Research Topics

Focus on *Popillia japonica*

Edited by
William D. Hutchison, David Held, Giselher Grabenweger
and Giulia Torrini

Coordinated by
Daniel A. Potter

Published in
Frontiers in Insect Science

ISSN 1664-8714
ISBN 978-2-8325-5975-8
DOI 10.3389/978-2-8325-5975-8

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Focus on *Popillia japonica*

Topic editors
William D. Hutchison — University of Minnesota Twin Cities, United States
David Held — Auburn University, United States
Giselher Grabenweger — Agroscope, Switzerland
Giulia Torrini — Council for Agricultural Research and Agricultural Economy Analysis | CREA, Italy

Topic coordinator
Daniel A. Potter — University of Kentucky, United States

Citation
Hutchison, W. D., Held, D., Grabenweger, G., Torrini, G., Potter, D. A., eds. (2025). *Focus on Popillia japonica*. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-8325-5975-8

Table of contents

frontiers | Frontiers in Insect Science

TYPE Editorial
PUBLISHED 24 January 2025
DOI 10.3389/finsc.2024.1532825

Editorial: Focus on *Popillia japonica*: New research for IPM of the Japanese beetle

Giselher Grabenweger[1]*, Giulia Torrini[2] and William D. Hutchison[3]

[1]Extension Arable Crops, Department of Plants and Plant Products, Agroscope, Zurich, Switzerland, [2]CREA - Research Center for Plant Protection and Certification, Florence, Italy, [3]Department of Entomology, University of Minnesota, St. Paul, MN, United States

KEYWORDS

***Popillia japonica*, Japanese beetle, invasive pest, management, editorial**

Editorial on the Research Topic
Focus on *Popillia japonica*: New research for IPM of the Japanese beetle

OPEN ACCESS

EDITED AND REVIEWED BY
Kris Braman,
University of Georgia, United States

*CORRESPONDENCE
Giselher Grabenweger
giselher.grabenweger@agroscope.admin.ch

RECEIVED 22 November 2024
ACCEPTED 20 December 2024
PUBLISHED 24 January 2025

CITATION
Grabenweger G, Torrini G and Hutchison WD (2025) Editorial: Focus on *Popillia japonica*: New research for IPM of the Japanese beetle. *Front. Insect Sci.* 4:1532825. doi: 10.3389/finsc.2024.1532825

The arrival of the Japanese Beetle, *Popillia japonica* Newman, in continental Europe (1) has sparked new interest in this well-known invasive species, and initiated international collaboration for its control, not only within Europe but also across the Atlantic. A session dedicated to this invasive pest at the International Congress of Entomology in Helsinki in 2022 enabled exchange between leading *Popillia* experts from the US and Canada and scientists involved in the containment of the Japanese Beetle in Europe. The most important work presented during this session is compiled in the presented e-book, along with other research updates regarding the biology, ecology and management of *P. japonica*, which was not included in previous reviews about this pest (2–4).

In this Research Topic, entitled "*Focus on Popillia japonica*," two articles deal with more robust estimates of *P. japonica* damage to important crops like wine grapes. Ebbenga et al. estimates the impact of *P. japonica* infestation on yield and grape quality. Although it is long known that grapes (cultivated and wild) is one of the beetle's preferred host plants, this paper is the first to assess the damage and impact on several berry/juice quality parameters. Straubinger et al. carried out a survey among Italian wine growers and attempted to put a price tag on the *P. japonica* invasion into the Piedmont wine growing region. Their findings show that increasing labor costs as a consequence of the invasion are responsible for about two thirds of the farmers' loss, while yield loss and costs of the plant protection treatments themselves are less important.

Investigations on the spatial distribution of the pest in certain crops as well on a wider scale are the topic of another two contributions. Henden and Guédot investigated how geographic, climatic, and landscape factors influence the spatial distribution of *P. japonica* abundance. They found that the abundance of Japanese beetles was higher in vineyards with pastures in the surrounding landscape, with higher temperatures, and located further east in the area of Southern Wisconsin. High leaf damage occurred at similar sites, but only when pesticide use was low. A tool that comes in handy in IPM against *P. japonica* was developed by (Toninato et al.). With their sequential sampling plan, farmers can estimate a *P. japonica* population density in raspberry fields, and consequently get a robust basis for a control decision, within an inspection time of about 11minutes per site only. They also could show that spatial patterns of the Japanese beetle abundance are not influenced by the use of insecticides, at least in cases where aggregation behavior may be triggered by stronger factors like host plant preference.

Alternatives to time-consuming monitoring of *P. japonica* with lure traps are presented in the following articles. Ribeiro et al. tested the feasibility of remote sensing for the detection of *P. japonica* in soybean. They found evidence that injury on leaves caused by *P. japonica* tends to reduce soybean canopy reflectance at wavelengths of 700-1000 nm. Such technology may come in handy for pest detection on large fields. Another paper by Ebbenga et al. describes a degree-day model to forecast flight periods of adult Japanese beetles. They were able to develop a straight forward model by summarizing degree-days starting from January 1st and setting lower and upper thresholds of 15 and 21.7°C, respectively. Upon reaching 257 and 345 degree-days, respectively, 10% and 50% of *P. japonica* adult emergence is forecasted to be underway.

Two contributions focus on the current invasion of *P. japonica* in continental Europe. Gotta et al. describe the many control attempts undertaken by Italian authorities and producers since the first detection of the pest in northern Italy in 2014. They review the strengths and weaknesses of chemical, physical, and biological control measures deployed for containment of *P. japonica* in the infested zones of the Lombardy and the Piedmont region. Poggi et al. performed a pest risk analysis for the pest-free (to date) region of Metropolitan France, which is quite close to infested regions geographically and also well-connected with infested zones by major routes of transport of humans and goods. They recommend early detection and early-stage eradication measures against *P. japonica* in outbreak zones as the most important measure to control the risk of pest invasion.

The development of environmentally friendly control measures against the invasive pest is the main goal of the last two contributions to this e-book. Graf et al. present experiments for biological control of *P. japonica* with entomopathogenic fungi (EPF). Their study gives evidence that Japanese beetle larvae are resistant to EPF infection, while adults are very susceptible. Consequently, more resources should be invested into the control of adult *P. japonica* with EPF. Carroll et al. tested gene silencing by feeding *P. japonica* with double-stranded RNA as a novel control approach. They show that fast degradation of dsRNA in the insect's gut may be avoided by micro-encapsulation, which increases gene knock-down and, consequently, efficacy of the control approach against the invasive pest.

As you read this summary, *P. japonica* continues to expand its range, slowly but steadily, in the US and Canada as well as in Italy and Switzerland, bringing new challenges to producers as well as plant health specialists on both continents. It is clear that single control measures will never stop this invasion, and that elaborate IPM strategies are necessary for successful *P. japonica* containment. We are hopeful that the contributions compiled in this e-book will contribute to the development of a sustainable response to the ongoing invasion.

Author contributions

GG: Writing – original draft, Writing – review & editing. GT: Writing – review & editing. WH: Writing – review & editing.

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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

1. Pavesi M. *Popillia japonica* specie aliena invasiva segnalata in Lombardia. *L'Informatore Agrario. Nr.* (2014) 32:S. 53–55.

2. Potter DA, Held DW. Biology and management of the Japanese beetle. *Annu Rev Entomology.* (2002) 47:175–205. doi: 10.1146/annurev.ento.47.091201.145153

3. Shanovich HN, Dean AN, Koch RL, Hodgson EW. Biology and management of Japanese beetle (Coleoptera: Scarabaeidae) in corn and soybean. *J Integ Pest Manage.* (2019) 10:9. doi: 10.1093/jipm/pmz009

4. Althoff ER, Rice KB. Japanese Beetle (Coleoptera: Scarabaeidae) invasion of North America: history, ecology, and management. *J Integ Pest Manage.* (2022) 13:2. doi: 10.1093/jipm/pmab043

frontiers | Frontiers in Insect Science

ORIGINAL RESEARCH
published: 26 April 2022
doi: 10.3389/finsc.2022.887659

Impact of Adult *Popillia japonica* (Coleoptera: Scarabaeidae) Foliar Feeding Injury on Fruit Yield and Quality of a Temperate, Cold-Hardy Wine Grape, 'Frontenac'

Dominique N. Ebbenga[1], Eric C. Burkness[1], Matthew D. Clark[2] and William D. Hutchison[1]*

[1] Department of Entomology, University of Minnesota, St. Paul, MN, United States, [2] Department of Horticultural Science, University of Minnesota, St. Paul, MN, United States

Edited by:
Michael Brewer,
Texas A&M AgriLife Research,
United States

Reviewed by:
David Gonthier,
University of Kentucky, United States
Adekunle W. Adesanya,
Washington State University,
United States

***Correspondence:**
Dominique N. Ebbenga
ebbe0031@umn.edu

Specialty section:
This article was submitted to
Invasive Insect Species,
a section of the journal
Frontiers in Insect Science

Received: 01 March 2022
Accepted: 30 March 2022
Published: 26 April 2022

Citation:
Ebbenga DN, Burkness EC, Clark MD and Hutchison WD (2022) Impact of Adult Popillia japonica (Coleoptera: Scarabaeidae) Foliar Feeding Injury on Fruit Yield and Quality of a Temperate, Cold-Hardy Wine Grape, 'Frontenac'. Front. Insect Sci. 2:887659. doi: 10.3389/finsc.2022.887659

Popillia japonica (Newman), is a highly polyphagous, invasive species, first recorded in the U.S. in 1916, and detected in Minnesota in the late 1960s. Historically, research on this pest in the Midwest U.S. has focused primarily on ornamental and turf crops, with little attention placed on adult feeding damage to fruit crops. Recently, wine grape producers in the region noted substantial increases in defoliation from *P. japonica* feeding, confirming concerns for this perennial high value crop. To address these concerns, studies were conducted during the summers of 2020–2021 to understand the impact of *P. japonica* foliar feeding on the quality and yield of wine grapes. Trials utilized vines of the wine grape variety, 'Frontenac.' In addition to open plots, whole vines were caged within fine mesh netting and infested with *P. japonica* at 0, 25, 50, and 100 beetles per meter-row of vine. Beetles used for infestations were collected from natural field populations of *P. japonica* and left to feed until grapes were ready for harvest. During harvest, data collection included leaf samples for obtaining average percent defoliation, cluster weights, and berry subsamples for soluble solid content, pH, titratable acidity, and phenolic compound measurements. Results from these studies demonstrated that as beetle population density and defoliation per m-row increases, at-harvest measurements of quality parameters are significantly and negatively affected ($P < 0.05$) when compared with uninfested vines. The negative impacts to fruit quality exhibited in these studies will be important in the development of future management strategies for *P. japonica* in 'Frontenac.'

Keywords: invasive species, defoliation, soluble solid content, titratable acidity, artificial infestation

INTRODUCTION

Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), is an invasive insect, native to Japan (1). The species was first detected in the United States in New Jersey in 1916, and eventually found in Minnesota in 1968 (2). Since the arrival in the U.S., *P. japonica* have become a major pest in turfgrass, ornamental and horticultural settings (3, 4). In response to the beetle's pest status in the U.S., a quarantine program has been in place for most western states to prevent further spread and establishment (5, 6). *Popillia japonica* exhibit a holometabolous life cycle. The larvae, or white grubs, live beneath the soil surface feeding on the roots of various grasses, potentially killing turfgrass and causing aesthetic damage to lawns and golf courses (7). Conversely, the adult life stage lives above ground and feeds on the fruit, flower, and foliage of more than 300 different plant species; primarily focusing on the foliage by defoliating the leaves in a characteristic pattern often referred to as skeletonization (3, 8).

Most of the published research on *P. japonica* has been focused on the biology, phenology, and control of the immature larval instars, particularly to lawns and golf courses (7, 9), while relatively little research has explored the impact of *P. japonica* adult feeding on horticultural crops (4, 5). When considering wine grapes (*Vitis vinifera* L. and *Vitis* hybrids), a highly preferred host of adult *P. japonica,* only a few studies have examined the impact of high defoliation rates on yield or juice quality (10–13). Because wine grapes depend on adequate leaf surface area for photosynthesis to provide sugars and non-structural carbohydrates vital to survival and fruit development (14), it is critical to understand the impacts of defoliation. As described by Pfeiffer (14), early season leaves are soft and delicate and photosynthesize sunlight to encourage shoot and cluster growth. Later in the season, during véraison (i.e., when fruit begins to change color), leaves become more tough and can tolerate more leaf feeding, and individual berries begin to become the main sink for sugars accumulated through photosynthesis (15). Understanding the susceptibility of different growth stages can be important when looking at *P. japonica* phenology in Minnesota. *Popillia japonica* adults typically emerge in late June to early July and populations persist until mid-October (16). This period of adult activity overlaps with most of the wine grape growth stages including when leaves are delicate, véraison, and through harvest. Because the foliar feeding activity of adult *P. japonica* and susceptible growth stages of wine grapes coincide, wine grapes in Minnesota may be at high risk for negative impacts to grape berry yield and quality.

Fruit quality refers to juice attributes of grapes both prior to and at harvest that determine the eventual quality of wine contributing to organoleptic properties, storage, stability, and color (15). Examples of this would be soluble solid content (SSC in °Brix) which estimates roughly as the percent sugar in a grape berry and depending on the value, can be a predictor of the percent alcohol in the subsequent wine and impacts flavor. SSC is typically measured prior to harvest in the field to inform growers when harvest should occur in addition to tasting and visual cues (17). Another quality parameter is total titratable acidity (TA), which measures the acid content in the grape juice and will impact the flavor of the subsequent wine. TA is typically measured after the grapes have been harvested (18) and if needed, wine-making practices can be used to adjust the wine flavor. A few other parameters include pH which will further describe the acidity, and finally phenolic compounds found in the grape skin and measured after grapes have been crushed to determine the color and sensory properties (tannin content) of the wine (19). Depending upon the region and the variety of grapes grown, different harvest recommendations are made to ensure the best quality wine possible (19). Furthermore, yield refers to the weight of clusters at the time of harvest.

In Minnesota, wine grapes account for over $80 million dollars in economic activity making it a high value crop that growers and other stakeholders wish to protect (20). The paucity of data for how defoliation by *P. japonica* affects wine grapes, both within season and over multiple growing seasons of a perennial crop, leads to many concerns for how to proceed agronomically and the financial impact that may occur. Current pest management strategies for *P. japonica* adults, in Minnesota wine grapes, rely heavily on the use of insecticides (3). However, there are currently no research-based action or economic thresholds for this pest in vineyards. If *P. japonica* adults are present at any level in the crop, insecticides may be applied for management. Furthermore, in times of high population density, insecticides can be sprayed as often as weekly intervals (personal observations). These excessive insecticide applications may cause unnecessary environmental impacts and gives cause to continue research on this pest's impact to wine grapes.

We therefore developed the following research objectives to better understand the degree to which *P. japonica* feeding activity may affect wine grape yield and juice quality under a midwestern U.S. climate, to better guide the development of integrated pest management (IPM) strategies. Our study focused specifically on a popular cold-hardy Minnesota wine grape variety, 'Frontenac' (21), to determine potential yield impacts, as well as fruit and juice quality, in response to a range of *P. japonica* infestation levels. However, results from these studies could also be beneficial and applicable to other regions *P. Japonica* has invaded.

MATERIALS AND METHODS

Impacts on Fruit Yield and Quality

Trials to assess the impact of *P. japonica* feeding and subsequent defoliation on fruit yield and quality were conducted at a 25-hectare production vineyard near Prior Lake, Minnesota (44.621776N, −93.442489W) in 2020 and 2021. Vines used in experimental plots were 6 or 7 years old depending on the year studies were conducted. Experimental plots were established on 19 June 2020, and 21 June 2021, during the wine grape growth stage known as 'pea-size berry' (22). This timing for plot establishment was selected because it occurs after flower pollination, so that netting would not interfere with fruit set, but before *P. japonica* emergence (16), to minimize any foliar injury from natural beetle populations prior to infestations of the netted vines. A total of 5 treatments were evaluated each year and consisted of: (1) an open plot to allow for natural beetle feeding, (2) a netted check plot with zero-beetle, (3) a netted plot infested with 25 beetles/m-row, (4) a netted plot infested with 50 beetles/m-row, and (5) a netted plot infested with 100 beetles/m-row. Each treatment was replicated 4 times in a randomized complete block design, within one trellised row measuring ∼156 m, of 'Frontenac' wine grapes selected each year. Netted plots each used a 3 m by 4 m piece of 80-gram mesh netting (ExcludeNet, Tek-knit Industries, Quebec, CA) to cage an entire vine (23, 24) measuring to be 2 m in length. A previous study, under Minnesota growing conditions, showed that the

FIGURE 1 | Example of exclusion netting plot used in trials. Netting was tossed over the top wire, rolled up on all edges and fastened with binder clips and wires to seal the netting. Poles were installed with wires attached to elevate the netting above the canopy.

same 80-gr mesh netting product did not significantly affect ambient temperatures during summer, compared to open plots (23). To keep the netting from resting on top of the plant canopy, 3.96 m poles were installed with wires attached that maintained the netting elevated above the canopy. To secure the netting around the vines, the ends of the netting were rolled into each other and then held together using large and medium steel binder clips (Office Depot Inc., Boca Raton, FL). In areas where clips did not create a tight enough closure around vines and trellis wires, 15- gauge wire was used to tightly wrap and secure netting (24). An example of a netted plot used in trials can be seen in **Figure 1**.

Infestations of treatments occurred on 15 and 9 July in 2020 and 2021, respectively, during the ‘pea-size berry’ growth stage. Beetles for infestations were collected from naturally occurring populations in a raspberry research field at UMore Park near Rosemount, MN (44.727839N, −93.097000W), placed in ventilated containers and transported immediately to the vineyard in Prior Lake for same-day release in the caged plots. Once infestation occurred, the beetles were allowed to feed on vines until harvest. After infestation, vines were observed weekly to assess the health and feeding activity of the beetles, ensure cages were securely sealed, and to take beetle counts in 1 m of row of each open plot.

Harvest of leaf, cluster and berry samples from experimental plots occurred on 5 Oct. 2020, and earlier on 9 Sept. 2021, due to drought conditions causing concern for loss of leaf samples; methodology was similar to that of Ebbenga et al. (25). For each plot, 10 randomly selected leaves were collected and placed in 20 cm by 25 cm re-closable bags (Minigrip Re-closable bags, Consolidated plastics, Stow, OH) to obtain average percent defoliation for each treatment using the LeafByte app (26). Additionally, all clusters were hand harvested from each plot and placed into 49 L plastic bags (Warp Bros., Chicago, IL) and weighed on a Doran 8000 digital scale (Doran Scales Inc. Batavia, IL) to obtain the 1-m row average weight. After weights were obtained, 5–7 clusters were randomly selected from the harvested clusters for each plot. Among these clusters, 10 randomly selected berries were removed from the remaining clusters from each plot and placed in individual 30-ml cups (Dart Container Corp., Mason, MI) and capped. The selected clusters and samples of berries were placed in coolers and immediately transported to the University of Minnesota’s Horticultural Research Center, Grape Breeding and Enology Laboratory in Excelsior, MN for processing.

Laboratory Processing for Quality Parameters

Once the 5–7 clusters of grapes arrived at the enology lab, the same day as harvest, they were juiced, utilizing methods similar to Ebbenga et al. (25). The juicing process consisted of placing the clusters from each plot in a 1-gallon Ziploc® bag (S.C. Johnson & Son, inc., Racine, WI), and crushed by hand. Once crushed, the subsequent juice was poured and pressed through a stainless-steel China Cap Strainer (New Star Foodservice, Chino, CA). The resulting juice was collected in a Falcon 50 ml Conical Centrifuge Tube (Fisher Scientific, Hampton, NH) and used to measure pH, and titratable acidity (25). In 2021, a second vial of juice was collected, labeled, and stored in a freezer at −62°C until the samples were delivered via overnight express shipment to the ETS laboratories (St. Helena, CA) to obtain measurements of tannin, polymeric anthocyanins, total anthocyanins, and polymeric anthocyanins/tannin index. Lastly, the 10 randomly selected individual berries placed in individual 30 ml plastic cups with lids (Dart Container Corp., Mason, MI) from each plot were used to obtain average SSC using a refractometer (Shen Zhen YIERYI Technology Co., Ltd Shen Zhen City, Guang Dong Province, China) (27).

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) with R statistical software (28). Trials were established in a randomized complete block design (RCBD). To achieve this, replications were made into four blocks and each of the five treatments were randomized within each block. Analysis comparing the treatments of the different beetle densities and replications were included in the linear model. When significant differences were found, a mean separation was conducted using Tukey’s honest significant difference test [Agricolae, HSD.test, (29)]. Analytical assumptions for a one-way ANOVA were met prior to analysis and no transformations were conducted on the data.

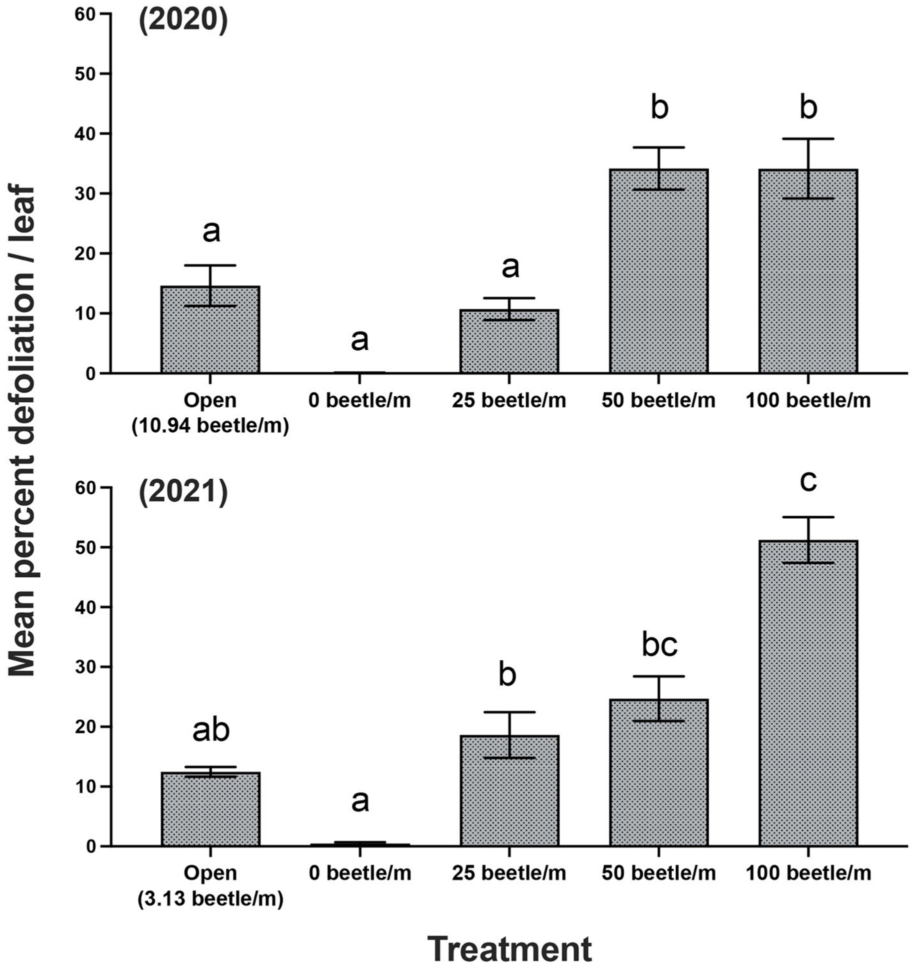


FIGURE 2 | Mean (±SEM) measurements of mean percent defoliation per leaf for years 2020 and 2021. Bars within a year followed by the same letter are not significantly different ($P > 0.05$); ANOVA with Tukey's HSD mean separation test.

RESULTS

For 2020, open plots averaged 10.94 *P. japonica* per m-row, observations of zero-beetle per m-row plots remained at zero, and all other treatments had the sufficient amount of beetles present in the cages based on infestations. Mean percent defoliation across plots based on leaf samples collected at harvest exhibited a significant increase [$F_{(4,12)} = 19.050$, $P < 0.001$] as beetle densities increased with the highest beetle density of 100 per m- row showing approximately 35% defoliation; this compared to near 0% defoliation with the zero-beetle per m-row treatment (**Figure 2**). Results from the fruit quality analysis indicated that several parameters were significantly affected by *P. japonica* defoliation. For the SSC results, we observed a significant decrease [$F_{(4,12)} = 5.514$, $P = 0.009$] in accumulation of SSC in grape samples as beetle densities and subsequent defoliation increased (**Figure 3A**). The TA parameter demonstrated a significant increase [$F_{(4,12)} = 8.4118$, $P = 0.002$] in acidity levels in treatments when higher densities of beetles and defoliation occurred (**Figure 3C**). Furthermore, SSC and TA parameters from these trials indicate that at about 50 beetles per m-row, and >30–35% defoliation we begin seeing undesirable trends where SSC values are lower and TA values are greater, relative to our treatments with little to no beetle feeding occurring. To further demonstrate the differences across treatments, a ratio of TA to SSC was also examined. Data from this analysis affirms that as treatments increase in beetle density and subsequent defoliation, there is a significant increase [$F_{(4,12)} = 19.938$, $P < 0.001$] in the TA/SSC ratio (**Figure 3E**). The pH results obtained for 2020 indicated a significant and negative trend in pH, or higher acidity [$F_{(4,12)} = 7.042$, $P = 0.004$], as percent defoliation increased (**Figure 3G**). In contrast to the juice quality parameters, however, yield in 2020, measured based on the mean weight of clusters per m-row, did not show significant differences [$F_{(4,12)} = 0.8650$, $P = 0.5124$] in response to infestation treatments. Mean (±SEM) weight (kg) for yield

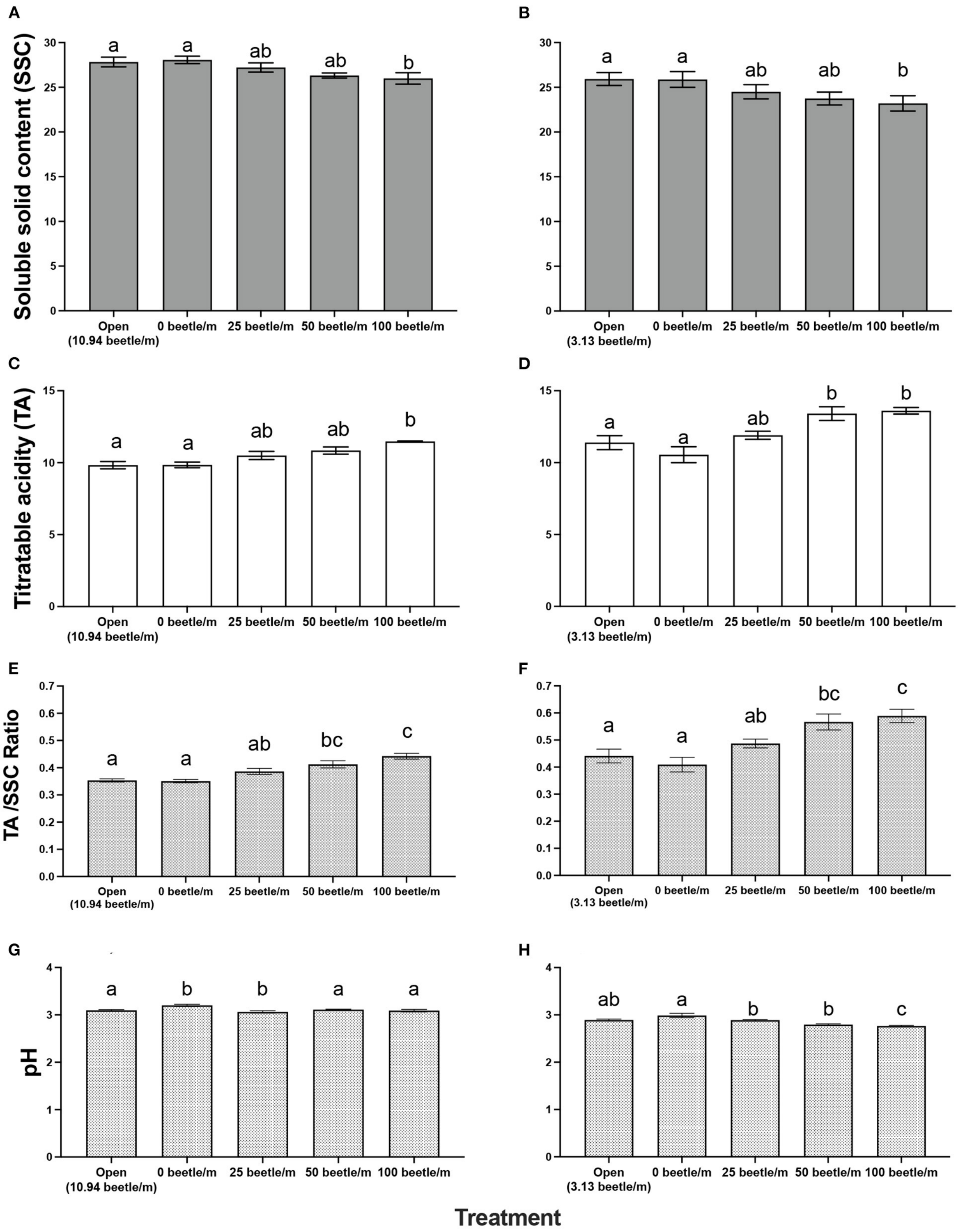


FIGURE 3 | Mean (±SEM) measurements of fruit quality for a 10-berry subsample for soluble solid content (SSC in °Brix) in 2020 **(A)** and 2021 **(B)**, titratable acidity in 2020 **(C)** and 2021 **(D)**, Acid/SSC Ratio in 2020 **(E)** and 2021 **(F)**, and pH in 2020 **(G)** and 2021 **(H)**. Bars within a year followed by the same letter are not significantly different ($P > 0.05$); ANOVA with Tukey's HSD mean separation test.

was 1.59(±0.01) kg/m-row for the open plot treatment, while the netted plots yielded 1.71 (±0.28), 1.61 (±0.26), 1.59 (±0.28), and 2.14 (±0.35) kg/m-row, for 0, 25, 50 and 100 beetles per m-row, respectively.

For 2021, the open plots averaged 3.13. *P. japonica* per m-row, while observations for the zero-beetle per m-row plots remained at zero, and all other treatments had the sufficient amount of beetles present in the cages based on infestations. Similar to results in 2020, leaf samples used to obtain average percent defoliation across plots showed significant differences [$F_{(4,12)} = 44.977, P < 0.001$]; as beetle densities increased with the highest beetle density at 100 per m- row showing 51% defoliation, compared to near 0% defoliation with the zero-beetle per m-row treatment (**Figure 2**). Results for fruit quality parameters again demonstrated significant differences in SSC [$F_{(4,12)} = 5.861$, $P = 0.009$] in accumulation of SSC in grape samples as beetle densities and subsequent defoliation increased (**Figure 3B**). Next, our TA parameters demonstrated a significant increase [$F_{(4,12)} = 8.649$, $P = 0.002$] in acidity levels as treatments increased to higher densities of beetles and defoliation (**Figure 3D**). For a second year, our SSC and TA parameters trials indicate that at about 50 beetles per m-row, and >30–35% defoliation we begin observing the negative impacts of decreased SSC and increased TA values. Ratios of TA to SSC data demonstrate again that as treatments increase in beetle density and subsequent defoliation, there is a significant increase [$F_{(4,12)} = 12.287$, $P < 0.001$] in the TA/SSC ratio (**Figure 3F**). Results obtained from our pH in 2021 observed a significant [$F_{(4,12)} = 10.815$, $P < 0.001$] and negative trend for pH values as percent defoliation increased (**Figure 3H**). Similar to 2020, there were no significant differences in yield [$F_{(4,12)} = 0.593$, $P = 0.674$] across infestation treatments. Mean (±SEM) weight (kg) for yield was 4.96 (±0.53) kg/m-row for the open plot treatment, while the netted plots yielded 5.44 (±0.88), 5.05 (±0.32), 4.48 (±0.58), and 4.43 (±0.13) kg/m-row, for 0, 25, 50 and 100 beetles per m-row, respectively.

Also in 2021, juice samples analyzed for measurements of phenolic compounds demonstrated additional negative impacts by *P. japonica*. For example, significantly lower polymeric anthocyanins [$F_{(4,12)} = 5.516$, $P = 0.009$], total anthocyanins [$F_{(4,12)} = 6.608$, $P = 0.005$], and polymeric anthocyanins/tannin index [$F_{(4,12)} = 4.882$, $p = 0.014$] were observed as beetle density and subsequent defoliation increased (**Table 1**). Phenolic compound measurements for the tannin index alone did not show significant differences across treatments [$F_{(4,12)} = 2.883$, $P = 0.069$]; however, we observed a decreasing trend in tannin values as beetle density increased.

DISCUSSION

In this study, we conceived research objectives to better understand the degree to which *P. japonica* feeding activity may have a negative impact on fruit yield and quality of wine grape, 'Frontenac,' and thus provide results that would be beneficial to growers who are motivated to improve their IPM programs. Overall, our results suggest that yield was not significantly impacted by an increase in *P. japonica* density. Any differences observed for yield in our study was more likely due to the natural variation in vine production or abiotic stressors for that given year, vs. the impacts of *P. japonica* treatments. However, data collected on the fruit quality parameters produced numerous significant impacts as beetle densities and subsequent defoliation increased.

Fruit quality parameters of 'Frontenac' at harvest demonstrated negative impacts to the SSC (°Brix) values among the different treatment levels of beetle infestations (**Figures 3A,B**). In a wine grape variety such as 'Frontenac,' grapes should measure between 22 and 25 °Brix and could be as high as 30 °Brix for dessert wines per recommendations from the University of Minnesota (30). While SSC for every treatment falls within this recommended harvest threshold, the data demonstrate that *P. japonica* feeding is impacting the plants' ability to properly photosynthesize and accumulate sugars in berries in an efficient manner (14). This becomes especially concerning in a cold climate such as Minnesota, because delaying harvest to boost SSC could place a grower at risk of losing the crop to an early frost, or berry breakdown for some varieties in some years.

For both years, treatments applied to TA resulted in a significant increase in acidity levels as beetle densities increased (**Figures 3C,D**). High TA in the grape juice can have an undesired impact on the flavor of the wine as it relates to the acid/sugar balance in the finished wine product. In a cold hardy wine grape variety such as 'Frontenac' with naturally high acidity, it is vital the acidity level remain below 15 g/L as described by Clark (30). Per these recommendations, we can note all our treatments remain below this threshold having little impact on the subsequent wine. However, it is still important to know that even though values remain within the recommended threshold, we again observe the negative impacts *P. japonica* feeding has on the wine grape berry quality.

