
. . . . . Rainfall April					33						1	
. . . . . Rainfall May					33						1	
. . . Soil compaction			43						5			
. . Cropping practices		**67**						**23**				
. . . Competition			**30**						**7**			
. . . . Ley/Cover crop				**71**						**5**		
. . . . . Crop type ley/cover crop					13						1	
. . . . . Ley/cover crop importance					**42**						**2**	
. . . . . . Ley/cover crop length						43						1
. . . . . . Return ley/cover crop						57						1
. . . . . Management frequency					26						1	
. . . . . Ley/cover crop termination					19						1	
. . . . Competitiveness of crop				**29**						**2**		
. . . . . Sown crop					50						1	
. . . . . Sowing density					50						1	
. . . Herbicide for Cirsium			35						8			
. . . Mechanical work			**35**						**8**			
. . . . Current crop mechanical work				**26**						**2**		
. . . . . Selective cutting					33						1	
. . . . . Interrow hoeing					67						1	
. . . . Stubble tillage effectiveness				41						3		
. . . . Plowing effectiveness				33						3		

The "local" and "global" weights, expressed in %, are calculated for each aggregated attribute separately and are distributed in six levels of aggregation. Bold and not in bold terms represent aggregated and basic attributes, respectively. Each additional dot in front of the attribute stands for a new lower level.

precision and recall, defined as:

$$F1 - score = \frac{2\sum_{i=1}^{N} \frac{p_i^{*} r_i}{p_i + r_i}}{N}$$

with N the number of class, p_i the precision of class i and r_i the recall for class i. These calculations were performed using RStudio© Version 1.1.456 (Studio, Inc., 2009–2018).

RESULTS

Presentation of ISPIM-*Cirsium* Model

Hierarchical Organization of Attributes

IPSIM-Cirsium was designed focusing on the *Risk of infestation* of *Cirsium arvense* and the *Initial infestation* level observed the year preceding the evaluation year. The possible evolutions from one level of infestation to another are described according to decision rules illustrated in **Figure 3**. The risk of infestation is calculated for June, before the harvest during summer. The risk is based on the two main sub-trees *Environment spring* describing the pedoclimate of the field during March, April and May of the evaluation year, and *Cropping practices* describing the crop management of the field to control *Cirsium arvense* during the four preceding years of the evaluation year.

The first sub-tree of IPSIM-*Cirsium* (**Figure 1**), *Environment spring* focuses on two main indicators:

(i) *Weather* during March, April, and May of the evaluation year. To describe the weather, two factors were chosen: the average *Temperature* and the accumulated *Rain*. These two factors are described per month and an aggregation of the 3 months was then calculated. Thresholds of the converter used to describe the average temperature and accumulated rain per month were defined according to literature and expert knowledge.

(ii) *Compaction of soil* of the evaluated field. This indicator describes the compaction of soil during March, April, and May. The compaction of soil is assumed to be constant during this period. Compaction of soil is here seen as an indirect factor favorable to *Cirsium arvense*, benefiting from the lack of competition provoked by compaction of soil. Type of soil is not directly used as an attribute in the model but is indirectly impacting the compaction of soil.

The second sub-tree describes the *Cropping practices* on a four-year period preceding the evaluation year, with the help of three main factors:

(i) *Competition* includes practices implemented before the evaluated year, such as *ley* or *cover crop,* and during the evaluated year, such as the crop competitiveness. *Ley* or *cover crop* are described with four indicators such as the species used in the ley or cover crop (*Crop type ley/cover crop*), the *Importance* of ley and cover crop calculated with the length of the ley or cover crop (*Length ley/cover crop*) and the length of time since the destruction of the last ley or cover crop (*Return ley/cover crop*), the number of *Management* of the ley or cover crop (e.g. chopping, mowing, and pasture), and finally the *Termination method* of the ley or cover crop (e.g., Frost, Plowing, etc.). The *Competitiveness of crop* is described with two indicators: the *Sown crop* which has a score of competitive level for each species described according to literature and expert knowledge; and the *Sowing density* relative to the regional recommendation of sowing for the concerned species. Competitiveness of crop might be impacted by the use of nutrients, however neither consensus between the experts during workshops, nor in the literature was found on the impact of nutrients on the benefit ratio between crop and weed. Indeed, while crop slightly benefit from the nutrient increase, *C. arvense* also benefit from the increase of nutrient (Hume, 1982; Edwards et al., 2000; Líška et al., 2007).

(ii) *Herbicide use frequency* is used in this model as a curative method. However, to be efficient, herbicide must target *Cirsium arvense* and be repeated several years. The description of the use of herbicide is only related to the number of years that an herbicide control is implemented. Thus, this attribute assumes that herbicides were applied in the best conditions and are efficient on controlling *Cirsium arvense*, i.e., regardless of the conditions of application (moisture, temperature, etc.), and whatever the dose applied.

(iii) *Mechanical operations* characterized the physical and mechanical management methods applied during the evaluation year (i.e., *Current crop mechanical work*) and the ones applied in the four preceding years of the evaluation year (i.e., *Stubble tillage effectiveness* and *Plowing effectiveness*). *Current crop mechanical work* is an aggregated attribute composed of two indicators: the *Selective cutting* which aims at the cutting of the aerial part of *Cirsium arvense,* and the *Interrow hoeing* which aims at the weeding of the superficial roots and aerial parts of *Cirsium arvense*. These two indicators are quantified according to the number of passes per year. The more the passes the more effective the practices. *Stubble tillage* considers several indicators such as the tools used, and the number of passes allocated per year for the stubble cultivation. These indicators however can vary along the four preceding years that are considered in the model. Therefore, it is not possible to assume a generic average stubble cultivation. The choice here was to consider each year only the stubble tillage that involves at least three repetitions between the harvest of the previous crop and the sowing of the new one. The number of stubble tillage per year that reach these conditions are counted and will enable to qualify the stubble tillage effectiveness. That way, all the information needed for the model is complete and the input requirement is simplified by omitting all the situations where "wrong" tools are used or the number of passes is too low. *Plowing effectiveness* considers the number of years that at least one inversion tillage is performed along the four preceding years. The IPSIM-Cirsium model has 33 attributes, of which are 13 aggregated and 20 basic attributes.

Selected Attributes and Their Relative Importance

Using weight calculation of attributes, each cropping practice and pedoclimate indicator can be described alone according to their importance to evaluate weed infestation. IPSIM-*Cirsium*, expert and literature-based model correctly reflects the knowledge available to build the model. Cropping practices were chosen to be more relevant in the explanation of *Cirsium arvense* than the environment of the field. This choice was supported both by literature and expert knowledge. Therefore, whenever cropping practices were rated as *Ineffective* to control *Cirsium arvense*, a mild or Favorable climate for the user did not influence the risk of weed infestation that was rated *High* already. *Environment* was thus accredited to a low weight by DEXi software, explained by the low number of rules directly influenced by the grade of its scale. The local normalized weights of *Environment* and *Cropping practices* are 33 and 67% respectively (**Table 3**).

Both *herbicide* control and *competition* control, by means of the use of ley, for example, enable a "cleaning" of the field by their curative aspect. These two methods are often chosen as the most effective practices to control *C. arvense* on a short-term basis. On the other hand, *mechanical* control of *C. arvense* is described as a method that will keep a constant pressure on this weed and more particularly on its sprouting capacity by exhausting root reserve. Therefore, *Competition*, *Herbicide,* and *Mechanical control* have a local normalized weight of 30, 35 and 35%, respectively. These weights match the perception of the expert's knowledge.

Scale of Attributes and the Use of Converters

The use of converters was needed for each input attribute except for the *compaction of soil,* which is qualitatively evaluated according to the observer. All the converters were simple: a book of rules (**Table 4** shows an example for *Cover crop or ley termination* converter rules) is written to describe each possible entry for the user. For each variable, quantitative (e.g.,

TABLE 4 | Converter of *Ley/Cover crop termination* attribute.

Ley/cover crop termination	Scale value
No ley/cover crop	Unfavorable termination
Frost	Unfavorable termination
Herbicide	Unfavorable termination
Rolling	Unfavorable termination
Chopping	Moderately favorable termination
Plowing	Favorable termination
Cultivator	Favorable termination
No termination	Unfavorable termination

A qualitative value is attached to each possible input of the user. Scale value is then used by the model as an input attribute value.

Temperature) or qualitative (e.g., *Cover crop or ley termination*), a qualitative value is associated to be directly used by the model. For some input attributes, regional context was important. Therefore, a regional threshold had to be specified for each location where the model is to be used. For example, the *Sowing density* is evaluated according to regional recommendations. This converter use quantitative references established by [Arvalis (2020); example on wheat sowing density in Centre region, France]. Converters are designed to have a certain genericity and apply to any pedoclimates and cropping practices. Some converters tackle several effects of the considered attribute. For example, *Sown crop* evaluate the competitiveness of the crop with a three-level scale: *Closed canopy, Moderately closed canopy, Open canopy*. To establish this scale, several components of the crop were studied: weed biomass (Gruber and Claupein, 2009; Thomsen et al., 2015), architecture of the plant (Edwards et al., 2000; Lukashyk et al., 2008), and growing speed (Weill, 2015, 2018).

Evaluation of the Predictive Quality

By means of the large dataset, gathering many sites and years (220 situations) with a wide diversity of cropping practices and pedoclimates, it was possible to perform a reliable evaluation of the predictive quality of the model. Calculated values of infestation were very similar to the observed values in field, resulting in a satisfactory evaluation (78.2% of the values were correctly calculated). **Figure 4** illustrates the confusion matrix between observed and calculated values of weed infestation. However, square weighted Cohen's kappa reached 0.543, meaning that slightly more than half of the variability of the observed values were explained by IPSIM-*Cirsium*. Here, kappa interprets the strength of agreement between calculated and observed values as moderate (Landis and Koch, 1977; Altman, 1999). Statistical results are presented in **Table 5**. The evaluation of the predictive quality of the model at the class-scale was less satisfactory. *Very low infestation* was the best evaluated class with 90% of correctly calculated values in this class (**Table 5**), followed by *High infestation* with 42% of correctly calculated values. However, *Low infestation* and *Intermediate infestation* obtained a F1-score of only 11 and 11%, respectively. It can be due to the low number of observations of *Low infestation* and *Intermediate infestation*, representing 5 and 3% of the observations, respectively; or it can also be due to a low predictive quality of the model. IPSIM-*Cirsium* seems to struggle with the evaluation of weed infestation from 1 to 7 shoots/m² (*Low infestation* and *Intermediate infestation*).

DISCUSSION

Interests and Limits of the Modeling of Canada Thistle Management Decisions

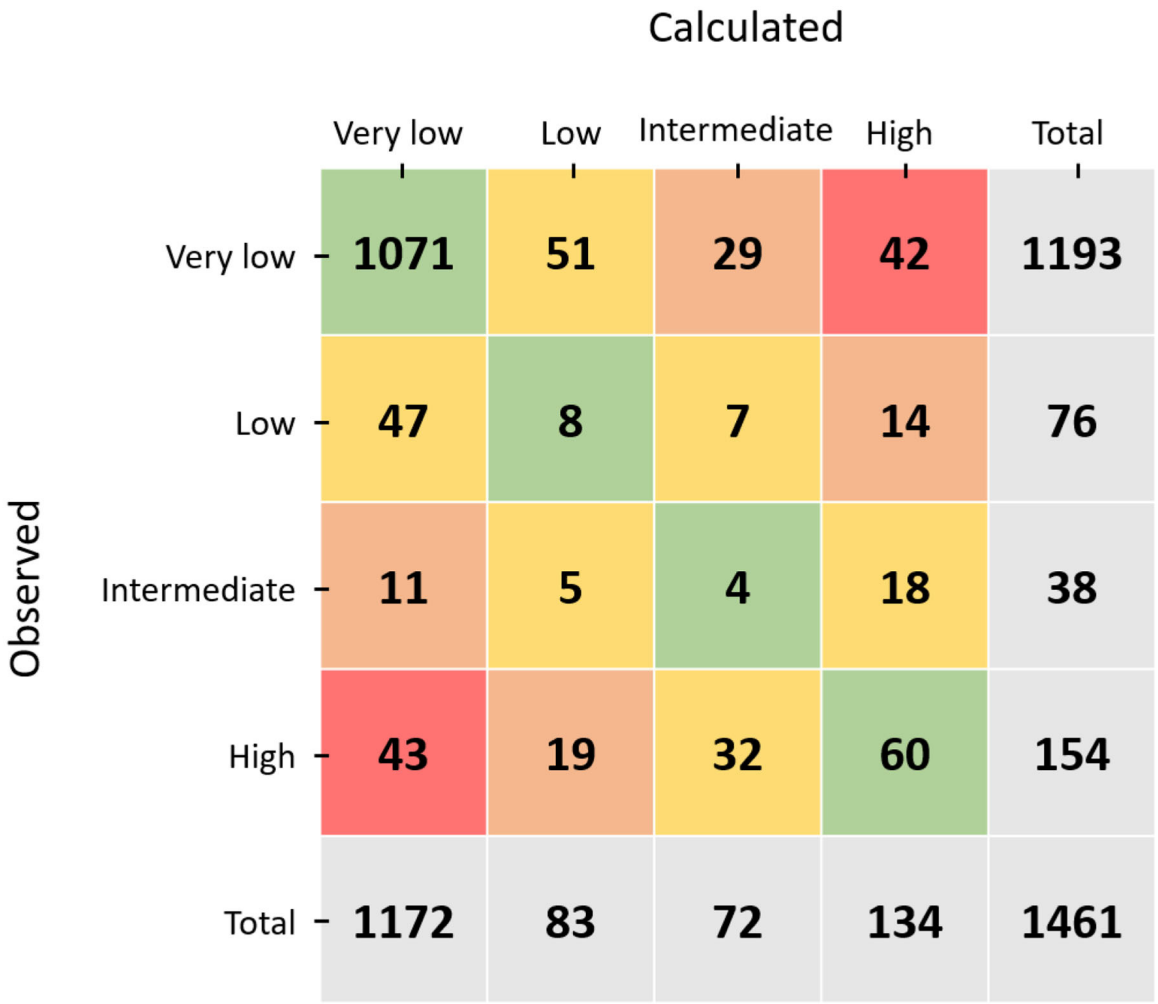
Interests of the Modeling

Multi-Attribute Qualitative Modeling, a Well-Suited Method to Tackle Agroecosystem Complexity

Agroecological management of pests relies on high complexity level systems. Agroecosystems require two integrations: a horizontal integration of the numerous populations of pests and a vertical integration of several combined management methods of pests (Aubertot et al., 2005; Malard et al., 2020). IPSIM-*Cirsium* only tackles the vertical integration of practices to control specifically *C. arvense*. The combination of partial effects practices and the interaction of *C. arvense* with the environment of the agroecosystem are the main bases of the agroecological management of pests. However, the impact of the combination of practices on pests is difficult to quantify because of the diversity and complexity of interactions of cropping practices, pedoclimate and field environment. It appears difficult to take all the possible interactions into account for the evaluation of *C. arvense* infestation.

Qualitative modeling approach enables the inclusion of numerous cropping practices, pedoclimates and field environments while considering their interactions. The DEX method used in the modeling approach permits to solve a complex decision problem by the evaluation of many simpler sub-problems. Furthermore, qualitative modeling is well suited to grasp large complex systems by reducing the complexity level of each attribute into a three or two levels scale. Integrated weed management gathers many cropping practices from soil cultivation to choice of sown crops (Rasmussen, 2011). It is important to focus on the aspect of each method that will determine its effectiveness (e.g., number of tillage instead of the type of tool used for cultivation) and to simplify it to a qualitative variable with a three- or two-level scale, i.e., *Effective, Moderately effective, Ineffective*. The interactions of cropping practices, pedoclimates and field environments are then easier to characterize with a defined number of rules according to aggregating tables. The IPSIM method rather focus on the accuracy of the model than on its precision (Aubertot and Robin, 2013).

The accuracy of IPSIM-*Cirsium* is 0.78, making IPSIM-*Cirsium* a highly accurate model of infestation of *C. arvense*. The precision of each control method alone is relatively low with a description of each control method made according to a single attribute (except for the description of ley and cover crop use, and competitiveness of crop), but the interactions between cropping practices, pedoclimate and field environment is well described. Attributes were first described with all the information available and then were simplified to a maximum to better discretize the



	Very low	Low	Intermediate	High	Total
Very low	1071	51	29	42	1193
Low	47	8	7	14	76
Intermediate	11	5	4	18	38
High	43	19	32	60	154
Total	1172	83	72	134	1461

FIGURE 4 | Confusion Matrix of IPSIM-Cirsium. Color of cells are determined with the level of difference between calculated and observed values of weed infestation: Green cells are perfectly matched (0 level of difference), Yellow cells have one level of difference, Orange cells have two levels of difference, and Red cells have three level of differences expressing the furthest distance between calculated and observed values of weed infestation.

multiplicity of complex interactions between attributes. Stubble tillage was for example hard to define because many factors impact its effectiveness (e.g., *Weather after cultivation, Number of passes, Choice of tools, Depth of tools, etc.*). Further, factors such as *Choice of tools* are described as non-significative (Moulin, 2011) or with marginal effect. Stubble tillage can then be simplified to the number of passes only. Simplifications of attributes might be seen as responsible for a reduction in accuracy of the model, by neglecting variability of the effectiveness of cropping practices, pedoclimates or field environments. Nevertheless, to widely integrate the vertical dimension of *C. arvense* control, it is necessary to tackle a large panel of control methods merely described.

Weed Infestation Indicator, Annual or Perennial

Cirsium arvense, as other perennial weeds is hardly manageable on a single year and requires a long-term approach to tackle a massive infestation (Weill, 2018). The IPSIM approach permits to take into account several years-factors. Some adaptations can be done by considering cropping practices on a wider temporal window and characterizing these cropping practices as *Favorable*, *Moderately favorable* or *Unfavorable* to weed control. This approach was applied for many cropping practices to ensure that the effectiveness of the practice was correctly evaluated in regard to the previous year's practices. Indeed, considering long term methods such as *Stubble tillage* on a 2-year period would have been marred with errors. Stubble tillage on perennial weeds is effective only after 2–3 years (Régis Hélias, personal communication, April 28, 2020), and needs to be repeated several years to reduce the population of perennial weeds. Therefore, stubble tillage was not here considered as a curative method in a year, but as a proper control method to maintain low level of infestation, planned for several years in the crop sequence. Control methods such as the introduction of *Ley* in the crop sequence were also implemented in IPSIM-*Cirsium* and ensure the possibility to plan

TABLE 5 | Metrics used per class to evaluate the predictive quality of IPSIM-Cirsium.

Weed Infestation level	Precision	Recall	F1-score
VL	0.898	0.914	0.906
L	0.105	0.097	0.101
I	0.105	0.056	0.073
H	0.390	0.448	0.417

The predictive quality of the four classes of infestation (H, High infestation; I, Intermediate infestation; L, Low infestation; VL, Very low infestation) are evaluated.

a control strategy of *Cirsium arvense* at the cropping system scale. Crop sequence on its own is not considered by the model. Only the current crop and the ley period in the crop sequence are considered.

IPSIM-*Cirsium* is a static deterministic model and is designed to be used on a single year to appreciate the infestation of *Cirsium arvense* in June, corresponding to the highest infestation of *Cirsium arvense* of the year. However, adaptations are possible here because the model is considering practices during the 4 years preceding the infestation evaluation. A visualization of the infestation as a function of the crop sequence to focus on "critical years", where level of infestation can increase according to "improper" cropping practices or decrease with effective cropping practices. It is interesting to consider a larger lapse of time than just one year to evaluate a system and find its weakness regarding weed management. Indeed, some crops require cropping practices that are not suitable for perennial weed management (e.g., Canada thistle is more easily controlled with a long bare soil period in summer, where many stubble cultivation passes can be performed). Using an effective herbicide on *C. arvense* can also be jeopardized with the sowing of crops, where authorized use herbicide is limited or absent (MacLaren et al., 2021). With an evaluation on a longer scale, we can focus on the years presenting a weakness due to improper cropping practices resulting from crops or pedoclimates and better anticipate and build the crop sequence to maintain a low level of weed pressure in the field.

Limitation to the Modeling of Perennial Weed Management

Construction Bias

IPSIM models are designed according to a large, detailed literature on one or several pests, to provide significant factors as indicators of the pest infestation level. Scientific consensus according to literature is often hard to obtain and leads to generalizing a specific information. The most dangerous generalization is the regional bias. In the building of IPSIM-*Cirsium*, *Cirsium arvense* genotypes were considered as identical, no matter the region it was observed. This hypothesis can lead to many mistakes; indeed, weeds are known to have different genotypes according to different episodes for invasive species or recombination (Gaskin et al., 2013). Considering two different genotypes can lead to uncertainties, such as thresholds for temperature or rainfall. The evolution of weed populations would be conditioned by its environment and would lead to different thermal time need for germination, for example. The response to cropping practices can also change between region and the evolution history of the considered genotype. The genericity of the model therefore suffers from few limitations to be applied in other regions of the world. Adjustments have to be made according to regional conditions.

This kind of mistake can be observed in the research of literature and parameterization of factors for the model, but also during co-design workshops with experts. Indeed, expert-based models rely on the experience of the experts involved. This experience can be affected by subjectivity of the expert and of the designers of the model. Expert knowledge will be conditioned by their experience, in a particular region with its pedoclimate or in a particular cropping system. Therefore, experts are also encountering non-consensus. It is important to have a wide diversity of experts to avoid this regional and system bias. Subjectivity of experts can also be observed when many factors are compared. It is hard for experts to consider a wide range of cropping practices or pedoclimates, and to consider their interactions to explain the output of the model. Hierarchical construction here helps us lower the level of complexity for each interaction by only considering interaction between attributes aggregated together. However, three attributes aggregated together, each having three levels per scale, leads to $3^3 = 27$ aggregating table rules to define. This kind of large consideration of attributes must be avoided at maximum to minimize uncertainties.

Outputs of the Model

Cirsium arvense has a distribution of patches in the field and present therefore a high heterogeneity of weed infestation level, except for low infestations where the level is homogeneously low among the field. It is hard to define a general level of threat or infestation of the weed, according to the observations of densities of *Cirsium arvense* at some punctual surveys in the field. Our first approach was to assess an average density of *Cirsium arvense*, considering the patches and the untouched areas. However, many uncertainties might come from this approach, and the average value might underestimate the infestation and the high density in patches, reducing drastically the yield in these areas. One of the methods for the evaluation of the weed infestation was to consider the distribution of *Cirsium arvense* among the field. This approach is addressed to tackle the heterogeneity of the distribution of *Cirsium arvense*. However, this approach is more complicated to apply for the user without a high number of observations in the field.

Crop losses due to weeds can be quantified according to the harmfulness of the weed in the field. However, to be able to express weed harmfulness in the field, it is necessary to describe its spatial distribution in the field according to patches for *C. arvense*. A relationship between the mapping of *Cirsium arvense* shoots and their impact on yield loss has been established for a few specific crops (Gee and Denimal, 2020; Rasmussen and Nielsen, 2020). Representing the patches of *Cirsium arvense* is not possible in IPSIM-*Cirsium* and the choice of representation of the infestation was done according to weed pressure. Weed pressure was evaluated by the mean value of all the density of *Cirsium arvense* observed in the field. To ensure the correct use of IPSIM-*Cirsium*, *Initial level of infestation* observed the year preceding the evaluation, it is necessary to assess an average density of *Cirsium arvense* according to the method widely used for weed pressure calculation in data collection protocol [Chicouène method; (Chicouene and Arbiotech, 2000); random quadrat collection, etc.]. This requirement can be a limit for the accuracy of the prediction of weed infestation by IPSIM-*Cirsium*. Still, it is possible for the user of the model to provide a qualitative value of the level of initial infestation without using a quantitative value converted into a qualitative value. This qualitative estimation might bring a bias of subjectivity related to the user appreciation of the infestation severity. The use of *Initial level of infestation* is a strength for the accuracy of the model, but it requires data that are sometimes difficult to obtain, which is a drawback of the model.

The output of the model aims at evaluating weed infestation in June. This is particularly relevant to characterize weed infestation in a French commercial field because it was mainly designed with the help of French experts and farmers. However, IPSIM-*Cirsium* lacks genericity in the yearly period considered. IPSIM-*Cirsium* considers first March, April and May temperature and rainfall, which are linked to the emergence of *C. arvense* in France, thereby evaluate a risk of infestation in June. This bias needs to be corrected for each country to consider three months of temperature and rainfall after the beginning of emergence of *C. arvense*. The evaluation of weed infestation is calculated for the fourth month following emergence of *C. arvense*. One way to calculate the time of emergence is to focus on thermal time (Donald, 2000). Here, the choice to use a specific month instead of the emergence month of *C. arvense* was done to simplify the model and to evaluate its predictive quality in French conditions. Furthermore, climate change might alter the phenology of *C. arvense* which could lead to an overestimation of the favorable mean temperature for its development. In case of new adaptations of the weed to temperature raise, or increase of drought frequency, the model structure, or its parameters, would have to be adapted.

Moreover, the specification of the output should be adapted to each type of user in order to provide an adequate level of complexity. A lot of information is available for the user of the model, from the infestation of *C. arvense* to the level of risk of increase of the weed population, detailed by cropping practices. The choice of information to communicate should be adaptable to the requirements of the user. Currently, the model provides an answer of *Cirsium arvense* infestation in June, detailed in four levels, and a grade for practices and field environment, which are ranked *Unfavorable*, *Moderately favorable*, or *Favorable* for the user. The model output enables the user to access his farming practices effectiveness and his environment's impact on the growth of *Cirsium arvense*.

Avenues for IPSIM-Cirsium Uses and Improvements

Current Use of the Model

Ex-ante and Ex-post Evaluation of Cirsium Infestations

IPSIM-*Cirsium* can be used to test and evaluate *ex-ante* several cropping systems on their *C. arvense* management on a specific crop or combination of crops. IPSIM-Cirsium, giving an infestation level, can be used as an indicator of the functioning of agroecosystems, for farmers, advisers or in experimental systems less reliant on herbicide and intensive plowing. The information of weed pressure that can be expected in June is a major information for farmers to better anticipate and tackle the issue of weed population increase. Used *ex-ante*, this tool enables farmers to adapt their cropping practices to the field environment and pedoclimate to try to reduce crop losses. According to their initial level of infestation, farmers can choose cropping practices that might reduce the risk of weed infestation or keep it under an acceptable level, in their specific conditions.