Values for pH in both years also demonstrate significant differences across treatments (**Figures 3G,H**). Current recommendations for pH are set to a value between 3 and 3.3 (30). In 2020, harvest of our trials occurred during the typical harvest window for 'Frontenac' in Minnesota. All pH values in 2020 were within the recommended threshold, but we still observed a negative trend for pH values as percent defoliation increased. In 2021, due to drought conditions during the season, harvest occurred sooner than recommended as to avoid loss of defoliated leaf samples. However, this does not negate the results collected from this trial. While we cannot comment on the pH being within recommended parameters, we see a similar pattern of negative trends as percent defoliation of the crop exceeded 30%, and >50 beetles per m-row were placed in a cage.

Finally, grape juice samples in 2021 that were analyzed to capture the phenolic compounds demonstrated significant differences in some measurements when compared to the zero-beetle treatment (**Table 1**). Like SSC, TA, and pH, these results alone do not make the wine unmarketable, but the impacts made by *P. japonica* feeding add an extra layer of work and consideration in the wine making process. For example, looking specifically at the tannin measurements, while it may not be statistically significant, we see a numerical trend for a decrease

TABLE 1 | Measures of juice quality parameters for the wine grape variety Frontenac, under different infestations with *P. japonica*, Prior Lake, MN, 2021.

Treatment	Tannin (mg/L)	Polymeric Anthocyanins (mg/L)	Total Anthocyanins (mg/L)	Polymeric anthocyanins/tannin index
	Mean (±SEM)	Mean (±SEM)	Mean (±SEM)	Mean (±SEM)
Open (3.13 beetle/m)	86.25 (±3.97) a	4.00 (±0.41) b	860.25 (±94.11) ab	0.046 (±0.003) b
Net 0 beetle/m	92.00 (±3.32) a	5.25 (±0.25) a	1073.50 (±136.74) a	0.057 (±0.001) a
Net 25 beetle/m	84.25 (±2.50) a	4.00 (±0.00) b	776.25 (±28.11) ab	0.048 (±0.001) ab
Net 50 beetle/m	81.25 (±1.98) a	4.00 (±0.00) b	559.75 (±49.14) b	0.049 (0±.001) ab
Net 100 beetle/m	81.75 (±1.11) a	3.74 (±0.25) b	516.50 (±61.62) b	0.046 (±0.003) b

^{a,b}*Means within columns followed by the same letter are not significantly different ($p > 0.05$); ANOVA with Tukey's HSD mean separation test. Results from ETS laboratory analysis, juice samples collected on Sept. 9, 2021.*

in tannin with the increase in beetle density treatments. Tannins not only impact the flavor and mouthfeel of the wine, but they also help wine retain its color, also referred to as anthocyanins, during the fermentation process (31). 'Frontenac' creates a red wine, and to achieve high quality, the grower must retain a deep red color. Given our results from our 2021 trial, it demonstrates that *P. japonica* feeding results in decreased tannins present, which in turn will impact the wines retention of anthocyanins. To correct this during the wine making process, growers will need to add additional tannins to ensure their red wine retains the proper anthocyanins. **Table 1** further demonstrates this phenomenon when we observe the anthocyanin measurements across treatments.

Results from these trials consistently demonstrate that as beetle density ($>$50 beetles per m-row) and defoliation ($>$30%) increase, there is a significant and negative impact on the quality of the fruit. Boucher et al. (32), concluded that just after a 20% leaf area loss, net photosynthesis began to decline quickly. This observation agrees with our results that when $>$30% defoliation by *P. japonica* is observed, negative impacts on the quality of the fruit begin to develop.

Thus far, only limited research has been conducted on this topic, but our studies agree with past literature from Boucher and Pfeiffer (10) where similar trials demonstrated that with high enough defoliation, fruit quality parameters such as SSC are negatively impacted in the wine grape variety 'Seyval Blanc.' Another study, published on the impact of *P. japonica* to young vines in Michigan, demonstrated little impact to the vine's vegetative growth (11). However, in the Michigan study, beetles were only exposed to the vines for 2 weeks starting at véraison and infested at much lower levels with the highest being 40 beetles per cage. Also, because the focus of these trials was on young vines that were not yet fruiting, it is difficult to compare our studies directly, as our vines were $\sim$7 years old and producing fruit. Furthermore, our trials had beetles feeding on the vine after the berries were at the pea-size stage and until harvest occurred creating a much longer window for beetle feeding to impact the crop. Finally, Hammons et al. (13) indicates how varieties may differ in both susceptibility and response to *P. japonica* foliar feeding. Our study focused exclusively on 'Frontenac' whereas Hammons et al. (12) compared yield and quality parameters of 5 different varieties. Results indicated yield impacts varied between the different varieties, meaning some varieties exhibited a decrease in cluster yield such as 'Cabernet Franc' and 'Norton,' while 'Concord' did not. Furthermore, none of the tested varities exhibited any significant impact to fruit quality. Results from this study emphasize the importance of continuing trials on other wine grapes to understand how different varieties respond to *P. japonica* foliar feeding.

In conclusion, results indicate that *P. japonica* feeding has a negative impact on the development of 'Frontenac' berries after $\sim$30–35% defoliation, and $>$50 *P. japonica* per m-row. These data support the use of a tentative action threshold of 25–30% defoliation or $>$25 *P. japonica* per m-row would warrant management action to minimize negative impacts to the quality parameters of the crop. Trials were conducted in an area of Minnesota where *P. japonica* has not yet exhibited high natural densities, which explains the low densities recorded in open plots. However, observations in other areas of Minnesota, where *P. japonica* is better established, often yield $>$25 beetles per-m row during peak beetle activity in mid to late summer (personal observation). This study is specific to 'Frontenac' and with the limited number of studies published on wine grapes (10–13), there is a critical need for additional research in other varieties. In areas where *P. japonica* continues to invade new regions, including Europe (33), it will be important to continue these studies on other varieties in different climates and potentially evaluate long term impacts to vines and berry quality after successive years of beetle injury. Moreover, in areas where *P. japonica* is now established such as Minnesota, climate change projections during the twenty first century of additional increases of 4°C increase summer months, and 6°C increase during winters (34) portend additional abiotic benefits to *P. japonica* population growth, overwintering success, and potential range expansion (33). Expanding our knowledge of the impact *P. japonica* has on high value crops such as wine grapes, will benefit growers and other stakeholders in finding sustainable IPM solutions for managing the pest now, and into the future as the pest is likely to invade new areas.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, upon request.

AUTHOR CONTRIBUTIONS

DE, EB, and WH: conceptualization. WH, EB, and MC: resources, project administration, and funding acquisition. DE, EB, and MC: methodology. DE: investigation and formal analysis. DE: writing original draft, and preparation. DE, EB, MC, and WH: writing. All authors provided a final review, and agreed to the published version of the manuscript.

FUNDING

The research was funded by the Rapid Agricultural Response Fund, Award 0081527, via the Minnesota Agricultural Experiment Station, University of Minnesota. The project was also supported by the McLaughlin Gormley King (MGK) Fellowship, and a University of Minnesota Doctoral Dissertation Fellowship.

ACKNOWLEDGMENTS

We thank Anh Tran, Adam Toninato, Sally Nelson, Samantha Meyer, and Oliver McEllistrem (UMN Entomology Fruit & Vegetable IPM lab) for assistance with data collection, and treatment applications. A special thanks to John and Jenny Thull, and Drew Horton (UMN Horticultural Research Center, HRC), for sharing their knowledge and expertise in wine grapes and assisting with data collection and trial processes. We also thank previous reviewers Dr. Robert Koch and Dr. Dan Cariveau from the University of Minnesota Department of Entomology, for their contributions to this manuscript.

REFERENCES

1. Fleming WE. *Integrating Control of the Japanese Beetle - A Historical Review*. Technical Bulletin No. 1545. Washington, DC: USDA (1976).
2. Hahn J, Weisenhorn J, Bugeja S. *Japanese Beetles in Yards and Gardens*. University of Minnesota Extension, St. Paul, MN (2020). Available online at: https://extension.umn.edu/yard-and-garden-insects/japanese-beetles (accessed December 23, 2021).
3. Potter DA, Held DW. Biology and management of the Japanese beetle. *Annu Rev Entomol.* (2002) 47:175–205. doi: 10.1146/annurev.ento.47.091201.145153
4. Shanovich HN, Ashley DN, Koch RL, Hodgson EW. Biology and management of Japanese beetle (Coleoptera: Scarabaeidae) in corn and soybean. *J Integ Pest Manage.* (2019) 10:1–14. doi: 10.1093/jipm/pmz009
5. Althoff EM, Rice KB. Japanese beetle (Coleoptera: Scarabaeidae) invasion of North America: history, ecology, and management. *J Integ Pest Manage.* (2022) 13:1–11. doi: 10.1093/jipm/pmab043
6. USDA: Animal, and Plant Health Inspection Service. Japanese Beetle Distribution in the U.S (2022). Available online at: https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/japanese-beetle/japanese-beetle. (accessed February 22, 2022).
7. Potter DA. *Destructive Turfgrass Insects: Biology, Diagnosis, and Control*. Chelsea, MI: Ann Arbor Press (1998). p. 368.
8. Fleming WE. *Biology of the Japanese Beetle*. Technical Bulletin No. 1449. Washington, DC: USDA (1972).
9. Vittum PJ, Villani MG, Tashiro H. *Turfgrass Insects of the United States and Canada*. 2nd ed. Ithaca, NY: Cornell University Press (1999).
10. Boucher TJ, Pfeiffer DG. Influence of Japanese beetle (Coleoptera: Scarabaeidae) foliar feeding on 'Seyval Blanc' grapevines in Virginia. *J Econ Entomol.* (1989) 82:220–5. doi: 10.1093/jee/82.1.220
11. Mercader RJ, Isaacs R. Damage potential of Rose Chafer and Japanese beetle (Coleoptera: Scarabaeidae) in Michigan vineyards. *Great Lakes Entomol.* (2003) 36:1–13.
12. Hammons DL, Kurtural SK, Potter DA. Japanese beetle defoliation reduces primary bud cold-hardiness during vineyard establishment. *Am J Enol Vitic.* (2010) 61:130–4.
13. Hammons DL, Kurtural SK, Potter DA. Impact of insecticide-manipulated defoliation by Japanese beetle (*Popillia japonica*) on grapevines for vineyard establishment through production. *Pest Manag Sci.* (2010) 66:565–71. doi: 10.1002/ps.1908
14. Pfeiffer DG. Japanese beetle and other Coleoptera Feeding on Grapevines in Eastern North America. In: Bostanian NJ, Vincent C, Isaacs R, editors. *Arthropod Management in Vineyards: Pests, Approaches, and Future Directions*. Dordrecht: Springer (2012). p. 403–29.
15. Ollat N, Carde J, Gaudillére J, Barrieu F, Diakou-Verden P, Moing A. Grape berry development: a review. *J Int Sci Vigne du Vin.* (2002) 36:109–31. doi: 10.20870/oeno-one.2002.36.3.970
16. Ebbenga DN, Wold-Burkness SJ, Burkness EC, Hutchison WD. *Japanese Beetle: An Emerging Pest of Fruit Crops*. Pest Profile, FruitEdge, University of Minnesota, St. Paul (2021). Available online at: http://www.fruitedge.umn.edu/japanese-beetle/japanese-beetle-emerging-pest-fruit-crops (accessed January 31, 2022).
17. Amerine MA, Roessler EB. Field testing grape maturity. *Hilgardia.* (1958) 28:93–114. doi: 10.3733/hilg.v28n04p093
18. Berg HW, Ough CS. The relation of balling to wine quality. *Am J Enol Viticult.* (1977) 28:235–8.
19. Du Plessis CS. Optimum maturity and quality parameters in grapes: a review. *S Afr J. Enol Viti.* (1984) 5:35–42. doi: 10.21548/5-1-2367
20. Tuck B, Gartner W, Appiah G. *Economic Contribution of Vineyards and Wineries of the North, 2015*. University of Minnesota Extension, St. Paul, MN (2017). Available online at: https://conservancy.umn.edu/bitstream/handle/11299/197808/2015-economic-contribution-wineries-and-grapes.pdf?sequence=1 (accessed December 23, 2021).
21. Hemstad P. Grapevine breeding in the Midwest. In: Reynolds AG, editors. *Grapevine Breeding Programs for the Wine Industry*. Cambridge: Woodhead (2015). p. 411–25.
22. Meier U. *Grapevine. Growth Stages of Mono- and Dicotyledonous Plants*. BBCH Monograph. Federal Biological Research Centre for Agriculture and Forestry, Berlin (2001). p. 204.
23. Rogers MA, Burkness EC, Hutchison WD. Evaluation of high tunnels for management of Drosophila suzukii in fall-bearing red raspberries: potential for reducing insecticide use. *J Pest Sci.* (2016) 89:815–21. doi: 10.1007/s10340-016-0731-1
24. Ebbenga DN, Burkness EC, Hutchison WD. Evaluation of exclusion netting for Spotted- wing drosophila (Diptera: Drosophilidae) management in Minnesota wine grapes. *J Econ Entomol.* (2019) 112:2287–94. doi: 10.1093/jee/toz143
25. Ebbenga DN, Burkness EC, Clark MD, Hutchison WD. Risk of spotted-wing drosophila injury and associated increases in volatile acidity in Minnesota wine grapes. *Am J Enol Vitic.* (2021) 72:106–12. doi: 10.5344/ajev.2020.20008
26. Getman-Pickering ZL, Campbell A, Aflitto N, Grele A, Davis JK, Ugine TA, et al. LeafByte: a mobile application that measures leaf area and herbivory quickly and accurately. *Methods Ecol Evol.* (2020) 11:215–21. doi: 10.1111/2041-210X.13340
27. Coombe BG. Research on development and ripening of the grape berry. *Am J Enol. Vitic.* (1992) 43:101–10.
28. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna (2017). Available online at: http://www.r-project.org/
29. Mendiburu FD. *Agricolae: Statistical Procedures for Agricultural Research*. R package version (2015). p. 2–3. Available online at: http://CRAN.R-project.org/package=agricolae (accessed February 20, 2021).
30. Clark MD. *Cold Hardy Wine Grapes: Frontenac*. University of Minnesota Extension, St. Paul, MN (2020). Available online at: https://enology.umn.

edu/sites/enology.umn.edu/files/2021-10/409_frontenacfactsheet_fin.pdf (accessed December 15, 2021).

31. Fournand D, Vicens A, Sidhoum L, Souquet J, Moutounet M, Cheynier V. Accumulation and extractability of grape skin tannins and anthocyanins at different advanced physiological stages. *J Agric Food Chem.* (2006) 54:7331–8. doi: 10.1021/jf061467h
32. Boucher TJ, Pfeiffer DG, Barden JA, Williams JM. Effects of simulated insect injury on net photosynthesis of potted grapevines. *Hortscience.* (1987) 22:927–8.
33. Kistner-Thomas EJ. The potential global distribution and voltinism of the Japanese beetle (Coleoptera: Scarabaeidae) under current and future climates. *J Insect Sci.* (2019) 9:1–13. doi: 10.1093/jisesa/iez023
34. Liess S, Twine TE, Snyder PK, Hutchison WD, Konar-Steenberg G, Keeler BL, et al. High-resolution climate projections over Minnesota for the 21st century. *Earth Space Sci.* (2022) 9:e2021EA.001893. doi: 10.1029/2021EA001893

Conflict of Interest: WH is a topic editor for the *P. japonica* RT, an independent Assoc. Editor was selected to manage the review process.

The remaining authors confirm that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Insect Science

TYPE Original Research
PUBLISHED 20 October 2022
DOI 10.3389/finsc.2022.1006092

OPEN ACCESS

EDITED BY
David Held,
Auburn University, United States

REVIEWED BY
Lara Maistrello,
University of Modena and Reggio Emilia, Italy
Véronique Martel,
Department of Natural Resources (Canada), Canada

*CORRESPONDENCE
Robert L. Koch
koch0125@umn.edu

†PRESENT ADDRESS
Theresa M. Cira,
Pesticide and Fertilizer Management Division, Department of Agriculture, Saint Paul, MN, United States

SPECIALTY SECTION
This article was submitted to Invasive Insect Species, a section of the journal Frontiers in Insect Science

RECEIVED 28 July 2022
ACCEPTED 05 October 2022
PUBLISHED 20 October 2022

CITATION
Ribeiro AV, Cira TM, MacRae IV and Koch RL (2022) Effects of feeding injury from *Popillia japonica* (Coleoptera: Scarabaeidae) on soybean spectral reflectance and yield. *Front. Insect Sci.* 2:1006092. doi: 10.3389/finsc.2022.1006092

Effects of feeding injury from *Popillia japonica* (Coleoptera: Scarabaeidae) on soybean spectral reflectance and yield

Arthur V. Ribeiro[1], Theresa M. Cira[1†], Ian V. MacRae[2] and Robert L. Koch[1*]

[1]Department of Entomology, University of Minnesota, Saint Paul, MN, United States, [2]Department of Entomology, University of Minnesota, Northwest Research and Outreach Center, Crookston, MN, United States

Remote sensing has been shown to be a promising technology for the detection and monitoring of plant stresses including insect feeding. *Popillia japonica* Newman, is an invasive insect species in the United States, and a pest of concern to soybean, *Glycine max* (L.) Merr., in the upper Midwest. To investigate the effects of *P. japonica* feeding injury (i.e., defoliation) on soybean canopy spectral reflectance and yield, field trials with plots of caged soybean plants were established during the summers of 2020 and 2021. In each year, field-collected *P. japonica* adults were released into some of the caged plots, creating a gradient of infestation levels and resulting injury. Estimates of injury caused by *P. japonica*, ground-based hyperspectral readings, total yield, and yield components were obtained from the caged plots. Injury was greatest in the upper canopy of soybean in plots infested with *P. japonica*. Overall mean canopy injury (i.e., across lower, middle, and upper canopy) ranged from 0.23 to 6.26%, which is representative of injury levels observed in soybean fields in the Midwest United States. Feeding injury from *P. japonica* tended to reduce measures of soybean canopy reflectance in near infra-red wavelengths (~700 to 1000 nm). These results indicate that remote sensing has potential for detection of injury from *P. japonica* and could facilitate scouting for this pest. Effects of *P. japonica* injury on total yield were not observed, but a reduction in seed size was detected in one of the two years. The threat to soybean yield posed by *P. japonica* alone appears minimal, but this pest adds to the guild of other defoliating insects in soybean whose combined effects could threaten yield. The results of this research will guide refinement of management recommendations for this pest in soybean and hold relevance for other cropping systems.

KEYWORDS

herbivory, integrated pest management, Japanese beetle, remote sensing, yield

Introduction

Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), is native to Japan and known to feed on more than 300 plant species (1). *Popillia japonica* has expanded its geographic range and became an invasive species in the United States in the early 1900s and Canada in 1998 (1, 2). More recently, *P. japonica* was also reported in mainland Europe (3) and it is now present in at least three European countries (4). Additionally, *P. japonica* has the potential to expand its range even further and invade Central and South America, Africa, and Oceania (5). In its region of origin, *P. japonica* is a minor agricultural pest, probably due to unfavorable environmental conditions and the presence of natural enemies (1). However, in invaded regions, *P. japonica* is an economically important pest of ornamental plants and turf in landscapes, and horticultural and field crops (1, 2, 6).

Soybean, *Glycine max* (L.) Merrill (Fabales: Fabaceae), is one of the most valuable crops worldwide due to its seed composition (i.e., oil and protein content) and versatility of end-use (7). The United States is the second largest soybean producer worldwide (8), but its production is compromised by the attack of insect pests (7). Defoliation caused by insect feeding is a common injury seen in soybean fields that can potentially lead to yield losses due to the reduction of plant photosynthetic area and disturbance of physiological processes (9). In soybean, *P. japonica* adults feed on leaves, creating a characteristic lace-like pattern of defoliation (6). Infestations of *P. japonica* alone can cause up to 20% loss of soybean yield (10), but *P. japonica* damage to soybeans can be even more problematic when combined with other defoliating insects (11). However, impacts of *P. japonica* feeding have not been well quantified in contemporary soybean varieties.

Current management of defoliating insects in soybean generally relies on the presence of the pest and estimation of percent defoliation across the whole field based on visual assessment of leaves from the top, middle, and bottom of plants selected from throughout the field (6). For *P. japonica*, assessment of the entire canopy of the crop is of particular importance because adults aggregate on the upper leaves of the plants and abundance tends to be higher at the edge of the fields, which can lead to overestimation of defoliation, especially at the field edges (6). Overall, traditional scouting and decision making for defoliating pests, like *P. japonica*, in soybean can be time consuming and therefore increase the overall cost of management.

Remote sensing became prominent in the past decades as a promising method for the detection and monitoring of plant stresses (e.g., insect feeding) (12–15). Remote sensing may be preferable to conventional scouting methods because it is faster and offers better coverage of the field (13, 14). Furthermore, remote sensing allows for early detection of diseases and pests (13). Typical applications of remote sensing consist of using sensors for contactless measurement of the electromagnetic radiation reflected from plants (12, 13). Numerous studies have documented the effects of pest injury and diseases on spectral reflectance of crops, mainly in the visible and near infra-red ranges of the electromagnetic spectrum (16–21). Studies assessing the effects of defoliation on plant spectral reflectance have focused mainly on forest areas (13–15), but field crops have also been investigated (22–26). In soybean, the normalized difference vegetation index (NDVI) was proposed for the detection of defoliation with simulated (22) and actual insect feeding by lepidopteran pests (25). More recently, Iost Filho et al. (2022) (26) evaluated the effects of defoliation by two lepidopteran pests on soybean leaf reflectance using individual wavelengths from the visible and near infra-red spectrum.

There is a lack of information of the effects of defoliation on the spectral reflectance of soybean that include individual wavelengths from the visible and near-infrared spectrum for other defoliators, such as *P. japonica*, especially under field conditions. Additionally, as abovementioned, a better understanding of the impacts of *P. japonica* feeding on yield of contemporary soybean varieties is also required. Thus, this study was done to assess the effects of feeding injury from *P. japonica* on the spectral reflectance, total yield, and yield components of soybean. Results of this study will help advance integrated pest management programs for *P. japonica* in soybean fields.

Methods

Field sites

This study was done in soybean fields of approximately 1 ha located at the University of Minnesota (UMN) Saint Paul campus (44.9898369° N, 93.1802096° W), and at the UMN Research and Outreach Center (44.7113597° N, 93.1041755° W) in Rosemount, Minnesota, United States, during 2020 and 2021, respectively. The soybean variety Stine '19EA32' was planted on 15 May 2020 and the variety Golden Harvest '1012E3' on 15 June 2021 with a seeding rate of 370,000 seeds/ha and row spacing of 0.76 m. When plants were at the V3 growth stage (plants with three fully expanded trifoliate leaves (27), plots of soybean were caged for manipulation of insect populations. Individual plots comprised two rows of soybean that were 1.5 m long (approximately 80 plants per plot), and caged with a 1.5×1.5×1.5-m polyvinyl chloride (PVC) frame covered with white no-see-um mesh (Quest Outfitters, Sarasota, FL, USA). A total of 32 and 24 plots (i.e., cages), arranged in 8 and 6 blocks, were caged in 2020 and 2021, respectively. Before caging, plants were visually inspected for the presence of insects and any individuals found on the plants were manually removed.

In each year, half of the plots in each block were randomly selected for infestation with field-collected *P. japonica* adults on four dates to create a gradient of insect injury. Adult *P. japonica* were collected from soybean fields using dual-lures (female sex pheromone and floral attractant) attached to Trécé Pherocon® standard traps (yellow top and green vented catch can) (Trécé Inc., Adair, OK, USA). Two days prior to each infestation, *P. japonica* were collected from traps 4-5 times per day to reduce insect mortality due to excess heat inside the traps. Trapped *P. japonica* adults were transferred to 34.29×34.29×60.96-cm pop-up insect cages (Bioquip, Rancho Dominguez, CA, USA). As a food source, each cage contained 3-4 field-collected soybean stems cut at the soil level with the cut end of the stems placed inside 20-mL tubes with pierceable caps containing water. For infestations, live (i.e., actively moving) *P. japonica* adults were manually collected from the pop-up cages and placed in containers to be transported to the field. The number of *P. japonica* adults in each container was estimated based on fresh biomass using the methods of Ebbenga et al. (2022) (28) and an analytical scale (Sartorius ENTRIS224-1S, Sartorius Lab Instruments GmbH & Co. KG, Goettingen, Germany). In 2020, *P. japonica* were released on 28 July (1037 individuals per plot), 3 August (224 individuals per plot), 13 August (212 individuals per plot), and 24 August (39 individuals per plot) for a total of 1512 individuals per plot. Similarly, in 2021, *P. japonica* were released on 4 August (831 individuals per plot), 9 August (877 individuals per plot), 16 August (945 individuals per plot), and 24 August (80 individuals per plot) for a total of 2734 individuals per plot.

Data collection

Spectral measurements were recorded within 2 h of solar noon (to reduce atmospheric and solar angle effects), with clear sky conditions or with low cloud cover (< 20%) and a clear view between the sun and the field. Measurements of canopy spectral reflectance were taken on 30 July and 4 September of 2020, and 17 August and 30 August of 2021, using a hyperspectral spectroradiometer (FieldSpec® HandHeld 2™ VNIR spectroradiometer, ASD Inc., Boulder, CO, USA) able to detect wavelengths ranging from 325 to 1075 nm with accuracy of ±1 nm. On each sample date, the spectroradiometer was calibrated immediately before the beginning of measurements and every 7-10 minutes throughout data collection with a Spectralon® reference standard (Labsphere, Inc. Sutton, NH, USA). In each plot, four spectral measurements were manually taken, two from each row, at approximately 0.5 m above the canopy. Cages were opened immediately before and closed immediately after measurements. Canopy-level spectral reflectance data were processed using the software ViewSpec Pro version 6.2.0 (ASD Inc., Boulder, CO, USA) and individual measures were averaged for each plot. Four vegetation indices were calculated from canopy-level spectral reflectance (Table 1) for each plot. These indices were selected because they were used in previous studies investigating the effects of insect feeding and diseases on soybean spectral reflectance (20, 29, 30).

To avoid effects on canopy reflectance measures caused by disturbing the soybean plant canopies, inspection of plants for other insects was performed one day before or one day after collection of spectral data. To do so, whole-plant counts of other insects were recorded for five plants per plot, which were later averaged for each plot. Insects observed on the plants were not removed. Estimates of injury from *P. japonica* were done one day before or after measurements of canopy spectral reflectance. Five leaflets were randomly selected and collected from the lower, middle, and upper portions of the canopy of each plot (i.e., 15 leaflets/plot). These leaflets were placed in individually-labeled 17×17-cm resealable plastic bags, which were placed in a cooler with ice packs for transportation to the laboratory where they were stored in a refrigerator at 5°C to avoid desiccation. To quantify *P. japonica* feeding injury (i.e., percentage of leaflet area removed), leaflets were placed individually on a white surface and were fully extended and flattened under a transparent glass circle (180 mm diameter). Measurements of injury were performed on pictures of each individual leaflet using the software LeafByte version 1.3.0 (31) with an iPad (A1893, Apple Inc., Cupertino, CA, USA). Mean canopy injury (%) for lower, middle, and upper canopy and total canopy (i.e., across lower, middle, and upper canopy) were obtained for each plot.

On 9 October 2020, plants were hand-harvested and seeds obtained with a threshing machine (LPR UMB, Almaco, Nevada, IA, USA). On 19 October 2021, plots were harvested with a small plot combine. Seeds were then manually inspected to remove debris, placed in individual paper bags for each plot and brought back to the laboratory for assessment of yield. Total yield (ton per ha) was obtained by weighing all the seeds from a plot on a

TABLE 1 Vegetation indices tested in this study for the detection of effects of *Popillia japonica* feeding injury in plots of caged soybean plants in the field during 2020 and 2021 in Saint Paul, MN and Rosemount, MN, respectively.

Index	Name	Equation*	Reference
NDVI	Normalized Difference Vegetation Index	$(R_{800}-R_{680})/(R_{800}+R_{680})$	(20)
NDRE	Normalized Difference Red Edge	$(R_{750}-R_{705})/(R_{750}+R_{705})$	(29)
GNDVI	Green Normalized Difference Vegetation Index	$(R_{801}-R_{550})/(R_{801}+R_{550})$	(29)
MCARI	Modified Chlorophyll Absorption Reflectance Index	$[(R_{700}-R_{670})-0.2\times(R_{700}-R_{550})]\times(R_{700}/R_{670})$	(30)

*R_x, reflectance at wavelength x.

scale (Scout Pro SP 4001, Ohaus Corp., Pine Brook, NJ, USA). Seed size (g) was estimated by separately weighing three subsamples of 100 seeds from each plot (i.e., 100-seed weight). Total number of seeds for each plot (i.e., seed number) was calculated using the total weight of seeds for each plot and the 100-seed weight.

Data analyses

As mentioned, plants in the caged soybean plots were inspected for the presence of other insects one day before or one day after collection of spectral data. *Aphis glycines* Matsumura (Hemiptera: Aphididae) was present in some of the cages, but no honeydew was observed on the plants. For the purposes of the present study, plots with average *A. glycines* densities above 20 aphids/plant were removed to avoid potential confounding effects of *A. glycines* feeding on soybean spectral reflectance. This threshold was used because previous research indicated that densities lower than 20 aphids/plant have negligible effects on soybean canopy reflectance (20, 32). A total of 3, 8 and 7 plots with average *A. glycines* densities above 20 aphids/plant were removed from 4 September 2020, 17 August 2021 and 30 August 2021, respectively. Thus, the total number of plots analyzed was 16 uninfested and 16 infested with *P. japonica* adults on 30 July 2020, 15 uninfested and 14 infested on 04 September 2020, 7 uninfested and 9 infested on 17 August 2021, and 10 uninfested and 7 infested on 30 August 2021. However, to account for the potential effects of *A. glycines*, even at low numbers (i.e., densities lower than 20 aphids/plant), on the spectral reflectance of soybean, *A. glycines* density was also included in the analyses (see below).

All analyses were performed and graphs made using the software R version 3.5.1 (33) and RStudio Desktop version 1.1.463 (34). For each date in each year, stratum-specific injury (i.e., lower, middle, and upper canopy), total canopy injury, and spectral reflectance of the canopy were evaluated using variable dispersion beta regression models with a logit link function (package, *code*: betareg, *betareg* (35)). These response variables were included in the models as proportions (i.e., values between 0 and 1). For stratum-specific injury, infestation status (i.e., uninfested or infested with *P. japonica*), canopy stratum (i.e., lower, middle, or upper canopy) and their interaction were included as explanatory variables; and infestation status was included as an additional regressor for the estimation of the model precision parameter. For total injury, infestation status was used both as the explanatory variable and for the estimation of the model precision parameter. For spectral reflectance of the canopy, wavelengths and vegetation indices were analyzed separately, with total canopy injury used both as the explanatory variable and for the estimation of the model precision parameter. The inclusion of a precision parameter significantly improved the models, which was checked *via* a likelihood-ratio test comparing the full model with and without this parameter (lmtest, *lrtest* (36)). Block, *Aphis glycines* density and its interaction with total canopy injury were initially included in the models as explanatory variables, but they were overall non-significant ($P > 0.05$) and therefore removed from the models. Model assumptions were assessed with diagnostic plots of residuals. Similarly to Geissinger et al. (2022) (37), the significance of explanatory variables for stratum-specific injury was obtained *via* sequential nested likelihood-ratio tests (lmtest, *lrtest* (36)), and mean separation tests with *P*-values of pairwise comparisons adjusted with the Tukey method were done using estimated marginal means ($\alpha = 0.05$) (emmeans, *emmeans* (38)). The significance of explanatory variables for total canopy injury and spectral reflectance of the canopy was estimated with partial Wald tests (stats, *summary* (33)).

Seed number, 100-seed weight, and total yield were analyzed with general linear models (stats, *lm* (33)) with injury (%) as the explanatory variable. Block was initially included in the models, but it was overall non-significant and therefore removed from the models. Linear model assumptions were visually checked with residual and quantile-quantile scatterplots, and formally with a global validation test (gvlma, *gvlma* (39)). The presence of outliers was assessed *a priori* with a Bonferroni outlier test (car, *outlierTest* (40)). One observation was indicated as an outlier and model assumptions were accepted after its removal.

Results

Feeding injury

The interaction between *P. japonica* infestation status and canopy stratum was significant on 4 September 2020 and 17 August 2021 ($P < 0.001$) (Table 2). However, on 30 July 2020 and 30 August 2021, the interaction between *P. japonica* infestation status and canopy stratum was not significant. On these two dates, canopy injury of soybean was significantly affected by *P. japonica* infestation status ($P < 0.001$) and canopy stratum (i.e., lower, middle, and upper canopy) ($P < 0.001$) (Table 2). Overall, injury in uninfested plots did not differ among canopy strata, but injury in plots infested with *P. japonica* was greater in the upper stratum of the soybean canopy (Figure 1). Across the two years, mean canopy injury ranged from 0.13 to 0.37%, 0.59 to 0.97%, and 0.25 to 1.40% in the lower, middle, and upper strata, respectively, in uninfested plots (Figure 1). For plots infested with *P. japonica* adults, mean canopy injury ranged from 0.95 to 1.35%, 1.58 to 5.45%, and 4.46 to 12.00% in the lower, middle, and upper strata, respectively, across the two years (Figure 1). Similarly, mean total canopy injury (i.e., across lower, middle, and upper strata of the canopy) ranged from 0.42 to 0.91% and from 2.28 to 6.51% in uninfested and infested plots, respectively, and was significantly higher in plots infested with *P. japonica* adults ($P < 0.001$) (Table 2 and Figure 1).

TABLE 2 Likelihood ratio tests of beta regression models testing the effect of *Popillia japonica* infestation status (i.e., infested or unifested), canopy stratum (i.e., lower, middle, or upper canopy) and their interaction on feeding injury within the canopy, and of *P. japonica* infestation status on total feeding injury in plots of caged soybean plants in the field during 2020 and 2021 in Saint Paul, MN and Rosemount, MN, respectively.