Ex-post evaluation can also be used by means of IPSIM-*Cirsium* to better understand and analyze the functioning of current agroecosystems, in experimental or commercial fields. This *ex-post* evaluation enables an understanding of the strengths and weaknesses of the current cropping practices by spotting the effective combination of factors that reduce the level of infestation of Canada thistle and the combinations that might enhance Canada thistle population, in a specific production situation (Aubertot and Robin, 2013). IPSIM-*Cirsium* can therefore be an important tool in the decisions of the selected control methods of *C. arvense* for farmers and advisers.

A Tool to Design Agroecological Cropping System Prototypes

Qualitative modeling enables users to understand the level of complexity of the considered agroecosystem. According to the multi-attribute approach of IPSIM-method models, many factors of cropping practices and pedoclimates are considered. The DEX method allows the description of all interactions between cropping practices. To control *Cirsium arvense* without herbicide, it is necessary to combine several control methods such as mechanical control, introduction of ley, or increase of competitiveness of the crop, planned for several years. These non-chemical methods are often providing low effectiveness to control *Cirsium arvense* and need to be seen as "many little hammers" methods. One use of this model is to provide a general picture of the effects of interactions of these only partly effective methods and the environment.

Multi-attribute hierarchical modeling in DEXi software perfectly fits the understanding of the complexity of agroecosystems, by reducing factors to only two to three scale levels. This approach greatly simplifies the conception of innovative agroecosystems by focusing on cropping practices that are directly described as efficient or not, depending on the chosen intensity of the implemented method. The interactions of simplified cropping practices are then described in aggregating tables, giving a new value to the aggregated attribute such as *Mechanical control* of *Cirsium arvense*. This value provides to the user a direct indicator of performance of the considered aggregated cropping practice. It is easier for the user to consider all the cropping practices instead of focusing on the improvement of one single practice that may not be sufficient to control weeds, even at high intensity. For example, it is not advised to perform every year only stubble tillage without inversion tillage to control *C. arvense* (Melander et al., 2013). IPSIM-*Cirsium* compiles expert-knowledge on the effect of individual tools and their interactions to manage *C. arvense* in interaction with pedoclimate conditions, so as to assess coherently design strategies to provide long-term control. Thus, this model gives practical answers to the question of whether or not the efficacy of *C. arvense* control can be increased through the combined use several non-chemical control methods at the same time, providing an indicator of risk of infestation, and an infestation level according to the initial infestation of the field.

Nonetheless, IPSIM-*Cirsium* is focused to help the design of agroecosystems less reliant on herbicide. It is not designed to address agronomic objectives such as conservation of soil, maintenance of biodiversity, yield, or economic return. Agroecosystem must be designed taking into account multiple objectives, which are not taken into account in this model. IPSIM-*Cirsium* can just provide an indicator of *Cirsium arvense* risk of infestation according to cropping practices and the considered production situation.

Education Tool

Model designing through co-design workshops emphasizes the need of knowledge transfer between agricultural actors. IPSIM-*Cirsium* was designed for farmers, technicians and advisers to evaluate *ex-post* or *ex-ante* weed infestation of a field, to develop innovative agroecosystems less reliant on herbicide. However, it can also be seen as an education tool for teachers and students in agriculture. In addition, this model can be seen as a communication educational tool for large groups of farmers, advisers, practitioners or students. IPSIM-*Cirsium* presents information in a user-friendly way through a range of colors, easily understood (i.e., from green being *Favorable* to the user, to red being *Unfavorable* to the user). The strength of this tool is its ability to transfer information and knowledge between actors of various fields, offering a support for interaction and communication between them.

Perspective for Improvement

IPSIM Perennial Weeds

IPSIM-Cirsium was built to represent specifically the infestation of *Cirsium arvense* according to cropping practices, pedoclimate and field environment. However, *Cirsium arvense* is not the only perennial weed that farmers are faced with. Two other perennial models have been built following the IPSIM method for *Sonchus arvensis* and *Elytrigia repens* evaluating their infestation levels according to cropping practices, pedoclimate and field environment. A first step to try to understand the perennial weed infestation of the field would be to combine these three qualitative models into a stand-alone model to represent an injury profile. This approach was first foreseen in the evaluation of severity of pests on wheat by Aubertot and Robin (2013). However, this approach implies to understand interactions between perennial weeds. Indeed, the three weeds here can benefit, ignore or suffer from the presence of other weeds. In order to grasp the interactions between weeds, additional aggregating tables would be required. In this multiple perennial weed approach, we would better take into account the horizontal dimension of agroecology.

Trait-Based Modeling Approach

Cirsium arvense, *Sonchus arvensis* and *Elytrigia repens* are not the only perennial weeds that can be found in an agroecosystem. Regrowth capacity according to the root reserve is not a specificity of *Cirsium arvense*. Therefore, it is important to aim at the generic traits that might distinguish two weeds from each other and describe weeds most efficiently. With accurate and specific traits, it would be possible to suggest a model that takes into account the response to pedoclimate, field environment and cropping practices. This trait modeling approach would not try to approach the assumption of plant diversity and ecosystem services of a field in response to pedoclimate and cropping practices, as many models are (Sande et al., 2017; Teixeira et al., 2021). This approach differs here with the use of traits as an input of the model to describe the pedoclimate and cropping practices that will reduce or enhance the weed infestation of one specific weed. This approach does not tend to represent weed ecology, but only the management effectiveness of one weed at a time. One of the main issues of developing a generic traits approach of weed management is the different thresholds of weed infestation levels. While keeping a qualitative modeling approach might help maintain an accurate evaluation of weed infestation by offering ranks of severity rate for each weed infestation, the use of convertors to describe this qualitative value into quantitative value such as abundance or biomass, might be a different kettle of fish.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

J-NA and M-HR designed the study and funded the research. OL, M-HR, and SC reviewed the literature on *Cirsium arvense*. OL led the workshops with experts and designed the IPSIM-Cirsium model based on principles developed by J-NA, M-HR, and MB. Field data were collected and gathered by SC. OL, J-NA, and DC analyzed the data. All authors were involved in the interpretation of the results and contributed to writing the original version of the manuscript and improving the subsequent ones.

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REFERENCES

Adeux, G., Giuliano, S., Cordeau, S., Savoie, J.-M., and Alletto, L. (2017). Low-input maize-based cropping systems implementing IWM match conventional maize monoculture productivity and weed control. *Agriculture* 7:74. doi: 10.3390/agriculture7090074

Adeux, G., Munier-Jolain, N., Meunier, D., Farcy, P., Carlesi, S., Barberi, P., et al. (2019). Diversified grain-based cropping systems provide long-term weed control while limiting herbicide use and yield losses. *Agron. Sustain. Dev.* 39:42. doi: 10.1007/s13593-019-0587-x

Altman, E. (1999). *Constrained Markov Decision Processes*. Leiden: CRC Press.

Armengot, L., Berner, A., Blanco-Moreno, J. M., Mäder, P., and Sans, F. X. (2015). Long-term feasibility of reduced tillage in organic farming. *Agron. Sustain. Dev.* 35, 339–346. doi: 10.1007/s13593-014-0249-y

Arvalis (2020). Déterminer la densité de semis du blé tendre 2020/2021 en Centre. Available online at: https://www.arvalis-infos.fr/ble-tendre-a-quelle-densite-semer--@/view-33421-arvarticle.html (accessed December 9, 2020).

Aubertot, J.-N., Barbier, J. M., Carpentier, A., Gril, J.-N., Guichard, L., Lucas, P., et al. (2005). *Pesticides, Agriculture and the Environment. Reducing the Use of Pesticides and Limiting Their Environmental Impact*. INRA-CEMAGREF. Available online at: https://hal.archives-ouvertes.fr/hal-01173732 (accessed March 2, 2021).

Aubertot, J.-N., and Robin, M.-H. (2013). Injury profile SIMulator, a qualitative aggregative modelling framework to predict crop injury profile as a function of cropping practices, and the abiotic and biotic environment. I. Conceptual Bases. *PLoS ONE* 8:e73202. doi: 10.1371/journal.pone.0073202

Bohanec, M. (2020). *DEXi: Program for Multi-Attribute Decision Making, User's Manual, Version 5.04. IJS Report DP-13100*. Available online at: http://kt.ijs.si/MarkoBohanec/pub/DEXiManual504p.pdf (accessed August 19, 2020).

Bostock, S. J. (1978). Seed germination strategies of five perennial weeds. *Oecologia* 36, 113–126. doi: 10.1007/BF00344576

Brandsæter, Bakken, A. K., Mangerud, K., Riley, H., Eltun, R., and Fykse, H. (2011). Effects of tractor weight, wheel placement and depth of ploughing on the infestation of perennial weeds in organically farmed cereals. *Eur. J. Agron.* 34, 239–246. doi: 10.1016/j.eja.2011.02.001

Brandsæter, L. O., Goul Thomsen, M., Wærnhus, K., and Fykse, H. (2012). Effects of repeated clover undersowing in spring cereals and stubble treatments in autumn on *Elymus repens*, *Sonchus arvensis* and *Cirsium arvense*. *Crop Protect.* 32, 104–110. doi: 10.1016/j.cropro.2011.09.022

Brandsæter, L. O., Mangerud, K., Helgheim, M., and Berge, T. W. (2017). Control of perennial weeds in spring cereals through stubble cultivation and mouldboard ploughing during autumn or spring. *Crop Protect.* 98, 16–23. doi: 10.1016/j.cropro.2017.03.006

Campiglia, E., Radicetti, E., and Mancinelli, R. (2012). Weed control strategies and yield response in a pepper crop (*Capsicum annuum* L.) mulched with hairy vetch (*Vicia villosa* Roth.) and oat (*Avena sativa* L.) residues. *Crop Protect.* 33, 65–73. doi: 10.1016/j.cropro.2011.09.016

Chauvel, B., Guillemin, J.-P., Gasquez, J., and Gauvrit, C. (2012). History of chemical weeding from 1944 to 2011 in France: changes and evolution of herbicide molecules. *Crop Protect.* 42, 320–326. doi: 10.1016/j.cropro.2012.07.011

Chicouene, D., and Arbiotech, B. (2000). *Methods for Assessing the Density of a Weed Peuplement Within a Field: Streamlined Procedures*. Phytoma La Defense des Vegetaux (France). Available online at: https://agris.fao.org/agris-search/search.do?recordID=FR2000003727 (accessed January 12, 2021).

Cohen, J. (1960). A coefficient of agreement for nominal scales. *Educ. Psychol. Meas.* doi: 10.1177/001316446002000104

Colbach, N., Colas, F., Cordeau, S., Maillot, T., Queyrel, W., Villerd, J., et al. (2021). The FLORSYS crop-weed canopy model, a tool to investigate and promote agroecological weed management. *Field Crops Res.* 261:108006. doi: 10.1016/j.fcr.2020.108006

Davis, S., Mangold, J., Menalled, F., Orloff, N., Miller, Z., and Lehnhoff, E. (2018). A meta-analysis of Canada Thistle (*Cirsium arvense*) management. *Weed Sci.* 66, 548–557. doi: 10.1017/wsc.2018.6

Donald, W. W. (1990). Management and control of Canada thistle (*Cirsium arvense*). *Rev. Weed Sci.* 5, 193–249.

Donald, W. W. (2000). A degree-day model of *Cirsium arvense* shoot emergence from adventitious root buds in spring. *Wees* 48, 333–341. doi: 10.1614/0043-1745(2000)048(0333:ADDMOC)2.0.CO;2

Donald, W. W., and Khan, M. (1996). Canada thistle (*Cirsium arvense*) effects on yield components of spring wheat (*Triticum aestivum*). *Weed Sci.* 44, 114–121. doi: 10.1017/S0043174500093632

Edwards, G. R., Bourdôt, G. W., and Crawley, M. J. (2000). Influence of herbivory, competition and soil fertility on the abundance of *Cirsium arvense* in acid grassland. *J. Appl. Ecol.* 37, 321–334. doi: 10.1046/j.1365-2664.2000.00495.x

Favrelière (2019). Gestion des adventices vivaces en Agriculture Biologique. *Agro Transfert*. Available online at: http://www.agro-transfert-rt.org/wp-content/uploads/2019/10/Gestion-des-adventices-vivaces-en-AB-oct-19.pdf (accessed July 23, 2020).

Favrelière, E., Ronceux, A., Pernel, J., and Meynard, J.-M. (2020). Nonchemical control of a perennial weed, *Cirsium arvense*, in arable cropping systems. A review. *Agron. Sustain. Dev.* 40:31. doi: 10.1007/s13593-020-00635-2

Favrelière, E., Ronceux, A., Pernel, J., and Rodriguez, A. (2016). Développement d'un outil d'aide à la décision pour la gestion du chardon des champs (*Cirsium arvense*) en systèmes de culture biologiques. Available online at: http://www.agro-transfert-rt.org/wp-content/uploads/2017/01/COLUMA-2016-outil-chardon_VF_FAVRELIERE_web.pdf (accessed December 11, 2020).

Fleiss, J. L., and Cohen, J. (1973). The equivalence of weighted kappa and the intraclass correlation coefficient as measures of reliability. *Educ. Psychol. Meas.* 33, 613–619. doi: 10.1177/001316447303300309

Gaskin, J. F., Schwarzlander, M., Hinz, H. L., Williams, L., Gerber, E., Rector, B. G., et al. (2013). Genetic identity and diversity of perennial pepperweed (*Lepidium latifolium*) in its native and invaded ranges. *Invasive Plant Sci. Manag.* 6, 268–280. doi: 10.1614/IPSM-D-12-00075.1

Gee, C., and Denimal, E. (2020). RGB image-derived indicators for spatial assessment of the impact of broadleaf weeds on wheat biomass. *Remote Sens.* 12:2982. doi: 10.3390/rs12182982

Graglia, E., Melander, B., and Jensen, R. K. (2006). Mechanical and cultural strategies to control *Cirsium arvense* in organic arable cropping systems. *Weed Res.* 46, 304–312. doi: 10.1111/j.1365-3180.2006.00514.x

Gruber, S., and Claupein, W. (2009). Effect of tillage intensity on weed infestation in organic farming. *Soil Tillage Res.* 105, 104–111. doi: 10.1016/j.still.2009.06.001

Hamdoun, A. M. (1972). Regenerative capacity of root fragments of cirsium arvense (l.) Scop. *Weed Res.* 12, 128–136. doi: 10.1111/j.1365-3180.1972.tb01196.x

Hansen, A. A. (1918). *Canada Thistle and Methods of Eradication*. Washington D.C.: U.S. Department of Agriculture.

Hausman, C. E., Jaeger, J. F., and Rocha, O. J. (2010). Impacts of the emerald ash borer (EAB) eradication and tree mortality: potential for a secondary spread of invasive plant species. *Biol. Invasions* 12, 2013–2023. doi: 10.1007/s10530-009-9604-3

Hochstrasse, M., Meerstetter, A., and Popow, G. (2012). Lutte contre le chardon des champs. *Agridea: Nature et agriculture* 1733. Available at: https://www.agridea.ch/old/fr/publications/publications/production-vegetale-environnement/faune-et-flore/lutte-contre-le-chardon-des-champs/ (accessed March 12, 2020).

Hodgson, J. M. (1968). The nature, ecology, and control of Canada thistle. Available at: https://core.ac.uk/display/66219670 (accessed August 19, 2020).

Hume, L. (1982). The long-term effects of fertilizer application and three rotations on weed communities in wheat (after 21–22 years at Indian Head, Saskatchewan). *Can. J. Plant Sci.* 62, 741–750. doi: 10.4141/cjps82-107

Landis, J. R., and Koch, G. G. (1977). An application of hierarchical Kappa-type statistics in the assessment of majority agreement among multiple observers. *Biometrics* 33, 363–374. doi: 10.2307/2529786

Liebman, M., Gallandt, E. R., and Jackson, L. (1997). "Many little hammers: ecological management of crop-weed interactions," in *Ecology in Agriculture*, ed L. E. Jackson (San Diego, CA: Academic Press), 291–343. doi: 10.1016/B978-012378260-1/50010-5

Liew, J., Andersson, L., Boström, U., Forkman, J., Hakman, I., and Magnuski, E. (2012). Influence of temperature and photoperiod on sprouting capacity of *Cirsium arvense* and *Sonchus arvensis* root buds. *Weed Res.* 52, 449–457. doi: 10.1111/j.1365-3180.2012.00936.x

Líška, E., Hunková, E., and Demjanová, E. (2007). Creeping thistle (*Cirsium arvense* (L.) Scop.) - an important competitor of nutrients consumption in grain maize stands (*Zea mays* L.). *J. Cent. Eur. Agric.* 8, 461–468.

Liu, C., Scursoni, J. A., Moreno, R., Zelaya, I. A., Sol Munoz, M., and Kaundun, S. S. (2019). An individual-based model of seed- and rhizome-propagated perennial plant species and sustainable management of *Sorghum halepense* in soybean production systems in Argentina. *Ecol. Evol.* 9, 10017–10028. doi: 10.1002/ece3.5578

Lukashyk, P., Berg, M., and Köpke, U. (2008). Strategies to control Canada thistle (*Cirsium arvense*) under organic farming conditions. *Renew. Agric. Food Syst.* 23, 13–18. doi: 10.1017/S1742170507002013

MacLaren, C., Labuschagne, J., and Swanepoel, P. A. (2021). Tillage practices affect weeds differently in monoculture vs. crop rotation. *Soil Tillage Res.* 205:104795. doi: 10.1016/j.still.2020.104795

Malard, J. J., Adamowski, J. F., Diaz, M. R., Nassar, J. B., Anandaraja, N., Tuy, H., et al. (2020). Agroecological food web modelling to evaluate and design organic and conventional agricultural systems. *Ecol. Model.* 421:108961. doi: 10.1016/j.ecolmodel.2020.108961

McHugh, M. L. (2012). Interrater reliability: the kappa statistic. *Biochem. Med.* 22, 276–282. doi: 10.11613/BM.2012.031

Melander, B., Holst, N., Rasmussen, I. A., and Hansen, P. K. (2012). Direct control of perennial weeds between crops—implications for organic farming. *Crop Protect.* 40, 36–42. doi: 10.1016/j.cropro.2012.04.029

Melander, B., Munier-Jolain, N., Charles, R., Wirth, J., Schwarz, J., van der Weide, R., et al. (2013). European perspectives on the adoption of nonchemical weed management in reduced-tillage systems for arable crops. *Weed Technol.* 27, 231–240. doi: 10.1614/WT-D-12-00066.1

Mézière, D., Petit, S., Granger, S., Biju-Duval, L., and Colbach, N. (2015). Developing a set of simulation-based indicators to assess harmfulness and contribution to biodiversity of weed communities in cropping systems. *Ecol. Indicat.* 48, 157–170. doi: 10.1016/j.ecolind.2014.07.028

Miller, T. W. (2016). Integrated strategies for management of perennial weeds. *Invasive Plant Sci. Manag.* 9, 148–159. doi: 10.1614/IPSM-D-15-00037.1

Moulin, V. (2011). *Maîtriser le chardon des champs (Cirsium arvense) en agriculture biologique*. Centre et Ile-de-France.

Nguwi, Y.-Y., and Cho, S.-Y. (2010). An unsupervised self-organizing learning with support vector ranking for imbalanced datasets. *Exp. Syst. Appl.* 37, 8303–8312. doi: 10.1016/j.eswa.2010.05.054

Pekrun, C., and Claupein, W. (2004). The effect of stubble tillage and primary tillage on population dynamics of Canada Thistle (*Cirsium arvense*) in organic farming. *J. Plant Dis. Protect.* Special Issue XIX, 483–490.

Powles, S. B., and Yu, Q. (2010). "Evolution in action: plants resistant to herbicides," in *Annual Review of Plant Biology, Vol. 61*, eds. S. Merchant, W. R. Briggs, and D. Ort (Palo Alto: Annual Reviews), 317–347. doi: 10.1146/annurev-arplant-042809-112119

Rasmussen (2011). "Use of competitive crops to reduce *Cirsium arvense*. organic is life—knowledge for tomorrow. Volume 1—organic crop production," *Proceedings of the Third Scientific Conference of the International Society of Organic Agriculture Research (ISOFAR)*, held at the 17th IFOAM Organic World Congress in cooperation with the International Federation of Organic Agriculture Movements (IFOAM) and the Korean Organizing Committee (KOC), 28. September 1. October 2011 in Namyangju, Korea Republic, 692–695.

Rasmussen, J., and Nielsen, J. (2020). A novel approach to estimating the competitive ability of Cirsium arvense in cereals using unmanned aerial vehicle imagery. *Weed Res.* 60, 150–160. doi: 10.1111/wre.12402

Sande, M. T., van der, Peña-Claros, M., Ascarrunz, N., Arets, E. J. M. M., Licona, J. C., Toledo, M., et al. (2017). Abiotic and biotic drivers of biomass change in a Neotropical forest. *J. Ecol.* 105, 1223–1234. doi: 10.1111/1365-2745.12756

Schreiber, M. M. (1967). Effect of density and control of Canada Thistle on production and utilization of Alfalfa pasture. *Weeds* 15, 138–142. doi: 10.2307/4041184

Sciegienka, J., Keren, E., and Menalled, F. (2011). Impact of root fragment dimension, weight, burial depth, and water regime on *Cirsium arvense* emergence and growth. *Can. J. Plant Sci.* 91, 1027–1036. doi: 10.4141/cjps2011-059

Stoate, C., Báldi, A., Beja, P., Boatman, N. D., Herzon, I., van Doorn, A., et al. (2009). Ecological impacts of early 21st century agricultural change in Europe —a review. *J. Environ. Manag.* 91, 22–46. doi: 10.1016/j.jenvman.2009.07.005

Taramarcaz, J. (2019). Ferme Pilote de Mapraz : Bilan 2000-2017. *Agridea: Nature et Agriculture*. Available online at: https://www.agridea.ch/old/fileadmin/user_upload/Mapraz_Bilan_CHARDONS_2000-2017.pdf (accessed March 12, 2020).

Tavaziva, V. J., Lundkvist, A., and Verwijst, T. (2019). Effects of selective cutting and timing of herbicide application on growth and development of *Cirsium arvense* in spring barley. *Weed Res.* 59, 349–356. doi: 10.1111/wre.12371

Teixeira, H. M., Bianchi, F. J. J. A., Cardoso, I. M., Tittonell, P., and Peña-Claros, M. (2021). Impact of agroecological management on plant diversity and soil-based ecosystem services in pasture and coffee systems in the Atlantic forest of Brazil. *Agric. Ecosyst. Environ.* 305:107171. doi: 10.1016/j.agee.2020.107171

Thomsen, M. G., Mangerud, K., Riley, H., and Brandsæter, L. O. (2015). Method, timing and duration of bare fallow for the control of *Cirsium arvense* and other creeping perennials. *Crop Protect.* 77, 31–37. doi: 10.1016/j.cropro.2015.05.020

Tiley, G. E. D. (2010). Biological flora of the British Isles: *Cirsium arvense* (L.) Scop. *J. Ecol.* 98, 938–983. doi: 10.1111/j.1365-2745.2010.01678.x

Verwijst, T., Tavaziva, V. J., and Lundkvist, A. (2017). Effects of selective cutting and herbicide use in spring barley on seed production of *Cirsium arvense*. *Acta Agric. Scand. Sect. B Soil Plant Sci.* 67, 562–570. doi: 10.1080/09064710.2017.1318164

Weill (2015). Effet de la jachère de printemps sur la répression du chardon, du laiteron et du tussilage. Available online at: https://www.cetab.org/system/files/publications/6_mars_gc_10h15_anne_weill_chardon_laiteron.pdf (accessed March 12, 2020).

Weill, A. (2018). *Répression du laiteron des* champs, du chardon des champs et du tussilage. Agri Réseau.

Wilson, R. G. (1979). Germination and seedling development of Canada Thistle (*Cirsium arvense*). *Weed Sci.* 27, 146–151. doi: 10.1017/S0043174500043708

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Long-Term Soil Nutrient Management Affects Taxonomic and Functional Weed Community Composition and Structure

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Weed communities can be influenced by nutrient availability, nutrient form (e. g., ammonium vs. nitrate), amendment timing, amendment type (e.g., organic vs. inorganic), and by immigration of seeds during amendment applications. The objective of this research was to compare the long-term effect of different fertility treatments in a corn (*Zea mays* L.)-alfalfa (*Medicago sativa* L.) rotation on taxonomic and functional structure and composition of weed communities by analyzing the soil weed seedbank. After 14 years of a long-term experiment in Aurora, NY, United States, soils were sampled in five fertility treatments for corn years in the rotation: liquid dairy manure, semi-composted separated dairy solids; or inorganic nitrogen (N) as starter fertilizer with either no sidedress N, a low rate or a high rate of inorganic N as sidedress fertilizer. Soil was collected in early spring 2015 and a greenhouse weed seed germination bioassay was used to quantify the germinable soil weed seedbank. Total weed seedbank density, species richness, and evenness did not vary by treatment. However, fertility treatments modified the ecological niche represented by 20 environmental descriptors, which filtered the weed community creating distinct functional group assemblages. A trait-based analysis revealed that nitrophilic dicotyledons preferring alkaline soil were associated with high concentrations of inorganic N fertilizer, whereas highly specialist monocotyledons preferring high amounts of light were associated with low concentrations of inorganic N fertilizer. Because fertility treatments affected weed community composition but not seed bank density and richness, results encourage the development of holistic management strategies that adopt coherent weed management and crop fertilization.

Keywords: agroecology, community assembly, fertilization, functional traits, seedbank

INTRODUCTION

Weeds are a major constraint to crop production (Adeux et al., 2019b). Concerns about herbicide resistance, environmental impacts, and human health effects associated with standard weed management strategies (e.g., herbicide use and soil tillage) have prompted renewed interest in ecological weed management (Liebman et al., 1997; Mortensen et al., 2000; Petit et al., 2018; Maclaren et al., 2020). A central theme in ecological weed management is using ecological knowledge to develop strategies to prevent weed problems rather than focusing narrowly on control tactics that aim to terminate emerged weeds. Community assembly theory has been proposed as a strategy for understanding and predicting weed species response to management practices (Booth and Swanton, 2002; Gaba et al., 2017; Smith and Mortensen, 2017). Environmental filters such as crop type, crop sequence (Fried et al., 2008; Mahaut et al., 2019), soil disturbance, and disturbance timing (Cordeau et al., 2017c) have been explored previously; however, limited information is available about the role of long-term nutrient management on weed community assembly in agroecosystems. Understanding the effects of different nutrient management strategies on weed community assembly can contribute to ecological weed management and reduce reliance on soil tillage and herbicides.