Date	Within canopy			Total
	Infestation status	Canopy stratum	Interaction	Infestation status
30 July 2020	$\chi^2_{(1)} = 13.04$ P < **0.001**	$\chi^2_{(2)} = 29.84$ P < **0.001**	$\chi^2_{(2)} = 4.77$ P = 0.092	$\chi^2_{(1)} = 16.11$ P < **0.001**
4 September 2020	$\chi^2_{(1)} = 33.92$ P < **0.001**	$\chi^2_{(2)} = 26.13$ P < **0.001**	$\chi^2_{(2)} = 13.38$ P = **0.001**	$\chi^2_{(1)} = 24.65$ P < **0.001**
17 August 2021	$\chi^2_{(1)} = 36.44$ P < **0.001**	$\chi^2_{(2)} = 21.48$ P < **0.001**	$\chi^2_{(2)} = 29.54$ P < **0.001**	$\chi^2_{(1)} = 28.45$ P < **0.001**
30 August 2021	$\chi^2_{(1)} = 28.67$ P < **0.001**	$\chi^2_{(2)} = 16.11$ P < **0.001**	$\chi^2_{(2)} = 3.28$ P = 0.194	$\chi^2_{(1)} = 24.21$ P < **0.001**

Significant P values are boldfaced.

Spectral reflectance

On 30 July 2020, spectral reflectance of the soybean canopy was not affected by injury from *P. japonica*. On 4 September 2020, a significant decrease in reflectance was observed at wavelengths above 723 nm. In 2021, a significant increase in reflectance was observed at wavelengths below 420 nm, and a significant decrease in reflectance from 722 to 898 nm on 17 August. On 30 August 2021, a significant increase in reflectance was generally observed from 427 to 529 nm and from 563 to 698 nm, and a significant decrease in reflectance from 735 to 944 nm (Figure 2). Across the three sample dates with significant effects of injury from *P. japonica* on spectral reflectance, the highest pseudo coefficients of determination (R^2) were observed for wavelengths around the 780 nm region (Figure 2).

The vegetation indices were generally not affected by injury from *P. japonica* in 2020, except for MCARI on 4 September 2020 (Table 3). On this date, injury from *P. japonica* significantly decreased MCARI. In 2021, a significant reduction was also observed for NDVI, GNDVI (Green Normalized Difference

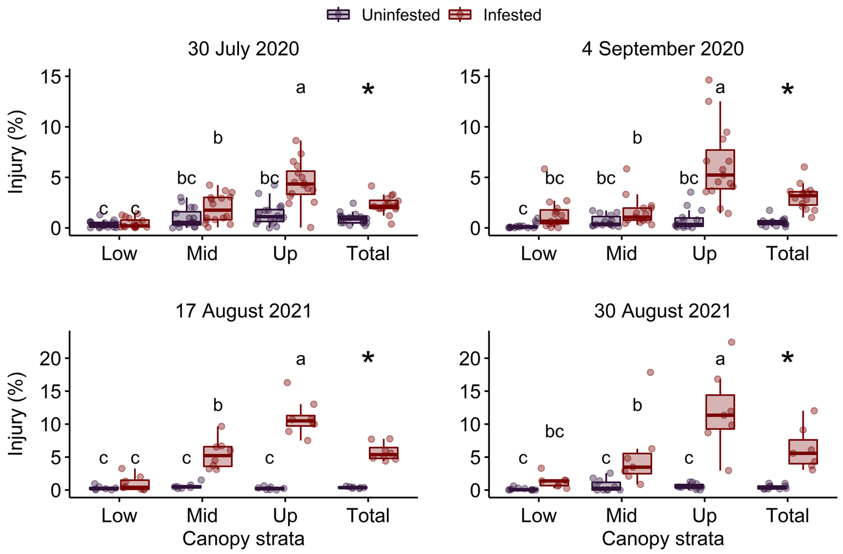


FIGURE 1
Percentage of leaf area injured in the lower (Low), middle (Mid), upper (Up) and total (i.e., across lower, middle, and upper) canopy in plots of caged soybean plants in the field that were uninfested or infested with *Popillia japonica* on two sample dates in each of 2020 and 2021. Different letters within each graph indicate significant differences among lower, middle, and upper canopy strata according to the Tukey's test ($P < 0.05$). Asterisks indicate significant differences within each graph between infestation treatments for total canopy injury according to the Tukey's test ($P < 0.05$).

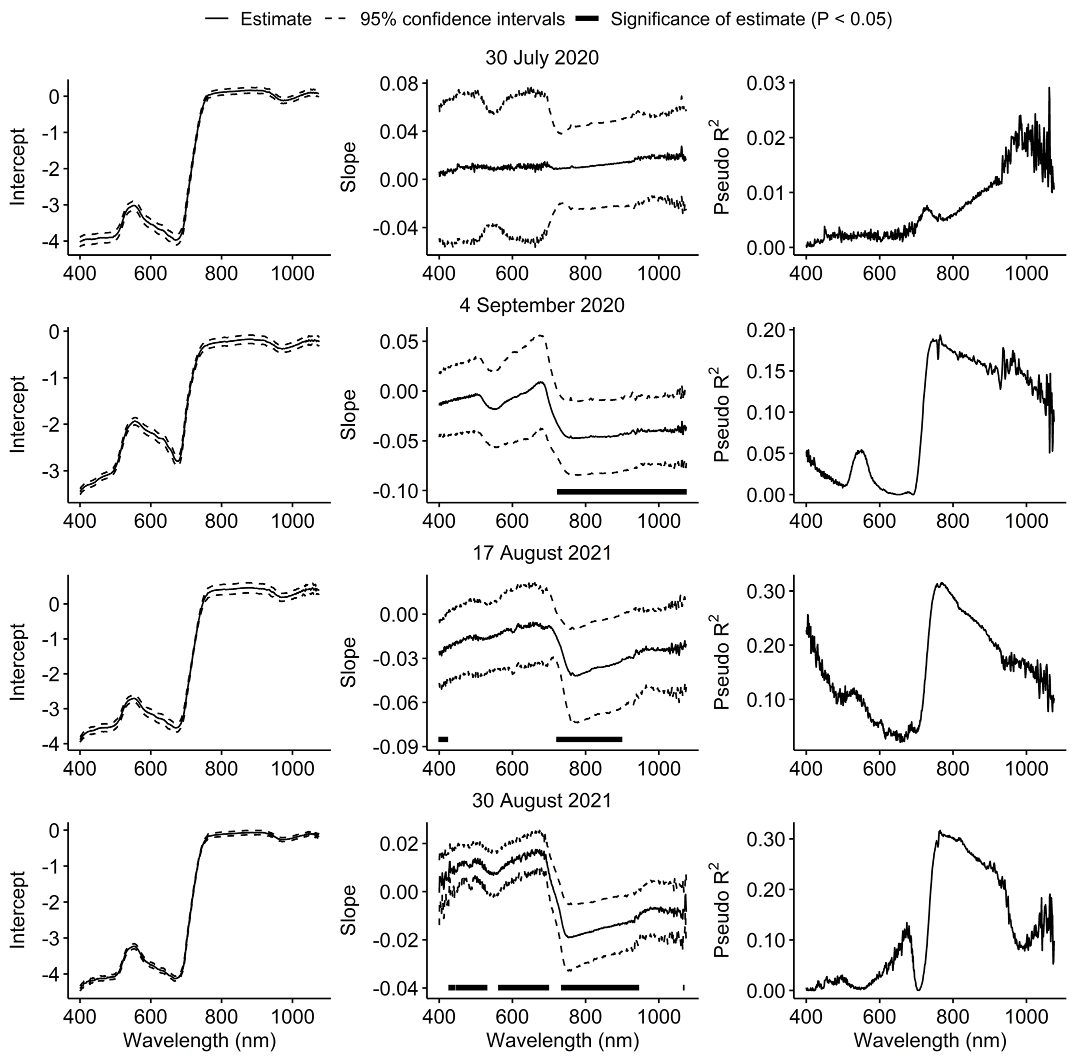


FIGURE 2
Estimates and 95% confidence intervals of intercepts and slopes, and pseudo coefficients of determination (R^2) from beta regression models testing the effect of feeding injury from *Popillia japonica* on canopy spectral reflectance in plots of caged soybean plants in the field across 676 narrowband wavelengths on two sample dates in each of 2020 and 2021. Black horizontal bars within graphs in the middle column indicate that estimates of slopes for wavelengths differ from zero according to partial Wald tests ($P < 0.05$).

Vegetation Index) and NDRE (Normalized Difference Red Edge) with increasing injury on 30 August (Table 3). Pseudo coefficients of determination (R^2) values ranged from 0.001 to 0.172 in 2020, and from 0.003 to 0.304 in 2021 (Table 3). Overall, higher pseudo R^2 were observed around 780 nm.

Total yield and yield components

In 2020, injury from *P. japonica* adults did not affect seed number (mean: 2.52 x 10^7; range: 1.86 x 10^5 – 3.04 x 10^7), 100-seed weight (mean: 16.60 g; range: 13.92 – 18.70 g), or total yield (mean: 4.17 ton per ha; range: 3.21 – 5.05 ton per ha) of soybean (Table 4). A similar result was found in 2021 for seed number (mean: 1.90 x 10^7; range: 1.39 x 10^7 – 2.28 x 10^7) and total yield (mean: 3.24 ton per ha; range: 2.55 – 4.01 ton per ha). However, a significant effect was detected for 100-seed weight (mean: 17.06 g; range: 15.55 – 18.36 g), which decreased 1.6 g for every 10% increase in injury (Table 4).

Discussion

Popillia japonica is an invasive insect species of global concern for food crops in North America (1, 2) and, more recently, Europe (4). In this study, feeding injury by *P. japonica*

TABLE 3 Summary outputs of beta regression models testing the effects of *Popillia japonica* feeding injury on vegetation indices from canopy spectral reflectance in plots of caged soybean plants in the field during 2020 and 2021 in Saint Paul, MN and Rosemount, MN, respectively.

Date	Index	Parameter	Estimate	Std. Error	z value	P value	Pseudo R^2
30 July 2020	NDVI	Intercept	2.58	0.06	41.33	**<0.001**	0.002
		Slope	-0.01	0.03	-0.26	0.792	
	NDRE	Intercept	1.11	0.05	23.19	**<0.001**	0.002
		Slope	-0.01	0.02	-0.37	0.713	
	GNDVI	Intercept	1.65	0.05	30.25	**<0.001**	0.001
		Slope	-0.01	0.03	-0.26	0.798	
	MCARI	Intercept	-2.66	0.06	-45.21	**<0.001**	0.003
		Slope	0.01	0.02	0.57	0.568	
4 September 2020	NDVI	Intercept	1.18	0.06	19.98	**<0.001**	0.076
		Slope	-0.04	0.03	-1.45	0.146	
	NDRE	Intercept	-0.48	0.06	-8.34	**<0.001**	0.037
		Slope	-0.03	0.02	-1.02	0.307	
	GNDVI	Intercept	0.23	0.04	5.55	**<0.001**	0.010
		Slope	-0.01	0.02	-0.47	0.639	
	MCARI	Intercept	-1.28	0.05	-24.07	**<0.001**	0.172
		Slope	-0.05	0.02	-2.60	**0.009**	
17 August 2021	NDVI	Intercept	2.28	0.09	26.72	**<0.001**	0.017
		Slope	-0.01	0.02	-0.72	0.473	
	NDRE	Intercept	0.95	0.07	12.79	**<0.001**	0.037
		Slope	-0.02	0.01	-1.25	0.210	
	GNDVI	Intercept	1.48	0.08	19.27	**<0.001**	0.003
		Slope	-0.01	0.01	-0.63	0.527	
	MCARI	Intercept	-2.48	0.06	-44.07	**<0.001**	0.047
		Slope	-0.01	0.01	-0.86	0.387	
30 August 2021	NDVI	Intercept	2.58	0.03	96.31	**<0.001**	0.304
		Slope	-0.02	0.00	-8.64	**<0.001**	
	NDRE	Intercept	1.12	0.03	37.46	**<0.001**	0.115
		Slope	-0.01	0.00	-3.82	**<0.001**	
	GNDVI	Intercept	1.74	0.03	60.07	**<0.001**	0.136
		Slope	-0.02	0.00	-5.81	**<0.001**	
	MCARI	Intercept	-2.82	0.05	-56.39	**<0.001**	0.079
		Slope	-0.01	0.01	-1.41	0.158	

Significant P values are boldfaced.

was detectable and greater in the upper stratum of the canopy of soybean, with potential effects on canopy spectral reflectance and minimal effects on total yield and seed quality. This is consistent with the top-down feeding patterns well documented for *P. japonica* on a range of host plants (1).

Overall mean canopy injury ranged from 0.23 to 6.26%. The levels of injury attained in this study are representative of levels of *P. japonica* injury observed in soybean fields in the Midwest (41, 42). Such levels of injury (i.e., overall mean canopy injury < 15%) are also commonly observed in other crops fed on by *P. japonica* (28, 43). In soybean, typical infestation levels of *P. japonica* may not be a threat to yield (11). However, *P. japonica* is part of a complex of defoliating pests that, in combination, can result in defoliation greater than the economic thresholds currently adopted in the Midwest (i.e., 30% before bloom or 20% from bloom to pod fill (6)).

Leaf injury reduces photosynthetic area of plants and can cause disturbance of physiological processes including water and nutrient transportation, as well as eliciting the expression of defense responses, which are energetically costly and therefore reduce plant efficiency (9). *Popillia japonica* feeding was previously found to increase transpiration and consequently water loss in soybean leaflets without affecting carbon assimilation rates or photosynthetic efficiency (9). However, physiological effects observed at the leaf level are not necessarily reflected at the canopy level. For example, Ostlie and Pedigo (1984) (44) observed higher transpiration from soybean leaflets following artificial defoliation; but artificial

TABLE 4 Summary outputs from general linear models estimating the effects of feeding injury (%) from *Popillia japonica* adults on seed number (seeds/ha x 10^6), 100-seed weight (g) and total yield (ton/ha) in plots of caged soybean plants in the field during 2020 (n = 28) and 2021 (n = 17) in Saint Paul, MN and Rosemount, MN, respectively.

Factor	Parameter	Estimate	Std. Error	t value	P value
2020					
Seed number	Intercept	24.44	0.78	31.23	< **0.001**
	Slope	0.39	0.33	1.20	0.242
100-seed weight	Intercept	16.97	0.30	56.68	< **0.001**
	Slope	-0.21	0.13	-1.63	0.114
Total yield	Intercept	4.14	0.13	31.09	< **0.001**
	Slope	0.01	0.06	0.23	0.819
2021					
Seed number	Intercept	19.46	0.70	27.71	< **0.001**
	Slope	-0.16	0.16	-1.05	0.309
100-seed weight	Intercept	17.46	0.14	120.07	< **0.001**
	Slope	-0.14	0.03	-4.37	< **0.001**
Total yield	Intercept	3.39	0.12	29.25	< **0.001**
	Slope	-0.05	0.03	-2.09	0.054

Significant P values are boldfaced.

defoliation or actual feeding by the green cloverworm, *Hypena scabra* (Fabricius) (Lepidoptera: Noctuidae), and the cabbage looper, *Trichoplusia ni* (Hubner) (Lepidoptera: Noctuidae), actually decreased canopy transpiration. Similarly, Klubertanz et al. (1996) (45) observed higher soil moisture in potted soybean plants following artificial defoliation.

The morphophysiological changes of plants caused by biotic or abiotic stresses can alter the spectral reflectance of plants including the visible and near infra-red ranges of the electromagnetic spectrum (15). For insect pests, this effect seems to be density-dependent as shown for *A. glycines* (20) and two lepidopteran pests (26) in soybean. In this study, a significant increase in soybean canopy reflectance of plants fed on by *P. japonica* at wavelengths in the visible range was observed, but this effect was inconsistent. However, a decrease in reflectance in the near infra-red associated with an increase in canopy injury from *P. japonica* was observed in three of the four dates across the two years. Lack of spectral response on the first sample date in 2020 was likely due to the low levels of injury observed on this date (Figure 1). Similarly, feeding injury by two lepidopteran species increased the visible and decreased near infra-red reflectance of soybean leaves in a greenhouse experiment (26). In contrast, an increase in both visible and near infra-red reflectance of leaves of peanut, *Arachis hypogaea* (L.) (Fabales: Fabaceae), was observed following injury by *Stegasta bosqueella* (Chambers) (Lepidoptera: Gelechiidae) and *S. cosmioides* (Walker) in the greenhouse (23).

Feeding injury from *P. japonica* to soybean tended to reduce spectral reflectance in the canopy in near infra-red wavelengths (~700 to 1000 nm). Furthermore, higher pseudo coefficients of determination (R^2) around 780 nm indicate that this region is optimal for the detection of *P. japonica* injury in soybean. Canopy reflectance at 780 nm has also been shown to be optimal for the detection of *A. glycines* in soybean (46). Although *A. glycines* was present at densities lower than 20 aphids per plant in some plots used in this study, this effect was non-significant across all dates. For this reason, the results presented here are due to the effects of *P. japonica* injury alone. This corroborates previous findings indicating that *A. glycines* at densities lower than 20 aphids per plant result in negligible effects on soybean canopy reflectance (20, 32). Nevertheless, such overlapping effects on canopy spectral reflectance around 780 nm suggests that *P. japonica* feeding resulting in mean canopy injury of ≥ 5% may confound the detection of *A. glycines* or other herbivores in soybean. Thus, further investigation of spectral data that includes *P. japonica* injury coincident with other pests such as *A. glycines* is needed.

Inconsistent results were observed for the vegetation indices evaluated for the effects of *P. japonica* injury on soybean canopy reflectance. This lack of a consistent effect is probably because the vegetation indices tested here incorporate reflectance from the visible spectrum. Previous studies found the vegetation index NDVI is associated with the distribution of three lepidopteran pests, but not with their feeding injury in soybean fields (25). In cotton, differences in NDVI were observed for plants fed on by *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae), but not for *T. ni* in the field (47).

Taken together, these findings indicate that plant spectral responses to defoliation are likely species-dependent, and care should be taken when generalizing across species of plants and defoliators. Furthermore, some of the results documented in the cited literature come from leaf measurements performed in the laboratory. Comparisons of canopy spectral reflectance obtained in the field to laboratory measurements can further create discrepancies between studies.

In this study, effects of *P. japonica* feeding injury on total yield were not observed, but a reduction in seed size was detected in one of the two years. Although soybean is tolerant to defoliation (48), the spatiotemporal distribution of canopy defoliation seems to have a differential impact on soybean yield. The intensity of feeding injury from *P. japonica* was quantified among strata of the soybean canopy more thoroughly than in previous studies. The lack of an effect of *P. japonica* feeding on total soybean yield confirms that contemporary soybean varieties likely respond similarly to this pest as those studied in the past.

In conclusion, near infra-red wavelengths may hold promise for remote sensing of *P. japonica* feeding injury in soybean. Because remote sensing can also be affected by other soybean pests, further studies incorporating near infra-red wavelengths and standard red, green and blue (RGB) imagery to differentiate *P. japonica* injury from that of other defoliators in soybean are needed. These results can facilitate refinement of management recommendations for *P. japonica* in soybean.

Data availability statement

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

Author contributions

AR, investigation, formal analysis, writing (original draft, review, and editing). TC, conceptualization, methodology, investigation, writing (review and editing). IM, conceptualization, writing (review and editing). RK, project administration, funding acquisition, conceptualization, methodology, writing (original draft, review, and editing). All authors contributed to the article and approved the submitted version.

Funding

Funding for this research was provided by the Minnesota Soybean Research and Promotion Council and the Minnesota Invasive Terrestrial Plants and Pests Center through the Minnesota Environment and Natural Resources Trust Fund.

Acknowledgments

We thank Rosa (Tina) Lozano, Rafael Carlesso Aita, James Menger, Grace Doyle, Samantha Janecek, Gunnar Morris, Ellen Adjeiwaa and Gloria Melotto for their help during data collection.

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.

Publisher's note

References

1. Potter DA, Held DW. Biology and management of the japanese beetle. *Annu Rev Entomol* (2002) 47:175–205. doi: 10.1146/annurev.ento.47.091201.145153

2. Althoff ER, Rice KB. Japanese Beetle (Coleoptera: Scarabaeidae) invasion of north America: History, ecology, and management. *J Integr Pest Manag* (2022) 13:2. doi: 10.1093/jipm/pmab043

3. EPPO. *First report of popillia japonica in Italy*. EPPO Report Service (2014) 10:179. Available at: https://gd.eppo.int/reporting/article-3272.

4. EPPO. *Popillia japonica. EPPO global database* (2022). Available at: https://gd.eppo.int/taxon/POPIJA/distribution.

5. Kistner-Thomas EJ. The potential global distribution and voltinism of the Japanese beetle (Coleoptera: Scarabaeidae) under current and future climates. *J Insect Sci* (2019) 19:16. doi: 10.1093/jisesa/iez023

6. Shanovich HN, Dean AN, Koch RL, Hodgson EW. Biology and management of Japanese beetle (Coleoptera: Scarabaeidae) in corn and soybean. *J Integr Pest Manag* (2019) 10:9. doi: 10.1093/jipm/pmz009

7. Shea Z, Singer WM, Zhang B. "Soybean production, versatility, and improvement". In: Hasanuzzaman M, editor. *Legume crops - prospects, production and uses*. London: IntechOpen (2020). doi: 10.5772/intechopen.91778

8. FAO. *FAOSTAT statistical database* (2022). Available at: https://www.fao.org/faostat/en/#data/QCL.

9. Aldea M, Hamilton JG, Resti JP, Zangerl AR, Berenbaum MR, DeLucia EH. Indirect effects of insect herbivory on leaf gas exchange in soybean. *Plant Cell Environ* (2005) 28:402–11. doi: 10.1111/j.1365-3040.2005.01279.x

10. Gould GE. Japanese Beetle damage to soybeans and corn. *J Econ Entomol* (1963) 56:776–81. doi: 10.1093/jee/56.6.776

11. Hammond RB. "Japanese beetle". In: Higley LG, Boethel DJ, editors. *Handbook of soybean insect pests*. Lanham, MD: Entomological Society of America (1994). p. 64–5.

12. Mulla DJ. Twenty five years of remote sensing in precision agriculture: key advances and remaining knowledge gaps. *Biosyst Eng* (2013) 114:358–71. doi: 10.1016/j.biosystemseng.2012.08.009

13. Zhang J, Huang Y, Pu R, Gonzalez-Moreno P, Yuan L, Wu K, et al. Monitoring plant diseases and pests through remote sensing technology: a review. *Comput Electron Agric* (2019) 165:104943. doi: 10.1016/j.compag.2019.104943

14. Abd El-Ghany NM, Abd El-Aziz SE, Marei SS. A review: application of remote sensing as a promising strategy for insect pests and diseases management. *Environ Sci pollut Res* (2020) 27:33503–15. doi: 10.1007/s11356-020-09517-2

15. Rhodes MW, Bennie JJ, Spalding A, ffrench-Constant RH, Maclean IMD. Recent advances in the remote sensing of insects. *Biol Rev* (2022) 97:343–60. doi: 10.1111/brv.12802

16. Reisig D, Godfrey L. Spectral response of cotton aphid-(Homoptera: Aphididae) and spider mite-(Acari: Tetranychidae) infested cotton: controlled studies. *Environ Entomol* (2007) 36:1466–74. doi: 10.1603/0046-225X(2007)36[1466:SROCAH]2.0.CO;2

17. Yang C-M, Cheng C-H, Chen R-K. Changes in spectral characteristics of rice canopy infested with brown planthopper and leaffolder. *Crop Sci* (2007) 47:329–35. doi: 10.2135/cropsci2006.05.0335

18. Huang J, Liao H, Zhu Y, Sun J, Sun Q, Liu X. Hyperspectral detection of rice damaged by rice leaf folder (*Cnaphalocrocis medinalis*). *Comput Electron Agric* (2012) 82:100–7. doi: 10.1016/j.compag.2012.01.002

19. Krishna G, Sahoo RN, Pargal S, Gupta VK, Sinha P, Bhagat S, et al. Assessing wheat yellow rust disease through hyperspectral remote sensing. *Int Arch Photogramm Remote Sens Spat Inf Sci* (2014) XL–8:1413–6. doi: 10.5194/isprsarchives-XL-8-1413-2014

20. Alves TM, Macrae IV, Koch RL. Soybean aphid (Hemiptera: Aphididae) affects soybean spectral reflectance. *J Econ Entomol* (2015) 108:2655–64. doi: 10.1093/jee/tov250

21. Ramos APM, Gomes FDG, Pinheiro MMF, Furuya DEG, Gonçalvez WN, Marcato Junior J, et al. Detecting the attack of the fall armyworm (*Spodoptera frugiperda*) in cotton plants with machine learning and spectral measurements. *Precis Agric* (2022) 23:470–91. doi: 10.1007/s11119-021-09845-4

22. Board JE, Maka V, Price R, Knight D, Baur ME. Development of vegetation indices for identifying insect infestations in soybean. *Agron J* (2007) 99:650–6. doi: 10.2134/agronj2006.0155

23. Pinto J, Powell S, Peterson R, Rosalen D, Fernandes O. Detection of defoliation injury in peanut with hyperspectral proximal remote sensing. *Remote Sens* (2020) 12:3828. doi: 10.3390/rs12223828

24. Furuya DEG, Ma L, Pinheiro MMF, Gomes FDG, Gonçalvez WN, Marcato Junior J, et al. Prediction of insect-herbivory-damage and insect-type attack in maize plants using hyperspectral data. *Int J Appl Earth Obs Geoinf* (2021) 105:102608. doi: 10.1016/j.jag.2021.102608

25. Greene AD, Reay-Jones FPF, Kirk KR, Peoples BK, Greene JK. Spatial associations of key lepidopteran pests with defoliation, NDVI, and plant height in soybean. *Environ Entomol* (2021) 50:1378–92. doi: 10.1093/ee/nvab098

26. Iost Filho FH, Pazini J de B, de MAD, DL R, Yamamoto PT. Assessment of injury by four major pests in soybean plants using hyperspectral proximal imaging. *Agronomy* (2022) 12:1516. doi: 10.3390/agronomy12071516

27. Fehr WR, Caviness CE. *Stages of soybean development*. Ames, IA: Cooperative Extension Service (1977).

28. Ebbenga DN, Burkness EC, Clark MD, Hutchison WD. Impact of adult *Popillia japonica* (Coleoptera: Scarabaeidae) foliar feeding injury on fruit yield and quality of a temperate, cold-hardy wine grape, 'Frontenac''. *Front Insect Sci* (2022) 2:887659. doi: 10.3389/finsc.2022.887659

29. Liu S, Yu H, Sui Y, Zhou H, Zhang J, Kong L, et al. Classification of soybean frogeye leaf spot disease using leaf hyperspectral reflectance. *PloS One* (2021) 16:e0257008. doi: 10.1371/journal.pone.0257008

30. Vigier BJ, Pattey E, Strachan IB. Narrowband vegetation indexes and detection of disease damage in soybeans. *IEEE Geosci Remote Sens Lett* (2004) 1:255–9. doi: 10.1109/LGRS.2004.833776

31. Getman-Pickering ZL, Campbell A, Aflitto N, Grele A, Davis JK, Ugine TA. LeafByte: a mobile application that measures leaf area and herbivory quickly and accurately. *Methods Ecol Evol* (2020) 11:215–21. doi: 10.1111/2041-210X.13340

32. Marston ZPD, Cira TM, Hodgson EW, Knight JF, Macrae IV, Koch RL. Detection of stress induced by soybean aphid (Hemiptera: Aphididae) using multispectral imagery from unmanned aerial vehicles. *J Econ Entomol* (2020) 113:779–86. doi: 10.1093/jee/toz306

33. R Core Team. *R: a language and environment for statistical computing* (2021). Available at: https://www.r-project.org/.

34. RStudio Team. *RStudio: integrated development for r* (2018). Available at: http://www.rstudio.com.

35. Cribari-Neto F, Zeileis A. Beta regression in r. *J Stat Softw* (2010) 34:1–24. doi: 10.18637/jss.v034.i02

36. Zeileis A, Hothorn T. Diagnostic checking in regression relationships. *R News* (2002) 2:7–10. Available at: https://cran.r-project.org/web/packages/lmtest/vignettes/lmtest-intro.pdf.

37. Geissinger EA, Khoo CLL, Richmond IC, Faulkner SJM, Schneider DC. A case for beta regression in the natural sciences. *Ecosphere* (2022) 13:e3940. doi: 10.1002/ecs2.3940

38. Lenth RV, Buerkner P, Herve M, Love J, Riebl H, Singmann H. *Emmeans: estimated marginal means, aka least-squares means. CRAN* (2020). Available at: https://cran.r-project.org/web/packages/emmeans/index.html.

39. Pena EA, Slate EH. *Gvlma: global validation of linear models assumptions. CRAN* (2019). Available at: https://cran.r-project.org/package=gvlma.

40. Fox J, Weisberg S. *An r companion to applied regression*. Sage: Thousand Oaks (2019). Available at: https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

41. Hodgson EW, VanNostrand GR, Dean A, Berns KR. *Japanese Beetle insecticide efficacy evaluation in Northeast Iowa* Vol. 1 2019. Ames: Iowa State Univ Res Demonstr Farms Prog Reports (2020). Available at: https://www.iastatedigitalpress.com/farmreports/article/id/11345/.

42. Hodgson EW, VanNostrand GR, Dean A. *Japanese Beetle insecticide efficacy evaluation in central Iowa* Vol. 1 2020. Ames:Iowa State Univ Res Demonstr Farms Prog Reports (2021). Available at: https://www.iastatedigitalpress.com/farmreports/article/id/12547/.

43. Shanovich HN, Ribeiro AV, Koch RL. Seasonal abundance, defoliation, and parasitism of Japanese beetle (Coleoptera: Scarabaeidae) in two apple cultivars. *J Econ Entomol* (2021) 114:811–7. doi: 10.1093/jee/toaa315

44. Ostlie KR, Pedigo LP. Water loss from soybeans after simulated and actual insect defoliation. *Environ Entomol* (1984) 13:1675–80. doi: 10.1093/ee/13.6.1675

45. Klubertanz TH, Pedigo LP, Carlson RE. Soybean physiology, regrowth, and senescence in response to defoliation. *Agron J* (1996) 88:577–82. doi: 10.2134/agronj1996.00021962008800040014x

46. Alves TM, Moon RD, MacRae IV, Koch RL. Optimizing band selection for spectral detection of *Aphis glycines* matsumura in soybean. *Pest Manag Sci* (2019) 75:942–9. doi: 10.1002/ps.5198

47. Sudbrink DL, Harris FA, Robbins JT, English PJ, Willers JL. Evaluation of remote sensing to identify variability in cotton plant growth and correlation with larval densities of beet armyworm and cabbage looper (Lepidoptera:Noctuidae). *Florida Entomol* (2003) 86:290–4. doi: 10.1653/0015-4040(2003)086[0290:EORSTI]2.0.CO;2

48. Turnipseed SG. Response of soybeans to foliage losses in south Carolina. *J Econ Entomol* (1972) 65:224–9. doi: 10.1093/jee/65.1.224

frontiers | Frontiers in Insect Science

TYPE Original Research
PUBLISHED 27 October 2022
DOI 10.3389/finsc.2022.961437

EDITED BY
Giulia Torrini,
Council for Agricultural and Economics Research (CREA), Italy

REVIEWED BY
Davide Martinetti,
Biostatistique et Processus Spatiaux (INRAE), France
Mattia Falaschi,
University of Milan, Italy

*CORRESPONDENCE
Christelle Guédot
guedot@wisc.edu

SPECIALTY SECTION
This article was submitted to Invasive Insect Species, a section of the journal Frontiers in Insect Science

RECEIVED 04 June 2022
ACCEPTED 12 October 2022
PUBLISHED 27 October 2022

CITATION
Henden J and Guédot C (2022) Effect of surrounding landscape on *Popillia japonica* abundance and their spatial pattern within Wisconsin vineyards. *Front. Insect Sci.* 2:961437.
doi: 10.3389/finsc.2022.961437

Effect of surrounding landscape on *Popillia japonica* abundance and their spatial pattern within Wisconsin vineyards

Jacob Henden and Christelle Guédot*

Department of Entomology, University of Wisconsin-Madison, Madison, WI, United States

Landscapes surrounding agroecosystems can provide resources that may benefit insect pests. This project examined the influence of the surrounding landscape on the abundance and spatial pattern of *Popillia japonica* (Coleoptera: Scarabaeidae) in vineyards. Twenty vineyards across Southern Wisconsin, spanning a gradient of 5-80% cropland in a 1.5km radius surrounding landscape, were sampled in 2017 and 2018 for *P. japonica* adults and leaf injury. The distribution of *P. japonica* and leaf injury was assessed by sampling along a transect at the edge, halfway from the edge to the center, and at the center of each vineyard. The proportion of cropland and pasture in the surrounding landscape along with abiotic factors of temperature, precipitation, longitude, and pesticide use (determined using Environmental Impact Quotient) were included in models to explain the variation of *P. japonica* abundance and leaf injury. No significant relationship was observed between proportion cropland in the surrounding landscape and *P. japonica* abundance or leaf injury. Combined effects of pasture, longitude, and temperature best explained variation in the abundance of *P. japonica* adults while longitude, temperature and EIQ best explained variability in leaf injury. Vineyards with more pastures in the surrounding landscape, located further east, and with higher temperatures, generally had more *P. japonica* adults and vineyards further east with higher temperature and lower EIQ pesticide use generally had higher levels of leaf injury. Additionally, variability in weekly temperature and precipitation influenced weekly abundance, with higher temperatures and less precipitation resulting in greater weekly abundance of *P. japonica* adults. Significantly more adult *P. japonica* and greater leaf injury were found at the edges than in the center of vineyards. Our results suggest beetles from the surrounding landscape likely contribute to populations of *P. japonica* adults found feeding on vines on vineyard edges, and *P. japonica* abundance and associated leaf injury are influenced by geographical location, local weather conditions, and pesticide use.

KEYWORDS
Japanese beetle, invasive, landscape effect, grape, EIQ, longitude, abiotic factors

Highlights

- The amount of pasture in the surrounding landscape, longitude and temperature best explained the abundance of *P. japonica* adults
- Vineyards further east with higher temperature and lower EIQ pesticide use experienced higher levels of leaf injury.
- Higher *P. japonica* populations and greater leaf injury were found at vineyard edges
- Management of *P. japonica* populations could be targeted towards vineyard edges

Introduction

The surrounding landscape can influence the abundance of pest insects within agricultural fields by providing shelter, refuge, and nutritional resources throughout the year (1, 2), by acting as natural barriers to movement (3), or by supporting populations of natural enemies which help suppress pest populations (4, 5). Insect diversity generally increases with a higher proportion of natural or uncultivated land in the surrounding landscape (6–8), but many insect pests may benefit from a higher proportion of cultivated land surrounding target crops (9, 10). Highly mobile and polyphagous insect pests can utilize a variety of alternative host plants across large areas and can be influenced by the availability of resources in surrounding cultivated and uncultivated land (11, 12). Understanding how the composition of landscape surrounding agroecosystems affects pest populations can be critical in assessing risk and implementing management strategies for particular pests.