Nutrient management practices can affect weed communities both directly through seed immigration and indirectly by altering competitive abilities. Weed seeds can immigrate to crop fields through manure application and increase the weed seedbank density and species diversity (Pleasant and Schlather, 1994). In some cases, new difficult-to-control weeds can be introduced with applications of manure. Composting manure has been shown to reduce weed seed viability and thus immigration into crop fields, but results vary with weed species, the temperature in the compost, and the duration of exposure (Larney and Blackshaw, 2003). Fertilization may also indirectly affect the weed community through resource modification by altering competition intensity from crops (i.e., crop: weed competition) and among different weed species (i.e., weed: weed competition). Depending on the crop, weed species, and soil conditions, N fertilizer can either give weeds a competitive advantage or disadvantage (Di Tomaso, 1995). Nitrogen is an essential plant nutrient that is known to impact plant community assembly, and previous research has documented that certain weed species (e.g., *Amaranthus retroflexus*, *Persicaria lapathifolia*, *Echinochloa crus-galli*) are more responsive to N than other species (Blackshaw and Brandt, 2008; Moreau et al., 2014). Phosphorus (P) and potassium (K) are also known to differentially impact weed species (Hoveland et al., 1976). For example, Tilman et al. (1999) suggested that *Taraxacum officinale* could be managed in lawns by reducing K fertilizer applications because it has high K requirements and is a poorer competitor than grasses.

Community assembly theory states that evaluating functional traits rather than species can provide important insights and lead to a more mechanistic understanding of plant community dynamics (Booth and Swanton, 2002). According to community assembly theory, abiotic and biotic factors, such as management practices and competition, act as filters preventing species with some traits while allowing species with other traits to exist in a community (Ryan et al., 2010). Fields with high concentrations of soil nutrients can select for weed species that favor high fertility, likewise fields that have low concentrations of soil nutrients tend to favor weeds that better tolerate low-fertility conditions (Tilman et al., 1999; Ryan et al., 2010). In addition, in low inorganic fertilizer conditions, weed communities often harbor fewer specialist species, i.e., with a higher ecological niche (Fried et al., 2010).

Assessing the soil seedbank is particularly relevant when studying the long-term effect of farming practices (Mahé et al., 2021). Soil weed seedbanks are the source of not only emerged weeds, but also weeds that establish at later times (Mirsky et al., 2010; Cordeau et al., 2017d). Soil weed seedbanks also carry legacy from past practices and can be considered as the "memory" of a weed community (Cavers, 1995; Buhler et al., 1997). The weed seedbank thus can provide greater insight into the effect of long-term weed management practices as there is typically more variation among emerged weeds than in the seedbank (Légère and Samson, 2004).

The objective of this research was to compare the effects of dairy manure and inorganic fertilizer on the soil weed seedbank in a long-term corn-alfalfa rotation experiment. We hypothesized that (1) weed seedbank density and weed species density are greater in the organic manure treatments (both liquid dairy manure and composted dairy solids) compared with the inorganic fertilizer treatments due to seed immigration, and (2) fertilizer type (liquid dairy manure vs. composted dairy solids vs. inorganic N fertilizer) shapes the soil ecological niche through modifications of soil parameters and nutrient concentrations, thus acting as weed community filters.

MATERIALS AND METHODS

Experimental Design and Treatments

This study was conducted within a field experiment initiated in 2001 in Aurora, NY, USA (42.73° N, 76.65° W). The soil type is a tile-drained Lima silt loam (a fine-loamy, mixed, active, mesic Oxyaquic Hapludalf), with pH of 7.5, organic matter of 35 g $kg-1$, N, P, and K, content of 21, 5.1, and 47 mg $kg-1$, respectively. It was classified, at the start of the experiment, as high in soil test P (Ketterings et al., 2003a) and medium in soil test K (Ketterings et al., 2003c). The experimental area had no manure applications for several decades and was under continuous corn production before 2001.

The field experiment was initiated in 2001 as a randomized complete block design with five fertility treatments and five replicates, cultivated in continuous silage corn for 5 years, rotated to alfalfa in April of 2006 for a period of 5 years, followed by corn grain from 2011 to 2015. We did not fertilize grain corn (because it was after 5 years of alfalfa) but corn was planted in 2011. Treatments were based on annual spring applications of semi-composted dairy solids, liquid dairy manure, and inorganic N fertilizer. The rates of dairy solids and liquid dairy manure were based on expected N needs of the crop [see details for rates used in 2001–2006 in Sadeghpour et al. (2016)]. Seedbed

TABLE 1 | Definitions, timings of sampling and ranges of values for environmental parameters.

Environmental variable	Meaning	Timing	Min–Max	Unit
Yield	Corn grain yield (0% humidity)	2014	1.02–1.78	t/ha
N_input	Total nitrogen fertilization in corn	2014	22–302	kg N ha^{-1}
N_initial20	Soil nitrate (0–20 cm)	Before planting corn, April 18, 2014	2.9–7	mg N kg^{-1} soil
N_planting20	Soil nitrate (0–20 cm)	At corn planting, May 29, 2014	6.4–23.2	mg N kg^{-1} soil
N_sidedress20	Soil nitrate (0–20 cm)	At sidedress fertilization in corn, June 27, 2014	2–17.5	mg N kg^{-1} soil
N_harvest20	Soil nitrate (0–20 cm)	At corn harvest, November 18, 2014	4–31.2	mg N kg^{-1} soil
N_initial30	Soil nitrate (0–30 cm)	Before planting corn April 18, 2014	1.6–6.9	mg N kg^{-1} soil
N_sidedress30	Soil nitrate (0–30 cm)	At sidedress corn fertilization, April 18, 2014	2.7–19.3	mg N kg^{-1} soil
N_harvest30	Soil nitrate (0–30 cm)	At harvest of corn, April 18, 2014	3.7–38.6	mg N kg^{-1} soil
pH	Soil pH (0–20 cm)	At seedbank sampling, April 28, 2015	6.5–7.8	unitless
OM	Organic matter (0–20 cm)	At seedbank sampling, April 28, 2015	24–49	g kg^{-1}
P	Phosphorus (0–20 cm)	At seedbank sampling, April 28, 2015	6.1–68.9	kg ha^{-1}
K	Potassium (0–20 cm)	At seedbank sampling, April 28, 2015	108–423	kg ha^{-1}
Mg	Magnesium (0–20 cm)	At seedbank sampling, April 28, 2015	524–949	kg ha^{-1}
Ca	Calcium (0–20 cm)	At seedbank sampling, April 28, 2015	3,543–11,993	kg ha^{-1}
Al	Aluminum (0–20 cm)	At seedbank sampling, April 28, 2015	9.0–21.3	kg ha^{-1}
B	Boron (0–20 cm)	At seedbank sampling, April 28, 2015	0.67–3.36	kg ha^{-1}
Fe	Iron (0–20 cm)	At seedbank sampling, April 28, 2015	0.9–2.8	kg ha^{-1}
Mn	Manganese (0–20 cm)	At seedbank sampling, April 28, 2015	50.1–80.4	kg ha^{-1}
Zn	Zinc (0–20 cm)	At seedbank sampling, April 28, 2015	0.56–3.36	kg ha^{-1}

preparation took place a minimum of 5 days after solid manure had been applied, to ensure loss of any inorganic N in the solids (Ketterings et al., 2003b). All plots received 22 kg ha^{-1} N in the starter band. The inorganic N sidedress applications took place when corn was at the V6 growth stage and applied in the form of urea ammonium nitrate. These treatments are hereafter referred to as: (1) Orgliquid (159 kL ha^{-1} liquid dairy manure); (2) Orgsolids (90 Mg ha^{-1} separated semi-composted dairy solids); (3) InorgN0 (no sidedress N); (4) InorgN1 (168 kg N ha^{-1} sidedress N); and (5) InorgN2 (280 kg N ha^{-1} sidedress N). No additional manure, compost or sidedress N was added in 2006–2010 when alfalfa was grown, or in 2011, the 1st year of corn after alfalfa. Rates of manure and dairy solids during the grain corn years from 2012 to 2015 were consistent with those in the earlier years and are documented in Sadeghpour et al. (2017). No data on micronutrients in manure were collected.

Soil Sampling and Soil Parameters

On April 28, 2015 a total of 30 randomly spaced soil cores (1.6 cm diameter) to 20 cm depth (maximum depth of tillage) were collected in each replicate of the five treatments to quantify the soil weed seedbank. The 30 soil cores were pooled and soil bulk density and percent moisture were determined using the pooled cores for each treatment replicate. Eleven soil parameters (**Table 1**) were determined at the University of Maine Analytical Laboratory and Maine Soil Testing Service (Orono, ME) from the soil sampled at seedbank collection. Seven soil parameters (**Table 1**) were determined from previous sampling events in 2014 and processed at Brookside Laboratories, Inc. (New Bremen, OH).

Weed Seedbank Assessment

The soil weed seedbank was quantified using a greenhouse emergence bioassay. A subsample of 1 kg of thoroughly mixed field soil was placed on top of 1–2 cm of vermiculite in a plastic tray (25 × 25 cm) and watered routinely in a greenhouse. The soil was spread in the tray to ensure a maximum of 1–2 cm of soil thickness to avoid non-emergence of germinated seeds (Mahé et al., 2021). Weed seedlings were identified to species, counted, and removed. After emergence ceased, the soil was allowed to dry for 1 month, homogenized, and the process was repeated for a second weed germination flush. Alternating dry and wet spells can cause seed envelopes to crack and initiate seed germination (Cordeau et al., 2018). A third and final flush occurred after soil flats had been stored in a cooler at 5°C for 3 months, because low temperature can break seed dormancy (i.e., stratification) (Baskin and Baskin, 1985). Emergence counts by species from the three flushes were pooled. The number of emerged seedlings was standardized to per kg dry weight soil using soil bulk density and gravimetric soil moisture.

Data Analysis

Total Abundance, Species Richness and Evenness

Weed species richness (S) and Pielou's evenness (J) were computed per plot with the "vegan" package (Oksanen, 2019) using R software. Evenness was computed as $J = H/\ln(S)$, where H is the Shannon-Weiner diversity index. Evenness is typically represented on a scale ranging from near 0, which indicates low evenness or high single-species dominance, to 1, which indicates equal abundance of all species or maximum evenness (Alatalo, 1981). Evenness was considered as 0 when only 1 species occurred. To test for differences in weed community structure among the different fertility treatments, weed species richness, total abundance, and evenness were modeled with a linear mixed model using the "lme4" package (Bates et al., 2015). The fertility treatment (5 levels) was considered a fixed effect, with block as a random effect. Type II Wald F tests were calculated for the three models using "Anova" in the "car" package (Fox and Weisberg, 2018).

Weed Community Composition

Associations between fertility treatments and weed seedbank community composition were assessed with a principal coordinates analysis (PCoA, a metric multidimensional scaling method), with a Bray-Curtis distance metric using the "vegan" package (Oksanen, 2019). Six species observed once were deleted from the dataset for PCoA (i.e., *Stellaria media*, *Senecio vulgaris, Lamium amplexicaule, Eragrostis* spp., *Chenopodium glaucum,* and *Atriplex patula*). The significance of fertility treatment was tested with a permutation-based multivariate analysis of variance, i.e., PERMANOVA (Anderson, 2001), using the "Adonis" function of the "FactoMineR" package (Husson et al., 2010). The analysis was conducted on the matrix of Bray-Curtis distance coefficients, and *P*-values were based on 999 permutations. The effect of fertility treatments on homogeneity of weed seedbank composition was assessed with the Betadisper function of the "FactoMineR" package. Betadisper is a multivariate analog of Levene's test for homogeneity of variances and computes non-Euclidean distances between objects (in our case, plots) and group centroids (fertility treatments), and tests for mean differences. A greater distance between dots and their fertility treatment centroid indicates greater heterogeneity (i.e., variance) in weed communities between plots belonging to the same fertility treatment. Fertility treatments with a high degree of filtering were expected to show homogeneity in weed communities.

Effect of Fertility Treatments on Soil Parameters

A matrix of the environmental variables (corn yield, soil N application and measurements, and soil parameters) by plots was submitted to a principal component analysis (PCA) using the package "FactoMineR." The significance of fertility treatments in shaping environmental variables was tested with a permutation-based multivariate analysis of variance (PERMANOVA with 999 permutations, using the "Adonis" function in the "vegan" package) using the matrix of Euclidean distances.

Relative Importance of Environmental Parameters on Weed Assembly

First, we used Mantel tests ("vegdist" function in the "vegan" package) to test for correlations among weed communities and environmental parameters in each of the 25 plots (Mantel, 1967). Correlation significance was assessed using permutation tests with 1,000 permutations. The entire data set (25 plots, 22 weed species described by their abundance, 20 explanatory variables) was subjected to canonical correspondence analysis (CCA) as implemented in the "vegan" package. All explanatory variables were continuous (**Table 1**). The ecological gradient length was assessed by a detrended correspondence analysis (DCA) performed on the overall plot-by-species dataset (Legendre and Legendre, 2012), dividing the first axis into 26 segments. The ecological gradient length was 4.8 standard deviation units, justifying the use of CCA, which assumes unimodal responses. Following the methodology of Cordeau et al. (2017b), net effects of the 20 explanatory variables (**Table 1**) on weed communities were calculated after partitioning out the effect shared with the other explanatory variables. The net effect of each particular variable was tested with a partial CCA (pCCA), proposed by Rao (1969), with a single explanatory variable and the other 20 variables used as covariates. The significance of the model was tested using a permutation based ANOVA ($N = 999$ permutations). The ratio of a particular eigenvalue to the sum of all eigenvalues (total inertia) was used as a measure of the proportion of variation (i.e., inertia) explained by each explanatory variable (Borcard et al., 1992).

Selection of Species Traits

Weed species traits were described using six descriptors that were extracted from online trait databases (**Table 2**). The six descriptors were: (1) cotyledon type (monocot vs. dicot) (Gaba et al., 2017); (2) specialization degree (generalist or specialist) (Fried et al., 2010); (3) Ellenberg-L (higher L values represent stronger affinity for light) (Bartelheimer and Poschlod, 2016); (4) Ellenberg-R (low and high R values represent affinity for acidic and alkaline soils, respectively) (Bartelheimer and Poschlod, 2016); (5) Ellenberg-N (low and high N values describe species growing on low-N and high-N soils, respectively) (Bartelheimer and Poschlod, 2016); and (6) seed weight (four categories for the mass of the seed in mg) (Gaba et al., 2017). Ellenberg values are simple ordinal classifications of plants according to the position of their realized ecological niche along an environmental gradient.

Grouping Species Into Functional Groups

Weed species were organized into functional groups following the methodology of Cordeau et al. (2017a) and Fried et al. (2009). A functional group is a set of species with common plant traits that behave in a similar way. First, a species-by-trait matrix was created to characterize the 22 weed species using the six biological descriptors (**Table 2**). Second, a multiple correspondence analysis (MCA) of this species-by-trait matrix was formed, using the "ade4" package (Dray and Dufour, 2007). The MCA is a data analysis technique for nominal categorical data, used to detect and represent underlying structures in data like a species-by-trait matrix. MCA places species in a multidimensional space with coordinates on each axis of the multidimensional space. The Euclidean distance between species in the multidimensional space represents the distance between species in terms of trait values representing their ecology. The closer the species are to each other in the multidimensional space, the more similar they are in terms of trait values. Third, the matrix of Euclidean distances between species was used to conduct a Ward hierarchical ascendant classification (HAC; i.e., cluster analysis). The HAC produced a dendrogram, which is a tree diagram frequently used to

TABLE 2 | Biological descriptors of weed species used for the functional grouping and count of weed species falling into each category.

Trait	References	Definition	Categories	Number of weed species
Cotyledon type	USDA-NRCS, 2017	Species that differ by root type and leaf form	Dicots	16
			Monocots	6
Specialization degree[a]	Fried et al., 2010	Generalist (adaptable) vs. specialist (with specific requirements)	Highly generalist (14–39)	3
			Moderately generalist (40–78)	4
			Moderately specialist (79–101)	4
			Highly specialist (102–122)	4
			Spec. degree not known	7
Ellenberg L	Ellenberg et al., 1992; Julve, 1998	Affinity for light	4	1
			6	1
			7	6
			8	9
			9	5
Ellenberg R	As above	Soil reaction gradient (affinity for certain soil pH)	4	1
			5	6
			6	3
			7	6
			8	6
Ellenberg N	As above	Affinity for soil N	6	9
			7	8
			8	5
Seed weight[a]	Klotz et al., 2002	Mass of seed in mg	(0.02–0.32)	5
			(0.33–0.64)	5
			(0.65–1.50)	5
			(1.51–10.6)	6
			NA	1

[a] *Degree of specialization and seed weight were continuous variables and transformed into four categories using quartiles.*

illustrate the arrangement of clusters produced by hierarchical clustering. Examination of the dendrogram allowed us to identify clear and non-redundant functional groups. Fisher's exact tests, performed with the "catdes" function (Husson et al., 2010) of the "FactoMineR" package, were used to classify those traits and their categories significantly associated with each functional group. The "catdes" function also provided "v.test values" that indicated trait categories that were overrepresented (v.test values > 0) in each functional group. Pearson's Chi-squared tests for count data were run on a contingency table for fertility treatment by abundance of each functional group, controlling for the abundance of weed species, to determine if the relative proportion of functional groups differed by fertility treatments.

RESULTS

Weed Species Abundance, Richness and Evenness

The total abundance of weed species ranged from 3 to 30 seedlings kg^{-1} of soil. A total of 22 weed species recorded across the whole experiment: *Chenopodium album* (Common lambsquarters, 100% of frequency of occurrence), *Taraxacum officinale* (Dandelion, 90.9%), *Digitaria sanguinalis* (Large crabgrass, 59.1%), *Oxalis* sp. (Woodsorrels, 59.1%), *Solanum* sp. (Nightshades, 50%), *Panicum dichotomiflorum* (Fall panicum, 36.4%), *Amaranthus* sp. (Pigweeds, 27.3%), *Plantago major* (Broadleaf plantain, 27.3%), *Setaria pumila* (Yellow foxtail, 27.3%), *Abutilon grandifolium* (Hairy Indian mallow, 27.3%), *Hibiscus trionum* (Venice mallow, 27.3%), *Veronica persica* (Persian speedwell, 13.6%), *Ambrosia artemisiifolia* (Common ragweed, 9.1%), *Juncus tenuis* (Path rush, 9.1%), *Panicum capillare* (Witchgrass, 9.1%), *Trifolium hybridium* (Alsike clover, 9.1%), *Atriplex patula* (Spear saltbush, 4.5%), *Chenopodium glaucum* (Oakleaf goosefoot, 4.5%), *Eragrostis* sp. (Lovegrasses, 4.5%), *Lamium amplexicaule* (Henbit, 4.5%), *Senecio vulgaris* (Common groundsel, 4.5%), *Stellaria media* (Common chickweed, 4.5%). Total abundance by treatment ranged from 10.1 ± 6.5 (OrgLiquid) to 15.4 ± 5.1 (InorgN2) individuals kg^{-1} soil (mean ± standard deviation). Species richness (S) by treatment ranged from 4.6 ± 0.9 (Org.Liquid) to 6.2 ± 2.4 (Org.Solids). Species evenness (J) by treatment ranged from 0.80 ± 0.10 (InorgN2) to 0.94 ± 0.03 (Org.Solids). No significant differences existed among fertility treatments for total abundance ($F = 0.77$, $Df = 4$, $P = 0.56$), species

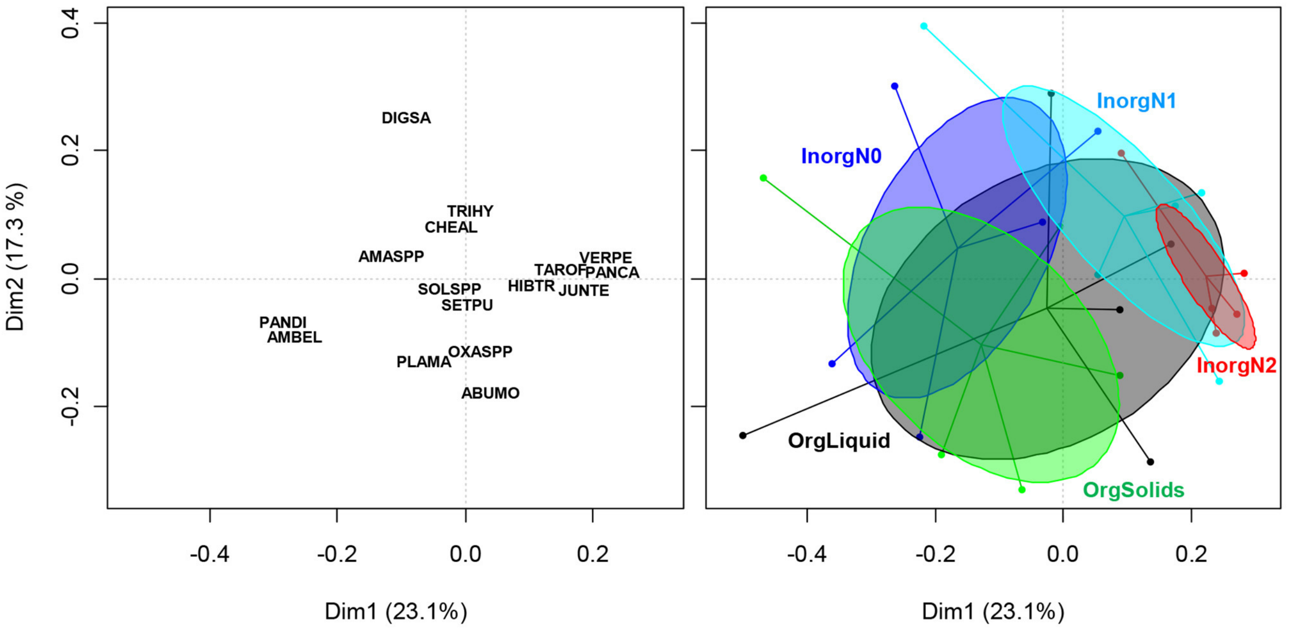

FIGURE 1 | Principal coordinate analysis (PCoA) with a Bray-Curtis distance metric of the weed seedbank community ($n = 25$ plots) under five different fertility treatments (right panel). Ellipses represent the 95% confidence interval. Species names (left panel) are coded with EPPO codes (https://gd.eppo.int/).

richness ($F = 0.95$, $Df = 4$, $P = 0.46$), or evenness ($F = 2.29$, $Df = 4$, $P = 0.10$).

Weed Communities Affected by Fertility Treatment

The first five axis of the PCoA explained 72.4% of the total inertia (**Figure 1**). Fertility treatments largely overlapped but significantly affected weed community assembly (PERMANOVA, $F = 1.57$, $Df = 4$, $P = 0.04$, $R^2 = 0.23$). Although treatment InorgN2 (the highest rate of inorganic N application) seemed to express a less heterogenous weed community than other treatments (i.e., smaller 95% confidence ellipse, **Figure 1**), no differences in variance were found among treatments (Betadisper multivariate homogeneity of variance, $F = 2.18$, $Df = 4$, $P = 0.10$).

Effect of Environmental Parameters on Weed Communities

The first five axes of the PCA explained 85% of the overall environmental variability (**Table 1**, **Figure 2**). Axis 1 (explaining 31% of the variability) separated organic from inorganic treatments and was positively correlated with pH, organic matter, P and K concentrations, and concentrations of other minor elements (e.g., B, Zn, Mg). Axis 2 (18%) separated the low inorganic N treatment (InorgN0) from the high inorganic N treatments (InorgN1 and InorgN2) and was correlated with corn yield, total N input, and soil nitrate at corn harvest. Fertility treatments significantly influenced the ecological niches that were described by the environmental variables (PERMANOVA, $F = 10.6$, $Df = 4$, $P = 0.001$, $R^2 = 0.68$) without affecting the variance within treatments (Betadisper multivariate homogeneity of variance, $F = 0.26$, $Df = 4$, $P = 0.90$).

The influence of fertility treatments on environmental parameters filtered weed communities. We observed a positive correlation between the distance matrices of weed communities and environmental variables (Mantel test, $r = 0.19$, $P = 0.04$), indicating that treatments with similar environmental parameters showed similar weed communities (composition and relative abundance).

The results of the CCA and pCCA weighted the relative importance of environmental parameters on weed assembly. The first five axis of the CCA explained 62% of the total inertia (**Figure 3**). The most complete model including all the environmental parameters (**Table 1**) explained 87% of the variation in weed communities ($F = 1.34$, $Df = 20$, $P < 0.05$). Individual parameters that offered the highest degree of explanation after partitioning out the effect of the other variables were the variables “total N input” and “calcium.” Total N input explained 7% of the variability in weed community ($P = 0.03$) and calcium explained 8% ($P = 0.01$).

Different Compositions of Functional Groups Among Fertility Treatments

Fertility treatments may have filtered certain species in similar ways because of the similarity of the species’ traits and biological characteristics. Here, we grouped species into functional groups and revealed changes in their relative abundances. The first five axes of the MCA (**Figure 4**) accounted for 63% of the total trait variation. The first axis (18% of the variation) separated dicot from monocot species (left-to right, **Figure 4**, left panels),

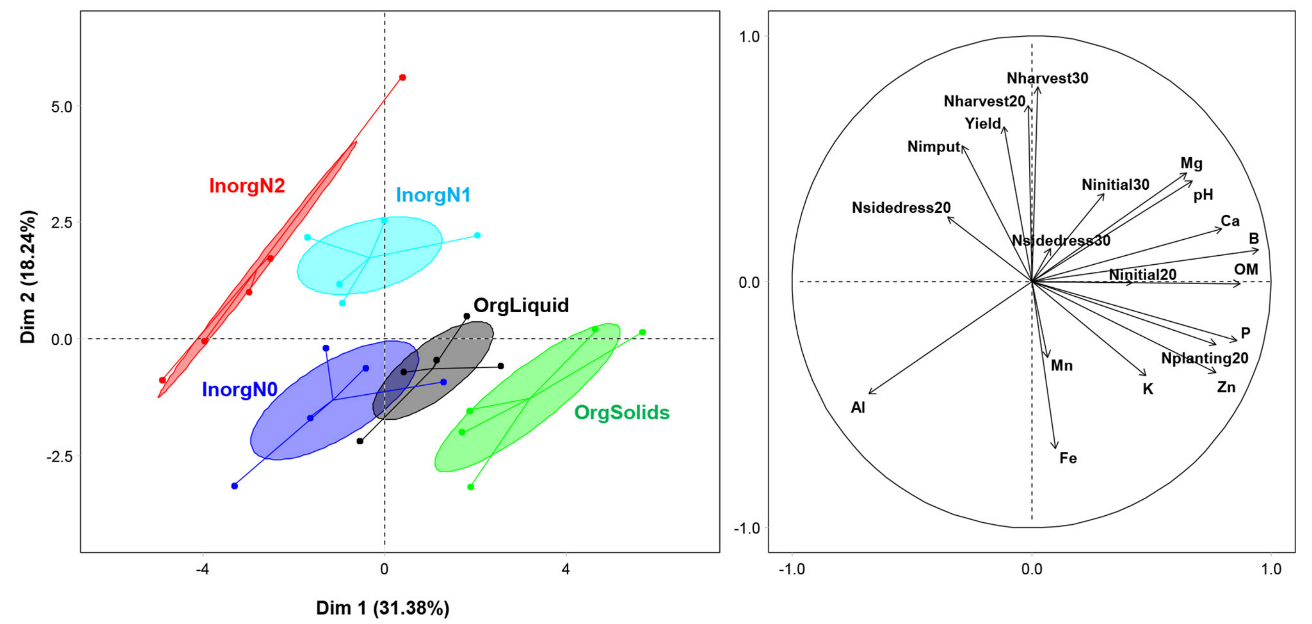

FIGURE 2 | Principal component analysis (PCA) of environmental variables (right panel, see **Table 1** for detailed description) of each fertility treatment (left panel, dots are plots, $n = 25$).

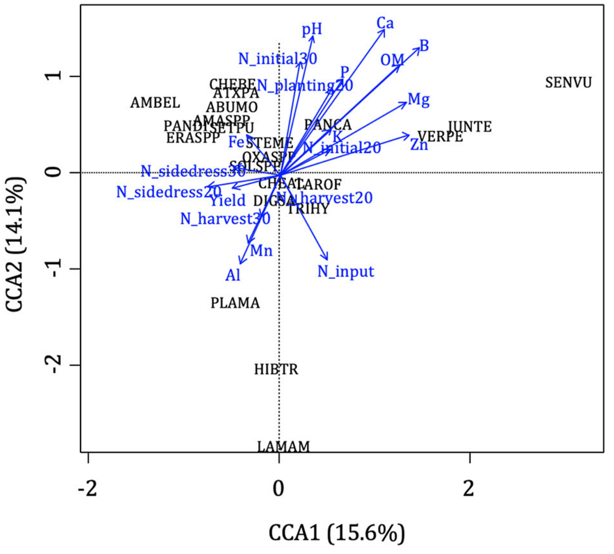

FIGURE 3 | Effect of environmental variables (in blue, see **Table 1** for a detailed description) on weed species (black, named by their EPPO codes, https://gd.eppo.int/) assessed by canonical correspondence analysis (CCA). Arrows indicate the direction and magnitude of responses.

generalists (large ecological niche) from specialists (reduced ecological niche) and more nitrophilic from less nitrophilic species (Ellenberg N values). The second axis (13% of the variation) separated large- from small-seeded species (bottom-to-top, **Figure 4**, left panels). Hierarchical Cluster Analysis (HAC) identified two clear and non-redundant functional groups (**Figure 4**, right panel). All traits, except seed weight ($\chi^2 = 6.2$, df $= 3$, $P = 0.10$), contributed to the grouping (Cotyledon type: Pearson's $\chi^2 = 7.1$, df $= 1$, $P = 0.007$; Specialization Degree: $\chi^2 = 10.8$, df $= 3$, $P = 0.01$; Ellenberg N: $\chi^2 = 8.2$, df $= 2$, $P = 0.02$; Ellenberg L: $\chi^2 = 10.6$, df $= 4$, $P = 0.03$; Ellenberg R: $\chi^2 = 15.9$, df $= 4$, $P = 0.003$).

Figure 5 presents trait descriptors of each functional group. Functional group 1 consisted of 10 weed species, among which were five monocot species (e.g., *Panicum capillare*), and five dicot species (e.g., *Ambrosia artemisiifolia*). Functional group 1 included species with highly specific requirements (highly specialist), those with lower affinity for soil N (Ellenberg N), species with higher affinity for light (Ellenberg L), and those preferring more alkaline and calcareous soils (Ellenberg R). Functional group 2 consisted of 11 weed species, comprised exclusively of dicot species (e.g., *Senecio vulgaris, Veronica peregrina*). Functional group 2 included species with higher affinity for soil N (Ellenberg N), and those preferring moderately alkaline and calcareous conditions (Ellenberg R).

No significant difference in the relative abundance of functional groups was found between organic treatments (i.e., liquid dairy manure and composted separated dairy solids) (**Figure 6**). This is congruent with the overlap of weed communities observed in the taxonomic analysis (PCoA, **Figure 1**). However, a significant difference was found among the three inorganic fertility treatments (**Figure 6**). The treatment with 280 kg N ha^{-1} of sidedressed urea ammonium nitrate (InorgN2), had a higher proportion of species belonging to functional group 2 (i.e., nitrophilic dicots) than did the other inorganic treatments. In contrast, the relative proportion

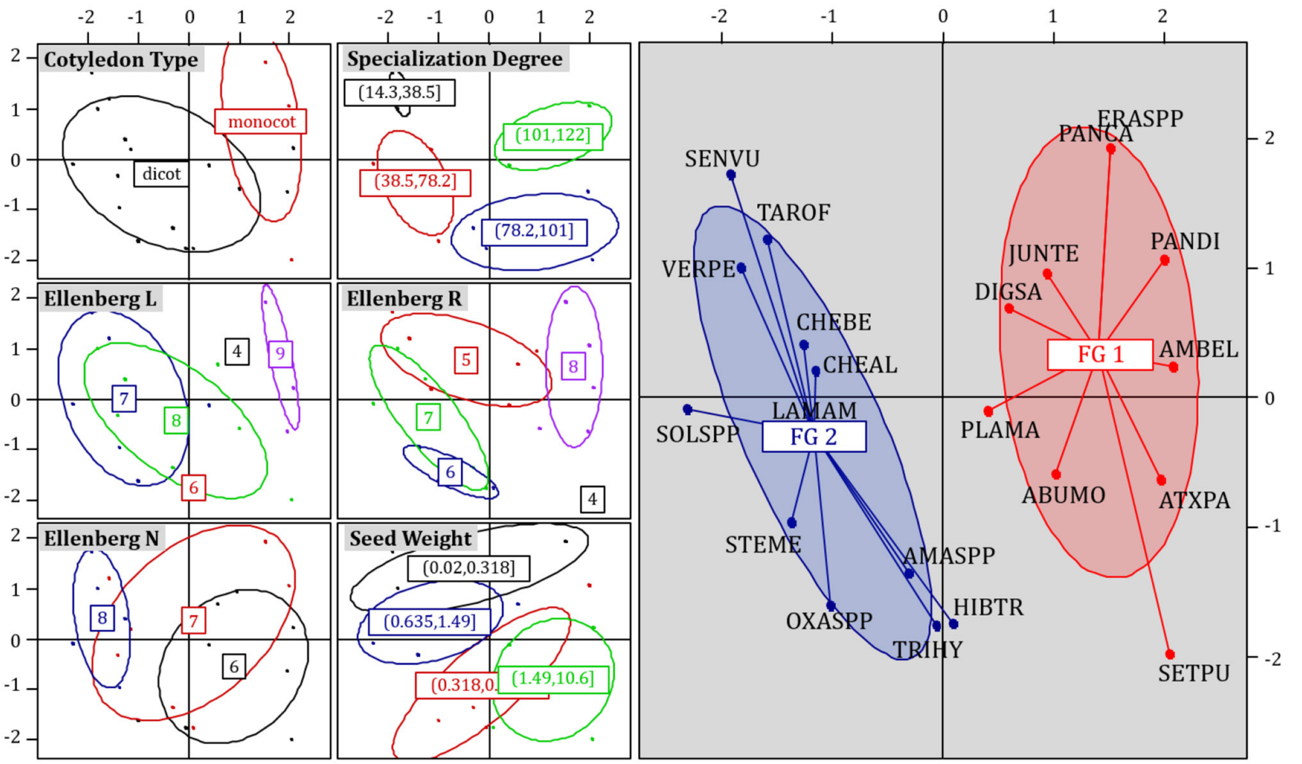

FIGURE 4 | Multiple correspondence analysis (MCA) ordination (axis 1: 18.1%; axis 2: 12.5%) of the weed species-by-trait matrix (**Table 2**). All seven ordination plots represent the same ordination solution. Weed species points are colored by different trait categories (see **Table 2** for category divisions). Ellipses show the 95% confidence interval (when no ellipse is visible, only one species belongs to this category). Trait category labels in the center of ellipses show the centroid of all species belonging to that trait category. The right panel shows weed species grouped into functional groups (FG 1 and FG 2). Species names are EPPO codes (https://gd.eppo.int/).

of functional group 1 (i.e., highly specialist monocots and dicots) in the InorgN2 treatment decreased as the level of N fertility increased.

DISCUSSION

The aim of this work was to compare the long-term effects of organic and inorganic fertilizers on weed seedbank communities through the analysis of their composition and structure. Overall, fertility treatments differentiated the ecological niches through modification of environmental parameters. Fertility treatments did not affect seedbank density, richness or evenness (refuting our first hypothesis) but shaped taxonomic and functional composition of weed communities (supporting our second hypothesis).

No Modification of Community Structure by Fertility Treatment

Our first hypothesis, that weed seedbank density and weed species richness are greater in the organic compared to the inorganic fertility treatments because of immigration of seeds, was not supported by our results. We can postulate that composting manure before application decreased the number of viable seeds (Larney and Blackshaw, 2003). Although studies agree that time and temperature requirements for thermal death vary considerably among species, temperatures of 50°C (Dahlquist et al., 2007) and sometimes less for certain species [i.e., 39°C (Larney and Blackshaw, 2003)] are lethal. Liquid dairy manure was not a source of seed immigration, because there was no increase in total weed density (Stevenson et al., 1997). Furthermore, because there were no differences in species richness among treatments (Stevenson et al., 1997), neither liquid nor composted manure introduced seeds not belonging to species already present in the site. Seed composition of the manure depends highly on the weediness of the fields where cattle graze or hay is harvested (Cudney et al., 1992; Pleasant and Schlather, 1994). Surprisingly, no significant differences existed in evenness among treatments, which is in contradiction with some studies [e.g., (Tang et al., 2014)]. This result indicates there were no major introductions of species that became dominant, which supports previous work showing that organic amendments have little influence on the soil seedbank (Pleasant and Schlather, 1994; Mccloskey et al., 1996; Stevenson et al., 1997).

Taxonomic Composition Shaped by Fertility Treatment

Our results showed that the ecological niche differed by fertility treatment, supporting our second hypothesis. The positive correlation between distance matrices of weed communities and

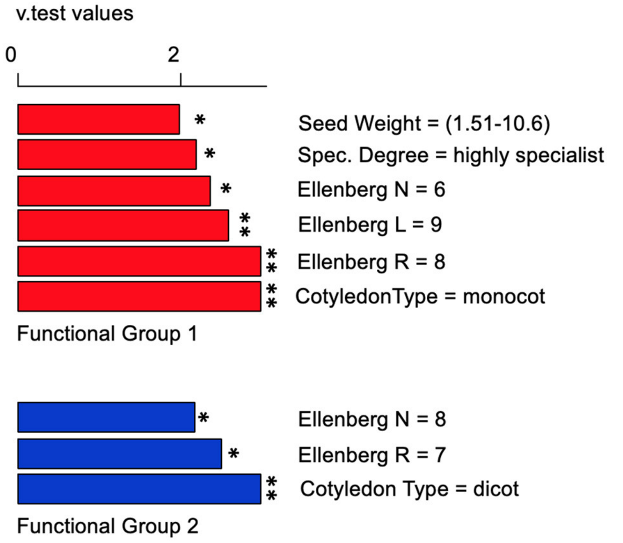

FIGURE 5 | Bars indicate the descriptor category within each trait that contributed to functional groups 1 (red) and 2 (blue). Fisher's exact tests were used to compare the allocation of the descriptor categories (see **Table 2**) within the functional groups (*$P < 0.05$; **$P < 0.01$). V.test values > 0 indicate an overrepresented category in the functional group. The "catdes" function of the "FactoMineR" package (Husson et al., 2010) provided v.test values (unitless).

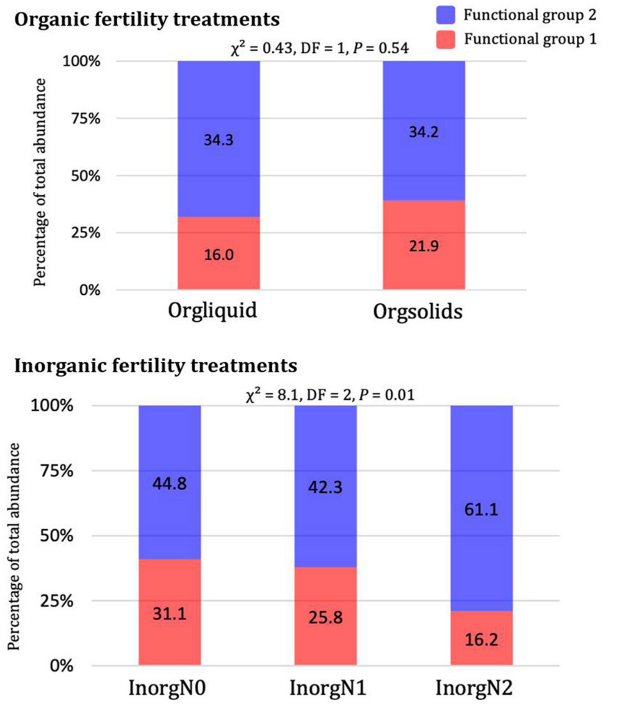

FIGURE 6 | Relative abundance of functional groups 1 (red) and 2 (blue) within organic fertility treatments (top) and inorganic fertility treatments (bottom). Chi-square tests on contingency tables of functional groups by fertility treatments are displayed above each graph. Values in the bars are total density of the species belonging to each functional group in each fertility treatment.

environmental variables confirmed that treatments with similar environmental parameters showed similar weed communities in composition and relative abundance. This correlation supports our hypothesis that management decisions related to fertility management (source, rates, etc.), repeatedly applied for years, can act as a filter on the weed community, as recently shown by Jiang et al. (2018) in winter wheat (*Triticum aestivum* L.).

We found that fertility treatments implemented during the corn years in the rotation affected taxonomic composition, which is congruent with previous results of Pyšek and Lepš (1991) who investigated the effect of type and amount of fertilizer in barley. For instance, the relative abundance of *Taraxacum officinale* may have been affected by fertility treatment (Tilman et al., 1999). However, our results did not show that treatments with high fertility homogenized the weed community by reducing the ecological niche. Thus, (i) continuous corn production before the start of the corn-alfalfa rotation experiment in 2001 favored species adapted to high fertility levels, as demonstrated by previous research (Fried et al., 2020); and (ii) since the start of the experiment, high weed diversity did not emerge in the low fertility treatment (InorgN0) as previous studies would suggest (Storkey et al., 2010). An alternative explanation is that herbicide weed management during the experiment might have kept new species from establishing or present species from dominating the community (Adeux et al., 2019a; Cordeau et al., 2020).

Disentangling the Effect of Environmental Parameters on Weed Communities

Of the 20 parameters used to describe the ecological niche in the analysis, only total N applied and calcium concentrations were significant in shaping weed communities after partialling out the effect of the other parameters. Calcium acts as a nutrient filler, to maintain balance among nutrients in the soil and occupy space on the soil adsorption complex which otherwise would be taken up by acid elements (i.e., H+ protons). In our experiment, calcium concentration is correlated with soil pH as shown by many previous studies [e.g., (Goto, 1985)] and might have selected for species preferring alkaline and calcareous soils (Fried et al., 2012) despite the relatively narrow range of pH in our experiment (i.e., 6.5–7.8). It has to be noticed that Ellenberg's values are discrete values but refer to qualitative descriptors of the plant species.

Some studies showed that these values cannot be directly related to particular values of soil parameters, because intraspecific variation in plant functional traits can be large (Zelený and Schaffers, 2012). However, they are widely used as they fairly indicate the range of these soil parameters (Wamelink et al., 2002). Total N input might have directly affected weed community, i.e., the amount of N available each year might have repeatedly favored nitrophilous species while filtering oligotrophic species (Pyšek and Lepš, 1991). Total N input might also have indirectly affected the weed community, by increasing corn growth (Sadeghpour et al., 2016) and strengthening crop: weed competition for light (Pyšek and Lepš, 1991; Yin et al., 2006), especially after corn canopy closure. Canopy height has often been considered a reliable proxy to assess competitive ability for light resources (Seavers and Wright, 1999; Norsworthy and Oliveira, 2004) under the theory of competitive hierarchies, where competitive outcome is determined by having better fitness of a specific trait (Funk and Wolf, 2016).

Although not significant, soil P and K concentrations explained 5 and 4%, respectively, of the variability in weed community assembly. Tang et al. (2014) showed that weed community assembly was influenced by nutrients in the order of P>N>K in winter wheat. With corn, Vengris et al. (1955) showed that relative plant nutrient uptake differed between weedy and weed-free plots, i.e., corn grown with weeds compared to corn alone took up only 47% as much K, 58% as much N, 63% as much as P, and 67% as much calcium. Vengris et al. (1955) also showed that some of the dominant weeds of our study (i.e., *Chenopodium album, Digitaria sanguinalis*) were between 20 and 57% more competitive for N and K than was corn, whereas all weeds were less competitive than corn for P. Our results from the pCCA analysis support that N remains the major nutrient driving weed community assembly, whether directly or indirectly.

Fertility Treatments Affect Functional Profiles

The trait-based approach provides important insights and leads to a more mechanistic understanding of the filtering effects of fertility treatments on weed communities (Booth and Swanton, 2002). Our results highlight that organic fertility treatments had few filtering effects on weed communities, probably because the nutrients were released slowly from the manure and did not directly affect weeds (Pleasant and Schlather, 1994; Mccloskey et al., 1996; Stevenson et al., 1997). In contrast to the findings in the organic fertility plots, high levels of inorganic fertility reflect the direct and long-term effects of increasing the proportion of nitrophilic species, supporting our second hypothesis. Our findings support the trait syndrome observed by Storkey et al. (2010) in the Broadbalk long-term experiment. Increasing the level of inorganic fertilizer reduces the ecological niche and selects against specialists and/or rare species (Fried et al., 2010). Specialist nitrophilic weeds may represent a threat of high weed:crop interference. The threat of interference may particularly be the case in less N-demanding crops such as wheat or alfalfa, because nitrophilic weeds (such as *Chenopodium glaucum* (Oakleaf goosefoot) and *Solanum* sp. (Nightshades) in our experiment) have been shown to respond to high soil N by producing large amounts of aboveground biomass, and then outcompeting crops for light (Moreau et al., 2013, 2014). In addition, according to the competitive hierarchy theory, over the long-term, nitrophilic weeds may dominate the community and thus represent a threat for preserving weed diversity that has been shown to mitigate yield loss (Adeux et al., 2019b).

CONCLUSIONS

The analysis of the effect of long-term fertility treatments on weed seedbank communities, comparing liquid dairy manure and semi-composted dairy solids to three levels of inorganic fertilizers (including a zero-N sidedress control), revealed no differences in total weed abundance, weed species richness, or weed species evenness. The ecological niches defined by 20 environmental variables differed among fertility treatments and acted as filters for weed communities, described in both the taxonomic and functional perspectives. The trait-based approach showed that species belonging to the functional group of nitrophilic dicots were more abundant with higher levels of inorganic N. These results encourage the development of holistic management strategies that optimize nutrient management for ecological weed management in order to reduce weed interference in the crop while meeting crop's nutrient requirements and building soil health.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

MR and QK designed the study and funded the research. SW, CP, and AS collected the data. SC analyzed the data. MR, QK, SW, AS, and SC were involved in the interpretation of the results, contributed to writing the original version of the manuscript, and improving subsequent versions. All authors contributed to the article and approved the submitted version.

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REFERENCES

Adeux, G., Munier-Jolain, N., Meunier, D., Farcy, P., Carlesi, S., Barberi, P., et al. (2019a). Diversified grain-based cropping systems provide long-term weed control while limiting herbicide use and yield losses. *Agronomy Sustain. Dev.* 39:42. doi: 10.1007/s13593-019-0587-x

Adeux, G., Vieren, E., Carlesi, S., Bàrberi, P., Munier-Jolain, N., and Cordeau, S. (2019b). Mitigating crop yield losses through weed diversity. *Nat. Sustain.* 2, 1018–1026. doi: 10.1038/s41893-019-0415-y

Alatalo, R. V. (1981). Problems in the measurement of evenness in ecology. *Oikos* 37, 199–204. doi: 10.2307/3544465

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Aust. Ecol.* 26, 32–46. doi: 10.1111/j.1442-9993.2001.01070.pp.x

Bartelheimer, M., and Poschlod, P. (2016). Functional characterizations of Ellenberg indicator values – a review on ecophysiological determinants. *Funct. Ecol.* 30, 506–516. doi: 10.1111/1365-2435.12531

Baskin, J. M., and Baskin, C. C. (1985). The annual dormancy cycle in buried weed seeds: a continuum. *BioScience* 35, 492–498. doi: 10.2307/1309817

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using {lme4}. *J. Statist. Softw.* 67, 1–48. doi: 10.18637/jss.v067.i01

Blackshaw, R. E., and Brandt, R. N. (2008). Nitrogen fertilizer rate effects on weed competitiveness is species dependent. *Weed Sci.* 56, 743–747. doi: 10.1614/WS-08-065.1

Booth, B. D., and Swanton, C. J. (2002). Assembly theory applied to weed communities. *Weed Sci.* 50, 2–13. doi: 10.1614/0043-1745(2002)050[0002:AIATAT]2.0.CO;2

Borcard, D., Legendre, P., and Drapeau, P. (1992). Partialling out the spatial component of ecological variation. *Ecology* 73, 1045–1055. doi: 10.2307/1940179

Buhler, D. D., Hartzler, R. G., and Forcella, F. (1997). Implications of weed seedbank dynamics to weed management. *Weed Sci.* 45, 329–336. doi: 10.1017/S0043174500092948

Cavers, P. B. (1995). Seed banks: memory in soil. *Canad. J. Soil Sci.* 75, 11–13. doi: 10.4141/cjss95-003

Cordeau, S., Baudron, A., and Adeux, G. (2020). Is tillage a suitable option for weed management in conservation agriculture? *Agronomy* 10:1746. doi: 10.3390/agronomy10111746

Cordeau, S., Ryan, M. R., Bohan, D. A., Reboud, X., and Chauvel, B. (2017a). Which traits allow weed species to persist in grass margin strips? *Weed Sci.* 65, 381–394. doi: 10.1017/wsc.2016.39

Cordeau, S., Smith, R. G., Gallandt, E. R., Brown, B., Salon, P., Ditommaso, A., et al. (2017b). Disentangling the effects of tillage timing and weather on weed community assembly. *Agriculture* 7:66. doi: 10.3390/agriculture7080066

Cordeau, S., Smith, R. G., Gallandt, E. R., Brown, B., Salon, P., Ditommaso, A., et al. (2017c). How do weeds differ in their response to the timing of tillage? A study of 61 species across the Northeastern United States. *Ann. Appl. Biol.* 171, 340–352. doi: 10.1111/aab.12377

Cordeau, S., Smith, R. G., Gallandt, E. R., Brown, B., Salon, P., Ditommaso, A., et al. (2017d). Timing of tillage as a driver of weed communities. *Weed Sci.* 65, 504–514. doi: 10.1017/wsc.2017.26

Cordeau, S., Wayman, S., Reibel, C., Strbik, F., Chauvel, B., and Guillemin, J. P. (2018). Effects of drought on weed emergence and growth vary with seed burial depth and presence of a cover crop. *Weed Biol. Manage.* 18, 12–25. doi: 10.1111/wbm.12136

Cudney, D., Wright, S., Shultz, T., and Reints, J. (1992). Weed seed in dairy manure depends on collection site. *CA. Agri.* 46, 31–32. doi: 10.3733/ca.v046n03p31

Dahlquist, R. M., Prather, T. S., and Stapleton, J. J. (2007). Time and temperature requirements for weed seed thermal death. *Weed Sci.* 55, 619–625. doi: 10.1614/WS-04-178.1

Di Tomaso, J. M. (1995). Approaches for improving crop competitiveness through the manipulation of fertilization strategies. *Weed Sci.* 43, 491–497. doi: 10.1017/S0043174500081522

Dray, S., and Dufour, A. B. (2007). The ade4 package: implementing the duality diagram for ecologists. *J. Statist. Softw.* 22, 1–20. doi: 10.18637/jss.v022.i04

Ellenberg, H., Weber, H., Düll, R., Wirth, W., and Paulissen, D. (1992). Zeigerwerte von Pflanzen in mitteleuropa. *Scrip. Geobotan.* 18, 1–248.

Fox, J., and Weisberg, S. (2018). *An R Companion to Applied Regression.* Thousand Oaks, CA: Sage publications.

Fried, G., Chauvel, B., Munoz, F., and Reboud, X. (2020). Which traits make weeds more successful in maize crops? Insights from a three-decade monitoring in France. *Plants* 9:40. doi: 10.3390/plants9010040

Fried, G., Chauvel, B., and Reboud, X. (2009). A functional analysis of large-scale temporal shifts from 1970 to 2000 in weed assemblages of sunflower crops in France. *J. Vegetat. Sci.* 20, 49–58. doi: 10.1111/j.1654-1103.2009.05284.x

Fried, G., Kazakou, E., and Gaba, S. (2012). Trajectories of weed communities explained by traits associated with species' response to management practices. *Agri. Ecosyst. Environ.* 158, 147–155. doi: 10.1016/j.agee.2012.06.005

Fried, G., Norton, L. R., and Reboud, X. (2008). Environmental and management factors determining weed species composition and diversity in France. *Agri. Ecosyst. Environ.* 128, 68–76. doi: 10.1016/j.agee.2008.05.003

Fried, G., Petit, S., and Reboud, X. (2010). A specialist-generalist classification of the arable flora and its response to changes in agricultural practices. *BMC Ecol.* 10:20. doi: 10.1186/1472-6785-10-20

Funk, J. L., and Wolf, A. A. (2016). Testing the trait-based community framework: Do functional traits predict competitive outcomes? *Ecology* 97, 2206–2211. doi: 10.1002/ecy.1484

Gaba, S., Perronne, R., Fried, G., Gardarin, A., Bretagnolle, F., Biju-Duval, L., et al. (2017). Response and effect traits of arable weeds in agro-ecosystems: a review of current knowledge. *Weed Res.* 57, 123–147. doi: 10.1111/wre.12245

Goto, K. (1985). Relationships between soil pH, available calcium and prevalence of potato scab. *Soil Sci. Plant Nutr.* 31, 411–418. doi: 10.1080/00380768.1985.10557448

Hoveland, C., Buchanan, G., and Harris, M. (1976). Response of weeds to soil phosphorus and potassium. *Weed Sci.* 194–201. doi: 10.1017/S0043174500065747

Husson, F., Josse, J., and Le, S. J. M. (2010). *FactoMineR: Multivariate Exploratory Data Analysis and Data Mining with R.* Available online at: http://CRAN.R-project.org/package=FactoMineR (accessed August 20, 2017).