Popillia japonica (Newman) (Coleoptera: Scarabaeidae), a widely established invasive herbivore across the Eastern and Central U.S (13). known to feed on over 300 plant species, has become the key pest of several small fruits, ornamentals, and field crops (14, 15). The larvae of *P. japonica* cause damage to grasses by feeding on roots and the adults through defoliation of leaves and sometimes direct feeding on fruits (16). The adult beetles have a strong flight capacity, being able to sustain flight for over 5 km (17), which allows them to move between habitats and feed on numerous host plant species including wild and cultivated species (16). Additionally, in the U.S. *P. japonica* does not appear to experience pressure from natural enemies that would significantly influence their populations (13). A previous study suggested that the density of *P. japonica* populations is largely determined by the total availability of preferred host plants in the area, and that emerging adults fly to and aggregate at sites with an abundance of preferred host plants (18). Landscapes with higher proportions of cropland in the surrounding area have previously been shown to result in higher number of adults captured in traps in Illinois, likely due to the availability of corn and soybean which are both suitable hosts to *P. japonica* adults (19). In addition to preferred adult host plants, *P. japonica* can also be influenced by the availability of optimal oviposition sites or areas where there is high soil moisture (20, 21) and where there is sunlight and short grass cover (22). These conditions are often found in pastures and turfgrasses, and the presence of these can also increase the prevalence of *P. japonica* (23, 24).

As *P. japonica* are generalists, suitable host plants and oviposition sites may be available within and outside of susceptible agricultural crops. For example, highbush blueberry fields offer suitable oviposition sites between rows of plants where larvae can overwinter, as well as preferred host plants for adults to feed on (14). Larvae and adults were found to be more abundant towards the edges of fields in blueberry (25) and soybean (26), and the number of larvae found in the spring in blueberry fields was correlated to the number of adults found later in the summer which suggests the overwintering larvae significantly contribute to the number of adults found feeding on blueberry plants in the summer (25). Grapes, which have an economic value estimated nationally at over $6 billion per year (27), are a preferred host of adults (28), and turfgrass, which is commonly used as ground cover within vineyards, is a highly suitable oviposition site for these beetles (16). It is unclear what proportion of *P. japonica* adults found feeding on grapevines move in from the surrounding landscape or overwinter as larvae within a vineyard. How different landscape types contribute to populations of *P. japonica* within agroecosystems is important to understanding their dispersal, and for growers to assess risk based on the composition of the landscape surrounding their farm.

Abiotic factors, such as precipitation and temperature, can affect *P. japonica* population abundance and feeding behavior. Higher precipitation can influence *P. japonica* abundance as beetles prefer to oviposit in wetter soils (20, 21), and variability in summer rainfall may explain year to year variability in *P. japonica* abundance as the larvae survive better in wetter conditions (29). Temperature may also influence *P. japonica* behavior as adults tend to have higher consumption rates at higher temperatures (30); however, no research has addressed the impact temperature and precipitation may have on adult abundance in vineyards.

The main objectives of this study were to assess how the surrounding landscape along with other abiotic factors influence *P. japonica* populations and to determine the distribution of adults from field edges to field interiors in vineyards. To address these objectives, we conducted a two-year, season-long study assessing *P. japonica* adult population abundance and leaf injury at selected vineyards across Southern Wisconsin with variable landscape composition surrounding vineyards. We expected to see a higher abundance of *P. japonica* and associated leaf injury as the amount of cropland increased in the landscape surrounding vineyards and higher population densities as well as increased leaf injury near field margins compared to field interiors in vineyards. A better understanding of how the surrounding landscape and other abiotic factors contribute to the abundance of *P. japonica* and how beetles are distributed within vineyards can provide valuable information for assessing risk and can allow growers to make more targeted applications of pesticides (31) to manage this detrimental pest.

Materials and methods

To assess the effect of the proportion of cropland in the surrounding landscape on *P. japonica* abundance in vineyards, we conducted a two-year study from June to September in 2017 and 2018, when *P. japonica* are known to occur in Southern Wisconsin (32). We monitored *P. japonica* adults as well as leaf injury caused by adults at 20 vineyards across Southern Wisconsin, USA (Supplemental Table 1). The average vineyard size was approximately 12000 m^2, with a range of 4000-32000 m^2, and the average transect length was 87 m, with a range of 50-150 m. Vineyards were all planted with a mixed variety of cold climate grapes.

Site selection

The surrounding landscape of vineyards were evaluated and described using satellite-derived Cropland Data Layer (NASS USDA 2017) using ArcMap 10.3 (ESRI, Redlands, CA). The composition of the surrounding landscape was calculated around a 1.5 km radius from the center of each vineyard based on typical dispersal of *P. japonica* shown in capture-mark-recapture experiments (17). Data from landcover classes was reclassified to focus on broader landscape categories of interest (33, 34): 1) Cropland, which included all cultivated crops excluding hay and non-alfalfa pastures; 2) Woodland, which included forests, deciduous forests, mixed forests and woody wetlands; 3) Non-pasture Grassland, which included non-alfalfa hay, shrublands, herbaceous wetlands, and fallow croplands, and; 4) Pasture, which included only the class specified as “Grassland/Pasture” in the Cropland Data Layer (NASS USDA 2017) (Supplemental Table 2).

In Southern Wisconsin, grassland and pastures were relatively uncommon land cover types surrounding potential vineyards we would sample at, while cropland and woodland were more prevalent landcover types. We selected 20 vineyards with less than 30% pasture and non-pasture grassland in the surrounding landscape and where at least 75% of the surrounding landscape was comprised of non-pasture grassland, pasture, cropland, and woodland (Figure 1). Selected vineyards spanned a gradient from low to high (4.8-78%) cropland in the surrounding landscape (Supplemental Table 1).

Experimental design

To assess the distribution from field edges to field interiors of *P. japonica* within vineyards, we set up a diagonal linear transect at each vineyard, which consisted of three sampling locations: one at the edge of the vineyard, one at the halfway point from the edge to the center, and one at the center of the vineyard (Figure 2). For each diagonal transect, the sampling location at the edge was established at a corner of the largest and most rectangular available field within the vineyard. Corners were selected which would allow for the maximum possible length of the diagonal transect. When multiple corners fit these criteria, we selected a corner that had at least two different types of bordering landscapes to reduce the impact of a single bordering landscape on the sampling design. Each vineyard was sampled for adult *P. japonica* and estimates of leaf damage were taken once a week between the hours of 09:00 and 17:00. Sampling began in mid-June, before the start of adult *P. japonica* emergence (32) and continued until no more adults were observed at the vineyards for two consecutive weeks, resulting in 17 weeks of sampling in 2017 and 16 weeks of sampling in 2018.

Adult sampling and leaf injury assessment

To determine the abundance of *P. japonica* adults in vineyards, we carefully hand-collected all adults present on sections of the 6 grapevines associated with each of the sampling locations, the first vine (out of 6 plants sampled at each sampling point) intersects with the established transect and the next 5 grape plants were located sequentially along the row of plants away from the edge of the vineyard where the transect begins (Figure 2). Collected adults were placed into a container with soapy water and were then placed in a freezer at -20°C upon return to the laboratory where they were later counted.

Each week, leaf injury was estimated at one of the six vines at each sampling location and 40 leaves on the closest shoot to the center of the vine were counted starting at the distal end of the shoot. For consistency across all of our counts and to avoid possible variability that may occur in different parts of the plant we always started with leaves on the most central shoot. If a shoot did not have 40 leaves, the next adjacent shoot was counted until 40 leaves were assessed. Each week, vines sampled were alternated as we would sample the first vine (out of the 6 per sampling point) on the first week of sampling, and then the second vine on the second week of sampling, until the seventh week where we would start again at the first vine and cycle through the vines again, additionally the shoots sampled were alternated (left or right of the center of the vine every other week). Leaf injury assessment was conducted following methods by Boucher and Pfeiffer (35) by counting the number of leaves visually estimated to have at least 10% area loss due to *P. japonica* feeding damage characterized by the skeletonization of leaves. Leaf counts and associated leaf injury estimates were removed from our dataset for the 3rd week of sampling in 2017 as the methodology used for leaf counts during this week was not consistent with our other weekly assessments.

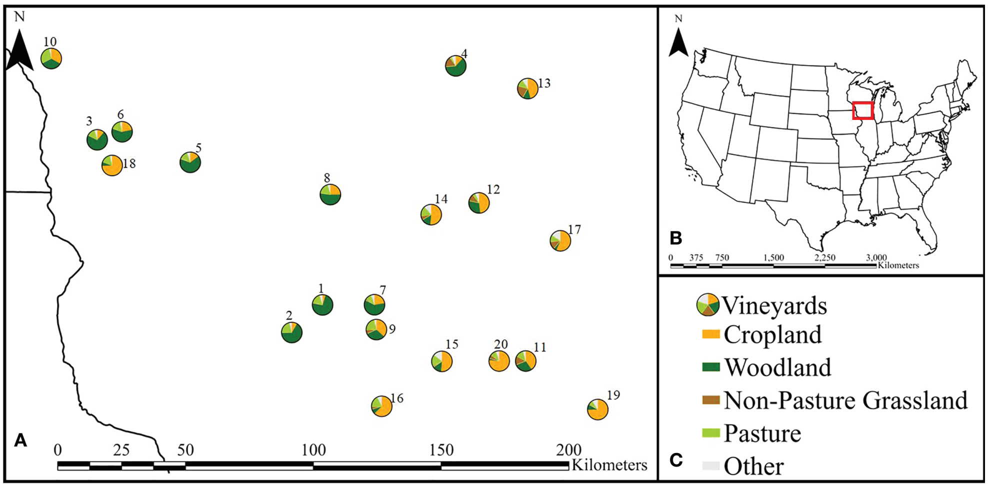


FIGURE 1
(A) Map showing the location of the 20 vineyards with pie charts of the composition of the respective 1.5 km surrounding landscapes for each vineyard across Southern Wisconsin where sampling occurred. Vineyards are labeled 1-20 and correspond to those listed in Supplemental Table 1. **(B)** Locator map to give spatial reference to map shown in panel **(A)**. **(C)** Legend indicating different landscape types (detailed in Supplemental Table 1).

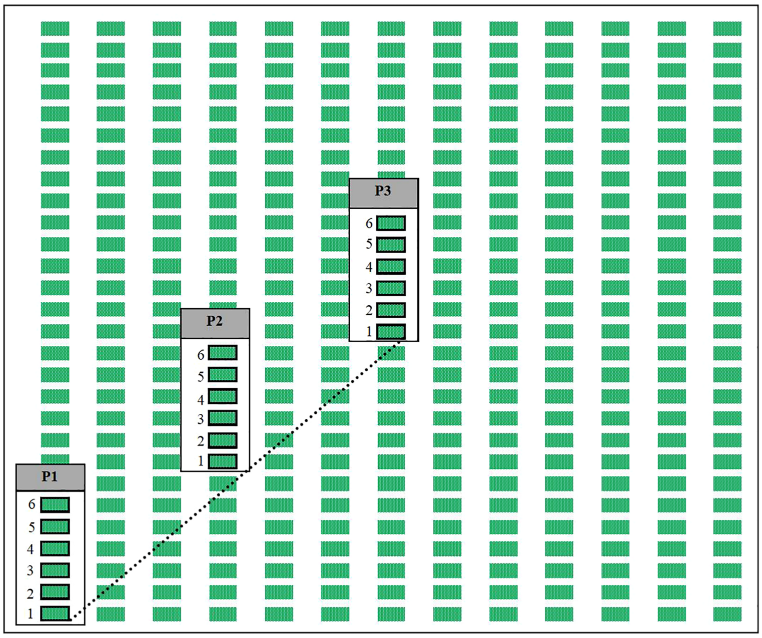


FIGURE 2
Simplified diagram of a vineyard field to show our experimental design. At each vineyard a diagonal transect (represented by the dashed line) was established from the corner of a field to its center, and we would sample from grape plants within the rows at three points (labeled as P1, P2, and P3) representing the edge of the field, a point halfway from the edge to center, and the center of the field respectively. Each week at every sampling point adult *P. japonica* would be collected from 6 vines (shown as rectangles outlined in black, labeled 1-6), and one vine per sampling point would be used for leaf injury assessment starting with the first vine (labeled as 1) on the first week, to the second vine (labeled as 2) on the second week, until the seventh week where we would start again at the first vine (labeled as 1) and cycle through again.

Weather conditions

To assess the impact that temperature and precipitation may have on the abundance of *P. japonica* adults throughout the season, estimates of weather conditions at each vineyard throughout the course of adult emergence were obtained from Oregon State University Parameter-elevation Regressions on Independent Slopes (PRISM) Model (http://prism.oregonstate.edu). The spatial resolution for this weather data was 4km. The average daily temperature and precipitation for vineyards from June to September in 2017 and 2018 were downloaded to evaluate conditions when adults were present.

Pesticide usage

To account for variable levels of insecticide applications between vineyards used to manage *P. japonica*, an Environmental Impact Quotient (EIQ) was calculated using an online field use EIQ calculator from Cornell University (nysipm.cornell.edu/eiq/calculator-field-use-eiq/) for each vineyard and each year of the study. The EIQ uses the rate and frequency of pesticide applications combined with toxicological and environmental data available on those pesticides to generate a standardized score that can be used to compare management programs (36). For our purposes, the average EIQ per year for each vineyard was calculated based only on insecticide applications and did not account for fungicide or herbicide applications. We produced yearly EIQ estimates per vineyard as each vineyard sprayed insecticides on their own schedule not allowing for weekly EIQ estimates across vineyards. No sampling occurred immediately following a pesticide application. The date, active ingredient, and application rate of all insecticides was self-reported by each vineyard manager for each year and the average EIQ values across the two years of the study for the different vineyards ranged from 0-140.

Vineyard location

We included longitude values for each vineyard in our data analysis, to account for the potential variations in levels of *P. japonica* establishment across Eastern to Western Wisconsin. As our vineyards were spread throughout Southern Wisconsin, they span different broader ecological landscapes with variable climate, soils, hydrology, and landcover (37), which could influence the establishment of *P. japonica* populations (17, 38).

Data analysis

Multiple regressions were used to build two sets of models to explain the effect of cropland, pasture, precipitation and temperature throughout the season (average of both years from June to September), the average EIQ of the vineyard (averaged across both years), and the longitude of the vineyard on the abundance of *P. japonica* adults (the average number of adult *P. Japonica* collected per vineyard per week across the two years) and estimated proportion of leaf injury (average estimated proportion of leaf injury recorded per vineyard per week across the two years). Interaction effects between variables were also included in these two sets of models.

Each set of models were evaluated based on Akaike Information Criterion (AIC) with a correction for small sample size (AICc), where models with lower AICc scores were considered to better fit the data (39). Models were also evaluated for multicollinearity, and models where any variable included had a variance inflation factor greater than 10 were excluded (40). Additionally, due to our relatively small sample size (n=20) we chose to include no more than three variables in a given model. A logarithmic transformation was also applied to the abundance of adults in order for the data to better fit assumptions of equal variance and normality (41). In each set of models we included all possible combinations of the independent variables and interaction effects that were not excluded by our previous mentioned criteria (Supplemental Tables 3 and 4). We additionally used this same model selection process, but modified the variables of cropland and pasture to reflect their proportion in the landscape surrounding vineyards at different buffer widths (0.5km, 1km, 1.5km, 2.5km, 5km, 10km). However, across different spatial scales there was no change in what individual factors were statistically significant or which models were evaluated to be better based on AICc, and therefore we chose to only present data for the 1.5 km spatial scale

Multiple regressions were also used to build a set of models to explain the effect of vineyard, week of sampling, year, weekly precipitation, and weekly temperature (for temperature and precipitation variables 7 days were averaged, consisting of the 6 days prior to when sampling occurred along with the day of sampling itself), on the weekly average abundance of *P. japonica* adults (Supplemental Table 5). A quadratic term for week sampled was also added to better fit the models to the seasonal variation in our data. We included vineyard, week of sampling, and year in all models built also testing possible combinations of precipitation, temperature, and interactions between precipitation and temperature. These models were evaluated based on Akaike Information Criterion (AIC) and models with lower AIC scores were considered to better fit the data. A logarithmic transformation was applied to the abundance of adults (per vineyard per week) in order for the data to better fit assumptions of equal variance and normality (41).

A Pearson's correlation test was used to assess correlations between the abundance of *P. japonica* adults and estimated proportion of leaf damage. The same test was used to assess the correlation between the proportion of cropland and the proportion of woodland in the surrounding landscape and the correlation between the longitude of the vineyard and the amount of cropland in the surrounding landscape.

One-way ANOVA tests were used to compare the abundance of adults and estimated proportion leaf injury between the three sampling points to determine if there were significant differences between the relative abundance of beetles and estimated leaf injury across different sampling points. Following a significant p-value for the ANOVA, a Tukey HSD test was used to perform pairwise comparisons between sampling points. All statistical analysis was performed in R (R Development Core Team 2021).

Results

The proportion of cropland and woodland surrounding vineyards were strongly negatively correlated (*r*(18)=-0.98, *p* <.001),indicating that from our selected sites, vineyards surrounded by less cropland generally had more woodland in the surrounding landscape. The proportion of cropland in the surrounding landscape and the longitude of the vineyard were somewhat positively correlated (*r*(18)=0.47, *p*<0.04), as selected vineyards located further east in Wisconsin had on average higher amounts of cropland in the surrounding landscape than vineyards further west.

The best-fitting model (lowest AICc) for the average adult *P. japonica* abundance included pasture, temperature, and longitude (R^2 = 0.75, *p*<0.001) (Table 1). This model shows adult abundance being higher in vineyards with more pastures in the surrounding landscape, with higher temperatures, and located further east in Southern Wisconsin.

The best-fitting model for the average estimated proportion of leaf injury included temperature, longitude, and EIQ (R^2 = 0.44, *p*=0.007) (Table 2), with vineyards located further east, with less intensive pesticide use (lower EIQ), and higher temperatures generally having higher levels of leaf injury than vineyards further west, with higher levels of pesticide use, and lower temperatures.

The best fitting model for weekly *P. japonica* abundance included year, week, vineyard, weekly precipitation, and weekly temperature (R^2 = 0.51, *p*<0.001) (Table 3) (Supplemental Table 6), with the models indicating that higher temperatures and less precipitation in the week leading up to sampling resulted in a greater weekly abundance of *P. japonica*.

The abundance of adults and the estimated proportion of leaf injury were positively correlated (*r*(18)=0.87, *p* <.001) indicating that there was more leaf injury when adult beetles were more abundant. There were significant differences in the proportion of adults collected at the different points along the transects ($F_{2,57}$ = 12.44, *p*<0.00001), with a higher proportion of adult *P. japonica* collected at the edges of the vineyards (Mean ± SEM: 0.46 ± 0.06) compared to the center (0.18 ± 0.03; t_{17} = 4.17, *p*<0.001) and halfway from the edge to the center (0.25 ± 0.03; t_{17} = 3.13, *p*=0.002) (Figure 3). Leaf injury varied between sampling locations ($F_{2,57}$ = 10.49, *p*<0.0001), with a significantly higher relative amount of estimated leaf injury at the edges of the vineyards (0.40 ± 0.02) compared to both the center (0.29 ± 0.14) (t_{17} = 1.23, *p*=0.0001) and halfway from the edge to the center of the vineyards (0.32 ± 0.01) (t_{17} = 2.06, *p*=0.008) (Figure 3).

TABLE 1 Parameter estimates ± SE of selected model (R^2 = 0.75, *p*<0.001) explaining variation in the average abundance of average *P. japonica* adults per vineyard per week (transformed by log base 10).

Coefficients	Parameter Estimate ± SE	t value	Pr>(|t|)
Intercept	71.84 ± 18.78	3.83	<0.01
Pasture	0.06 ± 0.02	2.38	0.03
Temp	1.09 ± 0.36	3.01	<0.01
Long	1.03 ± 0.17	5.94	<0.002

Variables included in the selected model were: 1) averaged daily temperature (°C) from June-September of 2017 and 2018 at the vineyard (temp) and 2) longitude value of the center of each vineyard (long).

TABLE 2 Parameter estimates ± SE of selected model (R^2 = 0.44, *p*=0.007) explaining variation in average leaf injury observed at vineyards.

Coefficients	Parameter Estimate ± SE	t value	Pr>(|t|)
Intercept	4.14 ± 2.89	1.43	0.17
Temp	0.15 ± 0.06	2.36	0.03
EIQ	-0.002 ± 0.0001	-2.87	0.01
Long	0.08 ± 0.03	2.67	0.02

Variables included in the selected model were: 1) averaged daily temperature (°C) from June-September of 2017 and 2018 at the vineyard (temp); 2) Environmental Impact Quotient score for the vineyard averaged across 2017 and 2018 (EIQ); and 3) longitude value of the center of each vineyard (long).

Discussion

Our results showed that the location of the vineyards, the proportion of pastures in the surrounding landscape, and temperature best explained the variability in *P. japonica* adult populations, while location, temperature, and pesticide usage best explained variability in leaf injury. Vineyards located further east with higher temperatures and more pasture in the surrounding landscape had more *P. japonica* adults present and vineyards further east with higher temperatures and lower EIQ pesticide usage had greater levels of leaf injury. We also found that week, year, and vineyard beetles were sampled from along with the prior week's variability in temperature and precipitation best explained weekly variability in *P. japonica* abundance across vineyards, with a greater abundance of *P. japonica* typically sampled when the temperature of the previous week was higher, and the precipitation was lower. Our results also showed an edge-biased distribution of *P. japonica* adults and leaf injury along our established transects, with more adults and leaf injury near the vineyard edge compared to the interior of the vineyards.

We hypothesized that more *P. japonica* adults would be more abundant as the amount of cropland increased in the landscape. Contrary to our expectation, the amount of cropland in the surrounding landscape, as defined in this study, did not have a significant effect on *P. japonica* abundance or leaf injury. The composition of the surrounding landscape can influence pest populations (2, 7, 8), and we did find landscape, specifically the proportion of pastures in the surrounding landscape, helped explain more variability in *P. japonica* abundance across vineyards. Our findings of greater *P. japonica* abundance at vineyards with more pasture in the surrounding landscape are consistent with previous research and may be driven by the availability of highly suitable oviposition sites for *P. japonica*

TABLE 3 Analysis of variance table for the selected model explaining variability in weekly abundance of *P. japonica* across 20 vineyards.

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Week^2	1	0.31	0.31	0.22	0.64
Week	1	351.80	351.80	243.80	<0.0001
Year	1	3.04	3.04	2.10	0.15
Vin	19	559.95	29.47	20.42	<0.0001
Precip	1	5.93	5.93	4.11	0.04
Temp	1	11.16	11.16	7.74	<0.01
Residuals	575	829.72	1.44		

The variables included in models include 1) Week (the week we sampled, 1st week for the year; 2)Year (year of sampling, 2017 or 2018); 3) Vin (vineyard sampled V01-V20); 4) Precip (average precipitation at the vineyard sampled for the previous 7 days); and 5) average temperature at the vineyard sampled for the previous 7 days.

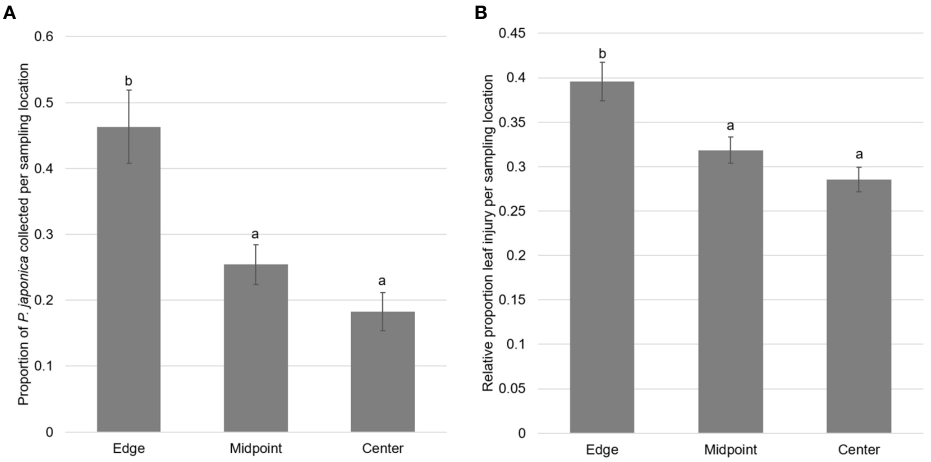


FIGURE 3
(A) Mean proportion ± SEM of adult *P. japonica* collected from three sampling locations along transects from field edges to field interiors at twenty vineyards. Different letters signify significant differences between the means of groups (*p*<0.05). **(B)** Mean relative estimated leaf injury ± SEM from three sampling locations along transects from field edges to field interiors at twenty vineyards. Different letters signify significant differences between the means of groups (*p*<0.05).

(23, 24). We had also expected to find an impact from the amount of cultivated land as previous studies suggest that more cultivated land in the surrounding landscape can increase population densities for a variety of insect pests (2, 9, 42). Here, the proportion of cropland in the surrounding landscape was less important than the geographical location, management practices of the vineyard, and proportion of pastures in the surrounding landscape in explaining the variation of *P. japonica* adults and leaf injury. As an invasive species with minimal pressure from natural enemies (13), *P. japonica* may likely not experience the indirect effects of uncultivated landscapes supporting larger populations of natural enemies that may contribute to pest suppression (e.g. 43–45), but would be expected to be influenced by resource availability from cultivated or uncultivated landscapes (42). *Popillia japonica* are extreme generalists and highly mobile (17, 46), which may allow them to utilize a wide variety of resources across landscapes with variable compositions (47), minimizing the variability in densities of *P. japonica* across different landscapes. The number of vineyards in our study did not enable us to perform a more complex analysis of multiple landscape characteristics, and the level of detail available to us from the Cropland Data Layer (NASS USDA 2017) did not allow us to calculate precise estimates of relative abundance of preferred host plants (19) and suitable oviposition sites (18), which could influence *P. japonica* populations. Future research with a greater number of study sites and a higher resolution of surrounding landscape composition may help elucidate finer scale landscape effects on *P. japonica* abundance.

Over the past century, *P. japonica* have gradually extended their range westward in the United States (38), and the larger abundance of adults observed in Eastern Wisconsin could be a result of an earlier establishment. A study on stink bug damage in Mid-Atlantic tomato fields observed a similar pattern with greater amounts of damage caused by the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), in fields further east, closer to the invasion epicenter for this pest (11). Invasive species often have lag times where population densities in newly invaded areas are initially low and then later dramatically increase (48), therefore the population densities we observed could be influenced by *P. japonica*'s invasion history.

Alternatively, the geographical differences in population density could be attributed to other abiotic or biotic conditions varying across the state. The vineyards sampled in this study span a distance of over 200 km and are located in different broader ecological landscapes across Southern Wisconsin which vary in climate, soils, hydrology, and landcover (37), and vineyards located further east did generally have a slightly higher percent cropland in their surrounding landscapes. The variable conditions throughout the state could influence the establishment of *P. japonica* and their relative abundance across these locations (16, 38), but further work is needed to evaluate the specific landscape features that affect the abundance of *P. japonica*.

Previous research has shown the defoliation caused by *P. japonica* can have a negative effect on the growth and productivity of grape vines, and that the susceptibility to leaf injury and the subsequent impact on the vines is determined by the time of the season when leaf injury occurs (49), the age of the vine (50), and the specific cultivar (28). Here we showed that a combined effect of longitude, EIQ, and temperature best explained the estimated proportion of leaf injury within vineyards, with more leaf injury seen at vineyards that were further east, that had not used pesticides or used pesticides with lower environmental impact quotients, and had higher temperatures compared to vineyards that were further west, that had greater or more environmentally impactful pesticide usage, and experienced lower temperatures. As expected, vineyards with a higher EIQ generally had a lower estimated proportion of leaf injury. The most common active ingredient applied across the vineyards sampled was carbaryl (Sevin), which contributed to the EIQ scores and has been shown to reduce defoliation from *P. japonica* (16, 51). Past research has also shown *P. japonica* adults tend to have higher consumption rates at higher temperatures (30), consistent with our findings of greater leaf injury, and our collection of more beetles on vines at vineyards with higher mean temperatures.

Our results show that the weekly variability in *P. japonica* abundance is influenced by the previous week's weather conditions of temperature and precipitation, with higher temperature and lower precipitation leading to higher adult abundance. Previous research has shown greater herbivory by *P. japonica* at higher temperatures in the laboratory (30) and that temperature and UV-radiation may encourage *P. japonica* adults to feed and aggregate on raspberry plants (52). Conversely, higher precipitation led to fewer *P. japonica* the following week on grapevines, consistent with historical observations of reduced flight activity and feeding of *P. japonica* during rain and dense cloud cover (53). A study that looked at variability between hourly abundance of *P. japonica* collected from traps with lures also found that flight activity and aggregation of *P. japonica* was sensitive to changes in weather conditions, capturing less beetles during hours with dense cloud cover and high winds (54).

As expected, our results showed a positive correlation between the abundance of *P. japonica* and estimated proportion of leaf injury and that more beetles and leaf injury was observed at the edges of vineyards compared to the interior of vineyards. This result is consistent with research in soybean and blueberry, which found a similar edge-biased spatial distribution for *P. japonica* (25, 26). One explanation for these results is that many of the adults found on grape vines moved in from the surrounding landscape, then started feeding on vines which immediately bordered the surrounding landscape. *Popillia japonica* respond to plant kairomones from feeding damage (55) and sex pheromones from female beetles, which can result in large aggregations of beetles feeding in concentrated locations

(56). Alternatively, *P. japonica* adults which emerge from within the vineyards may start to distribute outwards and may stop at the vineyard edges in response to the distinct change in habitat (57), so these aggregations could result from beetles which had overwintered further within the vineyards. Edge effects, or edge-biased distributions, have been widely observed across many insect species, but theories explaining this phenomenon have not been extensively tested (58). Understanding the spatial distribution of pest insects is important as it can influence management practices, such as encouraging more targeted applications of insecticides (31). For *P. japonica* adults in vineyards, our results suggest that it may be more effective to focus management specifically near vineyard edges.

Conclusions

Understanding the composition of the surrounding landscape can be important when assessing risk posed to crops by different pest insects. Our results showed that the abundance of *P. japonica* within vineyards was not significantly influenced by the amount of cropland in the surrounding landscape but rather was better explained by the amount of pasture, geographical location of the vineyard and temperature, and that leaf injury was influenced by geographical location of the vineyard, temperature, and pesticide usage. Additionally, we found weekly adult *P. japonica* abundance was greater when recent temperatures were higher and precipitation was lower. The edge-biased spatial distribution of *P. japonica* along linear transects within vineyards suggests that targeted management strategies to vineyard edges may be useful for reducing populations of adult beetles and their associated leaf injury.

Data availability statement

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

Author contributions

JH and CG designed the research. CG secured funding. JH collected and analyzed the data. JH and CG wrote the manuscript. All authors read and approved this manuscript.

Funding

This study was funded by the UW-Madison Department of Entomology Lilian and Alex Feir Fellowship and a USDA North Central Region Sustainable Agriculture Research and Education program grant project number GNC 17-239.

Acknowledgments

We give special thanks to all the participating growers for access to their vineyards to conduct this study. We also thank Sarah Woody, Michaela Taddeini, and Cybil Biehlmann for helping with sample collection and processing.

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/finsc.2022.961437/full#supplementary-material

SUPPLEMENTAL TABLE 1

This table describes various characteristics of the vineyards in our study, as well as a summary of collected data used in our analysis, the table includes the following columns: 1) A vineyard identification label for each vineyard V01-V20 (VID); 2) Latitude of the vineyard in decimal degrees (Lat); 3) Longitude of the vineyard in decimal degrees (Long); 4) Area of the vineyard in hectares [Area (ha)]; 5) Length of diagonal transect established at the vineyard [Transect (m)]; 6) Proportion of surrounding 1.5km landscape defined as cropland (Cropland); 7) Proportion of surrounding 1.5km landscape defined as woodland (Woodland); 8) Proportion of surrounding 1.5km landscape defined as Non-Pasture Grassland (Non-Pasture Grassland); 9) Proportion of surrounding 1.5km landscape defined as Pasture (Pasture); 10) Average precipitation (mm) from June-September for 2017 [2017 Precip (mm)]; 11) Average temperature(°C) from June-September for 2017 [2017 Temp (°C)]; 12) Average precipitation (mm) from June-September for 2018 [2018 Precip (mm)]; 13) Average temperature(°C) from June-September for 2018 [2018 Temp (°C)]; 14) Average precipitation (mm) from June-September for 2017 and 2018 [2017,2018 Precip (mm)]; 15) Average temperature(°C) from June-September for 2017 and 2018 [2017,2018 temperature(°C)]; 16) Environmental Impact Quotient Estimates for June-September 2017 (2017 EIQ); 17) Environmental Impact Quotient Estimates for June-September 2018 (2018 EIQ); Average Environmental Impact Quotient Estimates for June-September 2017 and 2018 (2017,2018 EIQ); 18) Number of collected *P. japonica* adults during sampling in 2017 (2017 P. *japonica*); 19) Number of collected *P. japonica* adults during sampling in 2018 (2018 P. *japonica*); 20) Average number of collected *P. japonica* adults during sampling in 2017 and 2018 (2017,2018 *P. japonica*); 21) Average estimated leaf injury in 2017 (2017 leaf injury); 22) Average estimated leaf injury in 2018 (2018 leaf injury); 23) Average estimated leaf injury across 2017 and 2018 (2017,2018 leaf injury).

SUPPLEMENTAL TABLE 2
Reclassification table of USDA NASS land cover classes.

SUPPLEMENTAL TABLE 3
The AIC, ΔAIC (difference between AIC of the model and AIC of the selected model), Adjusted R square, and p-values of the 37 simple or multiple regression models produced to examine variables to explain the variation in average adult P. japonica abundance (log transformed) across vineyards. Variables included in the model selection included: 1) proportion of the surrounding landscape covered in cropland (woodland); 2) averaged daily precipitation (cm) from June-September of 2017 and 2018 at the vineyard (precip); 3) averaged daily temperature (° C) from June-September of 2017 and 2018 at the vineyard (temp); 4) Environmental Impact quotient score for the vineyard averaged across 2017 and 2018 (EIQ); and 5) longitude value of the center of each vineyard (long). No more than three variables were included in single model. The selected model with the lowest AICc shown in bold.