Jiang, M., Liu, T., Huang, N., Shen, X., Shen, M., and Dai, Q. (2018). Effect of long-term fertilisation on the weed community of a winter wheat field. *Sci. Rep.* 8, 1–7. doi: 10.1038/s41598-018-22389-4

Julve, P. (1998). *Baseflor. Index Botanique, Ecologique et Chorologique de la Flore de France.* Available online at: http://perso.wanadoo.fr/philippe.julve/catminat.htm (accessed August 20, 2017).

Ketterings, Q. M., Czymmek, K. J., and Klausner, S. D. (2003a). *Phosphorus Guidelines for Field crops in New York.* Second *Release. Department of Crop and Soil Sciences Extension Series E03-15.* Ithaca, NY: Cornell University.

Ketterings, Q. M., Klausner, S. D., and Czymmek, K. J. (2003b). *Nitrogen Guidelines for Field Crops in New York.* Second *Release. Department of Crop and Soil Extension Series E03-16.* Ithaca, NY: Cornell University.

Ketterings, Q. M., Klausner, S. D., and Czymmek, K. J. (2003c). *Potassium Guidelines for Field Crops in New York.* Second *Release. Dept. Crop and Soil Sciences Extension Series E03-14.* Ithaca, NY: Cornell University.

Klotz, S., Kühn, I., Durka, W., and Hrsg. (2002). BIOLFLOR - *Eine Datenbank zu biologisch-ökologischen Merkmalen der Gefäßpflanzen in Deutschland. Schriftenreihe für Vegetationskunde* 38 - Bonn: Bundesamt für Naturschutz.

Larney, F. J., and Blackshaw, R. E. (2003). Weed seed viability in composted beef cattle feedlot manure. *J. Environ. Qual.* 32, 1105–1113. doi: 10.2134/jeq2003.1105

Legendre, P., and Legendre, L. (2012). *Numerical Ecology*. Amsterdam: Elsevier.

Légère, A., and Samson, N. (2004). Tillage and weed management effects on weeds in barley–red clover cropping systems. *Weed Sci.* 52, 881–885. doi: 10.1614/WS-04-011R

Liebman, M., Gallandt, E. R., and Jackson, L. (1997). Many little hammers: ecological management of crop-weed interactions. *Ecol. Agri.* 1, 291–343. doi: 10.1016/B978-012378260-1/50010-5

Maclaren, C., Storkey, J., Menegat, A., Metcalfe, H., and Dehnen-Schmutz, K. (2020). An ecological future for weed science to sustain crop production and the environment. a review. *Agronomy Sustain. Dev.* 40, 1–29. doi: 10.1007/s13593-020-00631-6

Mahaut, L., Gaba, S., and Fried, G. (2019). A functional diversity approach of crop sequences reveals that weed diversity and abundance show different responses to environmental variability. *J. Appl. Ecol.* 56, 1400–1409. doi: 10.1111/1365-2664.13389

Mahé, I., Cordeau, S., Bohan, D. A., Derrouch, D., Dessaint, F., Millot, D., et al. (2021). Soil seedbank: Old methods for new challenges in agroecology? *Ann. Appl. Biol.* 178, 23–38. doi: 10.1111/aab.12619

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Res.* 27, 209–220.

Mccloskey, M., Firbank, L. G., Watkinson, A. R., and Webb, D. J. (1996). The dynamics of experimental arable weed communities under different management practices. *J. Veget. Sci.* 7, 799–808. doi: 10.2307/3236458

Mirsky, S. B., Gallandt, E. R., Mortensen, D. A., Curran, W. S., and Shumway, D. L. (2010). Reducing the germinable weed seedbank with soil disturbance and cover crops. *Weed Res.* 50, 341–352. doi: 10.1111/j.1365-3180.2010.00792.x

Moreau, D., Busset, H., Matejicek, A., and Munier-Jolain, N. (2014). The ecophysiological determinants of nitrophily in annual weed species. *Weed Res.* 54, 335–346. doi: 10.1111/wre.12090

Moreau, D., Milard, G., and Munier-Jolain, N. (2013). A plant nitrophily index based on plant leaf area response to soil nitrogen availability. *Agronomy Sustain. Dev.* 33, 809–815. doi: 10.1007/s13593-013-0145-x

Mortensen, D. A., Bastiaans, L., and Sattin, M. (2000). The role of ecology in the development of weed management systems: an outlook. *Weed Res.* 40, 49–62. doi: 10.1046/j.1365-3180.2000.00174.x

Norsworthy, J. K., and Oliveira, M. J. (2004). Comparison of the critical period for weed control in wide-and narrow-row corn. *Weed Sci.* 52, 802–807. doi: 10.1614/WS-03-165R

Oksanen, J. (2019). *vegan: Community Ecology Package (R package Version 2.5-5).* Available online at: https://CRAN.R-project.org/package=vegan (accessed December 01, 2020).

Petit, S., Cordeau, S., Chauvel, B., Bohan, D., Guillemin, J.-P., and Steinberg, C. (2018). Biodiversity-based options for arable weed management. a review. *Agronomy Sustain. Dev.* 38:48. doi: 10.1007/s13593-018-0525-3

Pleasant, J. M., and Schlather, K. J. (1994). Incidence of weed seed in cow (*Bos* sp.) manure and its importance as a weed source for cropland. *Weed Technol.* 8, 304–310. doi: 10.1017/S0890037X00038823

Pyšek, P., and Lepš, J. (1991). Response of a weed community to nitrogen fertilization: a multivariate analysis. *J. Veget. Sci.* 2, 237–244. doi: 10.2307/3235956

Rao, B. R. (1969). Partial canonical correlations. *Trabajos Estadist. Investigación Oper.* 20, 211–219. doi: 10.1007/BF03028532

Ryan, M. R., Smith, R. G., Mirsky, S. B., Mortensen, D. A., and Seidel, R. (2010). Management filters and species traits: weed community assembly in long-term organic and conventional systems. *Weed Sci.* 58, 265–277. doi: 10.1614/WS-D-09-00054.1

Sadeghpour, A., Ketterings, Q. M., Godwin, G. S., and Czymmek, K. J. (2016). Nitrogen- vs. phosphorus-based manure and compost management of corn. *Agron. J.* 108, 185–195. doi: 10.2134/agronj2015.0218

Sadeghpour, A., Ketterings, Q. M., Godwin, G. S., Czymmek, K. J., and Vermeylen, F. (2017). Agro-environmental consequences of shifting from nitrogen- to phosphorus-based manure management of corn. *Soil Sci. Soc. Am. J.* 81, 1127–1138. doi: 10.2136/sssaj2016.03.0417

Seavers, G., and Wright, K. (1999). Crop canopy development and structure influence weed suppression. *Weed Res.* 39, 319–328. doi: 10.1046/j.1365-3180.1999.00148.x

Smith, R. G., and Mortensen, D. A. (2017). "A disturbance-based framework for understanding weed community assembly in agroecosystems: challenges and opportunities for agroecological weed management," in *Agroecological Practices for Sustainable Agriculture*, ed A. Wezel (London: World Scientific), doi: 10.1142/9781786343062_0005

Stevenson, F. C., Legere, A., Simard, R. R., Angers, D. A., Pageau, D., and Lafond, J. (1997). Weed species diversity in spring barley varies with crop rotation and tillage, but not with nutrient source. *Weed Sci.* 45, 798–806. doi: 10.1017/S0043174500088998

Storkey, J., Moss, S. R., and Cussans, J. W. (2010). Using assembly theory to explain changes in a weed flora in response to agricultural intensification. *Weed Sci.* 58, 39–46. doi: 10.1614/WS-09-096.1

Tang, L., Wan, K., Cheng, C., Li, R., Wang, D., Pan, J., et al. (2014). Effect of fertilization patterns on the assemblage of weed communities in an upland winter wheat field. *J. Plant Ecol.* 7, 39–50. doi: 10.1093/jpe/rtt018

Tilman, E. A., Tilman, D., Crawley, M. J., and Johnston, A. (1999). Biological weed control via nutrient competition: potassium limitation of dandelions. *Ecol. App.* 9, 103–111. doi: 10.1890/1051-0761(1999)009[0103:BWCVNC]2.0.CO;2

USDA-NRCS (2017). *Fact Sheets and Plant Guides is a Partnership of the National Plant Data Team and the Plant Materials Program*. Available online at: https://plants.usda.gov/java/factSheet (accessed August 20, 2017).

Vengris, J., Colby, W. G., and Drake, M. (1955). Plant nutrient competition between weeds and corn. *Agron. J.* 47, 213–216. doi: 10.2134/agronj1955.00021962004700050005x

Wamelink, G., Joosten, V., Van Dobben, H., and Berendse, F. (2002). Validity of Ellenberg indicator values judged from physico-chemical field measurements. *J. Veget. Sci.* 13, 269–278. doi: 10.1111/j.1654-1103.2002.tb02047.x

Yin, L., Cai, Z., and Zhong, W. (2006). Changes in weed community diversity of maize crops due to long-term fertilization. *Crop Protection* 25, 910–914. doi: 10.1016/j.cropro.2005.11.013

Zelený, D., and Schaffers, A. P. (2012). Too good to be true: pitfalls of using mean E llenberg indicator values in vegetation analyses. *J. Veget. Sci.* 23, 419–431. doi: 10.1111/j.1654-1103.2011.01366.x

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Assessing Physiological and Genetic Evidence for Evolution of Shared Weedy Rice Traits at the Vegetative Growth Stage

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Weedy rice (*Oryza* spp.) has successfully adapted to invasion of cultivated rice (*O. sativa* L.) fields by being a strong competitor from the early vegetative growth stages to crop harvest. While seed shattering and seed dormancy have been shown to contribute to competitiveness at the reproductive stage, much less is known about the traits that could contribute to weedy rice adaptation at the vegetative stage. We examined several growth and physiological traits in five different weedy rice lineages with different ancestral origins, and found that no single vegetative phenotype characterizes all weedy rice. Divergence in growth and physiological traits between weedy rice groups and their putative cultivated ancestors has been limited, suggesting that altered vegetative traits have not been a common path to weed adaptation. There is a lack of convergence in patterns of gene expression in two independent weedy rice lineages, suggesting that there are few shared genetic mechanisms in the evolution of vegetative traits. We conclude that it must not be assumed that all weedy rice groups necessarily have altered vegetative growth or physiological mechanisms compared to their ancestors, that facilitate their invasion of crop fields.

Keywords: red rice, convergent evolution, parallel evolution, weediness traits, RNA-Seq, *Oryza* groups

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INTRODUCTION

Agricultural weeds are plants adapted to infest crop fields and reduce agricultural production. The adverse effect of agricultural weeds stems from their competitiveness, which decreases crop yields. Competitiveness refers to the ability of an organism to reduce the fitness of another organism through its presence. A plant can be competitive by having an efficient reproductive strategy, capturing or using resources more efficiently (Radosevich et al., 1997), by having improved tolerance to abiotic stress (Nadir et al., 2017), or by having improved resistance to local pathogens and/or herbivores (Jia and Gealy, 2018). Understanding how competitiveness evolves is important for understanding how noxious agricultural weeds can be curtailed.

Weedy rice (*Oryza* spp.), a type of weed that infests cultivated rice (*O. sativa*) fields throughout the world, is a strong competitor of cultivated rice from the early vegetative growth stage to harvest. Competition between weedy rice and cultivars leads to severe rice yield losses (Caton et al., 2003), a serious concern in a crop that serves as the primary calorie source for more than one-third of the world's population (Bhullar and Gruissem, 2013). For example, in Malaysia, 74% of cultivated rice yield can be lost due to infestations of weedy rice (Karim et al., 2004). In the US, weedy rice infestations lead to estimated annual economic losses of over $45 million (Estorninos et al., 2005; Gealy and Yan, 2012). In China, infestations of as few as eight local weedy rice plants per square meter have the capacity to reduce yields of the rice cultivar "Nanjing 44" by almost two-thirds (Xu et al., 2018), although the impact of weedy rice on yield depends, among other factors, on the cultivar it infests (Ottis et al., 2005; Shivrain et al., 2009).

The two most well-studied weedy traits enhancing weedy rice competitiveness occur at the reproductive stage, and are seed shattering and seed dormancy. Seed shattering enables weedy rice seeds to escape the crop harvest and disperse in the field (Fischer et al., 1995; Li et al., 2006), while seed dormancy, and, in particular, mixtures of weedy rice types in fields with varying levels of dormancy, promote persistence of viable weed seeds in the soil through variable periods of time (Gu et al., 2005; Tseng et al., 2013). In contrast, mechanisms contributing to competitiveness at the vegetative growth stage have not been as fully studied in weedy rice, though some traits have been suggested to facilitate weed success. Weedy rice has been reported to grow taller and produce more tillers than cultivated crops in some world areas (Shivrain et al., 2010), and a field competition study in Arkansas in the US suggests that taller weedy rice stature is correlated with stronger negative impact on cultivar performance (Estorninos et al., 2005). There are also studies suggesting that some weedy rice lines may have more efficient nitrogen assimilation than cultivated rice under low nitrogen treatments (Sales et al., 2008), and that nitrogen applications under competition can lead to greater shoot biomass gain in some weedy rice lines compared to cultivated rice (Chauhan and Johnson, 2011). Based on studies such as these, weedy rice in general has been hypothesized to accumulate more nitrogen and to respond to higher nitrogen content better than cultivated rice (Burgos et al., 2006).

Although there is no established list of traits that determines the degree of competitiveness at the vegetative growth stage in weedy rice plants, previous research has shown that several traits in cultivated rice are associated with greater success in the field. For example, hybrid rice varieties in Brazil have higher photosynthetic capacity in terms of sub-stomatal CO_2 content, photosynthetic rate and CO_2 consumed, which results in higher dry mass accumulation compared to inbred cultivars (Concenco et al., 2011); hybrid varieties in the US have also been documented to produce more tillers and achieve canopy coverage faster, affecting their competitiveness favorably (Ottis et al., 2005). Chlorophyll and carbohydrate (CHOs) content are also often included among the traits affecting cultivated rice competitiveness (He et al., 2006). Because chlorophyll contains nitrogen, its content is a good indicator of nitrogen supply, and assessing chlorophyll content by measuring leaf color has long been used as a non-invasive criteria to determine the health and stress level of plants (Adhikari et al., 1999; Richardson, 2002). CHOs, transported as soluble sugars, and stored as starch constitute the main sources of energy supply and can have an impact on plant vitality (Zhang et al., 2012). Starch content in source leaves, and sucrose content in sink organs have been shown to be important markers of early vigor, defined as biomass accumulation ability during vegetative growth (Rebolledo et al., 2012). Percentage of total nitrogen in leaf tissue is considered a common marker for crop competitiveness. Many agronomic traits (i.e., crop growth rate, leaf area index, plant height, tiller number, spikelets per panicle, grain filling) and physiological processes (i.e., photosynthesis and respiration, nitrogen and carbon metabolism) are negatively affected under nitrogen-deficient conditions (Novoa and Loomis, 1981), and thus high nitrogen content in leaves is considered a sign of overall vitality.

Understanding the bases of weedy rice competitiveness is important not only for curtailing this noxious agricultural weed, but also because some weed competitive traits could be beneficial if transferred to crops. A complicating aspect of weedy rice studies is the independent evolution of multiple weedy rice populations around the world from diverse genetic backgrounds (Reagon et al., 2010; Huang et al., 2017; Vigueira et al., 2019). For example, in the US, two genetically differentiated weedy rice populations, the SH (straw hulled) and the BHA (black-hulled awned), have evolved from the *indica* and *aus* rice cultivar groups, respectively (Reagon et al., 2010). In South Asia, the wild ancestor of cultivated rice (*Oryza rufipogon/nivara*), together with *aus* and *indica* cultivars contribute to weedy rice origins, giving rise to wild-like, *aus*-like, and *indica*-like weedy rice (Huang et al., 2017). More populations along with other ancestries have been detected in other areas including Southeast Asia, Northeast Asia, China, Europe, Colombia and other parts of South America (Cao et al., 2006; Song et al., 2014; Qiu et al., 2017, 2020; Hoyos et al., 2019; Vigueira et al., 2019). While the reproductive competitiveness traits of seed shattering and seed dormancy have been found in most, if not all, populations of weedy rice worldwide, regardless of ancestral genetic background (Ziska et al., 2015), the shared prevalence of vegetative growth and physiological traits that could enhance competitiveness in independently evolved weed groups and the extent to which weedy rice has diverged from its ancestors for these traits is unknown.

To address this knowledge gap, we have assembled a panel of weedy rice from two geographic areas, the US and South Asia (SA), with three different ancestries, *aus*, *indica* and wild rice (*O. rufipogon/O. nivara)*. We have examined various growth-related traits at the vegetative stage and gene expression patterns of these weeds, and, when possible, compared these to representatives of the ancestral groups of each weedy population to determine the degree of change during weed evolution. We note that our trait selection is not exhaustive and that our conclusions are limited to the growth conditions of our study. However, within this framework, we attempt to answer the following three questions: (1) For what traits do weedy rice groups differ from related cultivated rice groups at the vegetative growth stage? (2) What vegetative traits are common to weedy rice groups from different ancestral backgrounds? and (3) What genes are associated with and could mediate patterns of weedy vegetative traits?

MATERIALS AND METHODS

Rice Plant Growth Conditions

We selected a panel of 14 accessions (genotypes) that included four US weedy rice [two each from the SH and BHA populations as identified by Reagon et al. (2010)], six South Asian weedy rice with *aus*, *indica* and wild rice ancestry (*O. rufipogon*/*O. nivara*) as identified by Huang et al. (2017), and two each of *aus* and *indica* cultivars (**Supplementary Table 1**). Three plants were grown for each accession in growth chamber conditions in a randomized block design, with an 11 h day length, day temperature of 30°C and night temperature of 27°C. Seeds were sown in 4″ × 4″ × 10″ pots (Treepots, Hummert International Missouri, USA). Rice Cornell mix soil (1 1/2 bales peat, 2 bags medium to course vermiculite, 5 lbs lime and 1 lb 30 z Peter's Unimix Plus III) was used as the starting soil with nitrogen content of 0.9%. A total of 0.26 g granule triple superphosphate (0-45-0) was applied to each plant at the day of sowing. Plants were fertilized weekly with a diluted content for a total of 0.049 g potassium chloride (0-0-62) and 0.03 g iron (Sprint 330) per plant before harvest. 0.337 g of total urea (converted to 150 kg N ha^{-1} content) per plant was applied with half of the amount at 15 days after emergence (DAE) and the other half at 37 DAE.

Plant Growth and Physiological Trait Measurements

For all plants, height, and chlorophyll status were measured at 10, 15, 20, 37, and 45 days after emergence (DAE), to capture aspects of plant growth before and after nitrogen application; however, measurements at different stages were highly correlated, so we proceeded only with the measurements at 45 DAE. Height was measured from soil surface to the tip of the longest leaf. SPAD value (Soil and Plant Analyzer Development) was used as an indicator of chlorophyll status, and was measured with the atLEAF+ chlorophyll meter Ver 1.0 (FT Green LLC, Wilmington, DE, USA) on a fully expanded healthy leaf from the main stem. To quantify the major growth period during the vegetative stage, the growth rate measured as centimeters of growth per day calculated between 15 DAE and 45 DAE (**Supplementary Table 2**).

Vegetative growth, defined as the phases from germination to panicle initiation, are the first phases of rice development. These developmental phases are usually marked as V stages. With V1 defined as when the first complete leaf pushes through the prophyll and forms a collar, and stages starting from V2 defined as the collar formation on leaf *n* on the main stem (Moldenhauer and Slaton, 2001). In this study, we recorded vegetative development rate as the days from emergence to different V stages. We recorded V1 and V8 as the initial and final vegetative development rate (**Supplementary Table 2**).

All plants were harvested at 55 DAE. Upon harvest, the second youngest fully expanded leaf on the main stem was cut fresh for chlorophyll content measurement, and the second youngest fully expanded leaf on a side tiller was collected for soluble and storage carbohydrate content measurements. The rest of the above ground plant tissue was collected and dried in an 80°C oven and dry biomass was measured 7 days after drying. One gram of ground dried plant tissue from each plant was sent to the University of Massachusetts soil and plant tissue testing laboratory for total nitrogen percentage test with catalytic combustion method (Pt/Al2O3) (**Supplementary Table 2**). Total nitrogen percentage in leaf tissue shows the amount of nitrogen in all formats including, ammonia, organic and reduced nitrogen, nitrates and nitrites (Ranker, 1925), remaining in plants.

Chlorophyll content was measured according to the protocol described by Inskeep and Bloom (Inskeep and Bloom, 1985), by immersing weighed fresh leaf tissue in DMF for 24 h at 4°C in dark, and then measuring $A_{664.5}$ and A_{657} with a spectrophotometer (**Supplementary Table 2**). Soluble carbohydrates were extracted by immersing ∼25 mg of dried and ground leaf tissue into 2 ml of water, followed by overnight shaking in an 80°C incubator. D-glucose, D-fructose and sucrose contents in supernatant were measured by Sucrose/D-Glucose/D-Fructose kit (catalog# 10716260035) (R-Biopharm AG, Darmstadt, Germany) (**Supplementary Table 2**). Starch was extracted by immersing ∼50 mg dried and ground leaf tissue into HCl (8M) and DMSO, incubating the mixture at 60°C for 60 min, adding 5 ml redistilled water, and then adjusting the pH value of the liquid to 4 or 5 with NaOH (5M). Starch content in the supernatant was measured by Starch kit (catalog# 10207748035) (R-Biopharm AG, Darmstadt, Germany) (**Supplementary Table 2**).

Photosynthesis Measurements

Photosynthesis (CO_2 update, $\mu mol\ m^{-2}\ s^{-1}$) was measured on two different days, 47 and 55 days after sowing, before any destructive sampling for other analysis. Plants measured were in development stages ranging from V8 to V10 (a few plants had already reached flowering and the vegetative growth had terminated). An infrared gas exchange analyzer (LiCor 6400; LiCor Inc., Lincoln, NE) was used for measurements. Photosynthesis (*A*), transpiration rate (*E*) and stomatal conductance (g_s) were measured simultaneously (Long and Bernacchi, 2003). Measurements with a relative humidity of over 55%, and stomatal conductance between 0.30 and 0.65 were considered effective. We used a 2 cm wide attachment chamber, and completely covered chamber space with the second and fourth youngest leaves on the main stem.

Phenotypic Data Analysis

We included the following physiological traits in the linear mixed model analysis: glucose, fructose, sucrose, total sugar, starch, SPAD at 45 DAE, chlorophyll concentration, height at 45 DAE, growth rate, V1, V8, dry biomass, total nitrogen percentage and photosynthesis rate. We performed linear mixed model fit of maximum likelihood (lmerMod) with the formula "trait ∼ ancestry_group + Oryza_type_group + (1 | genotype)" with the "lme4" package in R (R Core Team, 2014). When assessing fixed effects of group combinations in terms of each trait, we looked at the *t*-values against the baseline. *t*-values in linear mixed models are the standardized parameters [ß/SE(ß)] (Gałecki and Burzykowski, 2013).

We also performed pairwise comparisons between weeds and their crop ancestors. Due to the small sample size within each weed and ancestor group, the measurements for traits do not follow normal distribution. We thus performed Wilcoxon rank sum test to determine the significance of differences for weed-cultivar pairs. We made the following four weed-crop pairs, one for US SH weeds with *indica* ancestry (rr01, rr09) vs. *indica* cultivars (sin11, sin31), one for US BHA weeds with *aus* ancestry (rr05, rr20) vs. *aus* cultivars (sau71, sau76), one for South Asian *indica* ancestry weeds (arr29, arr74) vs. *indica* cultivars (sin11, sin31), and the last one for South Asian *aus* ancestry weeds (arr38, arr54) vs. *aus* cultivars (sau71, sau76).

RNA Extraction and Library Preparation

Due to the limitation of leaf tissue available in the physiology panel, we re-planted a subset of the panel using the same growth chamber conditions and nutrition treatment on July, 1st, 2015, and harvested the third and fourth fully expanded leaf from the top at 55 DAE for RNA-seq sampling. We picked four accessions to re-plant with three replicates each, including one *indica* cultivar (sin11), one US weed with *indica* ancestry that belongs to the group SH (rr09), one *aus* cultivar (sau76) and one US weed with *aus* ancestry that belongs to the group BHA (rr20). Collected leaf tissue was frozen at −80°C and ground with mortar and pestles. A Qiagen RNeasy Plant Mini Kit (Valencia, CA) was used to extract RNA. A Bioanalyzer 2100 (Agilent Technologies, Inc.) was used to qualify and quantify RNA samples. Only RNA samples with RNA Integrity Number (RIN) > 7.5 were used for library preparation. Libraries (a total of 12) were constructed with NEBNext mRNA Library Prep Master Mix Set for Illumina kit (New England BioLabs Inc.). Total RNA was fragmented into smaller pieces, and then synthesized into the first and second strand of cDNA with reverse transcriptase and random primers. NEBNext singleplex Oligos for Illumina as indexes of each library were added during the preparation (New England BioLabs Inc.). A Qubit fluorometer and Qubit dsDNA BR (broad-range) Assay Kit (Thermo Fisher Scientific, MA, USA) were used to quantify cDNA library. High quality cDNA libraries were normalized with $0.1 \times$ TE to 4 nM content before sequencing.