SUPPLEMENTAL TABLE 4
The AICc, ΔAICc (difference between AICc of the model and AICc of the selected model), Adjusted R square, and p-values of the 37 simple and multiple linear regression models with interaction effects produced to examine variables to explain the variation in average estimated proportion of leaf injury across vineyards. Variables included in the model selection included: 1) proportion of the surrounding landscape covered in cropland (woodland); 2) averaged daily precipitation (cm) from June-September of 2017 and 2018 at the vineyard (precip); 3) averaged daily temperature (°C) from June-September of 2017 and 2018 at the vineyard (temp); 4) Environmental Impact quotient score for the vineyard averaged across 2017 and 2018 (EIQ); and 5) longitude value of the center of each vineyard (long). No more than three variables were included in single model. The selected model with the lowest AICc shown in bold.

SUPPLEMENTAL TABLE 5
The following table represents models we built exploring weekly variability in adult P.japonica (log transformed to meet assumptions of normality) across 20 vineyards. The variables included in models were 1) Week (the week we sampled, 1st week for the year; 2) Year (year of sampling, 2017 or 2018); 3) Vin (vineyard sampled V01-V20); 4) Precip (average precipitation at the vineyard sampled for the previous 7 days); and 5) average temperature at the vineyard sampled for the previous 7 days. The AIC, ΔAIC (difference between AIC of the model and AIC of the selected model), Adjusted R square, and p-values are shown for all models.

SUPPLEMENTAL TABLE 6
Parameter estimates ± SE of selected model (R2=0.51, p<0.001) explaining variation in the average weekly abundance of average P. japonica adults per vineyard (transformed by log base 10). Variables included in the selected model were: 1) Week (quadratic term added to fit the data); 2)Year (2017 or 2018); 3) Vineyard (V02-V20) (vineyards were considered categorical in this model, so coefficients for vineyards V02-V20 are shown relative to V01); 4) average weekly temperature (°C) representing an average of 7 days of daily temperature consisting of the 6 days prior to when sampling occurred along with the day of sampling itself (Temp) and ; 5) average weekly precipitation (mm) representing an average of 7 days of daily temperature consisting of the 6 days prior to when sampling occurred along with the day of sampling itself (Precip).

SUPPLEMENTAL TABLE 7
This table includes all data collected from vineyards in our study for and includes the following columns: 1) Day of the year (Day); 2) Month of the year (Month); 3) Year of the study, 2017 or 2018 (Year); 4) Time of day when sampling occurred (Time); Week of the study in the given year (Week); Vineyard Identification label for the 20 vineyards, V01-V20 (VID); Point Identification for sampling points along the transect P1 = Edge of vineyard, P2 = halfway from edge to center, P2 = center of vineyard (PID); Indicated which of the 6 vines associated with each sampling point was used for leaf injury estimates, vines sequentially numbered along the row of plants starting at the beginning of the sampling point moving further into the vineyard (Vine Number); 9) Indicates where on a grape plant sampling took place from the perspective of the collector standing in front of the plant within the vineyard row, RC = Right cordons, or shoots on the right side of the plant, LC = Left Cordons, or shoots on the left side of the plant, EV = Entire Vine, or collecting from both left and right side of the plant in order to count up to 40 leaves (Vine Cordon); 10) Number of leaves counted, counts less than 40 indicate a limited number of leaves on the plant of adequate size to assess (Leaves Counted); 11) Out of all of the leaves counted, the number that were visually estimated to have at least 10% leaf area loss (Leaved Injured);12) The proportion of damaged leaves out of all leaves counted (Proportion Leaf Injury); 13) The number of *P. japonica* adults collected (*P. japonica*); 14) The number of *P. japonica* female adults collected (*P. japonica* Females); 15) The number of *P. japonica* male adults collected (*P. japonica* Males); 16) Average daily precipitation (mm) [Precip (mm)]; 17) Average daily temperature [Temp (°C)]; 18) Average weekly precipitation for the day of sampling and previous 6 days [Weekly Precip (mm)]; 19) Average weekly temperature for the day of sampling and previous 6 days [Weekly Temp (°C)].

References

1. Gurr GM, Wratten SD, Landis DA, You M. Habitat management to suppress pest populations: Progress and prospects. *Annu Rev Entomol* (2017) 62:91–109. doi: 10.1146/annurev-ento-031616-035050

2. Rusch A, Chaplin-Kramer R, Gardiner MM, Hawro V, Holland J, Landis D, et al. Agricultural landscape simplification reduces natural pest control: A quantitative synthesis. *Agriculture Ecosyst Environ* (2016) 221:198–204. doi: 10.1016/j.agee.2016.01.039

3. Bhar R, Fahrig L. Local vs. landscape effects of woody field borders as barriers to crop pest movement. *Conserv Ecol* (1998) 2:1–19.

4. Letourneau DK, Jedlicka JA, Bothwell SG, Moreno CR. Effects of natural enemy biodiversity on the suppression of arthropod herbivores in terrestrial ecosystems. *Annu Rev Ecology Evol Systematics* (2009) 40:573. doi: 10.1146/annurev.ecolsys.110308.120320

5. Petit S, Muneret L, Carbonne B, Hannachi M, Ricci B, Rusch A, et al. Landscape-scale expansion of agroecology to enhance natural pest control: A systematic review. *Adv Ecol Res* (2020) 63:1–48. doi: 10.1016/bs.aecr.2020.09.001

6. Tscharntke T, Steffan-Dewenter I, Kruess A, Thies C. Contribution of small habitat fragments to conservation of insect communities of grassland-cropland landscapes. *Ecol Appl* (2002) 12:354. doi: 10.2307/3060947

7. Rösch V, Tscharntke T, Scherber C, Batáry P. Landscape composition, connectivity and fragment size drive effects of grassland fragmentation on insect communities. *J Appl Ecol* (2013) 50:387–94. doi: 10.1111/1365-2664.12056

8. Zou Y, Bianchi FJJA, Jauker F, Xiao H, Chen J, Cresswell J, et al. Landscape effects on pollinator communities and pollination services in small-holder agroecosystems. *Agric Ecosyst Environ* (2017) 246:109–16. doi: 10.1016/j.agee.2017.05.035

9. Bianchi FJJA, Booij CJH, Tscharntke T. Sustainable pest regulation in agricultural landscapes: A review on landscape composition, biodiversity and natural pest control. *Proc Biol Sci* (2006) 273:1715–27. doi: 10.1098/rspb.2006.3530

10. Chaplin-Kramer R, O'Rourke ME, Blitzer EJ, Kremen C. A meta-analysis of crop pest and natural enemy response to landscape complexity. *Ecol Lett* (2011) 14:922–32. doi: 10.1111/j.1461-0248.2011.01642.x

11. Rice KB, Troyer RR, Watrous KM, Tooker JF, Fleischer SJ. Landscape factors influencing stink bug injury in mid-Atlantic tomato fields. *J Econ Entomol* (2017) 110:94–100. doi: 10.1093/jee/tow252

12. Sivakoff FS, Rosenheim JA, Dutilleul P, Carrière Y. Influence of the surrounding landscape on crop colonization by a polyphagous insect pest. *Entomologia Experimentalis Applicata* (2013) 149:11–21. doi: 10.1111/eea.12101

13. Althoff ER, Rice KB. Japanese Beetle (Coleoptera: Scarabaeidae) invasion of north America: History, ecology, and management. *J Integrated Pest Manage* (2022) 13(1):2. doi: 10.1093/jipm/pmab043

14. Isaacs R, Szendrei Z, Wise JC. Evaluation of new approaches for management of Japanese beetles in highbush blueberries. *Small Fruits Rev* (2004) 3:349–60. doi: 10.1300/J301v03n03_12

15. Pfeiffer DG. "Japanese beetle and other Coleoptera feeding on grapevines in eastern North America" In *Arthropod Management in Vineyards*. Dordrecht, ZH: Springer (2012) p. 403–29. doi: .1007/978-94-007-4032-7_17

16. Potter DA, Held DW. Biology and management of the Japanese beetle. *Annu Rev Entomol* (2002) 47:175–205. doi: 10.1146/annurev.ento.47.091201.145153

17. Fleming WE. *Biology of the Japanese beetle*. U.S: Department of Agriculture (1972). p. 129. Available at: https://play.google.com/store/books/details?id=cKYoAAAAYAAJ.

18. Régnière J, Rabb RL, Stinner RE. Popillia japonica (Coleoptera: Scarabaeidae): distribution and movement of adults in heterogenous environments. *Can Entomologist* (1983) 115:287–94. doi: 10.4039/ent115287-3

19. Hamilton RM, Foster RE, Gibb TJ, Sadof CS, Holland JD, Engel BA. Distribution and dynamics of Japanese beetles along the Indianapolis airport perimeter and the influence of land use on trap catch. *Environ Entomol* (2007) 36:287–96. doi: 10.1603/0046-225x(2007)36[287:dadojb]2.0.co;2

20. Allsopp PG, Klein MG, McCoy EL. Effect of soil moisture and soil texture on oviposition by Japanese beetle and rose chafer (Coleoptera: Scarabaeidae). *J Econ Entomol* (1992) 85:2194–200. doi: 10.1093/jee/85.6.2194

21. Régnière J, Rabb RL, Stinner RE. Popillia japonica: Effect of soil moisture and texture on survival and development of eggs and first instar grubs. *Environ Entomol* (1981) 10:654–60. doi: 10.1093/ee/10.5.654

22. Potter DA, Powell AJ, Spicer PG, Williams DW. Cultural practices affect root-feeding white grubs (Coleoptera: Scarabaeidae) in turfgrass. *J Economic Entomology* (1996) 89(1):156–64. doi: 10.1093/jee/89.1.156

23. Smitley DR. Incidence of popillia japonica (Coleoptera: Scarabaeidae) and other scarab larvae in nursery fields. *J Economic Entomology* (1996) 89:1262–6. doi: 10.1093/jee/89.5.1262

24. Ando Y. Seasonal prevalence and outbreaks of the Japanese beetle, Popillia japonica Newman (Coleoptera : Scarabaeidae). *Jap J Appl Entomol Zool* (1986) 30:111–16. doi: 10.1303/jjaez.30.111

25. Szendrei Z, Mallampalli N, Isaacs R. Effect of tillage on abundance of Japanese beetle, popillia japonica Newman (Col., scarabaeidae), larvae and adults in highbush blueberry fields. *J Appl Entomology* (2005) 129:258–64. doi: 10.1111/j.1439-0418.2005.00961.x

26. Sara SA, McCallen EB, Switzer PV. The spatial distribution of the Japanese Beetle,Popillia japonica, in soybean fields. *J Insect Sci* (2013) 13:1–9. doi: 10.1673/031.013.3601

27. USDA National Agricultural Statistics Service (NASS). U.S. Census of Agriculture: United States Summary and State Data, Vol. 1: *Geographic Series, Part 51* Report AC-17-A-51. US: Department of Agriculture (2019). Available at: https://www.nass.usda.gov/Publications/AgCensus/2017/Full_Report/Volume_1,_Chapter_1_US/usv1.pdf.

28. Gu S, Pomper KW. Grape cultivar feeding preference of adult Japanese beetles. *HortScience* (2008) 43:196–9. doi: 10.21273/hortsci.43.1.196

29. Hawley IM. The effect of summer rainfall on Japanese beetle populations. *J N Y Entomol Soc* (1949) 57:167–76.

30. Lemoine NP, Drews WA, Burkepile DE, Parker JD. Increased temperature alters feeding behavior of a generalist herbivore. *Oikos* (2013) 122:1669–78. doi: 10.1111/j.1600-0706.2013.00457.x

31. Brenner RJ, Focks DA, Arbogast RT, Weaver DK, Shuman D. Practical use of spatial analysis in precision targeting for integrated pest management. *Am Entomol* (1998) 44:79–102. doi: 10.1093/ae/44.2.79

32. Maier RM. *Alternative management strategies for Japanese beetles popillia japonica (Coleoptera: Scarabaeidae) in vineyards.* Williamson CR, editor. Ann Arbor, United States: The University of Wisconsin - Madison (2016). p. 83. Available at: https://ezproxy.library.wisc.edu/login?url=https://www.proquest.com/dissertations-theses/alternative-management-strategies-japanese/docview/1932285290/se-2.

33. Werling BP, Meehan TD, Gratton C, Landis DA. Influence of habitat and landscape perenniality on insect natural enemies in three candidate biofuel crops. *Biol Control* (2011) 59:304–12. doi: 10.1016/j.biocontrol.2011.06.014

34. Pelton E, Gratton C, Isaacs R, Van Timmeren S, Blanton A, Guédot C. Earlier activity of drosophila suzukii in high woodland landscapes but relative abundance is unaffected. *J Pest Sci* (2016) 89:725–33. doi: 10.1007/s10340-016-0733-z

35. Boucher JT, Pfeiffer DG. Influence of Japanese beetle (Coleoptera: Scarabaeidae) foliar feeding on "Seyval blanc" grapevines in Virginia. *J Econ Entomol* (1989) 82:220–5. doi: 10.1093/jee/82.1.220

36. Kovach J, Petzoldt C, Degni J, Tette J. *A method to measure the environmental impact of pesticides* (1992). Available at: https://hdl.handle.net/1813/55750.

37. Wisconsin Department of Natural Resources ed. "Southeast Glacial Plains Ecological Landscape" In *The ecological landscapes of Wisconsin: An assessment of ecological resources and a guide to planning sustainable management*. Madison, WI: Wisconsin Department of Natural Resources (2015). Available at: https://dnr.wi.gov/topic/Landscapes/documents/1805Ch18.pdf.

38. Allsopp PG. Japanese Beetle, popillia japonica Newman (Coleoptera: Scarabaeidae): Rate of movement and potential distribution of an immigrant species. *Coleopt Bull* (1996) 50:81–95.

39. Bedrick EJ, Tsai C-L. Model selection for multivariate regression in small samples. *Biometrics* (1994) 50:226–31. doi: 10.2307/2533213

40. Farrar DE, Glauber RR. Multicollinearity in regression analysis: The problem revisited. *Rev Econ Stat* (1967) 49:92–107. doi: 10.2307/1937887

41. Martin Bland J, Altman DG. Statistics notes: Transforming data. *BMJ* (1996) 312:770. doi: 10.1136/bmj.312.7033.770

42. Rand TA, Waters DK, Blodgett SL, Knodel JJ, Harris MO. Increased area of a highly suitable host crop increases herbivore pressure in intensified agricultural landscapes. *Agric Ecosyst Environ* (2014) 186:135–43. doi: 10.1016/j.agee.2014.01.022

43. Perdikis D, Fantinou A, Lykouressis D. Enhancing pest control in annual crops by conservation of predatory heteroptera. *Biol Control* (2011) 59:13–21. doi: 10.1016/j.biocontrol.2011.03.014

44. Martin EA, Reineking B, Seo B, Steffan-Dewenter I. Natural enemy interactions constrain pest control in complex agricultural landscapes. *Proc Natl Acad Sci USA* (2013) 110:5534–9. doi: 10.1073/pnas.1215725110

45. Blaauw BR, Isaacs R. Wildflower plantings enhance the abundance of natural enemies and their services in adjacent blueberry fields. *Biol Control* (2015) 91:94–103. doi: 10.1016/j.biocontrol.2015.08.003

46. Ladd TLJr. Japanese Beetle (Coleoptera: Scarabaeidae): Feeding by adults on minor host and nonhost plants. *J Econ Entomol* (1989) 82:1616–9. doi: 10.1093/jee/82.6.1616

47. Kennedy GG, Storer NP. Life systems of polyphagous arthropod pests in temporally unstable cropping systems. *Annu Rev Entomol* (2000) 45:467–93. doi: 10.1146/annurev.ento.45.1.467

48. Crooks JA. Lag times and exotic species: The ecology and management of biological invasions in slow-motion1. *Écoscience* (2005) 12:316–29. doi: 10.2980/i1195-6860-12-3-316.1

49. Mercader RJ, Isaacs R. Phenology-dependent effects of foliar injury and herbivory on the growth and photosynthetic capacity of nonbearing vitis labrusca (Linnaeus) var. *Niagara. Am J Enol Vitic* (2003) 54:252-60.

50. Mercader RJ, Isaacs R. Phenophase-dependent growth responses to foliar injury in vitis labruscana bailey var. *Niagara during Vineyard Establishment Am J Enol Vitic* (2004) 55:1–6.

51. Hammons DL, Kaan Kurtural S, Potter DA. Impact of insecticide-manipulated defoliation by Japanese beetle (Popillia japonica) on grapevines from vineyard establishment through production. *Pest Manag Sci* (2010) 66:565–71. doi: 10.1002/ps.1908

52. Cramer ME, Demchak K, Marini R, Leskey T. UV-Blocking high-tunnel plastics reduce Japanese beetle (Popillia japonica) in red raspberry. *HortScience* (2019) 54(5):903–9. doi: 10.21273/HORTSCI13820-18

53. Hawley IM, Metzger FW. *Feeding habits of the adult Japanese beetle*. Washington, DC: US Department of Agriculture (1940). Available at: https://play.google.com/store/books/details?id=MTioESjcr8UC.

54. Lacey LA, Amaral JJ, Coupland J, Klein MG. The influence of climatic factors on the flight activity of the Japanese-beetle (Coleoptera: Scarabaeidae): Implications for use of a microbial control agent. *Biol Control* (1994) 4(3):298–303. doi: 10.1006/bcon.1994.1038

55. Loughrin JH, Potter DA, Hamilton-Kemp TR. Volatile compounds induced by herbivory act as aggregation kairomones for the Japanese beetle (Popillia japonica Newman). *J Chem Ecol* (1995) 21:1457–67. doi: 10.1007/BF02035145

56. Ladd TLJr. Sex attraction in the Japanese beetle. *J Econ Entomol* (1970) 63:905–8. doi: 10.1093/jee/63.3.905

57. Bohlen PJ, Barrett GW. Dispersal of the Japanese beetle (Coleoptera: Scarabaeidae) in strip-cropped soybean agroecosystems. *Environ Entomol* (1990) 19:955–60. doi: 10.1093/ee/19.4.955

58. Nguyen HDD, Nansen C. Edge-biased distributions of insects. *A review Agron Sustain Dev* (2018) 38:11. doi: 10.1007/s13593-018-0488-4

frontiers | Frontiers in Insect Science

TYPE Mini Review
PUBLISHED 12 December 2022
DOI 10.3389/finsc.2022.1079756

OPEN ACCESS

EDITED BY
William D. Hutchison,
University of Minnesota Twin Cities,
United States

REVIEWED BY
Giulia Torrini,
Council for Agricultural and
Economics Research (CREA), Italy
Robert C. Venette,
Forest Service, United States
Department of Agriculture (USDA),
United States

*CORRESPONDENCE
Sylvain Poggi
sylvain.poggi@inrae.fr

SPECIALTY SECTION
This article was submitted to
Invasive Insect Species,
a section of the journal
Frontiers in Insect Science

RECEIVED 25 October 2022
ACCEPTED 25 November 2022
PUBLISHED 12 December 2022

CITATION
Poggi S, Desneux N, Jactel H, Tayeh C
and Verheggen F (2022) A nationwide
pest risk analysis in the context of the
ongoing Japanese beetle invasion in
Continental Europe: The case of
metropolitan France.
Front. Insect Sci. 2:1079756.
doi: 10.3389/finsc.2022.1079756

A nationwide pest risk analysis in the context of the ongoing Japanese beetle invasion in Continental Europe: The case of metropolitan France

Sylvain Poggi[1]*, Nicolas Desneux[2], Hervé Jactel[3], Christine Tayeh[4] and François Verheggen[5]

[1]IGEPP, Institut Agro, Univ Rennes, INRAE, Le Rheu, France, [2]University of Côte d'Azur, INRAE, CNRS, UMR ISA, Nice, France, [3]INRAE, University of Bordeaux, UMR Biogeco, Cestas, France, [4]Expertise and Biological Risks Unit (ERB), Plant Health Laboratory, French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Angers, France, [5]TERRA, Gembloux Agro-Bio Tech, University of Liege, Gembloux, Belgium

The Japanese beetle, *Popillia japonica*, is native to Japan and became established in North America in the early twentieth century. The beetle was detected in Europe, first in Italy in 2014 and then in Switzerland in 2017. Metropolitan France is at the forefront of the Japanese beetle threat, due to its geographical proximity to the European populations established in the Piedmont, Lombardy and Ticino regions. An express pest risk analysis for metropolitan France was therefore conducted. The most likely pathways for entry include (i) natural dispersion, (ii) trades of plant products with adherent soil and (iii) hitchhiking behaviour, leading to a high probability of entry. The spread rate of *P. japonica* was also evaluated as high, resulting from natural spread as well as human activities. Given the absence of significant limiting factors, the potential impacts of *P. japonica* in France will likely be as important as in its current geographic distribution. Although several sources of uncertainty were highlighted throughout the evaluation, none of them has significant impact on the conclusions of the present express pest risk analysis. Measures to prevent entry, establishment and spread of *P. japonica* are recommended and include surveillance with pheromone traps and control *via* integrated pest management strategies. However, most efforts should be concentrated on eradication measures while *P. japonica* is still in the early stages of invasion.

KEYWORDS

***Popillia japonica*, biological invasion, risk assessment, surveillance, monitoring, pest control, metropolitan France**

Introduction

The Japanese beetle, *Popillia japonica* Newman (Coleoptera, Scarabaeidae), is native to Japan (1). In the early twentieth century, it became established in North America, especially in the USA where it was initially introduced in the states bordering the Atlantic coast. Then, it quickly spread westwards (2) and became one of the worst invasive pests, inflicting severe damage to many cultivated and ornamental plants, trees, fruits, turfs and grasses. Its damage has been estimated to be $460 million per year in the USA (3).

Based on the severity of the economic, social and environmental impacts that *P. japonica* can cause in the European Union territory (4, 5), the European Commission classified *P. japonica* as a priority quarantine pest listed in Annex IIB of Regulation 2019/2072, subject to compulsory control and to a national sanitary emergency response plan, in accordance with European Regulation (EU) 2016/2031. Specific requirements are defined for imports of certain plants for planting because of their likelihood of harbouring *P. japonica*, depending on their origin ((EU) 2021/2285). Despite these efforts, the beetle was detected in Europe, first in Italy in 2014 (6, 7) and then in Switzerland in 2017 (8), two countries where it is now considered established. A few adults were also trapped in south-western Germany in 2021 and 2022 (9, 10), yet these sightings were considered as incursions without establishment.

The challenge for Europe is to counteract this invasion at a very early stage to significantly enhance the chances of successful eradication or containment. Because metropolitan France (i.e. mainland France and Corsica as well as nearby islands in the Atlantic Ocean, the English Channel and the Mediterranean Sea) is at the forefront of the *P. japonica* threat, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) was asked to carry out an express pest risk analysis (PRA thereafter; 11), based on the European and Mediterranean Plant Protection Organization (EPPO) express PRA scheme (12), in order to better and quickly prepare the State services for the implementation of measures in the case of a suspected and to implement control measures in the case of a confirmed outbreak. The presumed time horizon of the assessment is five years as suggested by EPPO (13). Following the recommended guidelines for performing an express PRA (12), we aimed at assessing the risks of entry, establishment, and spread of *P. japonica* in France as well as its potential impacts. Based on the overall risk, recommendations for surveillance and management measures in the event of an outbreak were formulated.

Assessment of the phytosanitary risk

Brief overview of the pest

Popillia japonica is generally a univoltine species (as observed in Japan, Italy and Switzerland) but its development can spread over two years under colder climates (1, 14, 15). The beetle spends most of its life underground in immature forms (eggs, three larval stages and pupae) and a few months above ground as an adult. In Italy, adults are active from June to September with an activity peak in mid-July (16). *P. japonica* is a dietary generalist: larvae can feed on the rootlets of all host plants while adults preferentially feed on leaves but also on fruits and flowers. The symptoms caused by *P. japonica* adults are easily observed and consist of skeletonization, adults chewing the leaf tissue between the veins. An updated overview of the host plants of *P. japonica* (17) emphasises how polyphagous the beetle is (Figure 1): the adults feed on at least 401 host plants belonging to 92 botanical families including fruit trees (e.g. apple, plum), forest species (e.g. maple, poplar), field crops (e.g. corn, soybean) or vegetables (e.g. asparagus, beans), ornamental plants (e.g. roses), herbaceous species (species of the genus *Festuca*, *Lolium* and *Poa* used in lawns and turfs), wild species (e.g. clovers, brambles) and vines. Among this great diversity of host plants, Tayeh et al. (17) identify 131 species as "main" hosts, insofar as they favour the survival and reproduction of *P. japonica*. We focused on these main hosts in the following pest risk analysis.

Pathways for entry

Entry of a pest is the movement of a pest into an area where it is not yet present, or present but not widely distributed and being officially controlled (19). For each pathway, a sequence of events was evaluated: (1) probability of the pest being associated with the individual pathway at origin, (2) probability of survival during transport or storage, (3) probability of surviving existing pest management procedures, and (4) probability of transfer to a suitable host or habitat. Six pathways were identified to assess the likelihood of entry of *P. japonica* into France: (1) import of plants for planting (except seeds, bulbs and tubers) with adhering soil, (2) natural spread, (3) hitchhiking behaviour, (4) import of soil (including potting soil and compost), (5) import of cut flowers and foliage, and (6) import of fruits. The in-depth assessment of each of these pathways of entry was based on data available in the scientific literature, the situation in the invaded countries and the flow of goods towards France (see Supplementary Material). We concluded that the likelihood of *P. japonica* entering metropolitan France is high with low uncertainty. The entry will likely occur through natural spread, since the beetle has high flight ability at the adult stage, or by hitchhiking behaviour, given the recent adult sightings in Basel (Switzerland, in 2021) and Baden-Württemberg (Germany, in 2021 and 2022) close to railway track or freight depot (https://gd.eppo.int/reporting/article-7240), in the Valle d'Aosta region (Italy, in 2021) near a motorway service area, and in Sardinia (Italy, in 2021) near the main airport of the island (20). If no regulations were in place, the likelihood of entry would be

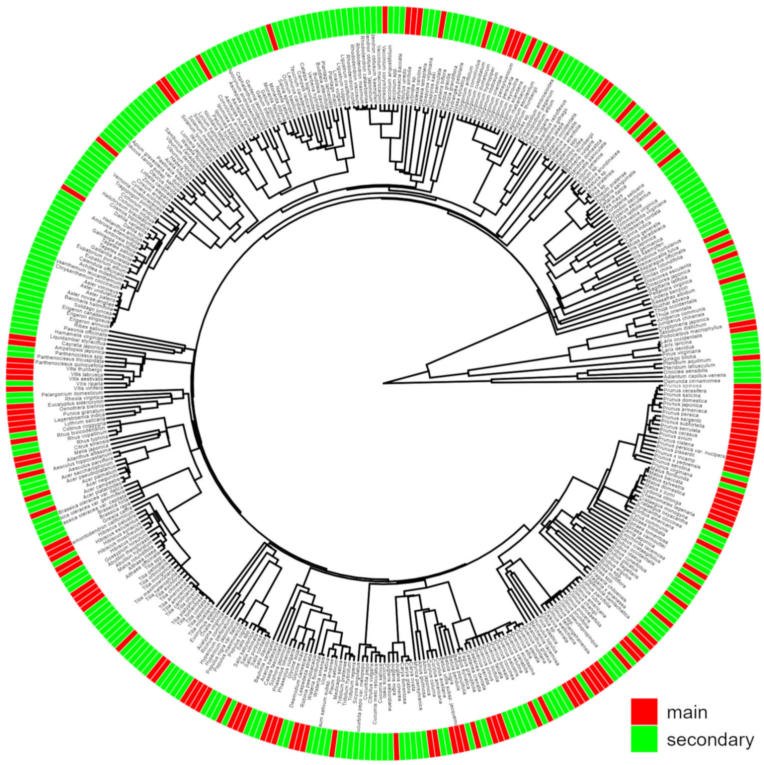


FIGURE 1
Phylogeny of the main and secondary host plants of *P. japonica* generated by the V.Phylomaker package (18). A total of 131 host plants, belonging to 39 families, are classified as "main" (17). This figure is also available as Supplementary Material to allow for magnification.

increased by imports of plants for planting with adherent soil from infested countries, taking into account the probability that the aerial and subterranean stages are associated at the origin and transported, the diversity and volume of the transported goods (such as roses and fruit tree plants especially from Italy), the abilities of the beetles to survive without food (21, 22) during transport, as well as their capacity of transfer to host plants cultivated in France.

Establishment

Establishment is the perpetuation, for the foreseeable future, of a pest within an area after entry (19). It depends mainly on the presence of host plants and a suitable climate in the PRA area. Four factors that can affect the establishment of *P. japonica* in a new territory have been identified: (1) mild temperature, (2) sufficient humidity for survival and development, (3) presence of host plants and (4) lack of natural enemies. The likelihood of outdoor establishment is considered high, based on the findings from species distribution models (23–26), the high diversity and abundance of host plants in metropolitan France (Figure 2), and the limited impact of natural enemies. The uncertainty is considered low. Indeed, the entire French territory, except mountainous areas, is suitable for the establishment of *P. japonica*, because summer rainfall is sufficient, temperature is favourable and many host plants are available. In addition, irrigation practices increase the likelihood of establishment in the less rainy areas of the Mediterranean region. In contrast, the likelihood of establishment in protected conditions (e.g. greenhouses) is considered low with a moderate level of uncertainty. This is due to several points: (1) the facilities concerned are generally small and subject to various pest management methods, (2) there have been no recent reports of *P. japonica* in greenhouses, (3) *P. japonica* populations seem unlikely to be overlooked during regular inspections by growers in indoor conditions.

Spread

Spread is defined as the expansion of the geographical distribution of a pest within an area (19) and relies on natural spread or human-assisted spread. All previous cases of establishment of *P. japonica* have been followed by spread activity. Natural spread of *P. japonica* is mainly achieved by

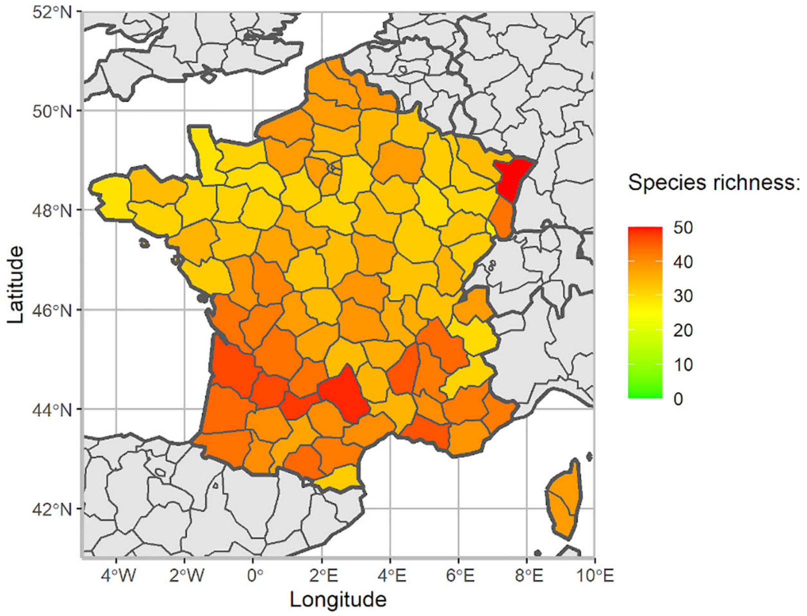


FIGURE 2
Species richness in main host plants of *P. japonica* in each department (NUTS3 region) of metropolitan France.

the flight of adults, with both male and female imagoes having functional wings. However, it appears that pioneer individuals are more likely to be females (27). Data on flight capabilities are essentially based on analyses of the speed of the colonisation front by *P. japonica*, especially in the USA and more recently in Italy. The progression of the invasion front is the result of natural spread, probably coupled with hitchhiking, and suggests spreading capabilities of about 10 km per year in the USA (1, 28, 29). Examining the dynamics of the invasion in Piedmont and Lombardy in Italy (30, 31) provides an equivalent estimate. Human activities, resulting in movements of goods and people, may also favour the long-distance spread of *P. japonica*. In international trade, larvae may be transported in soil attached to the roots of plants for planting, while adult beetles have been intercepted on agricultural products, on packaging and in ships and aircraft (16). Overall, the spread is expected higher than 10 km per year, which is considered a high magnitude according to the EPPO guidance (32). The uncertainty is low, as no barriers to spread within the metropolitan French territory have been identified.

Impact

Within its current invasion range (data collected from the USA and Italy), the magnitude of impact of *P. japonica* is considered high, with low uncertainty. This results from: (1) high direct damage in terms of loss of yield (fruit crops) and quality (ornamental crops), (2) high indirect costs related to control (especially chemical and biological), (3) the generalist diet of the insect that can affect many production sectors, with significant damage locally. The potential environmental (e.g. increase in phytosanitary treatments, competition with other species) and social impacts (e.g. human health risks, loss of availability of popular ornamental plants) were also considered. In the USA, efforts to control the larval and adult stages were estimated to be around $460 million in 2015 (3). No extensive damage has been recorded in Europe yet, but recent studies (5, 33) suggest substantial potential damage costs. In particular, Straubinger et al. (33) outlined that major grape and wine producing countries like France and Italy would have a potential economic damage of about €92 million and €68 million per year, respectively.

Within the French area at risk of establishment, the magnitude of impact is also considered high with a low uncertainty. This conclusion is mainly supported by (1) the importance of main host plants in terms of area, yield and export volumes, (2) the absence of currently deployed cultural practices that would significantly reduce the impact of *P. japonica* and (3) chemical control is mainly based on a single family of products (pyrethroids). The main point of uncertainty concerns the level of susceptibility of French varieties of the main host plants to *P. japonica*.

Overall, the risk posed by *P. japonica* for the threatened French metropolitan area is considered high with a low uncertainty. This risk is therefore considered as unacceptable and justifies the recommendation of management measures.

Recommendations for pest risk management

To prevent the entry of *P. japonica* on the French territory, an efficient surveillance strategy is needed to ensure the early detection and to allow the rapid implementation of eradication measures. Using semiochemical-based traps is recommended along the border with infested countries to detect natural spread, as well as near key entry points and transport networks (e.g. national interest markets, airports, air cargo entry points, railways, ports, road hubs, motorways service areas, and unloading areas) to take the risk of hitchhiking behaviour into account. Pheromone traps should be positioned in preferred habitats such as grasslands, vineyards and fruit crops fields located in the close vicinity of entry points. In addition, visual inspections of the aerial parts of the main host plants of *P. japonica* are recommended in these areas along the borders with infested countries. Awareness raising of target stakeholders, including for instance nurserymen and garden owners, is also recommended. The use of pheromone-baited traps is the most reliable monitoring method as it is selective and effective. Compared to visual inspection of aerial parts, trapping ensures permanent coverage of the area to be monitored with a higher level of sensitivity.