Next Generation Sequencing and RNA-seq Data Analysis

We used two Illumina NextSeq500 Mid Output Kits (150 cycles) for pair-end sequencing of six libraries on each flowcell in the Genome Resource Lab at the University of Massachusetts Amherst. The quality of raw sequencing reads was investigated with FastQC program (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). For sequences of samples with low per base sequence quality, FASTQ Groomer in Galaxy (https://usegalaxy.org/) was used to convert FASTQ files to FASTSANGER files and Trimmomatic in Galaxy was used to trim both forward and reverse sequences. Sequences of all samples were then concatenated with Galaxy.

We used the Tuxedo method (Trapnell et al., 2012) in the CyVerse Discovery Environment (http://www.cyverse.org/discovery-environment) for RNA-seq data analysis. Reads were mapped to the rice reference genome MSU7 (http://rice.plantbiology.msu.edu) with TopHat version 2.0.9 (Trapnell et al., 2009) with the reference genome annotation file using minimum intron length as 30, mate-pair inner distance as 100 and other settings as default. The aligned reads were assembled and transcript expression was quantified using FPKM (Fragments Per Kilobase of transcript per Million fragments mapped) by Cufflinks2 version 2.0.2 (Trapnell et al., 2010). We obtained a rice reference genome annotation file in gtf format from MSU7 (http://rice.plantbiology.msu.edu/annotation_pseudo_current.shtml), and a rice rRNA mask file in gff3 format from RAP-DB (http://rapdb.dna.affrc.go.jp/download/irgsp1.html). The rRNA mask file was then converted to gtf format using the gffread tool in Galaxy. Cuffmerge was used to create one gtf file of each sample from the three replicates. We then used Cuffdiff2 to detect differential expression of the four following sets of comparisons: (i) sin11 vs. rr09 (*indica* vs. SH weed), (ii) sau76 vs. rr20 (*aus* vs. BHA weed), (iii) sin11 vs. sau76 (*indica* vs. *aus*) and (iv) rr09 vs. rr20 (SH weed vs. BHA weed). We used two housekeeping genes, *UBQ5* (LOC_Os01g22490) and *eEF-1α* (LOC_Os03g08020), which have been shown to be the most stably expressed across rice genotypes, phases of development and different environment conditions, for normalization of expression data among genotypes (Jain et al., 2006). Transcripts and genes in the Cuffdiff2 results with FDR <0.01 and $\mathrm{abs}(\log_2 \mathrm{FC})>1$ were considered to be significantly differentially expressed and considered for further analysis. For each set of comparisons, we also considered as differentially expressed for genes that are not expressed in one genotype, but expressed with >1 FPKM in the other genotype.

In this project, we only take into consideration of transcripts and isoforms already annotated by the MSU7 (http://rice.plantbiology.msu.edu/) database. We focused on differential expression tests from the Cuffdiff2 outputs which test difference in the summed FPKM of transcripts sharing the same gene IDs.

GOterm Analysis and GOSlim Retrieval

We performed gene ontology (GO) term enrichment with agriGO (Du et al., 2010), using the *Oryza sativa* MSU7.1 non-TE genome as background. Significance was evaluated using a hypergeometric statistical test, with a Hochberg FDR multiple correction and a significant cutoff of 0.05; the minimum number of mapping entries was set to two. We used the Rice Genome Annotation Project database (http://rice.plantbiology.msu.edu/) for putative function and GOSlim (Harris et al., 2004) assignments retrieval for each gene of interest.

Analyzing Metabolic Pathways of Differentially Expressed Genes With MapMan

We used MapMan (version 3.5.1) (http://MapMan.gabipd.org), a software developed to annotate plant-specific biological processes, to analyze expression data at the pathway level. *Oryza* MSU7 annotation was used as the reference mapping file. Fold change data from the four comparisons, sin11 (*indica*) vs. rr09 (SH), sau76 (*aus*) vs. rr20 (BHA), rr09 (SH) vs. rr20 (BHA), and sin11 (*indica*) vs. sau76 (*aus*) were used for MapMan. For genes that expressed in only one genotype, we arbitrarily assigned

15 or−15 as log2(fold_change). We used a Benjamini Hochberg correction of multiple comparisons for the Wilcoxon rank sum test report of bins of interest.

RESULTS

Vegetative Growth Differences Among Ancestry and *Oryza*-Type Groups

We selected a panel of 14 accessions (genotypes) that included four US weedy rice (two each from the SH and BHA populations, which have *indica* and *aus* ancestry, respectively), six South Asian weedy rice with *aus* (two), *indica* (two) and wild rice (two) ancestry as identified by Huang et al. (2017), and two each of *aus* and *indica* cultivars (**Supplementary Table 1**). Accessions were selected to be genetically representative of their respective groups based on prior studies (Reagon et al., 2010; Huang et al., 2017). Wild rice was not grown as part of the panel due to the lack of sufficient available seeds and greater uncertainty about the wild subpopulation giving rise to these South Asian wild-like weeds (Huang et al., 2017). Each of the chosen weed groups represents an independent evolutionary origin. Three replicates were grown per accession in a growth chamber in a randomized block design, and with standard nitrogen application (see methods) that would mimic the optimal nitrogen condition found in crop fields. Multiple growth and physiological traits were measured at several developmental timepoints as shown in **Figure 1** for all samples (**Supplementary Tables 2, 3**). Chosen traits represent either plant growth or development parameters, or traits that have been associated with increased competitiveness in weedy or cultivated rice, as detailed in our introduction.

We first determined that single traits measured at multiple time points (10, 15, 20, 37, and 45 days after emergence) presented the same trends across genotypes (**Supplementary Table 2**). Thus, for plant height, growth rate, and Soil and Plant Analyzer Development (SPAD) index we proceeded only with measurements from 45 days after emergence (DAE). We applied linear mixed modeling to estimate the variances for the two fixed effect grouping factors: ancestry, which could be *aus*, *indica*, or wild, and *Oryza*-type, which could be either cultivated rice, US weedy rice, or SA weedy rice (**Table 1**, **Supplementary Table 1**). Since there are no cultivars with wild ancestry, and no US weeds with wild ancestry, two cells of the 3 × 3 table were considered empty (**Table 1**). The genotypes within each cell were considered random draws from the collection of all possible genotypes in that category, and hence random effects. *Aus* was set as the baseline to compare with *indica* and wild rice for the ancestry grouping, and cultivar as a baseline for *Oryza*-type (**Tables 1**, **2**). We considered t-values > 2 or smaller than-2 as significant (**Table 2**) (Luke, 2017).

For the five carbohydrate (CHO) content traits examined (glucose, fructose, sucrose, total soluble sugar and starch), the wild ancestry group had significantly higher content than the *aus* baseline (**Table 2**, **Figure 2**), while no differences were observed between *indica* and *aus* ancestry. Additionally, the US weed *Oryza*-type group showed significantly higher fructose and total soluble sugar content than the cultivar baseline (**Table 2**, **Figure 2**). In contrast, few differences were observed between groups for the two leaf chlorophyll related traits examined, in either of the grouping criteria. The single exception was the *indica* ancestry group, which displayed significantly higher SPAD at 45 days after emergence (DAE) than the *aus* baseline (**Table 2**, **Figure 2**). We found no correlation between SPAD results and those for chlorophyll or nitrogen content. US weeds had significantly less total nitrogen in leaves than the cultivar baseline (**Table 2**, **Figure 2**), though there is a general trend for both weed groups to contain less total N in leaves.

A few more differences between groups were evident for growth and developmental traits. Height at 45 DAE and growth rate were both significantly higher in the US weed group than the cultivar baseline, suggesting that weeds in this group grow more and faster during the vegetative stage than their cultivated relatives (**Table 2**, **Figure 2**). Additionally, growth rate was higher in the wild ancestry group than the *aus* baseline. Developmental rate differed among some groups, with plants with wild ancestry taking a longer time to reach the V8 stage (i.e., once collar formation on leaf 8 of the main stem has occurred) than the *aus* baseline, and South Asian weeds reaching V8 more quickly than cultivars (**Table 2**, **Figure 2**), although this latter trend seems to be driven by South Asian weeds with a domesticated background (**Figure 2**, **Supplementary Table 2**).

As a general trend we found that for ancestry group, wild ancestry accessions consistently performed better than the baseline in multiple traits including soluble and storage sugars and growth rate, suggesting that plants with wild ancestry tend to have a more optimal usage of the same amount of nutrients during the vegetative growth stage. On the other hand, for *Oryza*-type group, although US weeds seem to have less above-ground nitrogen than cultivars, they had higher levels of some sugars, were taller than cultivars at 45 DAE, and grew faster. These results suggest that US weeds can reach the same level of plant vigor with less nitrogen allocated to above ground tissues and thus may have higher nitrogen use efficiency (**Table 2**, **Figure 2**).

Comparative Transcriptomics of Weed and Crop Vegetative Tissue

We performed RNA sequencing on leaf tissue at the vegetative growth stage of a subset of our panel, including three replicates of one *aus* (sau76) and one *indica* (sin11) cultivar, as well as one US SH (rr09) weed and one US BHA (rr20) weed, to identify differentially expressed transcripts between weeds and ancestors that could account for growth trait differences. US weeds showed the most altered trait values compared to cultivars, so RNA-seq was limited to these weeds and their putative ancestors (**Supplementary Tables 2, 4**).

Total reads from the 12 libraries ranged from 13.3 million to 42.2 million with an average of 30.2 million (**Supplementary Table 5**). TopHat read alignments ranged from 79 to 96% (**Supplementary Table 5**). A comparison of expression levels of two major housekeeping genes (*UBQ5* and *eEF-1α*) (Jain et al., 2006) across the four accessions showed high FPKM values with no significant expression differences between

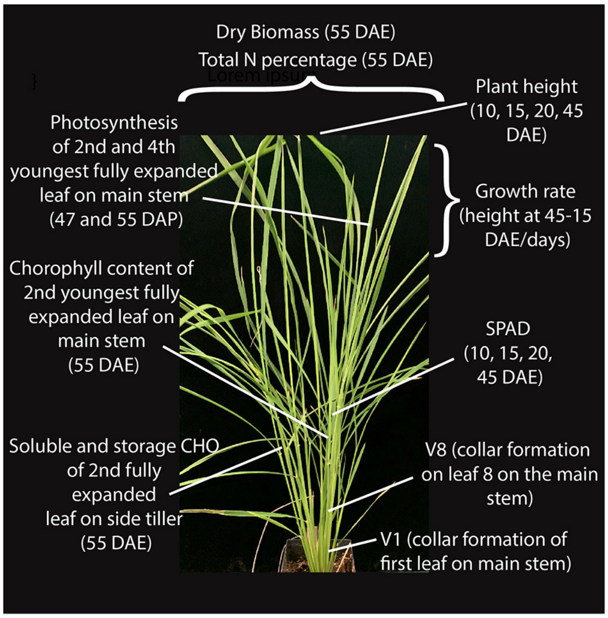

FIGURE 1 | Growth and physiological traits measured at different stages of vegetative growth in weedy rice.

TABLE 1 | *Oryza* genotypes included in the study and their ancestry and *Oryza*-type groupings.

Ancestry type	***Oryza*-type**		
	Cultivar[b]	**US weed**	**SA weed**
aus[a]	sau71, sau76	rr05, rr20	arr38, arr54
indica	sin11, sin31	rr01, rr09	arr29, arr74
wild			arr43, arr70

[a] *Aus was set as the baseline to compare among ancestry types.*
[b] *Cultivar was set as the baseline to compare among Oryza-types.*

samples (**Supplementary Table 6**), thus, we did not carry out normalization between samples.

We identified differentially expressed (DE) transcripts between each weed and their crop ancestor (*BHA* weed vs. *aus*, *SH* weed vs. *indica*), between cultivars (*aus* vs. *indica*) and between weeds with different ancestries (*BHA* vs. *SH*) (**Table 3**), including transcripts that lacked expression. Fewer transcripts were differentially expressed in the two weed-crop comparisons than in the weed-weed and crop-crop comparisons (**Table 3**; **Supplementary Tables 7–10**), consistent with the genetic background shared between weed and crop ancestors in each pair. A total of 73 DE transcripts were shared between the two weed-crop comparisons (**Supplementary Table 11**); however, 42 of these DE transcripts were shared among all four comparisons (**Supplementary Table 12**), and, of the remaining 31, none was exclusively to just the two weed-crop comparisons.

We performed gene ontology (GO) term enrichment analysis with agriGO (Du et al., 2010) for DE transcripts in all comparisons (**Supplementary Table 13**). Both weed-crop comparisons have a very limited number of biological processes (BP) terms significantly enriched ($P < 0.05$ with FDR correction) in DE transcripts, with the very high level terms "cellular process," "photosynthesis," and "metabolic process" shared between the two comparisons. However, no enriched terms were specific to weed-crop comparisons, with all these terms also appearing as enriched in either weed-weed or crop-crop comparisons (**Supplementary Table 13**).

We also looked at GO terms significantly enriched among DE transcripts that were shared across both weed-crop comparisons, and found much fewer but similar terms to those observed in individual comparisons and in transcripts that were DE in all four comparisons, with "cellular process," "response to abiotic stimulus," and "secondary metabolic process" dominating (**Supplementary Table 13**). We thus conclude that gene expression divergence between weedy rice and their cultivated ancestors occurs for the same general types of biological processes that are likely to change during general lineage divergence, regardless of whether diverging groups have a weedy, or cultivated status.

Metabolism Pathway Analysis of Differentially Expressed Genes

We used MapMan (Thimm et al., 2004) to attain a better understanding of the plant-specific functions of genes significantly differentially expressed between weeds and their crop ancestors, and to determine if any of these genes could explain the growth trait differences we had observed among groups. We also performed MapMan analysis on weed-weed and crop-crop comparisons to better understand the differences among same *Oryza*-type accessions. Since MapMan only recognizes gene IDs for MSU v7 genome (http://rice.plantbiology.msu.edu/) in the mapping file, we prepared input files with significant expression differences (as identified above) for each gene instead of each transcript. The total number of DE genes underscored, again, the greater expression similarity between weeds and their crop ancestors than between cultivars or weeds from different lineages, with 267 DE genes between SH and *indica,* 181 DE genes between BHA and *aus,* 412 DE genes between SH and BHA, and 327 DE genes between *indica* and *aus*.

In the SH vs. *indica* weed-crop comparison, the 267 DE genes mapped to 217 different pathway bins, with some genes mapping to multiple bins and some bins representing child categories of others, so that around 26 higher order pathway categories were represented (**Supplementary Table 14**; excluding the "not assigned" bins, which in all comparisons constituted the largest category). No bin was significantly overrepresented in this or other comparisons, but together they provide a view of the types of pathways for which gene expression differs in weed-crop, crop-crop, and weed-weed comparisons. As expected, due to the lower number of DE genes, fewer bins were mapped in the BHA weed vs. *aus* crop comparison (**Supplementary Table 14**; 154 total bins; 22 higher order pathway categories). Both the weed-weed (245 bins; 25 higher order pathway categories) and the crop-crop (232 bins; 25 higher order pathway categories) comparisons had genes mapping to a greater number of pathway bins, but

TABLE 2 | Linear mixed model analysis for genotypes from different ancestry and *Oryza* groups.

Trait	**Fixed effects**				**Random Effects**	
	Ancestry group *t*-value (*aus* as baseline)		***Oryza*-type group *t*-value (cultivar as baseline)**			
	***indica* to baseline**	**wild to baseline**	**US weed to baseline**	**SA weed to baseline**	**Genotype variance**	**Residual variance**
Glucose (g/100 g)	1.11	**4.57**	1.27	0.37	0	0.12
Fructose (g/100 g)	1.42	**3.42**	**2.25**	0.21	0	0.34
Sucrose (g/100 g)	0.74	**3.45**	0.98	0.26	0	1.63
Total sugar (g/100 g)	1.75	**6.47**	**2.43**	0.47	0	1.53
Starch (g/100 g)	0.91	**4.74**	0.85	0.18	2.94E-14	4.89E-01
SPAD_45DAE	**2.36**	−0.53	0.73	0.38	2.06	3.32
Chlorophyll concentration (mg/g)	0.69	−1.13	1.64	−0.19	0	0.76
Height_45DAE (cm)	0.44	1.78	**2.44**	−0.66	94.16	121.36
growth rate: (45–15)/30 (cm/day)	0.51	**2.27**	**2.44**	−0.42	0.074	0.11
V1 (days)	0.43	0.34	−0.27	0.27	0	0.59
V8 (days)	−0.71	**2.09**	−0.91	**−3.00**	0	15.07
Dry biomass (g)	−0.06	0.20	0.73	0.98	8,72	6.79
Photosynthesis rate	0.13	−0.53	0.80	−0.32	1.36	2.16
Total nitrogen percentage (%)	−0.1	1.51	**−3.15**	−1.80	0	0.085

t-values with absolute value >2 are in bold.

all comparisons had similar number of higher order pathway categories (**Figure 3**, **Supplementary Table 14**). Focusing on these higher level pathway categories, it is apparent that most of the mapped bins appear in all four comparisons, and there are no pathways for which gene expression differences are exclusive solely to weed-crop comparisons (**Figure 3**).

Despite a lack of exclusivity, we specifically compared the MapMan results of the two weed-crop comparisons to discover pathways or related genes possibly important for weed evolution from crop ancestors. For both weed-crop comparisons, pathways representing a large number of DE genes corresponded to transport, signaling, protein (primarily protein synthesis, degradation, and posttranslational modification), RNA (primarily RNA processing and regulation of transcription), and secondary metabolism (primarily phenlypropanoids and lignin, flavonoids, and isoprenoids) (**Supplementary Table 14** and **Figure 3**). However, the individual DE genes within each of these shared higher level pathway categories generally differed between the two weed-crop comparisons (**Supplementary Tables 14, 15**). The shared pathway category with the largest number of shared DE genes between the two weed-crop comparisons corresponded to secondary metabolism (**Supplementary Table 15**). However, for shared DE genes in any category there were no consistent trends in whether genes in weeds or crops were expressed more highly. For example, for the two shared DE genes mapping to photosynthesis pathways (LOC_Os04g59440 and LOC_Os12g19470), both were expressed at higher levels in the SH weed compared to the crop ancestor, but at lower levels in the BHA weed than its crop ancestor (**Supplementary Table 15**). We conclude that there is no evidence for an expression "syndrome" typical of weedy rice in comparison to its crop ancestors, or, if this exists, it involves the expression of only limited genes.

Associations Between Gene Expression and Phenotypic Differentiation

In our vegetative trait measurements for weedy and cultivated rice, we observed that US weedy rice has lower total nitrogen content than cultivars, higher fructose and total sugar content than cultivars, and faster growth rate and taller height than cultivars (**Supplementary Table 2**; **Supplementary Table 3**). We examined our list of differentially expressed transcripts and genes, the MapMan pathways they mapped to, and presumed functions based on literature searches, to identify candidates that could be responsible for the phenotypic differences we observed. Although we acknowledge the potential of annotation bias affecting our identification of interesting loci, we feel it would be remiss to not explore our dataset for potential candidates.

Beginning with nitrogen accumulation, we noted two N-response genes (LOC_Os01g48960 and LOC_Os04g56400) detected in the rr20 (*aus*-like weed) vs. sau76 (*aus*) and rr09 (*indica*-like weed) vs. sin11 (*indica*) comparisons (**Table 4**). These two genes fall in the MapMan bins of N-metabolism, ammonia metabolism, glutamate synthase (12.2.1) and glutamine synthase (12.2.2), respectively. They are part of conserved cross-species N-regulated network modules (Obertello et al., 2015). Both of the identified genes, which have higher expression in the weeds, have been implicated in ammonium assimilation and found to be sensitive to N treatments (Obertello et al., 2015) and could play a role in how much N uptake there is in weedy plants.

Multiple sugar and sugar derivative pathways related genes were detected as DE in the two weed-crop comparisons

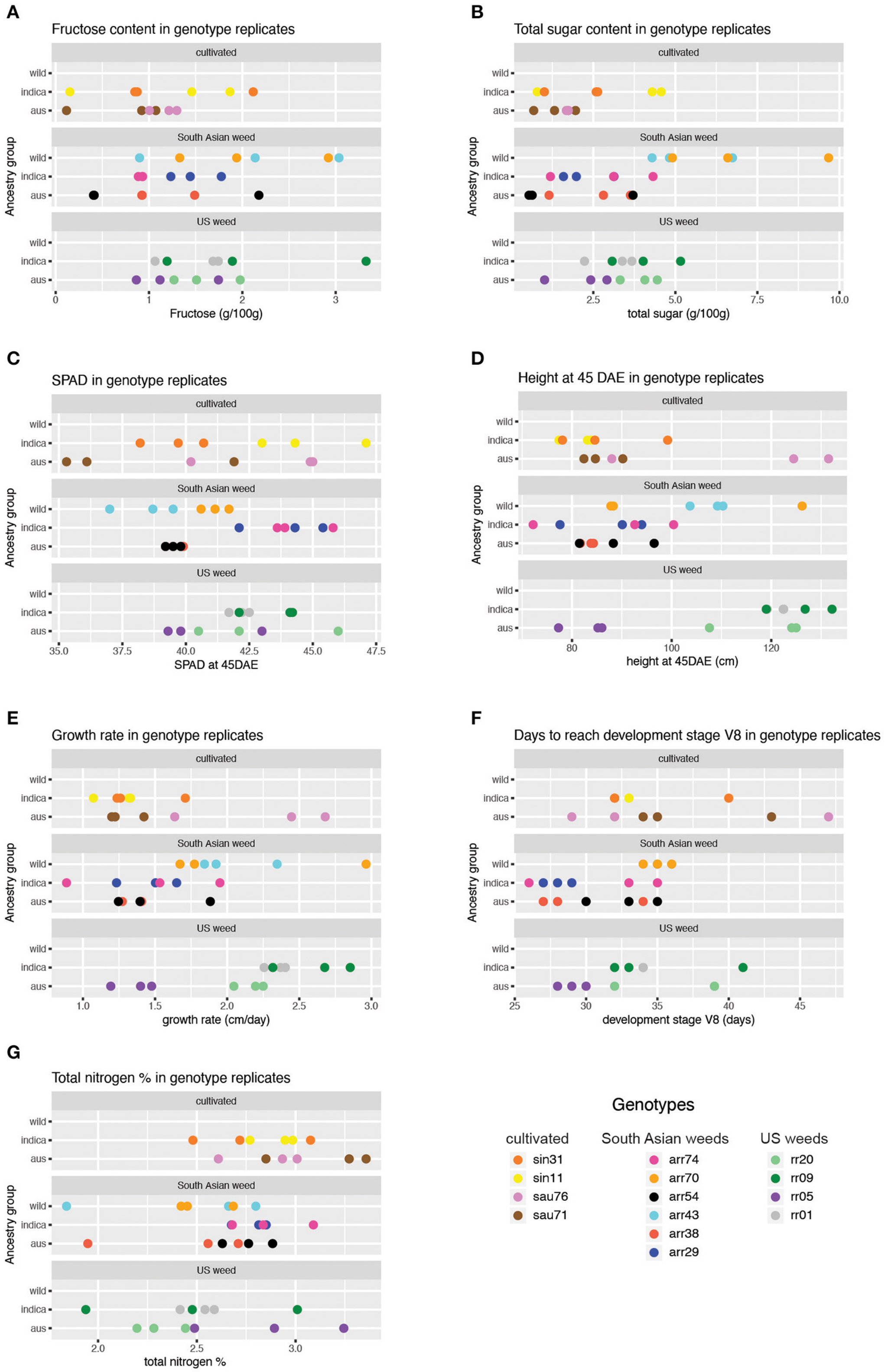

FIGURE 2 | Trait values obtained across genotypes and replicates for phenotypes showing significant results in the mixed model analysis (**Table 2**). Results for only two of the five sugar traits are shown, given similar results across sugar traits for the wild ancestry group. **(A)** Fructose content across genotype replicates. **(B)** Total sugar content across genotype replicates. **(C)** SPAD at 45 DAE across genotype replicates. **(D)** Height at 45 DAE across genotype replicates **(E)**. Growth rate across genotype replicates. **(F)** Days to reach V8 stage across genotype replicates. **(G)** Total nitrogen percentage across genotype replicates.

TABLE 3 | Summary of differentially expressed (DE) transcripts for the four RNA-seq comparisons.

Comparison	Expression G1>G2[a]	Expression G1<G2	Total expressed DE transcripts	G1, present G2, absent	G1, absent G2, present	Total present/ absent transcripts	Total DE transcripts
sin11 (*indica*) vs. rr09 (SH weed)	166	167	333	114	82	196	529
sau76 (*aus*) vs. rr20 (BHA weed)	100	121	221	94	118	212	433
rr09 (SH weed) vs. rr20 (BHA weed)	330	186	516	51	365	416	932
sin11 (*indica*) vs. sau76 (*aus*)	264	120	384	76	329	405	789

[a] *G1 represents the first genotype mentioned in the comparison, and G2 represents the second genotype mentioned in the comparison.*

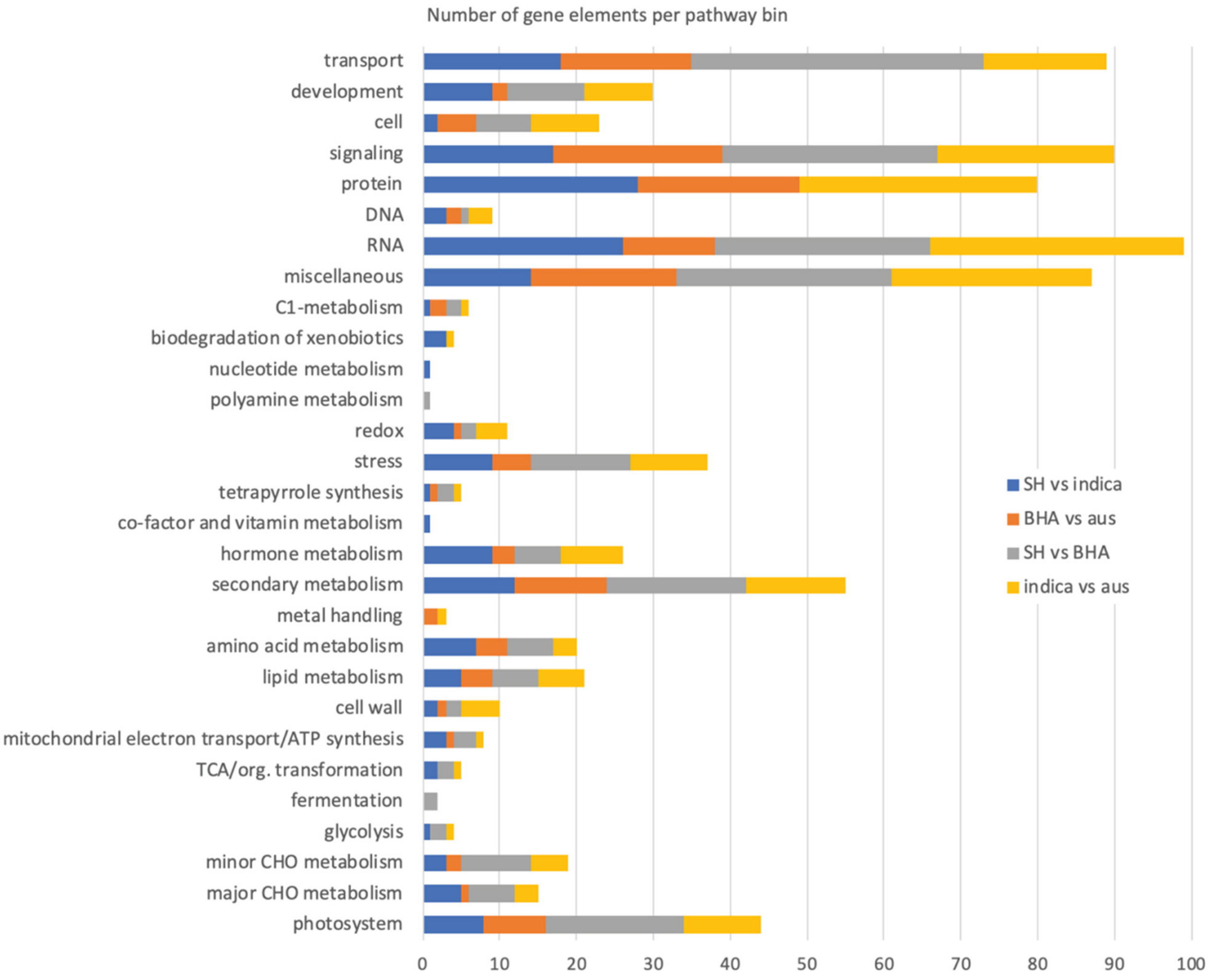

FIGURE 3 | Number of genes mapping to higher order MapMan plant pathway bins in the various weed-crop, crop-crop, and weed-weed comparisons. The "not assigned" pathway category, in all cases the largest category of mapped genes, is not included in the plot.