In case of first capture, we recommend the deployment of a systematic trapping network with one trap every 1 km on a 10 km square, centred on the trap with the first authenticated capture. This 100-km^2 area would constitute the reinforced surveillance zone within which an infested zone surrounded by a buffer zone would be delimited. We suggest that the delimitation of the infested zone is continuously adapted according to the locations and amount of new captures, following the concept of weighted barycentre (34). The limits of the buffer zone remain to be determined according to new knowledge on the dispersal capacities of the beetle in the colonised area. We currently suggest a 5-km wide buffer based on the dispersal capacity of *P. japonica* reported in recent studies (35, 36). Since the infested area is a dynamic surface, changing with each discovery of an infestation point, the boundaries of the demarcated area (infested area + buffer zone) would be updated accordingly.

Within the infested area, a combination of measures should be implemented rapidly as part of an eradication strategy: (1) chemical control with the use of authorized active substances against adults and larvae, (2) biological control when available [e.g. entomopathogens such as nematodes (37) or *Paenibacillus popilliae* (3)], (3) cultural practices involving reduced irrigation during the critical oviposition period (38) and tillage in the fall (39, 40). Importantly, many chemical molecules used in the USA to control *P. japonica* are banned in the European Union (notably neonicotinoids), restricting the available arsenal essentially to the family of pyrethroids.

The movement of rooted plants, soil and growing media as well as plant wastes originating from the infested area should be prohibited. The same should apply to plants originating from the buffer zone, which should not be moved out. These actions must be carried out within a short period to increase the chances of eradication. Otherwise, the containment strategy is both time consuming and has, in our opinion, little chance of success, since it would at most slow down the spread of *P. japonica*. Furthermore, the containment strategy involves suppression of *P. japonica* populations within the infested area by chemical control, biological control and mass trapping, whose effectiveness is reduced in case of heavy infestations.

Discussion and perspectives

Pest risk analyses commissioned at a national level bring out limitations to the precise evaluation of the threat associated with invasive pests and identify opportunities to control their impact. In our study, we addressed specifically the invasive Japanese beetle and its risk to metropolitan France. We assessed the phytosanitary risks in terms of pathways for entry, establishment, spread and economic impacts, and actionable recommendations to mitigate this risk. In this section, we highlight a few points that we believe deserve further attention.

Given the high capacity of *P. japonica* to hitchhike, it is crucial to identify and quantify the movements of goods and people from infested areas to susceptible regions. Eurostat, the statistical office of the European Union, provides extensive information on flows within well-defined pathways, notably those listed in "pathways for entry". However, specific custom codes do not allow discriminating goods of particular interest regarding the biology and ecology of *P. japonica*, making it difficult to capture only relevant information. Furthermore, whether on a national or European scale, if they exist, the accessibility and visibility of data describing the connectivity of means of transport deserve to be improved. Information on freight by road, rail, air, the road traffic during the period of beetle activity, the list of cars and trucks stop locations, unloading areas, etc., would be valuable to better assess the entry routes of the pest.

The biology and ecology of *P. japonica* have been extensively studied (see for example 1, 41–44). However, further knowledge would be useful, such as the attraction radius of pheromone traps for *P. japonica*. Simple methods exist to estimate this radius (45), useful information to optimise the density of trapping networks. A better understanding of the role of fruit odours on adult feeding attractiveness would also be helpful. Knowing the correlations between the level of susceptibility of host plants and the degree of defoliation should help in assessing the impact on crop yield. It would also be interesting to develop innovative and environmentally sound control strategies that are in line with the European Union commitment to reduce the use of pesticides, such as the use of biological control agents, and the use of attract-and-kill, attract-and-infest, or push-pull strategies.

So far, in continental Europe, *P. japonica* is still confined to a single and relatively small area of about 14000 square kilometres (20) overlapping northern Italy and southern Switzerland. This early stage of the invasion opens up opportunities for successful control, provided that there is greater harmonisation of the surveillance and control strategy on the European level. For example, the insect is rapidly spreading but information on its presence is not fully centralised, making it difficult to develop and share distribution maps. As pointed out by Thompson et al. (46), surveillance strategy and biosecurity measures should be informed by epidemiological processes rather than limited by administrative boundaries. The interception of an adult Japanese beetle in Basel (in 2021), at the intersection of three countries (France, Germany, Switzerland) illustrates these difficulties. In line with recent publications (47–49), we believe that biological invasions could be better regulated by promoting international scientific and technical collaboration to harmonise management practices and regulations.

Author contributions

This review was conducted by the expert working group "Popillia japonica" of the French Agency for Food, Environmental, and Occupational Health and Safety (ANSES). All authors have contributed equally to the risk assessment presented in this document. SP wrote the first draft and all other authors have provided significant inputs into the text. All authors contributed to the article and approved the submitted version.

Acknowledgments

We thank the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) that funded the Pest Risk Analysis conducted by the expert working group "Popillia japonica".

Conflict of interest

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/finsc.2022.1079756/full#supplementary-material

References

1. Fleming, WE. *Biology of the Japanese beetle, technical bulletin*. Washington, D.C.: U.S. Department of Agriculture (1972).
2. USDA. Japanese Beetle distribution in the U.S. APHIS plant protection & quarantine. (2018). Available at: https://www.aphis.usda.gov/plant_health/plant_pest_info/jb/downloads/jb-distribution-map.pdf (Accessed December 1, 2022).
3. USDA. Managing the Japanese beetle: a homeowner's handbook. *APHIS* (2015) 81:25–003.
4. Baker R, Gilioli G, Behring C, Candiani D, Gogin A, Kaluski T, et al. Report on the methodology applied by EFSA to provide a quantitative assessment of pest-related criteria required to rank candidate priority pests as defined by regulation (EU) 2016/2031. *EFSA J.* (2019) 17.EFS2. doi: 10.2903/j.efsa.2019.5731
5. Sanchez B, Barreiro-Hurle J, Soto Embodas I, Rogriguez-Cerezo E. *The impact indicator for priority pests (I2P2): a tool for ranking pests according to regulation (EU) 2016/2031. (EUR 29793 EN)*. Luxembourg: Publications Office of the European Union (2019).
6. EPPO RS. *EPPO reporting service no. 10. Num. article 2014/179 First report of Popillia japonica in Italy*. (2014).
7. Pavesi M. Popillia japonica specie aliena invasiva segnalata in Lombardia. *L'Informatore Agrario* (2014) 32:53–5.
8. EPPO RS. *EPPO reporting service no. 09. Num. article 2017/160 First report of Popillia japonica in Switzerland*. (2017).
9. EPPO RS. *EPPO reporting service no. 01. Num. article: 2022/010 First finding of Popillia japonica in Germany*. (2022).
10. EPPO RS. *EPPO reporting service no. 08. Num. article: 2022/165 new finding of Popillia japonica in Germany*. (2022).
11. ANSES. *Demande d'èvaluation du risque simplifiée (ERS) lié à popillia japonica, le sacarabée japonais, pour la France mètropolitaine. (Saisine 2021-SA-0090)*. Maisons-Alfort: Anses (2022). p. 202.
12. EPPO. Decision-support scheme for an express pest risk analysis. *EPPO Bull* (2012) 42:457–62. doi: 10.1111/epp.2591
13. EPPO. Decision-support scheme for quarantine pests. (2011). Available at: https://www.eppo.int/media/uploaded_images/RESOURCES/eppo_standards/pm5/pm5-03-05-en.pdf (Accessed December 1, 2022).
14. Clausen CP, King JL, Teranishi C. *The parasites of popillia japonica in Japan and chosen (Korea), and their introduction into the united states /*. Washington, D.C: U.S. Dept. of Agriculture (1927). doi: 10.5962/bhl.title.108287
15. King JL. The present status of the established parasites of popillia japonica Newman. *J Econ. Entomol.* (1931) 24:453–62. doi: 10.1093/jee/24.2.453
16. EPPO. PM 9/21(1) *Popillia japonica:* procedures for official control. *EPPO Bull* (2016) 46 (3):543–55. doi: 10.1111/epp.12345
17. Tayeh C, Poggi S, Desneux N, Jactel H, Verheggen F. Host plants of popillia japonica: a review. *Recherche Data Gouv.* (2022). doi: 10.57745/SXZNQF
18. Jin Y, Qian H. V.PhyloMaker: an r package that can generate very large phylogenies for vascular plants. *Ecography* (2019) 42:1353–9. doi: 10.1111/ecog.04434
19. IPPC Secretariat. *Glossary of phytosanitary terms. international standard for phytosanitary measures no. 5*. Rome: FAO on behalf of the Secretariat of the International Plant Protection Convention (2022).
20. EPPO RS. *EPPO reporting service no. 04. Num. article: 2022/081 Update of the situation of Popillia japonica in Italy*. (2022).

21. Keathley CP, Potter DA. Quantitative resistance traits and suitability of woody plant species for a polyphagous scarab, *Popillia japonica* Newman. *en* (2008) 37:1548–57. doi: 10.1603/0046-225X-37.6.1548

22. Pires EM, Koch RL. Japanese Beetle feeding and survival on apple fruits. *Biosci J* (2020) 36:1327–34. doi: 10.14393/BJ-v36n4a2020-50364

23. Borner L, Martinetti D, Poggi S. A new chapter of the Japanese beetle invasion saga: predicting suitability from long-infested areas to inform surveillance strategies in Europe. *bioRxiv* (2022). doi: 10.1101/2022.11.14.515960

24. Della Rocca F, Milanesi P. The new dominator of the world: Modeling the global distribution of the Japanese beetle under land use and climate change scenarios. *Land* (2022) 11:567. doi: 10.3390/land11040567

25. Kistner-Thomas EJ. The potential global distribution and voltinism of the Japanese beetle (Coleoptera: Scarabaeidae) under current and future climates. *J Insect Sci* (2019) 19:16. doi: 10.1093/jisesa/iez023

26. Zhu G, Li H, Zhao L. Incorporating anthropogenic variables into ecological niche modeling to predict areas of invasion of popillia japonica. *J Pest Sci* (2017) 90:151–60. doi: 10.1007/s10340-016-0780-5

27. Kowles KA, Switzer PV. Dynamics of aggregation formation in Japanese beetles, popillia japonica. *J Insect Behav* (2012) 25:207–21. doi: 10.1007/s10905-011-9291-7

28. Allsopp PG. Japanese Beetle, popillia japonica Newman (Coleoptera: Scarabaeidae): Rate of movement and potential distribution of an immigrant species. *Coleopt. Bull* (1996) 50:81–95.

29. Fox H. The distribution of the Japanese beetle in 1930 and 1931, with special reference to the area of continuous Infestation1. *J Econ. Entomol.* (1932) 25:396–407. doi: 10.1093/jee/25.2.396

30. Mondino EB, Lessio F, Bianchi A, Ciampitti M, Cavagna B, Alma A. Modelling the spread of popillia japonica Newman (Coleoptera: Scarabaeidae) from a recently infested area. *entomologia* (2022) 42:713–21. doi: 10.1127/entomologia/2022/1370

31. Poggi S, Roche J, Tayeh C, Borner L, Martinetti D. Biological invasion of the Japanese beetle in continental Europe at a glance. *Recherche Data Gouv.* (2022). doi: 10.57745/R18NGL

32. EPPO. Corrigendum-EPPO standard PM 5/5 (1) decision-support scheme foran express pest risk analysis. *EPPO Bull* (2020) 50:525. doi: 10.1111/epp.12713

33. Straubinger FB, Benjamin EO, Venus TE, Sauer J. The economic importance of early pest control: new insights from potential *Popillia japonica* infestation in Europe. *agriRxiv* (2022) 2022:20220316785. doi: 10.31220/agriRxiv.2022.00151

34. Nunes P, Branco M, Van Halder I, Jactel H, et al. Modelling Monochamus galloprovincialis dispersal trajectories across a heterogeneous landscape to optimize monitoring by trapping networks. *Landsc Ecol* (2021) 36:931–41. doi: 10.1007/s10980-020-01188-1

35. Caton BP, Fang H, Manoukis NC, Pallipparambil GR. Quantifying insect dispersal distances from trapping detections data to predict delimiting survey radii. *J Appl Entomol* (2021) 146:203–16. doi: 10.1111/jen.12940

36. Lessio F, Pisa CG, Picciau L, Ciampitti M, Cavagna B, Alma A. An immunomarking method to investigate the flight distance of the Japanese beetle. *entomologia* (2022) 42:45–56. doi: 10.1127/entomologia/2021/1117

37. Torrini G, Paoli F, Mazza G, Simoncini S, Benvenuti C, Strangi A, et al. Evaluation of indigenous entomopathogenic nematodes as potential biocontrol agents against popillia japonica (Coleoptera: Scarabaeidae) in northern Italy. *Insects* (2020) 11:804. doi: 10.3390/insects11110804

38. Potter DA, Powell AJ, Spicer PG, Williams DW. Cultural practices affect root-feeding white grubs (Coleoptera: Scarabaeidae) in turfgrass. *J Econ. Entomol.* (1996) 89:156–64. doi: 10.1093/jee/89.1.156

39. Szendrei Z, Mallampalli N, Isaacs R. Effect of tillage on abundance of Japanese beetle, popillia japonica Newman (Col., scarabaeidae), larvae and adults in highbush blueberry fields. *J Appl Entomol.* (2005) 129:258–64. doi: 10.1111/j.1439-0418.2005.00961.x

40. Tonhasca A. Response of soybean herbivores to two agronomic practices increasing agroecosystem diversity. *Agric. Ecosyst Environ* (1994) 48:57–65. doi: 10.1016/0167-8809(94)90075-2

41. Potter DA. *Destructive turfgrass insects: biology, diagnosis, and control*. Chelsea, Mich: Ann Arbor Press (1998).

42. Potter DA, Held DW. Biology and management of the Japanese beetle. *Annu Rev Entomol* (2002) 47:175–205. doi: 10.1146/annurev.ento.47.091201.145153

43. Shanovich HN, Dean AN, Koch RL, Hodgson EW. Biology and management of Japanese beetle (Coleoptera: Scarabaeidae) in corn and soybean. *J Integr. Pest Manage* (2019) 10:9. doi: 10.1093/jipm/pmz009

44. Vittum PJ, Villani MG, Tashiro H. *Turfgrass insects of the united states and Canada. 2nd ed.* Ithaca: Comstock Pub. Associates (1999).

45. Jactel H, Bonifacio L, van Halder I, Vétillard F, Robinet C, David G. A novel, easy method for estimating pheromone trap attraction range: application to the pine sawyer beetle *Monochamus galloprovincialis*: Estimating pheromone trap attraction range. *Agr For Entomol* (2019) 21:8–14. doi: 10.1111/afe.12298

46. Thompson RN, Cobb RC, Gilligan CA, Cunniffe NJ. Management of invading pathogens should be informed by epidemiology rather than administrative boundaries. *Ecol Model* (2016) 324:28–32. doi: 10.1016/j.ecolmodel.2015.12.014

47. Carvajal-Yepes M, Cardwell K, Nelson A, Garrett KA, Giovani B, Saunders DGO, et al. A global surveillance system for crop diseases. *Science* (2019) 364:1237–9. doi: 10.1126/science.aaw1572

48. Giovani B, Blümel S, Lopian R, Teulon D, Bloem S, Galeano Martínez C, et al. Science diplomacy for plant health. *Nat Plants* (2020) 6:902–5. doi: 10.1038/s41477-020-0744-x

49. Hulme PE. Advancing one biosecurity to address the pandemic risks of biological invasions. *BioScience* (2021) 71:708–21. doi: 10.1093/biosci/biab019

frontiers | Frontiers in Insect Science

TYPE Original Research
PUBLISHED 15 December 2022
DOI 10.3389/finsc.2022.1075807

OPEN ACCESS

EDITED BY
Tim Haye,
CAB International, United Kingdom

REVIEWED BY
Frank Chidawanyika,
International Centre of Insect Physiology and Ecology (ICIPE), Kenya
Leonardo Marianelli,
Council for Agricultural and Economics Research (CREA), Italy

*CORRESPONDENCE
Dominique N. Ebbenga
ebbe0031@umn.edu

SPECIALTY SECTION
This article was submitted to Invasive Insect Species, a section of the journal Frontiers in Insect Science

RECEIVED 20 October 2022
ACCEPTED 30 November 2022
PUBLISHED 15 December 2022

CITATION
Ebbenga DN, Hanson AA, Burkness EC and Hutchison WD (2022) A degree-day model for forecasting adult phenology of *Popillia japonica* (Coleoptera: Scarabaeidae) in a temperate climate.
Front. Insect Sci. 2:1075807.
doi: 10.3389/finsc.2022.1075807

A degree-day model for forecasting adult phenology of *Popillia japonica* (Coleoptera: Scarabaeidae) in a temperate climate

Dominique N. Ebbenga[1]*, A. A. Hanson[2], E. C. Burkness[1] and W. D. Hutchison[1]

[1]Department of Entomology, University of Minnesota, St. Paul, MN, United States, [2]University of Minnesota Extension, Extension Integrated Pest Management (IPM) Program, Morris, MN, United States

Japanese beetle, *Popillia japonica* (Newman), was first detected in the United States in New Jersey in 1916. The beetle gradually spread to the Midwest U.S. region, and was first confirmed in Minnesota in the late 1960's. *Popillia japonica* has subsequently become a major invasive insect pest in turfgrass and several agricultural crops. As *P. japonica* continues to spread throughout the U.S., and other countries, it is important to develop efficient ways to monitor adult populations, and where possible, forecast the phenology of adult population dynamics. During 2019-2021, field trials were conducted to develop a degree-day model that can be used to forecast *P. japonica* adult phenology under Minnesota, and Midwest summer climatic conditions in. We used commercially available traps and lures to monitor adult flight phenology, specifically beetle trap-catch, along with weather data at four locations in Minnesota, to relate ambient field temperatures to population phenology. The concordance correlation coefficient (*CCC*), an index of both precision and accuracy, was used to develop a final degree-day model. Model development included evaluation of simple and sine-wave degree-day calculation methods, start dates between 1 Jan. and 1 April, and a range of lower (0-15 °C) and upper (20-37 °C) thresholds. The optimum model was found to be a simple degree-day calculation, using a biofix date of 1 Jan, and lower and upper thresholds of 15 and 21.7 °C, respectively, for predicting 10% beetle trap-catch. The model will aid in future integrated pest management (IPM) and regulatory strategies by providing a tool for prediction of *P. japonica* adult flight phenology.

KEYWORDS

Japanese beetle, *Popillia japonica*, pest phenology, modeling, invasive species

Introduction

Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), is an invasive species first detected in 1916 in New Jersey, following an accidental introduction from Japan, on imported rootstock (1). *Popillia japonica* was first detected in Minnesota in 1968, gradually increased in abundance (2), and has only recently become a dominant pest, since 2010 (3, 4). The beetle is currently a major invasive insect pest of turf, residential ornamentals, and several agricultural crops in the Midwest U.S. region (5–7).

Popillia japonica typically exhibits a univoltine life cycle in the Midwest (8), where immature stages reside in the soil and overwinter as late instar larvae (9, 10). Once *P. japonica* has completed pupation in spring, adults eclose and emerge from the soil to seek mates, and host plants for nutrition; adult longevity ranges from 4-6 weeks (9). Although several trapping and behavior studies have been conducted to understand adult biology (6, 11), most of the research to date has been directed toward understanding the development of the immature stages and the larval damage inflicted upon turfgrass (11, 12). In recent years, however, concerns have been raised regarding the biology and impact of the adult beetles, particularly for several horticultural and agricultural crops in the Midwest region (5, 6). With over 300 different host plants, there are many opportunities, even in northern tier, temperate climates such as Minnesota, for *P. japonica* adults to cause substantial defoliation, mainly consuming leaf tissue within plant canopies (7, 9, 11). Adult feeding can be very concerning to producers as they observe heavy defoliation with little knowledge or predictability as to how the infestation may impact their crops.

Currently, *P. japonica* has spread to at least 36 states in the U.S. (6, 13). Since 2014, concerns of *P. japonica* invading new regions have caused some states, with no known established populations, to implement quarantine protocols for either early detection or to assist in mass trapping activities to help prevent an introduction of *P. japonica* (13). However, in areas where *P. japonica* has established, monitoring the pest has become important for determining the geographic extent of invaded areas, or used to inform producers of the potential threats to high-value crops. Commercially available traps are currently used for monitoring *P. japonica* (14–17) and the use of an in-field, volumetric approach for rapid processing of trap samples was recently validated (17). However, traps used for monitoring only give notification of pest activity following emergence or dispersal to specific field sites. Beyond weekly trapping, another way to utilize trap-catch data is the development of models for the purpose of forecasting emergence, or insect phenology throughout the season (18–20).

Degree-day models are a useful tool for growers, crop consultants and researchers to forecast or predict the phenology of various important insect life-stage events (e.g., 19, 21), such as first emergence or peak (50%) adult emergence. Clearly, access to local or regional ambient temperature data is also critical for accurate degree-day modeling and forecasts. For the Midwest climate, *P. japonica* adults typically becomes active from mid-June to early July and continue to feed on several crops through early September (4). However, as climates in the Midwest continue to moderate with milder winters, and warmer springs (22), this general timeframe of insect activity can differ greatly. Currently for *P. japonica*, there are no established degree-day models for adult activity in the Midwest region.

Most recently, research on *P. japonica* has increasingly focused on adult beetle feeding injury and the need for improved integrated pest management (IPM) strategies for several fruit and agricultural crops (5–7, 23). Given the extent of *P. japonica* feeding injury, it is critical to develop new tools that can be shared with growers, crop consultants, and researchers to monitor *P. japonica* before feeding damage occurs. Therefore, research was conducted during the summers of 2019–2021 to develop a model that can be used to better track and forecast *P. japonica* adult phenology throughout the growing season, in a temperate, Midwest U.S. climate. In this study, we used commercially available traps and lures to monitor adult *P. japonica* activity at four locations in southern Minnesota, to better define adult *P. japonica* population dynamics throughout the year. Specifically, our objective was to develop a degree-day model to improve current IPM strategies and regulatory planning for adult *P. japonica* monitoring.

Materials and methods

Monitoring adult *P. japonica*

During 2019- 2021, *P. japonica* Trécé$^{\text{TM}}$ traps (Trécé$^{\text{TM}}$, Adair, OK) were deployed near raspberry crops at both the Rosemount Research and Outreach Center, near Rosemount, MN (RROC), MN (44° 43' N, 93° 05' W), and Forest Lake, MN (45° 13' N, 92° 53' W). Two additional trapping sites in vineyards were located near Hastings (44° 41' N, 92° 52' W) and the Horticultural Research Center (HRC), University of Minnesota, in Excelsior (44°52' N, 93°38' W), MN. Soils at each location were classified as follows: RROC had approximately 0.1% sand, 62.4% silt, and 37.6% clay; Forest Lake had 58.8% sand, 15% silt, and 26.2% clay; Hastings had 71.2% sand, 5% silt, and 23.8% clay; HRC had 32.5% sand, 33.8% silt, and 33.8% clay. Trécé traps, paired with semiochemical based lures containing a blend of the *P. japonica* sex pheromone and floral compounds (7, 14–16), were used for all traps. The lure used in traps is the commercially available *P. japonica* dual lure system. The dual lure consists of a food bait (phenethyl propionate + eugenol + geraniol (3:7:3)) and the synthetic sex pheromone known as 'Japonilure' ((R,Z)-5-1-decenyl)dihydro-2(3H)-furanone) (16). Since the lure has both a food and pheromone component, it is highly effective in attracting and capturing both male and female *P. japonica*, making for an ideal lure to monitor populations (7, 14, 15).

Samples were collected twice per week in 2019 and 2021; however, due to COVID-19 restrictions in 2020, traps were collected once per week. Trap samples were processed in the field using a volumetric measurement method established by Ebbenga et al. (7). Trap contents were placed in an Accu-pour$^{\mathrm{TM}}$ measuring pitcher (Gemplers, Janesville, WI), with a capacity range of 100 – 2000 ml, rounded to the nearest 100 ml. For beetle samples that were <100 ml, a smaller Accu-pour beaker (Gemplers, Janeville, WI) was used, consisting of a range from 20 – 500 ml. In 2019, three traps were placed at each location on 3 Jun., and in 2020 and 2021, trap number was increased to 4 at each location and traps were deployed on 9 Jun. and 25 May, respectively, well before the first beetles were captured. Traps were secured to green metal stakes approximately 1 m above the soil surface, and were set approximately 10 m apart. Due to constraints with research locations and allowability of trap deployment, greater distances between traps were not achievable.

Temperature data

Each year, minimum and maximum daily temperatures were collected from local weather stations and HOBO temperature loggers (Onset Computer Corporation, Bourne, MA) depending on the location. Locations in Forest Lake and Hastings used weather station data collected from the Minnesota State Climatology office (https://climateapps.dnr.state.mn.us/index.htm) operated and maintained by the Department of Natural Resources, Division of Ecological and Water Resources. For these locations, all weather stations were within 16 km of trap locations. For the HRC, an onsite weather station was used, and data were collected from the NEWA website operated and maintained by Cornell (newa.cornell.edu), which is the preferred weather station used on this research site. Finally, temperatures collected from RROC were collected using an Onset HOBO MX2303 wireless temperature data logger (Onset Computer Corporation, Bourne, MA). The Onset HOBO MX2303 was set to read ambient air temperature every 2 hours 7 days per week. The temperature probe recording ambient air temperatures was secured to a green metal stake adjacent to a raspberry patch and sheltered from direct sunlight. Temperature data collected at RROC was compared to nearby local weather stations to confirm daily minimums and maximums were similar, prior to use in model development.

Model development and validation

Model development and validation was based on the approach developed by Hanson et al. (19), where field-based trap-catch pest data are used to seek optimal model solutions. This was done by using multiple locations to reflect natural variability in pest phenology, and for multiple years to reflect variable weather scenarios. Model development was conducted using multiple site-year data sets, by iterating through all possible combinations of model start dates, lower and upper thresholds, and calculation methods for degree-days (19). Separate, independent data sets were selected for several site-years for model validation. All calculations and analyses were performed according to Hanson et al. (19), using R version 4.1.2 (24).

To partition model development and validation data, the 12 site-years were divided in half and randomly selected for model development (n=6), while the remaining data sets were used for model validation (n=6) (Table 1). For model building, four start dates often used in the Midwest U.S., were included, Jan. 1, Feb. 1, March 1, and April 1, and converted to Julian dates of 1, 32, 50, and 91, respectively (19). Lower and upper threshold parameters, respectively, ranged from 0-15 °C (32-59°F) and 20- 37 °C (68-98.6°F), respectively, increasing by 0.56°C (1°F) increments. Degree-day calculations were performed using both a simple average degree-day method (25, 26) and the half-day sine-wave method (27), for a total of 7,392 degree-day model parameter combinations. Simple degree-days were calculated according to McMaster and Wilhelm (28) using the average of observed daily maximum and minimum temperatures minus the lower developmental threshold (i.e., method 1), which is not to be confused with another common simple-degree method (i.e., method 2) that, before averaging, changes the observed daily maximum or minimum temperature to equal to the lower developmental threshold if either falls below that threshold.

For each combination of start date, upper and lower threshold, and calculation method, logistic regression [eq. 1] was performed using the six-model development site-years

TABLE 1 Randomized location datasets used for *P. japonica* model development and validation, Minnesota, 2019-2021.

Location	Development dataset year	Validation dataset year
Rosemount	2020	2019, 2021
Hastings	2020	2019, 2021
Forest Lake	2019, 2021	2020
Excelsior	2019, 2021	2020

$$prop\,.\,emergence = \frac{e^{\ln(D)s+i}}{1+e^{\ln(D)s+i}} \qquad \text{eq. 1}$$

where D = degree-days, s = slope, and i = intercept. Degree-days were natural-log transformed to use a log-logistic distribution for improved model fit with accumulated annual degree-days as an independent variable and proportion cumulative percent adult emergence as the dependent variable. Observed degree-days for 10% trap-catch were determined from each regression model to generate predicted dates of 10% trap-catch activity in the model development data.

To assess the performance of the models, *via* predicted versus observed dates of 10% trap-catch, the concordance correlation coefficient (*CCC*) was used (19). The *CCC* was selected because it reflects both precision (*r*) and accuracy (*A*) where *CCC*= *rA*. (29–31). Typical *CCC* values range between 0 and 1 with 1 being a perfectly precise and accurate model, and 0 representing no precision or accuracy (19, 32).

During development, the model with the highest *CCC* value was selected and used for model validation. Model validation used the target date of 10% trap-catch to determine the best performing model, given observed trap-catch and utilized data from the remaining 6 site-years (Table 1). While *CCC* was primarily used to rank models for 10% trap-catch, the Akaike information criterion (*AIC*) was also used to compare fit across the entire logistic distribution. When comparing *AIC* values across models, the lowest value indicates for best agreement across the distribution, and these values are on an unbounded scale, so *AIC* is only used for relative comparisons between models rather than absolute measures of fit (33).

Results

Monitoring adult *P. japonica*

The 3-year study provided high *P. japonica* adult populations, with 111,497 beetles caught in semiochemical based traps across all site years. Mean beetle phenology (mean beetle trap catch/week), across the 4 locations and 3 years, is illustrated in Figure 1. Given the 3-year study, total beetles captured in datasets for model development were 42,968, whereas validation datasets included 68,571 beetles. Across all years, and for all locations, peak trap-catch on a calendar time scale varied considerably. Mean trap catch was lowest in 2020.

Model development and validation

Development of the model indicated that the best model for simple average and sine-wave calculation methods performed similarly at 10% emergence (*CCC* = 0.899 and 0.895

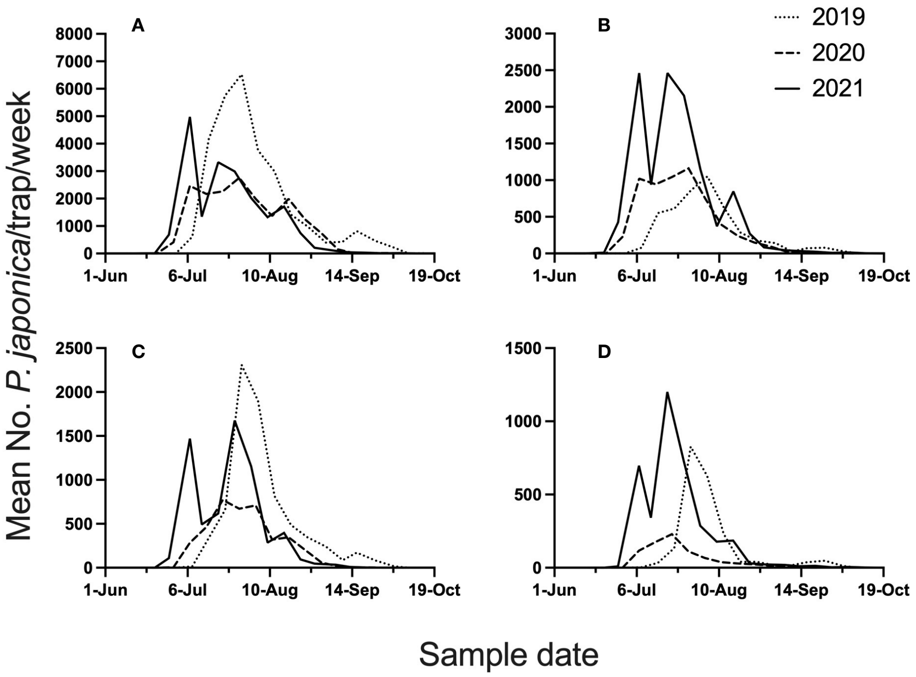


FIGURE 1
Mean number of *P. japonica* adults per trap, per week, for 2019-2021. Traps were deployed at Rosemount **(A)**, Excelsior **(B)**, Hastings **(C)**, and Forest Lake **(D)**, MN.

respectively), and both had near-perfect agreement based on *CCC* alone (Table 2). However, when considering both the highest *CCC* and lowest *AIC* values to measure fit across the entire distribution (Table 2), the simple degree-day method, along with recommended lower and upper thresholds, was selected as the most robust model (Figure 2A).

The best-performing, or optimum model using simple degree-days, was based on lower and upper thresholds of 15 and 21.7 °C, respectively, with a start date of Jan. 1. All start dates used in model development did not exhibit any differences in *CCC*, so the Jan. 1 start date was selected as the biofix date (Table 2). Compared to this simple method model (*CCC* = 0.899), multiple sine-wave models did have a similar *CCC* value (*CCC* = 0.895) at 10% emergence. However, *AIC* values (i.e., smaller values indicate better agreement) for these sine-wave models indicated relatively poorer fit across the entire distribution of emergence (Table 2). The selected simple method model had an *AIC* of 46.23, which ranked 1,137 out of 7,392 total models for fit across the entire distribution or fell in the top 89% of the range of *AIC* values of 44.46 to 60.61. The top ranked model based on *AIC* alone (*AIC* = 44.46) had a slightly better AIC than the simple model, but also had a poorer *CCC* of 0.745 at 10% emergence. The top-ranked sine-wave model for *CCC* however, had an *AIC* of 51.26 and ranked much lower for 6,195 out of 7,392 models when sorted by *AIC* alone.

Using these selected parameters for simple degree-days, no significant differences were observed when adding a site-year covariate interaction to intercept [*F* (5, 141) = 1.03, *P* = 0.40] or the degree-day effect [*F* (5, 141) = 0.11, *P* = 0.99], which indicated similar model performance of the degree-day effect alone across site-years. When performing logistic regression using only natural-log transformed degree-days as an independent variable, intercept and slope terms were -43.34 and 7.41, respectively [eq. 1]. Additionally, mean differences between predicted and observed days for 10% trap-catch, for model development and validation was limited to -1.4 d (Table 3).

Datasets used for model validation indicated a lower *CCC* of 0.785 (Figure 2B) but were still in strong agreement with an *r* of 0.837 and an *A* of 0.938. When degree-day accumulation to proportion trap-catch data were plotted on the log-logistic distribution, we observe a good fit for both model development and validation data sets (Figures 3A, B). Finally, fitting the data to the proposed model indicates that at 257 degree-days (Celsius), adult *P. japonica* populations will have reached 10% trap-catch for the season. Furthermore, this model can be utilized to create spatio-temporal maps adult trap catch phenology, *via* the University of Minnesota VegEdge website (https://vegedge.umn.edu/degree-day-models-select-insect-pests-midwest-region). The software collects 2.5-km^2 resolution daily temperature data to generate 7-day forecasts, provided through the National Phenology Network (https://www.usanpn.org/data/agdd_maps), and is used to produce pest development maps (Figure 4). For the 2022 example shown in Figure 4A, the

TABLE 2 Modeling results to determine the optimum simple *vs.* sine-wave degree-day models, for 4 Minnesota locations, 2019-2021.

Lower threshold (°C)	Upper threshold (°C)	Method	Start date	*AIC*	*CCC*
15.0	21.7	Simple	1	46.226	0.899
15.0	21.7	Simple	32	46.226	0.899
15.0	21.7	Simple	50	46.226	0.899
15.0	21.7	Simple	91	46.226	0.899
15.0	22.2	Simple	1	47.068	0.899
15.0	22.2	Simple	32	47.068	0.899
15.0	22.2	Simple	50	47.068	0.899
15.0	22.2	Simple	91	47.068	0.899
15.0	22.8	Simple	1	47.979	0.899
15.0	22.8	Simple	32	47.979	0.899
15.0	22.8	Simple	50	47.979	0.899
15.0	22.8	Simple	91	47.979	0.899
14.4	23.3	Simple	1	48.599	0.899
14.4	23.3	Simple	32	48.599	0.899
14.4	23.3	Simple	50	48.599	0.899
14.4	23.3	Simple	91	48.599	0.899
11.7	34.4	Sine-wave	50	51.261	0.895
11.7	34.4	Sine-wave	1	51.262	0.895
11.7	34.4	Sine-wave	32	51.262	0.895
11.7	35.0	Sine-wave	50	51.308	0.895

Best performing model was determined by first selecting for the highest CCC, and then lowest AIC values. This table shows the top 20 models out of the 7,392 iterations created.

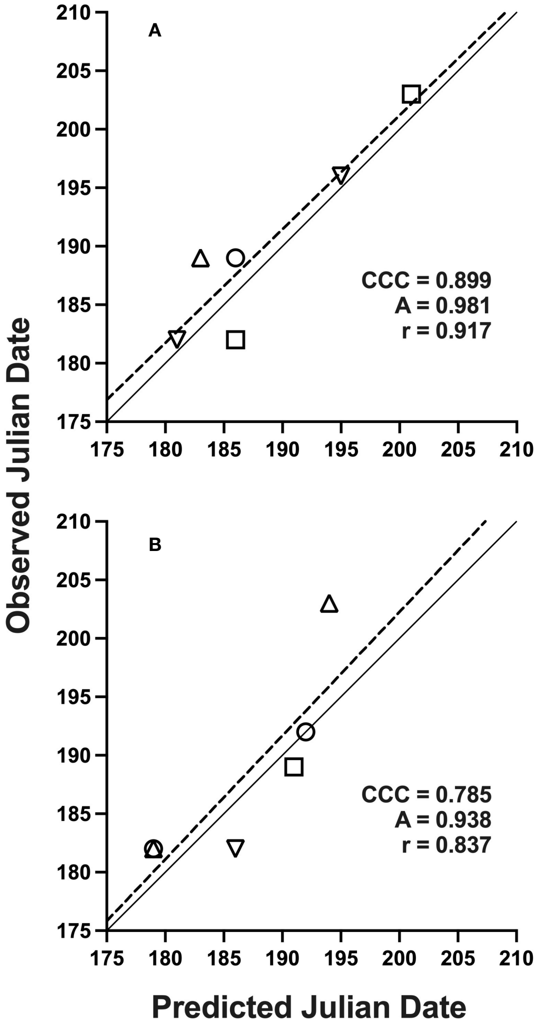


FIGURE 2
Solid lines indicate perfect agreement. Dashed lines are least squares regression lines that indicate deviations from agreement. Overall agreement is characterized by concordance correlation coefficients (*CCC*), which depend on component measures of precision (Pearson's correlation coefficient, *r*) and accuracy **(A)**, as defined in text. Data represents Julian date of 10% trap-catch with included *CCC* values using a lower threshold of 15 °C and upper threshold of 21.7 °C for model development **(A)** and validation **(B)**; locations are represented by: (○) Rosemount, (□) Forest Lake, (△) Hastings, (▽) Excelsior, MN, 2019-2021.

degree-day forecast illustrates an early "hot spot" of adult activity in the 7-county, Minneapolis-St. Paul, metro area, compared to several surrounding rural areas.

Discussion

Development and validation of a degree-day model for *P. japonica* indicated that a biofix date of 1 Jan., using the simple average degree-day method was the most precise and accurate model at 10% emergence, had improved agreement across the entire emergence curve compared with the best 10% emergence sine-wave model (Table 2). Overall, for the simple average model, start dates did not differ in their *CCC* values (Table 2); thus, Jan. 1 was selected as a start date to simplify when degree days should begin to be monitored for the season.

In some cases, sine-wave models did have a better fit across the whole emergence distribution than the selected simple model when sorting by *AIC* alone. However, using only this metric sacrificed fit at the target 10% emergence needed to alert growers in a timely fashion. Instead, prioritizing fit at 10% emergence by first sorting by *CCC* resulted in a simple model providing the best prediction out of all models at 10% emergence while still providing good prediction across the entire distribution where the selected model ranked 1,137 out of 7,392 total models for *AIC* (Table 2; Figure 3). *AIC* values do not indicate absolute measures of agreement like *CCC*, but are useful for making relative comparisons between models. These results also help illustrate the need to use multiple measures of agreement in modeling, especially when needing to optimize targeting a specific point on the emergence curve for IPM planning versus modeling the whole emergence period for wider uses. In addition to increased overall model performance in this instance, the simple degree-day calculations are less complex than sine-wave, so the simple degree-day model is likely to have a higher chance of adoption for use by growers (34). We therefore recommend beginning the simple average degree-day accumulation on Jan. 1 using a lower threshold of 15°C (59 °F) and upper threshold of 21.7 °C (71 °F).

This model will be useful in predicting 10% beetle trap-catch (257 degree-days, C), which should be early enough in the season to give growers and crop consultants ample time to

TABLE 3 Summary statistics for predicted versus observed days when 10% trap-catch by *P. japonica* occurred among 12 site-years for both model development and validation using the top simple degree-day model.

	Mean error (predicted-observed)	Std. Dev	Min	Max
Development	-1.5	3.271085	-6	4
Validation	-1.5	4.593474	-9	4

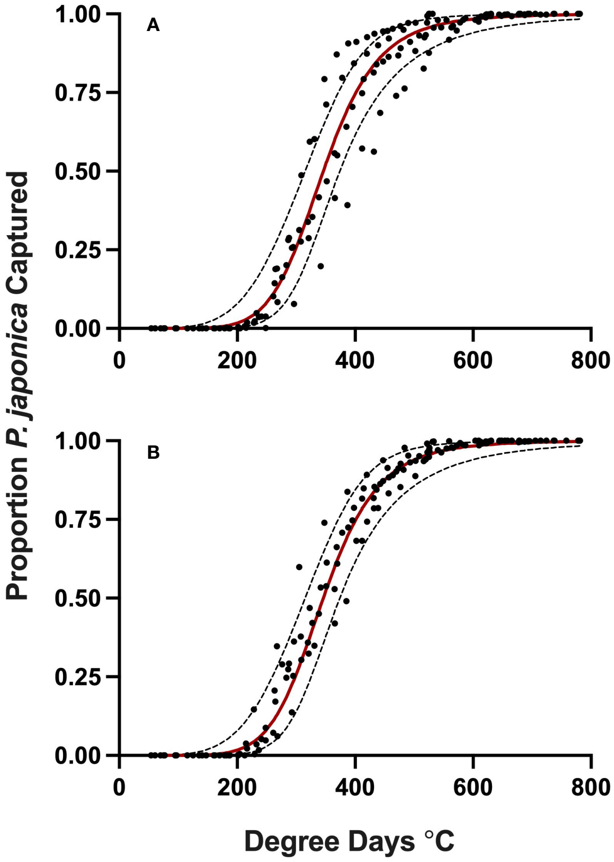


FIGURE 3
Proportion *P. japonica* adult trap-catch for development **(A)** and validation **(B)** of a degree-day model, in relation to cumulative degree-days, with 95% confidence intervals (red line, predicted model) simple average degree-day model, with a 1 Jan. start date, lower threshold of 15 °C and an upper threshold of 21.7 °C, MN, 2019-2021.

prepare for in-field sampling, and advance warning of peak beetle activity. The model can also forecast a reasonable estimate of beetle trap-catch at 25%, 50% (peak), 75% and 90%, for degree days of 298, 346, 401, and 465 (°C), respectively (Table 4). Knowledge of these degree-day forecasts will be useful for estimating the onset and phenology of trap-catch within a given year, and for comparing phenology across years.

To date, only a few previous studies have used laboratory-based developmental rates, and modeling to predict development of immature stages, to estimate *P. japonica* adult emergence or phenology (12, 35, 36). While these studies provided new insights into specific developmental thresholds for *P. japonica* life stages, it is difficult to compare the previous results with our field-based results. Our study, conducted in 2019-2021, used only ambient air temperatures collected from nearby weather stations and trap-catch data in the modeling analysis to attempt to characterize development of individuals in the soil. Studies conducted by Régniére et al. (12) placed larvae in individual cups and used laboratory-controlled temperatures to record the immature development of *P. japonica.* Further studies in the laboratory were conducted to attempt to assess adult maturation and time to emergence from rearing medium for several constant temperatures (12). While this information is important to understanding the biology of larvae, and adult eclosion, our research aimed to assist with predicted timely beetle emergence and phenology for IPM applications under field conditions, often involving multiple unknown variables.

In our study, we initiated data collection after *P. japonica* adults eclosed and found that this approach could be useful in forecasting the adult life stage and are meant to be used as a tool for monitoring and tracking the adult population, versus previous studies which looked to measure development. Our study was designed to allow potential confounding factors from field conditions that can act as nuisance variables to be incorporated as background variation across the selected variables: calculation method, upper and lower threshold, and biofix date. This allowed us to use air temperature, a more accessible type of weather data than soil temperature and

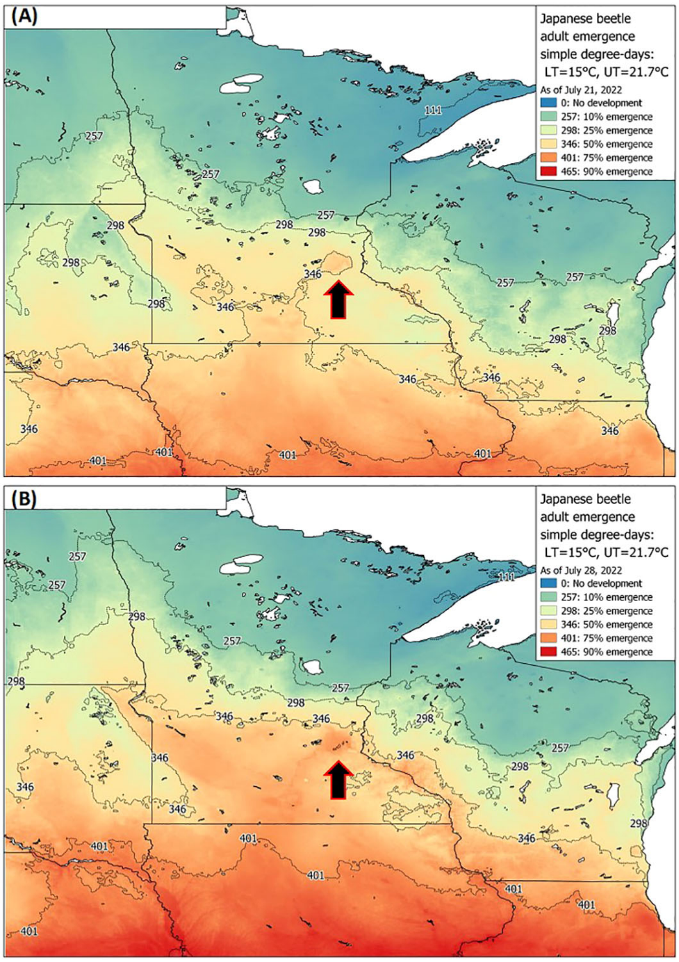


FIGURE 4
Example of a *P. japonica* predictive tool available to growers and crop consultants, *via* UMN Extension, showing real-time degree-day forecasts for 10-75% emergence as of July 21st **(A)** and July 28th **(B)** based on adult trap-catch, across Minnesota and nearby states during 2022. Maps are based on the simple average degree-day model, with lower and upper thresholds of 15 °C and 21.7 °C, respectively. Arrows indicate the 7-county metro area of Minneapolis-St. Paul, MN, which illustrates earlier beetle trap-catch **(A, B)**, compared to many of the surrounding rural areas.

determine if air temperature alone could reliably predict development while immature stages develop in the soil. However, the tradeoff is that is our model parameters, such as lower thresholds, are purposely confounded either with environmental effects, such as differences in soil and air temperatures or soil moisture, or as interactions with the other model terms. While this allows development of models with simple inputs to represent complex field conditions, our

TABLE 4 Degree-day estimates for the simple model, in °C and °F in relation to predicted adult trap-catch of *P. japonica**.

Proportion trap-catch	Degree-days °C	Degree-days °F
0.10	257	463
0.25	298	537
0.50	346	623
0.75	401	722
0.90	465	837

*Simple degree-day model using lower and upper thresholds of 15 and 21.7C, respectively (see Table 2). Proportion catch can be calculated using eq. 1 with °C degree-days, intercept -43.34 and slope 7.41. Degree-day requirements, from C to F, where F = C * 9/5 (19, 34).

parameters will not necessarily be directly comparable to previous studies in controlled laboratory conditions.

Understanding the differences in the aim of each study helps to better understand why lower and upper thresholds appear to be so different across the studies. Ambient air and soil temperatures are closely related, but depending on the height both above and below the soil surface where measurements are taken, there can be substantial differences observed between air temperature and what the insect actually experiences. These differences, particularly in temperate regions, often occur during spring, when soils warm relatively slowly compared to ambient air temperatures (19). Also, air temperatures can change drastically depending on cloud cover, precipitation events and changes in solar radiation (37). By contrast, each of these factors demonstrate what soil-dwelling insects are going to experience in different developmental environments when compared to adult life stages that have completed development and emerged from the soil (38). Similar to traditional regression analyses, background variation in an iterative modeling study such as this can cause parameters, such as lower thresholds based on air or soil temperatures, to deviate from thresholds determined in controlled laboratory studies (e.g. 19). For instance, the best sine-wave model did have a lower threshold of 11.7°C, which is closer to the lower threshold of 10°C described by Régnière et al. (12) than the simple model's lower threshold of 15°C. The thresholds in our models may be different than laboratory studies due to the buffering effects of the soil, differences in how simple and sine-wave calculations accumulate degree-days, the interaction of those two effects (e.g., how well each method accounts for soil buffer effects), or any number of other field or model parameter combinations. Because each model's performance is dependent on the combination of calculation method, upper and lower threshold, and to a lesser extent for the top models in Table 2, start date, the effect of a single parameter cannot be easily compared in isolation to laboratory study thresholds. Other approaches could include measuring soil temperature directly in a study such as this. However, ambient air temperature measurements are much more accessible relative to observed or modeled soil temperatures, which makes it easier for growers and producers to efficiently monitor their own degree-days.

Additionally, understanding the behavior of adult *P. japonica* after emergence can help explain why in Figures 3A, B, we observed a slight increase in the spread of observed data points as the model approaches 50% trap-catch. We speculate that this occurs for at least two reasons. At 10% trap-catch, or soon after adult eclosion and emergence, male beetles have specific tendencies to find a newly emerged, virgin female for mating (12), and will soon be attracted to the pheromone baited traps, following mating. These behaviors may explain why we see such a tight fit to the predicted model as both males and females will emerge from the soil and stay close to their emergence site for mating and initial flights to traps. Once mating has occurred and virgin females are no longer the majority, males will begin to expand their behavior to prioritize feeding on suitable hosts, and potentially move significant distances to seek preferred host plants such as wild grapes, wine grapes, or raspberry (9, 39–41). The variation observed in data point spread is an indication of this phenomenon as trap catch may now be accounting for beetles that have immigrated from other emergence sites to find suitable hosts for feeding and mating. Furthermore, progressing through the season, beetle behavior and flight is heavily dependent on other environmental factors such as cloud cover, wind, rainfall, and humidity (9, 42). While these environmental factors are still important after first emergence, beetles may not be traveling to other host sites as much due to their priority of mating with virgin females in the immediate area. However, even with the wider distribution of data points as degree-days reach the 50% activity, the model is still a beneficial predictive tool, especially considering how indiscriminate beetle behavior and dispersal becomes after initial mating goals have been met. In addition, the increase in variation is occurring after the primary target of predicting 10% trap-catch, as an early warning forecast prior to peak beetle trap-catch at 50%.

Efficient monitoring of crop pests is fundamental to the success of IPM programs, particularly for invasive arthropod species (43). Development of this degree-day model for *P. japonica* was created with the objective to produce an additional tool to assist growers, crop consultants, and regulatory staff, with an early-warning and predictive method for tracking beetle population phenology more efficiently. Calculations using a simple degree-day method, with the recommended lower (15 °C) and upper (21.7°C) thresholds, and a biofix date of 1 Jan. will provide growers and producers an early warning forecast for when 10% trap-catch will occur, and when crops should be monitored more closely for potential feeding damage (e.g., 17). Even with potential background sources of environmental variation, this model had very high agreement at the target 10% emergence predictions, and was one of the better performing models across the entire emergence period, which indicates this model can be a reliable and tool with relatively simple inputs for growers.

As the accumulation of degree-days reaches 257 °C, growers and regulatory staff can also prepare for the predicted peak beetle trap-catch, at 346 °C. The recent example, *via* the University of Minnesota Extension, VegEdge website (https://vegedge.umn.edu/degree-day-models-select-insect-pests-midwest-region), is the regional map of *P. japonica* beetle phenology, illustrated in Figure 4. The regional map is updated daily during the growing season using the simple average degree-day model presented in Figure 4. The degree-day maps provided real-time updates during the growing season for both 10% and 50% trap-catch targets; in addition, 7-day forecasts are also provided (e.g., www.fruitedge.umn.edu), to assist growers in tracking *P. japonica* adult activity. Although our model will be of immediate use in Minnesota, and likely perform well in other temperate climates, future applications beyond the region may require additional validation. Additional research could also be directed toward improved understanding of the development and survival of the larval instars during spring in relation to warming soil temperatures, and for a variety of climate regions. This could assist with further understanding of the efficacy of various management strategies against the larval stage, the role of overwintering soil temperature stress the larval stage (44), as well as improvements in phenology models, particularly in newly invaded regions or countries. Finally, the degree-day model presented here should continue to be useful to growers for *P. japonica* management in the future, given the context of global climate change for the Midwest region. For example, in Minnesota, ambient temperatures over next 80 years are projected to increase by 4 to 6 °C, during summer and winter periods, respectively (22).

Data availability statement

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

Author contributions

DE, AH and WH: conceptualization. WH and EB: resources, project administration, and funding acquisition. DE, AH, EB: methodology. DE and AH: investigation and formal analysis. DE and AH: writing original draft, and preparation. EB, and WH: writing and reviewing. All authors contributed to the article and approved the submitted version.

Funding

The research was funded by the Rapid Agricultural Response Fund, Award 00081823, *via* the Minnesota Agricultural Experiment Station, University of Minnesota, and a Minn. Dept. of Agric. (MDA), Specialty Crop Block Grant (00070811). The project was also supported by a McLaughlin Gormley King (MGK) Fellowship to DE, a University of Minnesota Doctoral Dissertation Fellowship to DE, and support for AH's effort by USDA-NIFA, Crop Protection and Pest Management-Extension Implementation Program award (2021-70006-35481).

Acknowledgments

We thank Adam Toninato, Anh Tran, Sally Nelson, Samantha Meyer, and Oliver McEllistrem (UMN Entomology Fruit and Vegetable IPM lab), for assistance with data collection. A special thanks to John and Jenny Thull (UMN Horticultural Research Center, HRC), and cooperating berry growers at Forest Lake (The Berry Patch), Hastings (Alexis Bailly's Vineyard), and Waverly, MN (Untiedt's Vegetable Farm), for allowing us to establish trapping studies at each site. We also thank previous reviewers Drs. Robert Koch and Dan Cariveau (Dept. of Entomology, UMN), and Dr. Matt Clark (Horticultural Science, UMN), for their previous reviews that improved the manuscript.

Conflict of interest

Although WH is an Editor for the *P. japonica* research topic, an independent Assoc. Editor was selected to manage the review process.

The remaining 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.

Publisher's note

References

1. Fleming WE. Integrating control of the Japanese beetle - A historical review. Technical Bulletin No. 1545. Washington, DC: USDA (1976).

2. Wawrzynski RP, Ascerno ME. Mass trapping for Japanese beetle (Coleoptera: Scarabaeidae) suppression in isolated areas. *J Arboric* (1998) 24:303–7. doi: 10.48044/jauf.1998.038

3. Hahn J, Weisenhorn J, Bugeja S. *Japanese Beetles in yards gardens* (2020). St. Paul, MN: University of Minnesota Extension. Available at: https://extension.umn.edu/yard-and-garden-insects/japanese-beetles (Accessed December 23, 2021).

4. Ebbenga DN, Wold-Burkness SJ, Burkness EC, Hutchison WD. *Japanese Beetle: An emerging pest of fruit crops* (2021). University of Minnesota, St. Paul. Available at: http://www.fruitedge.umn.edu/japanese-beetle/japanese-beetle-emerging-pest-fruit-crops (Accessed February 7, 2022).

5. Shanovich HN, Ashley DN, Koch RL, Hodgson EW. Biology and management of Japanese beetle (Coleoptera: Scarabaeidae) in corn and soybean. *J Integ Pest Manage* (2019) 10:1–14. doi: 10.1093/jipm/pmz009

6. Althoff EM, Rice KB. Japanese Beetle (Coleoptera: Scarabaeidae) invasion of north America: history, ecology, and management. *J Integ Pest Manage* (2022) 13 (1):1–11. doi: 10.1093/jipm/pmab043

7. Ebbenga DN, Burkness EC, Clark MD, Hutchison WD. Impact of adult Popillia japonica (Coleoptera: Scarabaeidae) foliar feeding injury on fruit yield and

quality of a temperate, cold-hardy wine grape, 'Frontenac'. *Front Insect Sci* (2022) 2:887659. doi: 10.3389/finsc.2022.887659

8. Kistner-Thomas EJ. The potential global distribution and voltinism of the Japanese beetle (Coleoptera: Scarabaeidae) under current and future climates. *J Insect Sci* (2019) 19(2). doi: 10.1093/jisesa/iez023

9. Fleming WE. *Biology of the Japanese beetle*. Washington, DC: USDA (1972).

10. Potter DA. *Destructive turfgrass insects: Biology, diagnosis, and control*. Chelsea, MI: Ann Arbor Press (1998). 368 p.

11. Potter DA, Held DW. Biology and management of the Japanese beetle. *Annu Rev Entomol* (2002) 47:175–205. doi: 10.1146/annurev.ento.47.091201.145153

12. Régniére J, Rabb RL, Stinner RE. *Popillia japonica*: Simulation of temperature-dependent development of the immatures, and prediction of adult emergence. *Environ Entomol* (1981) 10:290–6. doi: 10.1093/ee/10.3.290

13. USDA: Animal and Plant Health Inspection Service (APHIS). *Japanese Beetle distribution in the U.S* (2022). Available at: https://www.aphis.usda.gov/plant_ _health/plant_pest_info/the Japanese beetle/downloads/the Japanese beetle-distribution-map.pdf (Accessed 7 February 2022).

14. Tumlinson JH, Klein MG, Doolittle RE, Ladd TL, Proveaux AT. Identification of the female Japanese beetle sex pheromone: Inhibition of male response by an enantiomer. *Science* (1977) 197:789–92. doi: 10.1126/science.197.4305.789

15. Ladd TL, Klein MG, Tumlinson JH. Phenethyl propionate + eugenol + geraniol (3:7:3) and japonilure: A highly effective joint lure for Japanese beetle. *J Econ Entomol* (1981) 74:665–7. doi: 10.1093/jee/74.6.665

16. Klostermeyer LE. Japanese Beetle (Coleoptera: Scarabaeidae) traps: Comparison of commercial and homemade traps. *J Econ Entomol* (1985) 78:454–9. doi: 10.1093/jee/78.2.454

17. Ebbenga DN, Burkness EC, Hutchison WD. Optimizing the use of semiochemical based traps for efficient monitoring of popillia japonica (Coleoptera: Scarabaeidae): Validation of a volumetric approach. *J Econ Entomol* (2022) 115(3):869–76. doi: 10.1093/jee/toac049

18. Hoard MW, Weiss MJ. Influence of post-diapause development on the voltinism of the European corn borer (Lepidoptera: Pyralidae) in north Dakota. *Environ Entomol* (1995) 24:564–70. doi: 10.1093/ee/24.3.564

19. Hanson AA, Moon RD, Wright RJ, Hunt TE, Hutchison WD. Degree-day predictions for the flight phenology of Western bean cutworm (Lepidoptera: Noctuidae) assessed with the concordance correlation coefficient. *J Econ Entomol* (2015) 108(4):1723–38. 10.1093/jee/tov110

20. Wilson J, Gut L, Grieshop M, Shane W. *Managing brown marmorated stink bug in Michigan orchards* (2020). Michigan State University. Available at: https://www.canr.msu.edu/resources/managing-brown-marmorated-stink-bug-in-michigan-orchards (Accessed 7 February 2022).

21. Smyers EC, Urban JM, Dechaine AC, Pfeiffer DG, Crawford SR, Calvin DD. Spatio-temporal model for predicting spring hatch of the spotted lanternfly (Hemiptera: Fulgoridae). *Environ Entomol* (2021) 50:126–37. doi: 10.1093/ee/nvaa129

22. Liess S, Twine TE, Snyder PK, Hutchison WD, Konar-Steenberg G, Keeler BL, et al. High-resolution climate projections over Minnesota for the 21st century. *Earth Space Sci* (2022) 9:1–16. doi: 10.1029/2021EA001893

23. Burkness EC, Ebbenga DN, Toninato AG, Hutchison WD. Exclusion and repulsion of *Popillia japonica* (Coleoptera: Scarabaeidae) using various coverings on high tunnel structures for primocane raspberry. *Insects* (2022) 13:771. doi: 10.3390/insects13090771

24. R Core Team. *R: A language and environment for statistical computing*. Vienna, Austria (2022). Available at: http://www.r-project.org/.

25. Roltsch WJ, Zalom FG, Strawn AJ, Strand JF, Pitcairn MJ. Evaluation of several degree-day estimation methods in California climates. *Int J Biometeorol.* (1999) 42:169–76. doi: 10.1007/s004840050101

26. UC-IPM. *Degree-day calculation methods* (2022). Davis, CA: University of California, Davis, Integrated Pest Management Program. Available at: http://ipm.ucanr.edu/WEATHER/ddconcepts.html (Accessed 21 February 2022).

27. Allen JC. A modified sine wave method for calculating degree days. *Environ Entomol* (1976) 5:388–96. doi: 10.1093/ee/5.3.388

28. McMaster GS, Wilhelm WW. Growing degree-days: One equation, two interpretations. *Agric For Meteorol* (1997) 87:291–300. doi: 10.1016/S0168-1923(97)00027-0

29. Lin L. A concordance correlation coefficient to evaluate reproducibility. *Biometrics* (1989) 42:255–68. doi: 10.2307/2532051

30. Lin L, Hedayat AS, Sinha B, Yang M. Statistical methods in assessing agreement: Models, issues, and tools. *J Am Stat Assoc* (2002) 97:386–8.

31. Liao JZ. An improved concordance correlation coefficient. *Pharm Stat* (2003) 2:253–61. doi: 10.1002/pst.52

32. Meek DW, Howell TA, Phene CJ. Concordance correlation for model performance assessment: An example with reference evapotranspiration observations. *Agron J* (2009) 101:1012–8. doi: 10.2134/agronj2008.0180x

33. Burnham KP, Anderson DR. *Model selection and multimodel inference: A practical information-theoretic approach. 2nd ed.* New York, NY: Spring-Verlag (1998).

34. Pruess KP. Day-degree methods for pest management. *Environ Entomol* (1983) 12:613–9. doi: 10.1093/ee/12.3.613

35. Régniére J, Rabb RL, Stinner RE. *Popillia japonica* (Coleoptera: Scarabaeidae): Distribution and movement of adults in heterogeneous environments. *Can Entomol* (1983) 115:287–94. doi: 10.4039/Ent115287-3

36. Ludwig D. The effects of temperature on the development of an insect (Popillia japonica Newman). *Physiol. Zool* (1928) 1:358–89.

37. Campbell GS, Norman JM. *An introduction of environmental biophysics. 2nd ed.* New York, NY: Springer-Verlag (1998).

38. Gilioli G, Sperandio G, Simonetto A, Colturato M, Battisti A, Mori N, et al. Modeling diapause termination and phenology of the Japanese beetle, popillia japonica. *J Pest Sci* (2021) 95:869–80. doi: 10.1007/s10340-021-01434-8

39. Barrows EM, Gordh G. Sexual behavior in the Japanese beetle, *Popillia japonica*, and comparative notes on sexual-behavior of other scarabs (Coleoptera: Scarabaeidae). *Behav Biol* (1978) 23:341–54. doi: 10.1016/S0091-6773(78)91365-2

40. Régniére J, Rabb RL, Stinner RE. *Popillia japonica* (Coleoptera: Scarabaeidae): A mathematical model of oviposition in heterogenous ecosystems. *Can Entomol* (1979) 111:1271–80. doi: 10.4039/Ent1111271-11

41. Hamilton RM, Foster RE, Gibb TJ, Sadof CS, Holland JD, Engel BA, et al. Distribution and dynamics of Japanese beetles along the Indianapolis Airport perimeter and the influence of land use on trap catch. *Environ Entomol* (2007) 36:287-96. doi: 10.1603/0046-225x(2007)36[287:dadojb]2.0.co;2

42. Lacey LA, Amaral JJ, Coupland J, Klein MG. The influence of climatic factors on the flight activity of the Japanese beetle (Coleoptera: Scarabaeidae): Implications for use of a microbial control agent. *Biol Control* (1994) 4:298–303. doi: 10.1006/bcon.1994.1038

43. Venette RC, Hutchison WD. Invasive insect species: Grand challenges, strategies and opportunities. *Front Insect Sci* (2021) 1:650520. doi: 10.3389/finsc.2021.650520

44. Ebbenga DN. *Understanding the biology and management of popillia japonica in a northern tier, temperate climate*. PhD Dissertation, University of Minnesota: St. Paul (2022). p. 115.

Frontiers in Insect Science

TYPE Original Research
PUBLISHED 10 May 2023
DOI 10.3389/finsc.2023.1138427

OPEN ACCESS

EDITED BY
Frank H. Koch,
Southern Research Station, Forest Service (USDA), United States

REVIEWED BY
Renata Bažok,
University of Zagreb, Croatia
Cody Prouty,
University of Minnesota Twin Cities, United States

*CORRESPONDENCE
Tanja Graf
tanja.graf@agroscope.admin.ch

RECEIVED 05 January 2023
ACCEPTED 11 April 2023
PUBLISHED 10 May 2023

CITATION
Graf T, Scheibler F, Niklaus PA and Grabenweger G (2023) From lab to field: biological control of the Japanese beetle with entomopathogenic fungi.
Front. Insect Sci. 3:1138427.
doi: 10.3389/finsc.2023.1138427

From lab to field: biological control of the Japanese beetle with entomopathogenic fungi

Tanja Graf[1,2]*, Franziska Scheibler[1,3], Pascal A. Niklaus[2] and Giselher Grabenweger[1]

[1]Extension Arable Crops, Department of Plants and Plant Products, Agroscope, Zurich, Switzerland, [2]Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland, [3]Department of Environmental Systems Science, ETH Zurich, Zurich, Switzerland

The Japanese beetle, *Popillia japonica*, is an invasive scarab and listed as quarantine organism in many countries worldwide. Native to Japan, it has invaded North America, the Azores, and recently mainland Europe. Adults are gregarious and cause agricultural and horticultural losses by feeding on leaves, fruits, and flowers of a wide range of crops and ornamental plants. Larvae feed belowground and damage grassland. To date, no efficient and environmentally friendly control measure is available. Larval populations of other scarab species such as *Phyllopertha horticola* and *Melolontha melolontha* are controlled by applying spores of the entomopathogenic fungi *Metarhizium brunneum* and *Beauveria brongniartii* to larval habitats. Here, we tested this control strategy against Japanese beetle larvae in grasslands, as well as spore spray applications against adults in crops. Using both, large-scale field experiments and inoculation experiments in the laboratory, we assess the efficacy of registered fungal strains against Japanese beetle larvae and adults. *Metarhizium brunneum* BIPESCO 5 established and persisted in the soil of larval habitats and on the leaves of adult's host plants after application. However, neither larval nor adult population sizes were reduced at the study sites. Laboratory experiments showed that larvae are not susceptible to *M. brunneum* ART 212, *M. brunneum* BIPESCO 5, and *B. brongniartii* BIPESCO 2. In contrast, adults were highly susceptible to all three strains. When blastospores were directly injected into the hemolymph, both adults and larvae showed elevated mortality rates, which suggests that the cuticle plays an important role in determining the difference in susceptibility of the two life stages. In conclusion, we do not see potential in adapting the state-of-the-art control strategy against native scarabs to Japanese beetle larvae. However, adults are susceptible to the tested entomopathogenic fungi in laboratory settings and BIPESCO 5 conidiospores survived for more than three weeks in the field despite UV-radiation and elevated temperatures. Hence, control of adults using fungi of the genera *Beauveria* or *Metarhizium* is more promising than larval control. Further research on efficient application methods and more virulent and locally adapted fungal strains will help to increase efficacy of fungal treatments for the control of *P. japonica*.

KEYWORDS

***Popilla japonica*, *Beauveria brongniartii*, *Metarhizium brunneum*, field experiments, virulence, adult, larva, spore injection**

1 Introduction

The Japanese beetle (*Popillia japonica*) is one of the most important invasive insects threatening the agricultural and horticultural sectors in its invasive range, and is listed as a priority quarantine pest in the European Union (1) and other countries (2, 3). It was accidentally introduced to the USA in the beginning of the 20th century (4) and spread from New Jersey to the west coast, up to Canada, and to the south of the USA (5). Since 2014, the Japanese beetle is present on mainland Europe (6), where it has spread from northern Italy (Piedmont and Lombardy) into southern Switzerland (Ticino), expanding its range each year. Both countries have designated infested zones where phytosanitary measures are in place to limit its spread (7–9).

Unlike many other insect pests, Japanese beetle cause significant damage as both larvae and adults. Larvae are white grubs that feed preferentially on grass roots (4), causing damage to grasslands, football fields, golf courses and other areas with turf (10–12). After emerging in early summer, adults move from the larval habitats to feed on their host plants, where they live for four to six weeks. They are gregarious and polyphagous, feeding on leaves, flowers, and fruits of more than 300 plant species including grapevines, stone fruits, berries, maize, soybean, roses and forest trees (4). Currently, the control of Japanese beetle adults and larvae mainly depends on the use of synthetic insecticides (13, 14), with the cost of damage and control measures estimated at more than $400 million per year in the USA alone (15). Besides these monetary costs, large-scale insecticide applications to control this invasive pest pose a risk to the environment and human health (16–18).