(**Table 4**), many of these falling within the major and minor CHO metabolism MapMan pathway bins (**Supplementary Table 15**). The gene, LOC_Os01g64660 (Zhu et al., 2018; Wang et al., 2020), annotated as fructose-1,6-bisphosphatase, could be related to the higher level of fructose in leaf tissue in SH weeds when compared to *indica* cultivars (**Table 2**). Several DE genes were also implicated in metabolism of sugar and sugar derivatives (**Supplementary Table 15**); these include the sucrose hexokinase (LOC_Os05g09500) (Cho et al., 2006), expressed at lower levels in the SH weed than

TABLE 4 | *Oryza* differentially expressed genes with potential associations with the observed phenotypic differentiation.

Rice transcript	Rice gene description	References	MapMan bin	Type	Comparisons[a]
N-response genes					
LOC_Os01g48960.1	Glutamate synthase, chloroplast precursor, putative, expressed	Obertello et al., 2015	N-metabolism.ammonia metabolism.glutamate synthase (12.2.1)	DEG	**rr20 > sau76**[b]
LOC_Os04g56400.1	Glutamine synthetize, catalytic domain containing protein, expressed	Obertello et al., 2015	N-metabolism.ammonia metabolism.glutamine synthase (12.2.2)	DEG	**rr09 > sin11**, rr09 > rr20, sin11 < sau76
Sugar and sugar derivative pathways/signals					
LOC_Os01g64660.1	Fructose-1,6-bisphosphatase, putative, expressed	Zhu et al., 2018; Wang et al., 2020	PS.calvin cycle.FBPase (1.3.7)	DEG	**rr09 > sin11**, rr09 > rr20, sin11 < sau76
LOC_Os05g09500.1	Hexokinase, putative, expressed	Cho et al., 2006	Major CHO metabolism.degradation.sucrose.hexokinase (2.2.1.4)	DEG	**rr09 < sin11,** sin11 > sau76
LOC_Os03g07480.1	Sucrose transporter, putative, expressed	Chen et al., 2019	Transport.sugar.sucrose (34.2.1)	DEG	**rr09 < sin11**, rr09 > rr20
LOC_Os03g22120.1	Sucrose synthase, putative, expressed	Chen et al., 2019	Major CHO metabolism.degradation.sucrose.Susy (2.2.1.5);	DEG	sin11 > sau76
LOC_Os03g52460.1	Glucose-1-phosphate adenylyltransferase large subunit, putative, expressed	López-González et al., 2019; Meng et al., 2019	Major CHO metabolism.synthesis.startch.AGPase (2.1.2.1)	DEG	rr09 > rr20
LOC_Os04g53310.1	Soluble starch synthase 3, chloroplast precursor, putative, expressed	Kharabian-Masouleh et al., 2011; López-González et al., 2019	Major CHO metabolism.synthesis.startch.starch synthase (2.1.2.2)	DEG	rr09 > rr20
LOC_Os06g06560.1	Starch synthase, putative, expressed	López-González et al., 2019	Major CHO metabolism.synthesis.startch.starch synthase (2.1.2.2)	DEG	**rr09 > sin11**, rr09 > rr2
LOC_Os06g14510.3	Glucose-6-phosphate isomerase, putative, expresse	Bagnaresi et al., 2012	Glycolysis.plastid branch.glucose-6-phosphate isomerase (4.2.3)	DEG	rr09 > rr20
LOC_Os06g22060.1	Pyrophosphate–fructose 6-phosphate 1-phosphotransferase subunit alpha, putative, expressed	Chen et al., 2020	Not assigned.unknown (35.2)	DEG	rr09 > rr20, sin11 > sau76
LOC_Os07g22930.3	Starch synthase, putative, expressed	López-González et al., 2019	Major CHO metabolism.synthesis.startch.starch synthase (2.1.2.2)	DEG	**rr09 > sin11**, rr09 > rr20
LOC_Os10g32810.1	Beta-amylase, putative, expressed	Kim et al., 2017	Major CHO metabolism.degradation.starch.starch cleavage.beta.amylase (2.2.2.1.2)	DEG	**rr09 > sin11**, rr09 > rr20
LOC_Os11g07020.1	Fructose-bisphospate aldolase isozyme, putative, expressed	Zhou et al., 2017	PS.calvin cycle.aldolase (1.3.6)	DEG	**rr09 > sin11**, **rr20 < sau76**, rr09 > rr20, sin11 < sau76
LOC_Os11g24240.1	Sinapoylglucose choline sinapoyltransferase, putative, expressed	Campo et al., 2014	Not assigned.unknown (35.2	transcript present in rr20 and absent in rr09	rr09 vs. rr20
LOC_Os12g20150.1	Phosphoglucan, water dikinase, chloroplast precursor, putative, expressed	Mahlow et al., 2016	Major CHO metabolism.degradation.starch.glucan water dikinase (2.2.2.3)	DEG	**rr09 > sin11**
LOC_Os08g02120.1	Kinase, pfkB family, putative, expressed	Zhang et al., 2016	Major CHO metabolism.degradation.sucrose.fructokinase (2.2.1.1)	DEG	**rr20 > sau76**

(Continued)

TABLE 4 | Continued

Rice transcript	Rice gene description	References	MapMan bin	Type	Comparisons[a]
Plant growth					
LOC_Os03g19520.2	Cyclin-related protein, putative, expressed	Liu et al., 2016	Cell.cycle (31.3)	DEG	**rr20 < sau76**, sin11 < sau76
LOC_Os03g54084.1	Phytochrome C, putative, expressed	Pareek et al., 2006	Signaling.light (30.11)	DEG	sin11 > sau76
Phytohormone					
LOC_Os01g48060.1	Auxin response factor, putative, expressed	Zhang et al., 2018	RNA.regulation of transcription.ARF, Auxin Response Factor family (27.3.4)	DEG	sin11 < sau76
LOC_Os02g57250.1	OsIAA10—Auxin-responsive Aux/IAA gene family member, expressed	Hoang et al., 2019	RNA.regulation of transcription.Aux/IAA family (27.3.40)	DEG	**rr09 < sin11**
LOC_Os03g22270.1	Auxin-repressed protein, putative, expressed	Arenhart et al., 2014	Not assigned.unknown (35.2)	DEG	**rr09 < sin11**
LOC_Os03g53150.1	OsIAA13—Auxin-responsive Aux/IAA gene family member, expressed	Kitomi et al., 2012	RNA.regulation of transcription.Aux/IAA family (27.3.40)	DEG	**rr09 > sin11**, rr09 > rr20
LOC_Os05g33900.1	Auxin-induced protein 5NG4, putative, expressed	Arbelaez et al., 2017	Development.unspecified (33.99)	DEG	**rr09 > sin11**
LOC_Os11g44810.1	Auxin-repressed protein, putative, expressed	Arenhart et al., 2014	Hormone metabolism. auxin.induced-regulated-responsive-activated (17.2.3); development. unspecified (33.99); not assigned. unknown (35.2)	DEG	**rr09 < sin11**, rr09 < rr20, sin11 > sau76
LOC_Os02g43790.1	Ethylene-responsive transcription factor, putative, expressed	Bargsten et al., 2014; Zhang et al., 2017; Malukani et al., 2019	hormone metabolism.ehylene.signal.transduction (17.5.2)	Transcript present in sin 11 and absent in rr09	**rr09 vs. sin11**
LOC_Os11g15040.4	S-adenosyl-L-methionine:benzoic acid/salicylic acid carboxyl methyltransferase, putative, expressed	Hsieh et al., 2018	Hormone metabolism.salicylic acid.synthesis-degradation (17.8.1)	DEG	**rr09 < sin11**, sin11 vs. sau76
LOC_Os02g12890.1	Cytochrome P450, putative, expressed	Xiu-mei et al., 2015	Hormone metabolism.auxin.signal transduction (17.2.2)	DEG	**rr09 > sin11, rr20 > sau76**
LOC_Os02g47510.1	9-cis-epoxycarotenoid dioxygenase 1, chloroplast precursor, putative, expressed	Wang et al., 2015; Borah et al., 2017	Hormone metabolism.abscisic acid.synthesis-deggratdation.synthesis.9-cis-epoxycarotenoid dioxygenase (17.1.1.1.10)	DEG	**rr09 < sin11**
LOC_Os05g28740.1	Universal stress protein domain containing protein, putative, expressed	Sudo et al., 2008	Hormone metabolism.ethylene.induced-regulated-responsive-activated (17.5.3)	DEG	**rr09 < sin11**, sin11 > sau76
LOC_Os05g05680.1	1-aminocyclopropane-1-carboxylate oxidase, putative, expressed	Yang et al., 2015	Hormone metabolism.ethylene.synthesis-degradation (17.5.1)	DEG	**rr09 < sin11**, rr09 < rr20
LOC_Os10g39140.1	Flavonol synthase/flavanone 3-hydroxylase, putative, expressed	Galland et al., 2014	Hormone metabolism.ethylene.synthesis-degradation (17.5.1)	DEG	**rr09 < sin11**, sin11 > sau76
LOC_Os03g08500.2	AP2 domain containing protein, expressed	González-Schain et al., 2019	Hormone metabolism.ethylene.signal transduction (17.5.2)	DEG	**rr09 < sin11**
LOC_Os08g26820.1	Plant protein of unknown function domain containing protein, expressed	NA	Hormone metabolism.ethylene.signal transduction (17.5.2)	DEG	**rr20 > sau76**
LOC_Os03g28940.1	ZIM domain containing protein, putative, expressed	Jisha et al., 2015	Hormone metabolism.jasmonate.signal transduction (17.7.2)	DEG	**rr20 > sau76**

[a] *> or < signs are used in the comparisons to show which genotype expressed higher or lower.*

[b] *Bolded text highlights weed-cultivar comparisons.*

the crop (**Table 4**, **Supplementary Table 15**), two starch synthase genes (LOC_Os07g22930 and LOC_Os06g06560) (López-González et al., 2019) expressed more highly in the SH weeds (**Table 4**, **Supplementary Table 15**), a starch cleavage gene (LOC_Os10g32810) (Kim et al., 2017) and starch glucan water dikinase gene (LOC_Os12g20150) (Mahlow et al., 2016), expressed more highly in the SH weed (**Table 4**, **Supplementary Tables 14, 15**). In contrast, fewer genes obviously involved in sugar metabolism were differentially expressed in the BHA-*aus* weed-crop comparison (**Supplementary Table 15**), with only one (LOC_Os08g02120) (Zhang et al., 2016), a sucrose fructokinase, differentially expressed in the whole sucrose-starch pathway (**Table 4**), even though total sugar and fructose levels in leaves were also highly differentiated in this weed-crop pair.

In our set of samples, faster growth rate and greater height in weeds seemed primarily driven by SH in comparison to *indica* crops (**Figures 1**, **2**, **Supplementary Table 2**), and height maybe driven in part by the occurrence of semi-dwarf *indica* cultivars, of which sin11 is likely one. Determining the type of loci likely to affect growth rate is less straightforward, but phytohormones, including auxin and ethylene, can trigger phytochrome-interacting factors (PIFs) (Leivar and Quail, 2011), which in turn regulate plant growth (Liu et al., 2011; Stewart et al., 2011), making phytohormone-related genes good candidates. Our literature searches implicated several phytohormone genes differentially expressed in SH vs. *indica* (**Table 4**), including five auxin related genes (LOC_Os02g57250, LOC_Os03g22270, LOC_Os03g53150, LOC_Os05g33900 and LOC_Os11g44810) (Kitomi et al., 2012; Arenhart et al., 2014; Arbelaez et al., 2017; Hoang et al., 2019), one ethylene gene (LOC_Os02g43790) (Bargsten et al., 2014; Zhang et al., 2017; Malukani et al., 2019), and one salicylic acid gene (LOC_Os11g15040) (Hsieh et al., 2018). Several other DE phytohormone genes were found assigned to the hormone metabolism MapMan bin (**Table 4**, **Supplementary Table 15**), including LOC_Os02g12890, a cytochrome P450 gene related to auxin signal transduction (Xiu-mei et al., 2015), LOC_Os02g47510, a gene related to abscisic acid (Wang et al., 2015; Borah et al., 2017) and ethylene related genes (LOC_Os05g28740, LOC_Os05g05680, LOC_Os10g39140 and LOC_Os03g08500) (Sudo et al., 2008; Galland et al., 2014; Yang et al., 2015; González-Schain et al., 2019). We detected fewer growth-related DE genes in the BHA-*aus* comparison (**Table 4**, **Supplementary Table 15**), but note a cyclin related protein gene (LOC_Os03g19520) (Liu et al., 2016), which has been implicated in abaxial-side leaf development, and two hormone metabolism genes (LOC_Os08g26820 and LOC_Os03g28940) (Jisha et al., 2015).

DISCUSSION

Weedy Rice Lineages Have Not Evolved a Single Vegetative Weed Phenotype

Weeds compete for space and nutrients starting early in their vegetative growth stages. Such vegetative competitiveness can be manifested through various traits such as fast growth, increased biomass, better nutrient uptake, and more efficient metabolism. Although weedy rice is often described as being more competitive than cultivated rice, to our knowledge no studies have investigated how weedy rice lineages from different and known genetic backgrounds compare amongst themselves and how they compare with their crop ancestors in terms of vegetative traits that could enhance competitiveness. In an effort to determine how traits at the vegetative stage and possible underlying genes differ between cultivated rice and its related noxious weed, we characterized a panel of weedy and cultivated *Oryza* from different geographic regions and ancestry (**Figure 1**). We found much variation among weedy rice groups in how their vegetative traits compared to non-weedy groups, indicating that there is no single vegetative phenotype that characterizes weedy rice.

This variation in traits is evident at various levels. For example, despite some overlap in ancestry, South Asian and US weeds do not show similar trends in vegetative traits when compared to *aus* and *indica* cultivars (**Table 2** and **Figure 2**). Additionally, weedy lineages within each world region also show divergence in some traits. For example, US weedy rice grows taller and has a higher growth rate in the vegetative stages we characterized than cultivars we examined (**Table 2**), however, this trend seems to be driven primarily by SH weeds at this stage (**Figure 2**). US weeds also contain more fructose and total sugars in leaves, a trend also likely driven by SH weeds (**Figure 2**). Interestingly, these enhanced growth traits in US weeds occur even while containing less total nitrogen in above ground tissue than cultivars (**Table 2**, **Figure 2**), which could indicate more nitrogen use efficiency. Unlike US weeds, SA weeds taken as a whole do not seem to perform differently from cultivars in measured traits, except for a somewhat faster developmental time that seems specific to cultivar-derived weeds (**Table 2**, **Figure 3**, **Supplementary Table 3**). It is possible that the more hand-intensive farming practices in South Asia could select for weedy rice that is less distinguishable from the crop. Overall, however, no single measured trait stands out as indicative of all weedy groups, suggesting that none of the traits we studied are essential for the origin and evolution of all weedy rice lineages.

Although greater nitrogen accumulation has been previously proposed to be a characteristic of weedy rice (Burgos et al., 2006; Sales et al., 2008), our study shows that there is no nitrogen-related phenotype that characterizes all weedy rice in our panel. Nitrogen content differed significantly only between US weeds and cultivars, and, in this case, there was evidence for lesser rather than greater accumulation in weeds (**Table 2**, **Figure 2**). Nitrogen accumulation in plants may differ under direct competition conditions (Chauhan and Johnson, 2011), which our plants were not subjected to, or when nitrogen is scarce (Burgos et al., 2006), which may account for different reports of nitrogen content in weedy rice. However, we urge caution in assuming that more efficient nitrogen assimilation is a hallmark adaptive trait in weedy rice, and recommend that this trait needs to be evaluated in each independently evolved weedy rice group. More remarkable than total nitrogen content is the high growth rate exhibited by US weedy rice, despite lower N accumulation.

Among the weedy lineages we studied, weedy rice with primarily wild ancestry is the rarest, having only been detected in South Asia so far (Huang et al., 2017), though wild contributions to weedy rice via hybridization have been detected elsewhere (Vigueira et al., 2019). However, this type of weedy rice was striking in its high levels of CHOs (glucose, fructose, sucrose and starch) in leaf tissue (**Table 2**) compared to other groups. High fructose and total sugars were also evident for the SH group of US weeds. As CHOs are the major sources of energy supply, high levels could be indicative of higher early vigor in weeds (Zhang et al., 2012). For example, high levels of soluble sugars in corn stalks have been correlated to plant vitality (Mortimore and Ward 1964). In both of these weed groups, the higher CHO content co-occurs with faster growth rate. Various other studies also have reported that sugar levels are a determinant of vegetative growth levels (Deprost et al., 2007; Smeekens et al., 2010; Robaglia et al., 2012), such as longer leaf phenotypes (Hakata et al., 2012), and sugar metabolizing enzymes and sugar transporters have been discovered to interconnect with plant growth (Tiessen and Padilla-Chacon, 2012). Sugar availability in leaf tissue is also a major sensor to regulate cell divisions, a necessary process in plant growth (Osuna et al., 2007; Usadel et al., 2008). Thus, the high levels of sugars in wild-like weeds may represent a near-unique vegetative advantage in this group.

Faster plant growth has long been considered as an important factor of plant competitiveness (Lastdrager et al., 2014), since when competing for scarce resources including nutrients and light, faster growth is beneficial. The fact that this trait was not a universal feature of weedy groups in our study is thus surprising. Likewise, photosynthesis-related traits (SPAD, chlorophyll and photosynthetic rate), thought to enhance growth, did not seem remarkable across weedy rice lineages in this study.

Some important caveats of our study include the small number of genotypes characterized per *Oryza* and ancestry group, which was due to growth chamber size limitations and the extreme labor-intensiveness of the traits collected. While we worked with accessions typical of their populations based on genetic assessment, more robust conclusions would be obtained with a greater number of samples. Additionally, it should be noted that we focused primarily on traits in weeds and how they compare to their ancestral cultivar lineages, rather than performance under field conditions. Some traits and gene expression patterns could change under field conditions affected by the presence of biotic or abiotic stresses, or under direct competition conditions due to changes in resource distribution. Additionally, under field conditions, weeds and crops will be growing closely, giving rise to possible interactions via allelopathy or rhizospheric microorganisms. It should also be noted that the exact cultivar weedy rice will compete with can vary widely from field to field. SA weeds should most often directly compete with *aus* and *indica* cultivars. However, US weeds are most likely to compete with *tropical japonica* cultivars, a completely different evolutionary lineage, as this is the main group grown in the US. How vegetative traits in SH and BHA weeds compare to *tropical japonica* is not examined here, but our results suggest that, for most traits we studied, divergence in weeds from ancestral cultivars has been minimal.

Differentially Expressed Genes Between Weedy Rice and Cultivated Ancestors Differ Among Weedy Lineages

The genetic changes that make the transition from cultivated rice, which is under constant human selective pressure, to weedy rice are key to understanding the repeated evolution of this noxious weed. We thus examined patterns of gene expression in US weeds and their cultivated ancestors, and searched for convergence that could possibly indicate common genetic mechanisms in the evolution of weed vegetative competitiveness. Our consistent results showing less expression differences in weed-crop pairs compared to crop-crop or weed-weed pairs indicate that the gene expression divergence needed to evolve from a cultivated plant to an agricultural weed is not as great as that occurring between *Oryza* accessions of historically diverged lineages. This is evident in the numbers of differentially expressed transcripts (**Supplementary Tables 7–10**), the number of significantly enriched biological process GO terms (**Supplementary Table 13**) and overall functional bins with DE genes (**Supplementary Table 14**). The *aus* and *indica* lineages are thought to have diverged 12,000 years ago (Civán et al., 2015; Choi et al., 2017), while weed-crop divergence for both SH and BHA occurred after the establishment of agriculture (Reagon et al., 2010; Li et al., 2017), thus gene expression differentiation among our *Oryza* pairs seems more highly correlated with time of divergence rather than whether plants have a cultivated or weedy status, suggesting that only modest genetic changes are necessary for weed adaptation.

Despite the modest number of DE genes between weeds and crop ancestors, we searched for genes that could be involved in sugar assimilation, growth, and nitrogen accumulation—the traits we found to show the greatest differentiation between US weedy rice and crops. Different nitrogen related genes showing differential expression were found in each weed-crop comparison (**Table 4**), but these nevertheless provide candidates that may account for the one physiological trait showing convergence between SH and BHA weeds.

Among the several DE genes possibly correlated with sugar-mediated plant growth regulation were genes related to the synthesis of fructose, sucrose, and starch (**Table 4**; **Supplementary Table 15**). Consistent with observed phenotypic values, most sugar pathway genes were differentially expressed between the SH weed and *indica*, suggesting that changes in sugar metabolism may be primarily implicated in the adaptation of the SH weedy group.

Despite the inherent difficulties in defining genes that are involved in a complex trait like growth rate and plant height, we found several phytohormone and growth-related candidate genes that differ between US weeds and crops (**Table 4**). As for sugars, gene expression differences were more common for SH weedy rice and *indica*, which, along with the observed phenotypic differentiation between these groups, leads us to suggest that more evolutionary change has occurred in the transition to weediness in SH than it has in BHA. This is curious, given that SH is thought to have a more recent origin than the BHA weed group (Reagon et al., 2010). It is possible that *indica* cultivars harbor phenotypes that are less adaptive for weedy rice, thus requiring greater evolutionary change in weed-adaptive traits. A similar explanation has been proposed for the evolution of shattering in US weed groups, with SH weedy rice showing less phenotypic overlap in this trait with *indica* cultivars, compared to BHA weedy rice and *aus* cultivars (Thurber et al., 2010, 2013).

CONCLUSIONS

The adaptation of agricultural weeds to crop fields has been thought to comprise many traits that could enhance competitiveness of weedy plants, collectively sometimes considered a "weed syndrome" (Vigueira et al., 2013). The remarkable recurrent evolution of weedy rice around the world (Ziska et al., 2015, Huang et al., 2017) offers a unique opportunity to determine which traits contribute consistently to weedy rice adaptation. Our finding of no overlap in various growth and physiological traits, nor in gene expression patterns, among independently evolved weedy rice lineages suggests that the vegetative traits we studied here may not be as universally crucial to weedy rice success. This is in contrast to the reproductive traits of seed shattering and seed dormancy, which have been found to occur in most populations of weedy rice around the world (Ziska et al., 2015). We caution that the presence of a single strategy for vegetative growth should not *a priori* be assumed for weedy rice. To better understand the circumstances that lead to the evolution of weedy groups, we encourage careful characterization to ascertain the adaptive traits comprising the weedy syndrome in each separate weedy rice lineage, as well as further expansion of such weedy rice comparative studies to other environmental conditions, including direct competition.

DATA AVAILABILITY STATEMENT

Raw reads of the RNAseq data from this article can be found in the NCBI SRA under Bioproject ID PRJNA699874. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/, SAMN17813093; https://www.ncbi.nlm.nih.gov/, SAMN17813094; https://www.ncbi.nlm.nih.gov/, SAMN17813095; https://www.ncbi.nlm.nih.gov/, SAMN17813096.

AUTHOR CONTRIBUTIONS

ZH and JR conducted the experiments. ZH and RM performed data analysis. ZH and ALC designed the study and wrote the paper. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2021.601414/full#supplementary-material

REFERENCES

Adhikari, C., Bronson, K. F., Panuallah, G. M., Regmi, A. P., Saha, P. K., Dobermann, A., et al. (1999). On-farm soil N supply and N nutrition in the rice–wheat system of Nepal and Bangladesh. *Field Crops Res.* 64, 273–286. doi: 10.1016/S0378-4290(99)00063-5

Arbelaez, J. D., Maron, L. G., Jobe, T. O., Piñeros, M. A., Famoso, A. N., Rebelo, A. R., et al. (2017). Aluminum Resistance Transcription Factor 1 (ART1) contributes to natural variation in aluminum resistance in diverse genetic backgrounds of rice (*O. sativa*). *Plant Direct* 1:e00014. doi: 10.1002/pld3.14

Arenhart, R. A., Bai, Y., Oliveira, L. F. V., de, Neto, L. B., Schunemann, M., Maraschin, F., et al. (2014). new insights into aluminum tolerance in rice: the ASR5 protein binds the STAR1 promoter and other aluminum-responsive genes. *Mol. Plant* 7, 709–721. doi: 10.1093/mp/sst160

Bagnaresi, P., Biselli, C., Orrù L., Urso, S., Crispino, L., Abbruscato, P., et al. (2012). Comparative transcriptome profiling of the early response to Magnaporthe oryzae in durable resistant vs susceptible rice (Oryza sativa L.) genotypes. *PLoS ONE*. 7:e51609. doi: 10.1371/journal.pone.0051609

Bargsten, J. W., Nap, J.-P., Sanchez-Perez, G. F., and van Dijk, A. D. (2014). Prioritization of candidate genes in QTL regions based on associations between traits and biological processes. *BMC Plant Biol.* 14:330. doi: 10.1186/s12870-014-0330-3

Bhullar, N. K., and Gruissem, W. (2013). Nutritional enhancement of rice for human health: The contribution of biotechnology. Biotechnol. *Adv*. 31, 50–57. doi: 10.1016/j.biotechadv.2012.02.001

Borah, P., Sharma, E., Kaur, A., Chandel, G., Mohapatra, T., Kapoor, S., et al. (2017). Analysis of drought-responsive signalling network in two contrasting rice cultivars using transcriptome-based approach. *Sci. Rep.* 7:42131. doi: 10.1038/srep42131

Burgos, N. R., Norman, R. J., Gealy, D. R., and Black, H. (2006). Competitive N uptake between rice and weedy rice. *Field Crops Res.* 99, 96–105. doi: 10.1016/j.fcr.2006.03.009

Campo, S., Baldrich, P., Messeguer, J., Lalanne, E., Coca, M., and San Segundo, B. (2014). Overexpression of a calcium-dependent protein kinase confers salt and drought tolerance in rice by preventing membrane lipid peroxidation. *Plant Physiol.* 165, 688–704. doi: 10.1104/pp.113.230268

Cao, Q., Lu, B.-R., Xia, H., Rong, J., Sala, F., Spada, A., et al. (2006). Genetic diversity and origin of weedy rice (*Oryza sativa* f. spontanea) populations found in North-eastern China revealed by simple sequence repeat (SSR) markers. *Ann. Bot.* 98, 1241–1252. doi: 10.1093/aob/mcl210

Caton, B. P., Cope, A. E., and Mortimer, M. (2003). Growth traits of diverse rice cultivars under severe competition: implications for screening for competitiveness. *Field Crops Res.* 83, 157–172. doi: 10.1016/S0378-4290(03)00072-8

Chauhan, B. S., and Johnson, D. E. (2011). Competitive interactions between weedy rice and cultivated rice as a function of added nitrogen and the level of competition. *Weed Biol. Manag*. 11, 202–209. doi: 10.1111/j.1445-6664.2011.00421.x

Chen, C., He, B., Liu, X., Ma, X., Liu, Y., Yao, H. Y., et al. (2020). Pyrophosphate-fructose 6-phosphate 1-phosphotransferase (PFP1) regulates starch biosynthesis and seed development via heterotetramer formation in rice (Oryza sativa L.). *Plant Biotechnol. J*. 18, 83–95. doi: 10.1111/pbi.13173

Chen, Z., Qin, C., Wang, M., Liao, F., Liao, Q., Liu, X., et al. (2019). Ethylene-mediated improvement in sucrose accumulation in ripening sugarcane involves increased sink strength. *BMC Plant Biol*. 19:285. doi: 10.1186/s12870-019-1882-z

Cho, J.-I., Ryoo, N., Ko, S., Lee, S.-K., Lee, J., Jung, K.-H., et al. (2006). Structure, expression, and functional analysis of the hexokinase gene family in rice (*Oryza sativa* L.). *Planta* 224, 598–611. doi: 10.1007/s00425-006-0251-y

Choi, J. Y., Platts, A. E., Fuller, D. Q., Hsing, Y.-I., Wing, R. A., and Purugganan, M. D. (2017). The rice paradox: Multiple origins but single domestication in Asian rice. *Mol. Biol. Evol.* 34:969–979. doi: 10.1093/molbev/msx049

Civán, P., Craig, H., Cox, C. J., and Brown, T. A. (2015). Three geographically separate domestications of Asian rice. *Nat. Plants* 1:15164. doi: 10.1038/nplants.2015.164

Concenco, G., Aspiazu, I., Galon, L., Ferreira, E. A., Freitas, M. (2011). Photosynthetic characteristics of hybrid and conventional rice plants as a function of plant competition. *Planta Daninha* 29, 803–809. doi: 10.1590/S0100-83582011000400010

Deprost, D., Yao, L., Sormani, R., Moreau, M., Leterreux, G., Nicola,ï, M., et al. (2007). The Arabidopsis TOR kinase links plant growth, yield, stress resistance and mRNA translation. *EMBO Rep*. 8, 864–870. doi: 10.1038/sj.embor.7401043

Du, Z., Zhou, X., Ling, Y., Zhang, Z., and Su, Z. (2010). agriGO: a GO analysis toolkit for the agricultural community. *Nucleic Acids Res*. 38, W64–W70. doi: 10.1093/nar/gkq310

Estorninos, L. E., Gealy, D. R., Gbur, E. E., Talbert, R. E., and McClelland, M. R. (2005). Rice and red rice interference. II. rice response to population densities of three red rice (*Oryza sativa*) ecotypes. *Weed Sci*. 53, 683–689. doi: 10.1614/WS-04-040R1.1

Fischer, A., Chatel, M., Ramirez, H., Lozano, J., and Guimaraes, E. (1995). Components of early competition between upland rice (*Oryza sativa* L.) and Brachiaria brizantha (Hochst. ex A.Rich*) Stapf. Int. J. Pest Manag*. 41, 100–103. doi: 10.1080/09670879509371931

Gałecki, A., and Burzykowski, T. (2013). "Linear mixed-effects model," in *Linear Mixed-Effects Models Using R: A Step-by-Step Approach*, eds A. Gałecki and T. Burzykowski (New York, NY: Springer). doi: 10.1007/978-1-4614-3900-4_13

Galland, M., Boutet-Mercey, S., Lounifi, I., Godin, B., Balzergue, S., Grandjean, O., et al. (2014). Compartmentation and dynamics of flavone metabolism in dry and germinated rice seeds. *Plant Cell Physiol*. 55, 1646–1659. doi: 10.1093/pcp/pcu095

Gealy, D. R., and Yan, W. (2012). Weed Suppression Potential of 'Rondo' and Other Indica Rice Germplasm Lines. *Weed Technol*. 26, 517–524. doi: 10.1614/WT-D-11-00141.1

González-Schain, N., Roig-Villanova, I., and Kater, M. M. (2019). Early cold stress responses in post-meiotic anthers from tolerant and sensitive rice cultivars. *Rice N. Y. N* 12, 94–94. doi: 10.1186/s12284-019-0350-6

Gu, X. Y., Kianian, S. F., Hareland, G. A., Hoffer, B. L., and Foley, M. E. (2005). Genetic analysis of adaptive syndromes interrelated with seed dormancy in weedy rice (Oryza sativa). *Theor. Appl. Genet.* 110, 1108–1118. doi: 10.1007/s00122-005-1939-2

Hakata, M., Kuroda, M., Ohsumi, A., Hirose, T., Nakamura, H., Muramatsu, M., et al. (2012). Overexpression of a rice TIFY gene increases grain size through enhanced accumulation of carbohydrates in the stem. *Biosci. Biotechnol. Biochem*. 76, 2129–2134. doi: 10.1271/bbb.120545

Harris, M. A., Clark, J., Ireland, A., Lomax, J., Ashburner, M., Foulger, R., et al. (2004). The gene ontology (GO) database and informatics resource. *Nucleic Acids Res.* 32, D258–D261. doi: 10.1093/nar/gkh036

He, H., Zhiting, X., Minjing, L., Shuanglian, X, Shenglan, L., and Obono Mba, F. (2006). Effect of cadmium and herbicides on the growth, chlorophyll and soluble sugar content in rice seedlings. *Wuhan Univ. J. Nat. Sci.* 11, 742–748. doi: 10.1007/BF02836701

Hoang, G. T., Van Dinh, L., Nguyen, T. T., Ta, N. K., Gathignol, F., Mai, C. D., et al. (2019). Genome-wide association study of a panel of vietnamese rice landraces reveals new QTLs for tolerance to water deficit during the vegetative phase. *Rice* 12:4. doi: 10.1186/s12284-018-0258-6

Hoyos, V., Plaza, G., and Caicedo, A. L. (2019). Characterization of the phenotypic variability in Colombian weedy rice (*Oryza* spp.). *Weed Sci*. 67, 441–452. doi: 10.1017/wsc.2019.18

Hsieh, P.-H., Kan, C.-C., Wu, H.-Y., Yang, H.-C., and Hsieh, M.-H. (2018). Early molecular events associated with nitrogen deficiency in rice seedling roots. *Sci. Rep*. 8:12207. doi: 10.1038/s41598-018-30632-1

Huang, Z., Young, N. D., Reagon, M., Hyma, K. E., Olsen, K. M., Jia, Y., et al. (2017). All roads lead to weediness: patterns of genomic divergence reveal extensive recurrent weedy rice origins from South Asian Oryza. *Mol. Ecol*. 26, 3151–3167. doi: 10.1111/mec.14120

Inskeep, W. P., and Bloom, P. R. (1985). Extinction coefficients of chlorophyll a and b in N,N-Dimethylformamide and 80% acetone. *Plant Physiol*. 77, 483–485. doi: 10.1104/pp.77.2.483

Jain, M., Nijhawan, A., Tyagi, A. K., and Khurana, J. P. (2006). Validation of housekeeping genes as internal control for studying gene expression in rice by quantitative real-time PCR. *Biochem. Biophys. Res. Commun*. 345, 646–651. doi: 10.1016/j.bbrc.2006.04.140

Jia, Y., and Gealy, D. (2018). ScienceDirect Weedy red rice has novel sources of resistance to biotic stress. *Crop J.* 6:443–450. doi: 10.1016/j.cj.2018.07.001

Jisha, V., Dampanaboina, L., Vadassery, J., Mithöfer, A., Kappara, S., and Ramanan, R. (2015). Overexpression of an AP2/ERF Type transcription factor OsEREBP1 confers biotic and abiotic stress tolerance in rice. *PLoS ONE* 10:e0127831. doi: 10.1371/journal.pone.0127831

Karim, R. S. M., Man, A. B., and Sahid, I. B. (2004). Weed problems and their management in rice fields of Malaysia: an overview. *Weed Biol. Manag*. 4, 177–186. doi: 10.1111/j.1445-6664.2004.00136.x

Kharabian-Masouleh, A., Waters, D. L., Reinke, R. F., and Henry, R. J. (2011). Discovery of polymorphisms in starch-related genes in rice germplasm by amplification of pooled DNA and deeply parallel sequencing. *Plant Biotechnol. J.* 9, 1074–1085. doi: 10.1111/j.1467-7652.2011.00629.x

Kim, S.-W., Lee, S.-K., Jeong, H.-J., An, G., Jeon, J.-S., and Jung, K.-H. (2017). Crosstalk between diurnal rhythm and water stress reveals an altered primary carbon flux into soluble sugars in drought-treated rice leaves. *Sci. Rep*. 7:8214. doi: 10.1038/s41598-017-08473-1

Kitomi, Y., Inahashi, H., Takehisa, H., Sato, Y., and Inukai, Y. (2012). OsIAA13-mediated auxin signaling is involved in lateral root initiation in rice. *Plant Sci. Int. J. Exp. Plant Biol*. 190, 116–122. doi: 10.1016/j.plantsci.2012.04.005

Lastdrager, J., Hanson, J., and Smeekens, S. (2014). Sugar signals and the control of plant growth and development. *J. Exp. Bot*. 65, 799–807. doi: 10.1093/jxb/ert474

Leivar, P., and Quail, P. H. (2011). PIFs: pivotal components in a cellular signaling hub. *Trends Plant Sci*. 16, 19–28. doi: 10.1016/j.tplants.2010.08.003

Li, C., Zhou, A., and Sang, T. (2006). Rice domestication by reducing shattering. *Science* 311, 1936–1939. doi: 10.1126/science.1123604

Li, L.-F., Li, Y.-L., Jia, Y., Caicedo, A. L., and Olsen, K. M. (2017). Signatures of adaptation in the weedy rice genome. *Nat. Genet*. 49, 811–814. doi: 10.1038/ng.3825

Liu, X., Li, M., Liu, K., Tang, D., Sun, M., Li, Y., et al. (2016). Semi-Rolled Leaf2 modulates rice leaf rolling by regulating abaxial side cell differentiation. *J. Exp. Bot*. 67, 2139–2150. doi: 10.1093/jxb/erw029

Liu, Z., Zhang, Y., Liu, R., Hao, H., Wang, Z., and Bi, Y. (2011). Phytochrome interacting factors (PIFs) are essential regulators for sucrose-induced hypocotyl elongation in Arabidopsis. *J. Plant Physiol*. 168, 1771–1779. doi: 10.1016/j.jplph.2011.04.009

Long, S. P., and Bernacchi, C. J. (2003). Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *J. Exp. Bot*. 54, 2393–2401. doi: 10.1093/jxb/erg262

López-González, C., Juárez-Colunga, S., Morales-Elías, N. C., and Tiessen, A. (2019). Exploring regulatory networks in plants: transcription factors of starch metabolism. *PeerJ* 7:e6841. doi: 10.7717/peerj.6841

Luke, S. G. (2017). Evaluating significance in linear mixed-effects models in R. *Behav. Res. Methods* 49, 1494–1502. doi: 10.3758/s13428-016-0809-y

Mahlow, S., Orzechowski, S., and Fettke, J. (2016). Starch phosphorylation: insights and perspectives. *Cell. Mol. Life Sci. CMLS* 73, 2753–2764. doi: 10.1007/s00018-016-2248-4

Malukani, K. K., Ranjan, A., Jyothi, H. S., Patel, H. K., and Sonti, R. V. (2019). Dual activities of receptor-like kinase OsWAKL21.2 induce immune responses. *Plant Physiol.* 183, 1345–1363. doi: 10.1104/pp.19.01579

Meng, D., Liang, A., Wei, X., You, C. (2019). Enzymatic characterization of a thermostable phosphatase from Thermomicrobium roseum and its application for biosynthesis of fructose from maltodextrin. *Appl. Microbiol. Biotechnol.* 103, 6129–6139. doi: 10.1007/s00253-019-09917-6

Moldenhauer, K., and Slaton, N. (2001). "Rice growth and development," in *Rice Production Handbook* (Little Rock, AR: Coop. Ext. Service, University of Arkansas), 7–14.

Nadir, S., Xiong, H.-B., Zhu, Q., Zhang, X.-L., Xu, H.-Y., Li, J., et al. (2017). Weedy rice in sustainable rice production. a review. *Agron. Sustain. Dev.* 37:46. doi: 10.1007/s13593-017-0456-4

Novoa, R., and Loomis, R. S. (1981). Nitrogen and plant production. *Plant Soil* 58, 177–204. doi: 10.1007/BF02180053

Obertello, M., Shrivastava, S., Katari, M. S., and Coruzzi, G. M. (2015). Cross-species network analysis uncovers conserved nitrogen-regulated network modules in rice. *Plant Physiol.* 168, 1830–1843. doi: 10.1104/pp.114.255877

Osuna, D., Usadel, B., Morcuende, R., Gibon, Y., Bläsing, O. E., Höhne, M., et al. (2007). Temporal responses of transcripts, enzyme activities and metabolites after adding sucrose to carbon-deprived Arabidopsis seedlings. *Plant J. Cell Mol. Biol.* 49, 463–491. doi: 10.1111/j.1365-313X.2006.02979.x

Ottis, B. V., Smith, K. L., Scott, R. C., and Talbert, R. E. (2005). Rice yield and quality as affected by cultivar and red rice (*Oryza sativa*) density. *Weed Sci.* 53, 499–504. doi: 10.1614/WS-04-154R

Pareek, A., Singh, A., Kumar, M., Kushwaha, H. R., Lynn, A. M., and Singla-Pareek, S. L. (2006). Whole-genome analysis of Oryza sativa reveals similar architecture of two-component signaling machinery with Arabidopsis. *Plant Physiol.* 142, 380–397. doi: 10.1104/pp.106.086371

Qiu, J., Jia, L., Wu, D., Weng, X., Chen, L., Sun, J., et al. (2020). Diverse genetic mechanisms underlie worldwide convergent rice feralization. *Genome Biol.* 21:70. doi: 10.1186/s13059-020-01980-x

Qiu, J., Zhou, Y., Mao, L., Ye, C., Wang, W., Zhang, J., et al. (2017). Genomic variation associated with local adaptation of weedy rice during de-domestication. *Nat. Commun.* 8:15323. doi: 10.1038/ncomms15323

R Core Team (2014). R*: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Radosevich, S. R., Holt, J. S., and Ghersa, C. (1997). *Weed Ecology: Implications for Management.* New York, NY: John Wiley & Sons.

Ranker, E. R. (1925). Determination of total nitrogen in plants and plant solutions: a comparison of methods with modifications. *Ann. Mo. Bot. Gard.* 12, 367–380. doi: 10.2307/2394062

Reagon, M., Thurber, C. S., Gross, B. L., Olsen, K. M., Jia, Y., and Caicedo, A. L. (2010). Genomic patterns of nucleotide diversity in divergent populations of U.S. weedy rice. *BMC Evol. Biol.* 10:180. doi: 10.1186/1471-2148-10-180

Rebolledo, M. C., Dingkuhn, M., Clément-Vidal, A., Rouan, L., and Luquet, D. (2012). Phenomics of rice early vigour and drought response: are sugar related and morphogenetic traits relevant? *Rice.* 5:22. doi: 10.1186/1939-8433-5-22

Richardson, A. D., Duigan, S. P., and Berlyn, G. P. (2002), An evaluation of noninvasive methods to estimate foliar chlorophyll content. New Phytol. 153, 185–194. doi: 10.1046/j.0028-646X.2001.00289.x

Robaglia, C., Thomas, M., and Meyer, C. (2012). Sensing nutrient and energy status by SnRK1 and TOR kinases. *Curr. Opin. Plant Biol.* 15, 301–307. doi: 10.1016/j.pbi.2012.01.012

Sales, M. A., Shivrain, V. K., Burgos, N. R., and Kuk, Y. I. (2008). Amino acid substitutions in the acetolactate synthase gene of red rice (*Oryza sativa*) confer resistance to imazethapyr. *Weed Sci.* 56, 485–489. doi: 10.1614/WS-07-165.1

Shivrain, V. K., Burgos, N. R., Gealy, D. R., Smith, K. L., Scott, R. C., Mauromoustakos, A., et al. (2009). Red rice (*Oryza sativa*) emergence characteristics and influence on rice yield at different planting dates. *Weed Sci.* 57, 94–102. doi: 10.1614/WS-08-112.1

Shivrain, V. K., Burgos, N. R., Scott, R. C., Gbur, E. E., Estorninos, L. E., McClelland, M. R. (2010). Diversity of weedy red rice (*Oryza sativa* L.) in Arkansas, U.S.A. in relation to weed management. *Crop Prot.* 29, 721–730. doi: 10.1016/j.cropro.2010.02.010

Smeekens, S., Ma, J., Hanson, J., and Rolland, F. (2010). Sugar signals and molecular networks controlling plant growth. *Curr. Opin. Plant Biol.* 13, 274–279. doi: 10.1016/j.pbi.2009.12.002

Song, B.-K., Chuah, T.-S., Tam, S. M., and Olsen, K. M. (2014). Malaysian weedy rice shows its true stripes: wild Oryza and elite rice cultivars shape agricultural weed evolution in Southeast Asia. *Mol. Ecol.* 23, 5003–5017. doi: 10.1111/mec.12922

Stewart, J. L., Maloof, J. N., and Nemhauser, J. L. (2011). PIF genes mediate the effect of sucrose on seedling growth dynamics. *PLoS ONE* 6:e19894. doi: 10.1371/journal.pone.0019894

Sudo, E., Itouga, M., Yoshida-Hatanaka, K., Ono, Y., and Sakakibara, H. (2008). Gene expression and sensitivity in response to copper stress in rice leaves. *J. Exp. Bot.* 59, 3465–3474. doi: 10.1093/jxb/ern196

Thimm, O., Bläsing, O., Gibon, Y., Nagel, A., Meyer, S., Krüger, P., et al. (2004). mapman: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *Plant J.* 37, 914–939. doi: 10.1111/j.1365-313X.2004.02016.x

Thurber, C. S., Jia, M. H., Jia, Y., and Caicedo, A. L. (2013). Similar traits, different genes? Examining convergent evolution in related weedy rice populations. *Mol. Ecol.* 22, 685–698. doi: 10.1111/mec.12147

Thurber, C. S., Reagon, M., Gross, B. L., Olsen, K. M., Jia, Y., and Caicedo, A. L. (2010). Molecular evolution of shattering loci in U.S. weedy rice. *Mol. Ecol.* 19, 3271–3284. doi: 10.1111/j.1365-294X.2010.04708.x

Tiessen, A., and Padilla-Chacon, D. (2012). Subcellular compartmentation of sugar signaling: links among carbon cellular status, route of sucrolysis, sink-source allocation, and metabolic partitioning. *Front. Plant Sci.* 3:306. doi: 10.3389/fpls.2012.00306

Trapnell, C., Pachter, L., and Salzberg, S. L. (2009). TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics* 25, 1105–1111. doi: 10.1093/bioinformatics/btp120

Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D. R., et al. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat. Protoc.* 7, 562–578. doi: 10.1038/nprot.2012.016

Trapnell, C., Williams, B., Pertea, G., et al. (2010). Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nat. Biotechnol.* 28, 511–515. doi: 10.1038/nbt.1621

Tseng, T. M., Burgos, N. R., Shivrain, V. K., Alcober, E. A., and Mauromoustakos, A. (2013). Inter- and intrapopulation variation in dormancy of *Oryza sativa* (weedy red rice) and allelic variation in dormancy-linked loci. *Weed Res.* 53, 440–451. doi: 10.1111/wre.12044

Usadel, B., Bläsing, O. E., Gibon, Y., Retzlaff, K., Höhne, M., Günther, M., et al. (2008). Global transcript levels respond to small changes of the carbon status during progressive exhaustion of carbohydrates in arabidopsis rosettes. *Plant Physiol.* 146, 1834–1861. doi: 10.1104/pp.107.115592

Vigueira, C. C., Olsen, K. M., and Caicedo, A. L. (2013). The red queen in the corn: agricultural weeds as models of rapid adaptive evolution. *Heredity (Edinb).* 110, 303–311. doi: 10.1038/hdy.2012.104

Vigueira, C. C., Qi, X., Song, B.-K., Li, L.-F., Caicedo, A. L., Jia, Y., et al. (2019). Call of the wild rice: *Oryza rufipogon* shapes weedy rice evolution in Southeast Asia. *Evol. Appl.* 12, 93–104. doi: 10.1111/eva.12581

Wang, F., Ning, D., Chen, Y., Dang, C., Han, N.-S., Liu, Y., et al. (2015). Comparing gene expression profiles between bt and non-bt rice in response to brown planthopper infestation. *Front. Plant Sci.* 6:1181. doi: 10.3389/fpls.2015.01181

Wang, G., Li, H., Wang, K., Yang, J., Duan, M., Zhang, J., et al. (2020). Regulation of gene expression involved in the remobilization of rice straw carbon reserves results from moderate soil drying during grain filling. *Plant J.* 101, 604–618. doi: 10.1111/tpj.14565

Xiu-mei, W., Yue-yang, L., Ling, L., Chang-wei, G., Hai-peng, W., Xiao-xi, H., et al. (2015). Identification and cloning of tillering-related genes OsMAX1 in rice. *Rice Sci.* 22, 255–263. doi: 10.1016/j.rsci.2015.06.001

Xu, X. M., Li, G., Su, Y., and Wang, X. L. (2018). Effect of weedy rice at different densities on photosynthetic characteristics and yield of cultivated rice. *Photosynthetica* 56, 520–526. doi: 10.1007/s11099-017-0707-2

Yang, S., Hao, D., Song, Z., Yang, G., Wang, L., and Su, Y. (2015). RNA-Seq analysis of differentially expressed genes in rice under varied nitrogen supplies. *Gene* 555, 305–317. doi: 10.1016/j.gene.2014.11.021

Zhang, F., Zhou, Y., Zhang, M., Luo, X., and Xie, J. (2017). Effects of drought stress on global gene expression profile in leaf and root samples of dongxiang wild rice (*Oryza rufipogon*). *Biosci. Rep*. 37:BSR20160509. doi: 10.1042/BSR20160509

Zhang, K., Song, Q., Wei, Q., Wang, C., Zhang, L., Xu, W., et al. (2016). Down-regulation of OsSPX1 caused semi-male sterility, resulting in reduction of grain yield in rice. *Plant Biotechnol. J*. 14, 1661–1672. doi: 10.1111/pbi.12527

Zhang, N., Yu, H., Yu, H., Cai, Y., Huang, L., Xu, C., et al. (2018). A core regulatory pathway controlling rice tiller angle mediated by the LAZY1-dependent asymmetric distribution of auxin. *Plant Cell* 30, 1461–1475. doi: 10.1105/tpc.18.00063

Zhang, Z., Qiang, L. I. U., Hai-xing, S., Xiang-min, R., and Ismail, A. M. (2012). Responses of different rice (*Oryza sativa* L.) genotypes to salt stress and relation to carbohydrate metabolism and chlorophyll content. *Afr. J. Agric. Res*. 7, 19–27. doi: 10.5897/AJAR11.834

Zhou, S., Jiang, W., Long, F., Cheng, S., Yang, W., Zhao, Y., et al. (2017). Rice homeodomain protein WOX11 recruits a histone acetyltransferase complex to establish programs of cell proliferation of crown root meristem. *Plant Cell* 29, 1088–1104. doi: 10.1105/tpc.16.00908

Zhu, X., Shen, W., Huang, J., Zhang, T., Zhang, X., Cui, Y., et al. (2018). Mutation of the OsSAC1 gene, which encodes an endoplasmic reticulum protein with an unknown function, causes sugar accumulation in rice leaves. *Plant Cell Physiol*. 59, 487–499. doi: 10.1093/pcp/pcx203

Ziska, L. H., Gealy, D. R., Burgos, N., Caicedo, A. L., Gressel, J., Lawton-Rauh, A. L., et al. (2015). "Weedy (Red) rice: an emerging constraint to global rice production," in *Advances in Agronomy*, ed D. L. Sparks (Academic Press). doi: 10.1016/bs.agron.2014.09.003

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