To date, no efficient and environmentally friendly control strategy exists against Japanese beetles (14). However, several biological agents have the potential to control this species, including parasitic nematodes (*Steinernema* sp. and *Heterorhabditis* sp.), bacteria (*Paenibacillus popilliae* and *Bacillus thuringiensis* var. *galleriae*), and entomopathogenic fungi (*Metarhizium* spp. and *Beauveria* spp.; 13, 14, 19–21). Entomopathogenic fungi have proved effective against many insect pests in the Coleoptera, Lepidoptera and Diptera, and fungi of the genera *Beauveria*, *Metarhizium*, *Isaria* and *Lecanicillium* are commercially applied worldwide (22–26). These fungi infect their insect hosts by attaching to and breaching the cuticle (27). For successful infection, the number of adhering spores is crucial, as mortality is dose dependent (28, 29). Once the fungi have reached the hemolymph of the insect, they form blastospores and exploit the nutrients of the insect (30). Upon the death of the host the fungus grows out of the insect to form new conidiospores (31, 32). The duration of fungal incubation can vary from a few days to a couple of months, and depends on the insect species, the virulence of the fungus and environmental conditions (32, 33).

In Switzerland, adults and larvae of native scarab beetles such as the cockchafer (*Melolontha* spp.), the June beetle (*Amphimallon* spp.), and the garden chafer (*Phyllopertha horticola*) are highly susceptible to different strains of *Beauveria brongniartii* or *Metarhizium* sp., depending on the scarab species (33–36). In contrast to the Japanese beetle, these native scarabs almost exclusively cause damage in their larval stages. Thus, the state-of-the-art control strategy against native scarabs consists of culture of the appropriate fungal strain on sterilized barley kernels and application of fungus-colonized barley kernels (FCBK) with a no-till seeder to the larval habitats (meadows, pastures, or turf; 36–38). The Japanese beetle is closely related to these native scarabs and shares a similar ecological niche and life cycle. Under laboratory conditions, several fungal strains (*Metarhizium* spp. and *Beauveria* spp.) have proven successful in infecting and killing Japanese beetle larvae or adults (39–42). Furthermore, Behle et al. (21) achieved a moderate to good control of larvae when applying the fungal strain *Metarhizium brunneum* BIPESCO5/F52 (Bip5) to turf in well-controlled small scale-field experiments. However, there remains a lack of research to test the efficacy of large-scale field application of entomopathogenic fungi against Japanese beetle larvae and adults.

Here, we tested the application of Bip5 against Japanese beetle larvae and adults in the field. We hypothesized that the Japanese beetle can be controlled in a similar way to its native relatives in Europe. Thus, we tested the application of FCBK for control of larvae on the Japanese beetle. Furthermore, we also assessed the efficacy of a spray application of a Bip5 conidiospore suspension to control adult populations. The objective of the field experiments was to monitor the ability of Bip5 to establish and survive in both adult and larval habitats and to measure its effect on insect survival and the damage caused by adult beetles.

Additionally, we carried out two sets of laboratory experiments with the overall aim of assessing the susceptibility of Japanese beetle larvae and adults to three commercially available fungal strains, *M. brunneum* ART 212, *M. brunneum* BIPESCO 5, and *B. brongniartii* BIPESCO 2, under standard laboratory conditions. These experiments revealed host stage-related differences in susceptibility to fungal infection and we hypothesized that they may be explained by cuticular defense mechanisms. We anticipated that larvae have a stronger cuticular defense against soil-borne pathogens than adults, owing to the long subterranean development of the former. Moreover, we expected blastospores to induce faster speed of kill under laboratory conditions due to their fast germination and growth, while more robust conidiospores may be slower in infesting their host.

Our experiments help to assess the potential of entomopathogenic fungi as biocontrol agents against Japanese beetles in the field, and to better understand the mechanisms underlying host-stage-specific virulence of the applied fungal strains.

2 Material and methods

2.1 Fungal strains

All fungal strains used in the field and laboratory experiments are commercially available. *Beauveria brongniartii* BIPESCO2 (Bip2) was originally isolated by H. Strasser from infected *Melolontha melolontha* (43), *Metarhizium brunneum* BIPESCO5 (Bip5) was isolated in Austria from infected *Cydia pomonella* (44), and ART 212 was isolated from *Agriotes* sp. at Agroscope (Switzerland). To ensure the fitness of the fungal strains, we isolated spores from mycosed cadavers of Japanese beetle (Bip5 and ART 212) and cockchafer (Bip2) larvae from previous inoculation experiments and plated them on selective medium plates (SM: sabouraud 2% glucose agar (SDA) supplemented with cycloheximide (0.05 g/l), streptomycin sulfate (0.6 g/l), tetracycline (0.05 g/l), and dodine (50 mg/l); 45). The F2 generation was grown for two weeks at 22°C and 80% RH in darkness and stored at 5°C after the fungi fully sporulated on the plates.

2.2 Meadow field experiments

To test whether the state-of-the-art control of native relatives is effective for Japanese beetles, we carried out two field experiments on meadows infested with Japanese beetle larvae in Piedmont, Italy. Each experiment consisted of 18 plots (9 × 10 m). Experiment 1 (45.6373°N, 8.6087°E, 311 m a.s.l.) was set up in September 2018 when second and third instar larvae were present. Three treatments (six plots each) were established: Bip5 FCBK (applied with a no-till seeder at an equivalent concentration of 10^{14} conidiospores ha^{-1}), treatment control (treatment with no-till seeder without FCBK), and a control (untreated). Experiment 2 (45.6354°N, 8.6377°E, 191 m a.s.l.) was set up in May 2019 before adults emerged and laid new eggs. Nine plots received the Bip5 FCBK treatment, the other nine plots were left untreated as control. This second meadow was irrigated once with 30 mm water during peak flight in July 2019 because the soil was very dry.

2.2.1 Fungal inoculum

Bip5 was grown on sterilized barley kernels in polypropylene zipper filter bags (Sac O_2, Deinze, Belgium; 2 kg unpeeled barley, 1.5 L tap water per bag). The bags were inoculated with sporulating Bip5 culture (F3 generation) that had been grown on complete medium plates (CM: 10 g glucose, 0.36 g KH_2PO_4, 1.78 g Na_2HPO_4, 1 g KCl, 0.6 g $MgSO_47H_2O$, 0.6 g NH_4NO_3, 5 g yeast extract, 20 g agar per 1 L distilled water; 46; one plate per bag). Bags were incubated for 6–8 weeks at 23°C in the dark and were mixed after approximately two weeks to increase conidiospore production. When fully sporulating, FCBK bags were stored at 5°C until use. Conidiospore concentration was determined by washing the spores from a subsample of each bag with 0.1% aqueous Tween 80 (Sigma-Aldrich, Seelze, Germany) and counting them with a hemocytometer.

2.2.2 Sampling

Soil samples were taken to estimate the number of *Metarhizium* sp. colony forming units (CFU) in the soil before and after the application of treatments. Four soil samples per plot (6 cm diameter × 10 cm depth) were pooled, mixed, and stored in plastic bags at 5° C until processing. The *Metarhizium* sp. CFU g^{-1} of soil were estimated as described by Kessler et al. (37). From each pooled sample, three subsamples of 20–24 g were taken, suspended, and plated on SM. After two weeks of incubation (22°C, 70% RH, darkness), we counted the *Metarhizium* sp. colonies on each plate and determined CFU g^{-1} of soil dry weight. We measured the water content of the soil samples gravimetrically. The mean of the three subsamples per plot was used for statistical analyses.

Larval density in the meadows was estimated by counting all Japanese beetle larvae present in five 20 × 20 × 10–15 cm soil blocks per plot. For statistical analysis, we used the sum of the number of larvae found in the five soil blocks.

2.3 Soybean field experiment

To assess whether Bip5 conidiospores can provide control against Japanese beetle adults, we carried out an experiment on an infested soybean field in northern Italy (45.5354°N, 8.6512°E, 177 m a.s.l.). We compared four treatments: 1) Bip5 conidiospore suspension (10^{14} conidiospores ha^{-1} suspended in 600 L ha^{-1} water with 2% Telmion (Omya International AG, Oftringen, Switzerland) as surfactant); 2) a surfactant control (water with 2% Telmion); 3) an untreated control; and 4) a reference application of the insecticide Karate Zeon 1.5 (15 g L^{-1} Lambda-Cyhalothrin, Syngenta, Basel Switzerland, 1.5 L ha^{-1}). All treatments were applied with an air-supported trail sprayer. We divided the field into 24 plots (6 plots per treatment) of 21×21 m and assigned the treatments randomly to plots. Treatments were applied at the beginning of the peak flight in early July 2019 in the morning and a second time 6 days later in the evening.

2.3.1 Fungal inoculum

Bip5 conidiospores were produced on barley kernels as described above. Bags with fully sporulating Bip5 were opened and air dried for 2-3 weeks, then conidiospores were removed from the barley kernels (mycoharvester VBS (Agriculture) Ltd., Beaconsfield, United Kingdom), and stored at 5°C. The spore concentration of the powder was determined using a hemocytometer (with the powder in 0.1% aqueous Tween 80 solution). Immediately before application, the conidiospores were formulated with water and 2% Telmion at the field site.

2.3.2 Sampling

To estimate the number of viable *Metarhizium* sp. spores on soybean leaves, we removed nine randomly chosen healthy leaves from all plots except the insecticide treated plots and stored them at 5°C in extraction bags (forming subsamples of three leaves per bag; Bioreba AG, Reinach, Switzerland) until processing. We added 10 mL buffer solution (0.01M PBS, 0.05% (v/v) Tween 20) to each extraction bag and homogenized the leaf samples with the buffer in each extraction bag using a grinding machine. Aliquots of 100 µL of each sample were pipetted and spread on SM plates for incubation (two weeks at 23°C, 80% RH, darkness). We counted *Metarhizium* sp. CFU on each plate, distinguishing them morphologically from other fungi. For statistical analysis, the mean of the three subsamples per plot was used.

To assess the effects on the Japanese beetle population, we counted all adult beetles along 5 m transects of two rows of soybean plants per plot. The sum of all counted beetles per plot was used for statistical analysis. In addition, we scored damage on five randomly selected plants per plot (0: no damage; 1: visible damage but less than half a leaf skeletonized; 2: leaves are green but at least half a leaf is skeletonized; 3: at least one fully skeletonized brown leaf). For each plot, the damage scores of the five plants were summed for statistical analysis.

The effect of the Bip5 conidiospore treatment and the surfactant control on the mortality of beetles was assessed immediately after the first spray application. Ten adults per plot were collected, placed individually in 90 mL plastic tubes filled with moist peat and hazelnut leaves as food, closed with a perforated lid, and incubated for five weeks (23°C, 60% RH, day–night cycle of 16:8 h). We replaced food weekly and checked for mortality at the same time.

2.4 Laboratory inoculation experiments

We carried out two sets of laboratory experiments where we combined different application methods (spraying, injection) with different fungal strains (Bip2, Bip5 and ART 212), spore types, and respective control treatments (Table 1). All treatments were applied to either Japanese beetle larvae or adults and the timing of the experiments was adjusted to the life cycle of the insect.

2.4.1 Insects

Japanese beetle adults and larvae were collected from wild populations in an infested area in the Swiss-Italian border region. Prior to experiments, adults were kept for 2–9 days in a refrigerator (5–6°C, to maintain their fitness) in groups of approximately 50 animals, in plastic containers containing moist peat and vine or hazelnut leaves as food. Larvae were kept for 26–32 days (experiment 2020) and 3–9 days (experiment 2021), individually in six-well cell culture plates filled with moist peat and slices of carrot as food (23°C, 60% RH, day–night cycle of 16:8 h). Before exposure to treatments, larvae were cooled in a refrigerator (5–6°C, for approximately 2 h).

2.4.2 Spore suspensions

F2 generation plates of the fungal strains served as starting material for all spore suspensions. Conidiospore suspensions were prepared by re-plating the F2 generation on SM plates and washing spores off fully sporulating cultures (F3) with 15 mL of sterile Tween 80 solution (0.1% v/v). We removed mycelium and other large particles from the suspensions by vacuum-filtration (Miracloth filter, Merck KGaA, Darmstadt, Germany). Final spore concentration was adjusted to 10^7 conidiospores mL^{-1} by adding sterile deionized water after counting using a hemocytometer.

Blastospore suspensions were obtained from liquid medium cultures (medium: 3% sucrose, 2.5% yeast extract, 1% peptone and 1% barley flour in 500 mL deionized water) inoculated with six to eight 7 mm-diameter plugs from F2 generation plates. After incubation on an orbital shaker at 250 rpm and 28°C for 3 days for Bip5, and 25°C for 4 days for Bip2, we filtered the liquid cultures through Miracloth to remove the mycelium and washed the remaining blastospores to remove the ingredients of the medium and mycotoxins produced by Bip2 and Bip5 (centrifugation at 1174g for 20 min, removal of supernatant, resuspension in deionized water, second centrifugation, resuspension in deionized

TABLE 1 Treatment combinations in the laboratory inoculation experiments.

	Treatment	Spore type	Application method
Experiment 2020			
	Control	None	None
	Tween 0.01%	None	Spray
	Bip2	Conidiospores	Spray
	Bip5	Conidiospores	Spray
	ART 212	Conidiospores	Spray
Experiment 2021			
	Control	None	None
	Tween 0.01%	None	Spray
	H_2O	None	Injection
	Bip2	Conidiospores	Spray
		Blastospores	Spray
		Blastospores	Injection
	Bip5	Conidiospores	Spray
		Blastospores	Spray
		Blastospores	Injection

Experiments were performed using third instar larvae and adult Japanese beetles (Experiment 2020, adults in June/July, larvae in October; experiment 2021, adults in July, larvae in September). We used five replicates per treatment combination, and 15 individuals per replicate. For the spray applications, we used 10^7 spores mL^{-1} or 0.01% (v/v) Tween 80. For injections, we used 0.2 µL of a suspension containing 10^6 spores mL^{-1} (approx. 200 spores per insect) or deionized water.

water and filtration using Miracloth). We determined the spore concentration using a hemocytometer and adjusted to 10^7 blastospores mL^{-1} by adding deionized water. For the injection of blastospores, we diluted 1 mL of the blastospore suspension to 10^6 spores mL^{-1}.

2.4.3 Experimental procedure

For the spray treatments, we applied the respective spore solution using a 30 mL spray flask; each insect received one spray dose from each side. For the injection treatments, we used microsyringes (10 µL, G31 injection needle, Hamilton Company, Reno, Nevada) to inject 0.2 µL of the respective suspension behind the third leg of the insect. Germination rates were higher than 95% for all spore solutions (quantified by pipetting three times 50 µL of spore suspension on CM, incubation for 24 h (conidiospores) or 12–18 h (blastospores), and counting 100 spores at 40× magnification).

After inoculation, insects were held individually in insect tubes filled with moist peat and hazelnut leaves (adults) or carrot slices (larvae) as food and closed with a perforated lid. All tubes from one treatment and one replicate were kept together in a plastic box, and these boxes were randomly placed on two racks in a climate-controlled room (23°C, 60% RH, day–night cycle 16:8 h). We assessed the mortality and fungal infection of the insects weekly and replaced food at the same time (adults 4 weeks, larvae 10 weeks). Mycosed cadavers were stored at 5°C and fungal spores were isolated from the cadavers on SM and grown for 2 weeks. The isolates were kept at 5°C until used for genetic analysis.

2.4.4 Genetic analysis

From the experiments in 2021, we selected fungal isolates according to morphology from each treatment to confirm their identity using genetic analysis (simple sequence repeats; SSR). Isolates were spread on CM plates covered with filter paper. After 4–5 days of incubation, the mycelia were scraped off the filters, transferred to 2 mL Eppendorf tubes and frozen at -70°C. The frozen mycelia were lyophilized, and cells were disrupted with glass beads (3 mm and 1 mm) in a FastPrep-24 homogenizer (MP Biomedicals, Eschwege, Germany; 25 s at 6 m s^{-1}). We extracted the DNA (sbeadex plant kit and King Fisher Flex Purification system, Thermo Fisher Scientific, Waltham, Massachusetts) and standardized the samples to 5 ng DNA μL^{-1}.

We used six SSR markers in two primer pair sets for each species to analyze fungal genotypes (Bb1F4, Bb2A3, Bb2F8, Bb4H9, Bb5F4, Bb8D6 for *B. brongniartii*; 47; Ma2049, Ma2054, Ma2063, Ma2287, Ma327, Ma195 for *M. brunneum*; 48, 49). Reference strains were included for both species (*B. brongniartii*, Bip2 and Bip4; *Metarhizium* spp., Ma714, Ma500 and Bip5). Multiplex PCRs and fragment size analyses were performed as described by Mayerhofer et al. (50) and Fernandez-Bravo et al. (51).

2.5 Data analysis

For all field experiments, data collected after treatment at multiple time points were aggregated at plot level (the unit of replication of the applied treatments). We modeled the variables in dependence of the applied treatments by using linear models summarized in analysis of variance (ANOVA) tables. These analyses were performed for all CFU data, data on larval densities in the soil of the meadow field experiments, and the abundance of adult beetles and damage rate of the soybean field experiment. The dependent variables of the two meadow field experiments were square-root transformed before aggregation to increase homoscedasticity. To test for different temporal dynamics in the different treatments, we first regressed the dependent variables against time, and then analyzed the temporal trends (slopes) with a one-sample t-test. This two-step procedure avoids the modeling of serial residual correlation structure, without loss of information. CFU data from both meadow field experiments were square-root-transformed before regression to increase homoscedasticity.

For the meadow field experiment 2 and the soybean field experiment, we expected a natural gradient in the insect populations on the study sites due to their surroundings. Therefore, we added to each of the datasets the variables X and Y to account for the order of the plots. We included those variables as factors into our analysis and fitted them before the applied treatments.

Insect mortality was analyzed using discrete-time hazard models. Specifically, we used a binomial generalized linear model with mortality measured during intervals as dependent variable, with complementary log–log link and the logarithm of the interval length as offset (ASReml-R V4 package, VSNi, Hemel Hempstead, UK). The fixed-effect terms were, in this sequence, interval, the experimental treatments, and the interaction between interval and these treatments. Note that an interaction between interval and any of the treatment indicates a deviation from proportional hazards. The box that harbored the group of initially 15 insects was fitted as random effect. To further inspect effects of the experimental treatments, we decomposed these into a series of individual contrasts and interactions with the time intervals. See results for details.

3 Results

3.1 Meadow field experiments

The application of Bip5 FCBK increased the abundance of *Metarhizium* sp. CFU in the soil in both experiments (Figures 1A, C; experiment 1, $F_{1,\ 15} = 145$, $P < 0.001$; experiment 2, $F_{1,\ 14} = 51$, $P < 0.001$). Despite increased CFU densities, Bip5 FCBK applications failed to reduce the larval populations significantly (Figures 1B, D). In experiment 1, we found no effect of Bip5 FCBK treatment on the larval population ($F_{2,\ 15} = 0.5$, $P > 0.5$); however, the population decreased over winter by approximately 50% irrespective of the treatment ($t_{17} = 10.2$, $P < 0.001$). In experiment 2, the larval population was in general lower in Bip5 FCBK treated plots compared to the control, but the effect was non-significant ($F_{1,\ 14} = 3.3$, $P = 0.089$). We did not find high winter mortality in experiment 2. *Metarhizium* sp. CFU numbers decreased over winter in Bip5 FCBK treated plots in both experiments (slopes differ from 0, experiment 1, $t_5 = -2.6$, $P <$

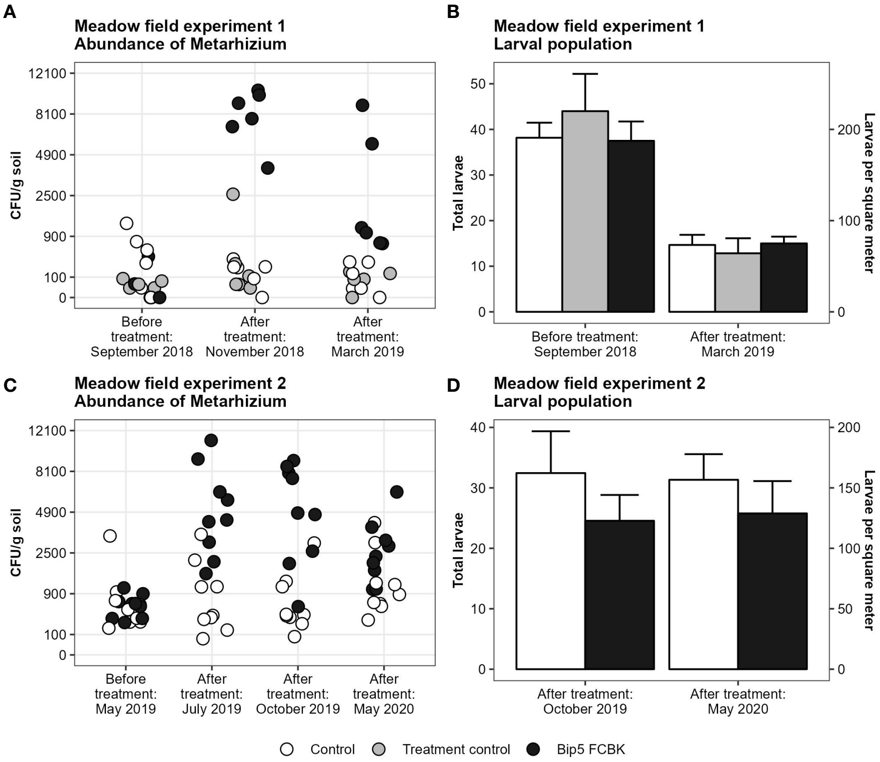


FIGURE 1
Number of *Metarhizium* CFU g^{-1} soil on square-root transformed y-axis **(A, C)** and mean number of larvae with standard error **(B, D)** before and after treatments.

0.05; experiment 2, t_8 = -2.7, $P < 0.05$) but remained constant in the control and treatment control (slopes do not differ from 0, experiment 1, t_{11}= -1.4, $P > 0.1$; experiment 2, t_8= 0.8, $P > 0.1$).

3.2 Soybean field experiment

No *Metarhizium* sp. CFU were found on soybean leaves before treatment and the application of Bip5 conidiospores increased the number of *Metarhizium* sp. CFU g^{-1} leaf tissue significantly (Figure 2A; Table 2). CFU numbers decreased in Bip5 condiospore treated plots over time (slope differs from 0, t_5 = -3.1, $P < 0.05$), but the treatment effect persisted to the last sampling at day 29.

The insecticide Karate Zeon significantly reduced the number of Japanese beetle adults on the soybean plants during the peak flight period (Figure 2B; Table 2). The Bip5 conidiospore suspension and the surfactant control had a moderate effect on beetle abundance on the soybeans, but there was no additive effect of the Bip5 conidiospores above that of the surfactant alone (Table 2). The leaf damage score of the insecticide treated plots was significantly lower than for all other treatments, while the Bip5 conidiospore treatment and the surfactant control did not differ from the untreated control (Table 2). Damage rates remained at pre-infestation levels when the insecticide Karate Zeon was applied (Figure 2C; slopes do not differ from 0, t_5 = 0.9, $P > 0.1$). In contrast, leaf damage increased in all other treatments (slopes differ from 0, Bip5 conidiospore suspension, t_5 = 3.2, $P < 0.05$; surfactant control, t_5 = 7.6, $P < 0.001$; untreated control, t_5 = 4.0, $P < 0.05$). The Bip5 conidiospore application in the field had a strongly significant effect on the mortality of the beetles when incubated in the laboratory, in comparison with the surfactant control (Figure 2D; $F_{1,\ 37}$ = 11.5, $P < 0.01$).

3.3 Laboratory inoculation experiments

3.3.1 Mortality

In general, adult Japanese beetles were more susceptible than larvae to the application of conidio- or blastospores of Bip2, Bip5 and ART 212. The spray application of conidio- or blastospores of all three fungal strains did not affect the mortality of larvae over the 10 weeks of the experiment (Figures 3A, C; Table 3). In contrast, fungus-treated adults showed elevated mortality already seven days after infection, with a clear effect on day 14 (Figures 3B, D; Table 3). All mortality effects were due to the applied fungal spores, with the corresponding control treatments having no statistically significant effects (Table 3). Neither fungal strains nor spore types differed in the mortality rates provoked in adults or larvae (Table 3). Injecting blastospores directly into the insects significantly increased mortality rates of both adults and larvae compared to H_2O injection (Figure 3; Table 3). In all experiments, mortality differed between time intervals (Table 3). However, we found an interaction between all treatments with blasto-or conidiospores and time intervals only for adults and not for larvae (Table 3).

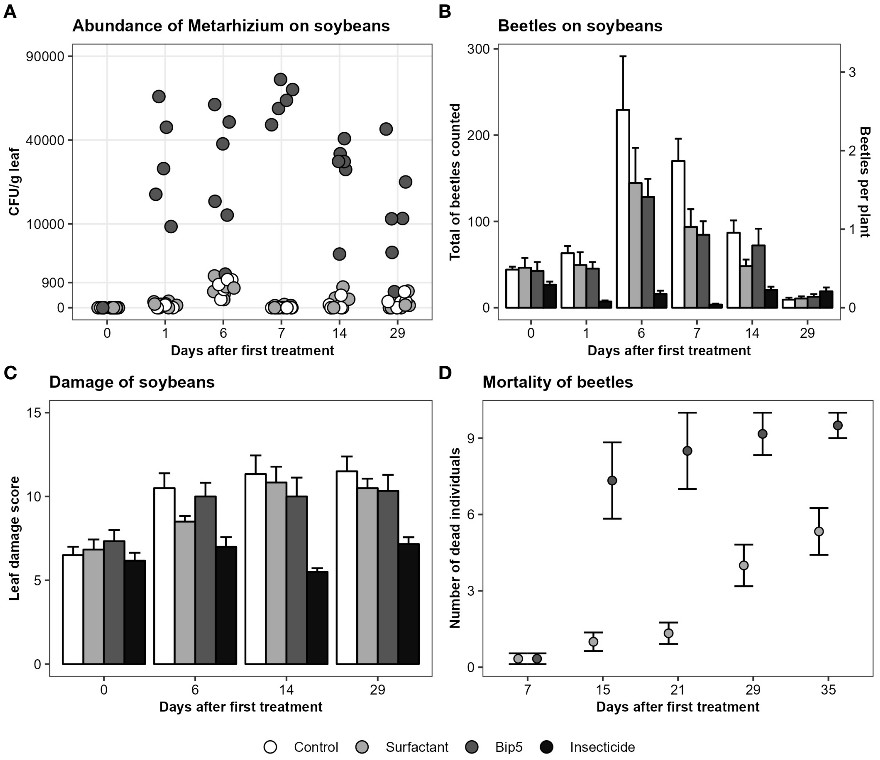


FIGURE 2
(A) *Metarhizium* CFU g^{-1} leaf tissue on the square root transformed y-axis, showing the mean values of the three samples per plot. **(B)** Mean and standard error of total number of beetles counted per plot. **(C)** Mean and standard error of leaf damage score per treatment. **(D)** Mortality of beetles (mean and standard error) collected from the experimental field after the first spray application and incubated in the laboratory. Bip5: Bip5 conidiospore suspension, Control: untreated control, Insecticide: Karate Zeon, Surfactant: surfactant control.

TABLE 2 Analysis of the soybean field experiment.

Dependent variable	Term	df	ddf	*F*	*P*
Log (CFU)					
	Bip5 ↔ Surfactant, Control	1	15	145	**<0.001**
	Control ↔ Surfactant	1	15	0.06	>0.5
Damage					
	X	1	18	1.04	>0.1
	Y	1	18	23.6	**<0.001**
	Insecticide ↔ all other treatments	1	18	29.0	**<0.001**
	Bip5 ↔ Surfactant ↔ Control	2	18	2.30	>0.1
Log (total beetles)					
	X	1	17	1.87	>0.1
	Y	1	17	47.6	**<0.001**
	X × Y	1	17	6.07	**<0.05**
	Insecticide ↔ all other treatments	1	17	156	**<0.001**
	Bip5, Surfactant ↔ Control	1	17	19.3	**<0.001**
	Bip5 ↔ Surfactant	1	17	0.04	>0.5

Bip5, Bip5 conidiospore suspension; Control, untreated control; Insecticide, Karate Zeon; Surfactant, surfactant control. In bold are the *P*-values showing significant differences.
Effect of the different treatments on the dependent variables Metarhizium sp. CFU g^{-1} leaf tissue, damage rating, and the total number of beetles on the soybeans. To disentangle the effects of the different treatments, we applied contrasts. For the analysis of the total number of beetles, we excluded data from day 29, because peak flight ended prior to that date.

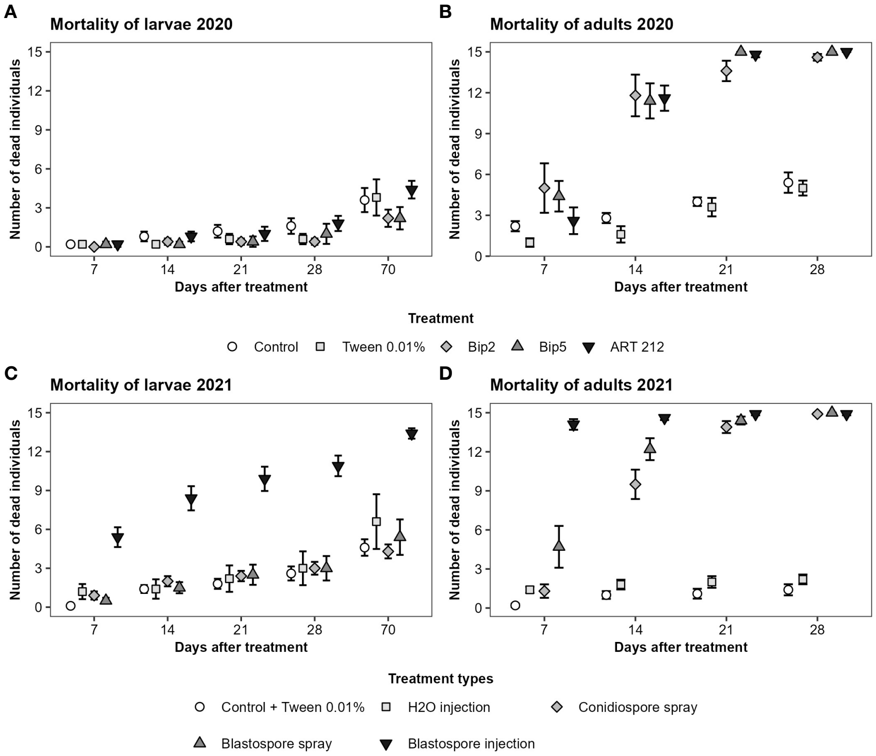


FIGURE 3

Mortality of Japanese beetle adults and larvae over time. The mortality was assessed over 4 weeks for adults and over 10 weeks for larvae at weekly time intervals. The figures show the mean number of dead individuals and the standard error. For larvae, results of the first 4 weeks and week 10 are displayed. **(A, B)** show the results from the experiments conducted in 2020 with the three fungal strains applied superficially as conidiospores. **(C, D)** show the results from the experiments conducted in 2021. Bip2 and Bip5 were statistically indistinguishable from each other; therefore, we show the aggregated data, broken down by spore type and application method (treatment types).

3.3.2 Mycosis

In the experiment in 2020, *Metarhizium* sp. were observed sporulating on one larval cadaver treated with Bip2 and three cadavers each treated with ART 212 and Bip5. *Beauveria* sp. did not sporulate on any larval cadavers. In contrast, all fungal strains were able to successfully sporulate on the cadavers of adult Japanese beetles. Bip2 (58 mycosed of 73 dead beetles) was slightly more successful in colonizing the treated adults than Bip5 (47 mycosed of 75 dead beetles) and ART 212 (43 mycosed of 75 dead beetles). We found *Beauveria* sp. on two cadavers of adults treated with ART 212. We did not find *Beauveria* sp. sporulating on any cadavers of adults in the control groups, but *Metarhizium* sp. were found on five adults treated with Tween 0.01%.

In the experiment in 2021, all control treatments together contained only three mycosed cadavers (one larva and two adults). One of those isolates was genetically identical with Bip5, the other two isolates differed genetically from Bip2 and Bip5. A few larval cadavers from the spray treatments (blasto- or conidiospores) showed mycosis of *Beauveria* sp. (10 cadavers) or *Metarhizium* sp. (16 cadavers; Supplementary Table 1). In contrast, most adult cadavers from the spray treatments were successfully colonized by the respective fungal strain (111 with *Metarhizium* sp., 133 *Beauveria* sp.; Supplementary Table 1). The results were similar for adults and larvae in the blastospore injection treatments: Bip2 and Bip5 grew on most of the cadavers of larvae and adults. SSR analysis revealed that the fungal isolates from the cadavers matched genetically with the applied fungal strains in most cases (Supplementary Table 1). We found only two unknown *Metarhizium* sp. strains, one each on an adult and larval cadaver treated with Bip5.

4 Discussion

Japanese beetle adults and larvae cause major damage in their invasive range. To efficiently protect valuable crops and grassland, a control strategy targeting both life stages is crucial. We tested the impact on Japanese beetle larvae in infested meadows of an application method using *M. brunneum* Bip5 that is well established for the control of scarab larvae native to Europe. In addition, we targeted adults feeding on a soybean field with Bip5 conidiospore spray applications. We found that Bip5 established and persisted in the soil of larval habitats and on the leaves of soybeans. However, neither larval nor adult population sizes were reduced by these treatments at the study sites. Subsequent laboratory experiments revealed that young third instar larvae were not susceptible to superficially applied blasto- or conidiospores of two *M. brunneum* (Bip5 and ART 212) and one *B. brongniartii* (Bip2) strain. In contrast, all spray applications of blasto- or conidiospores of the same strains increased the mortality of adults. Both life stages were susceptible to Bip5 and Bip2 when blastospores were injected directly into the hemolymph of the

TABLE 3 Analysis of the laboratory inoculation experiments. Effect of the different treatments on the mortality of Japanese beetle adults and larvae.

Term	Larvae				Adults			
Experiment 2020	df	ddf	*F*	*P*	df	ddf	*F*	*P*
Time								
Interval	9	178.2	2.88	**<0.01**	3	51.0	17.05	**<0.001**
Controls								
Control ↔ Tween 0.01%	1	16.1	0.18	>0.5	1	35.1	0.06	>0.5
Interval × Control ↔ Tween 0.01%	9	178.2	1.38	>0.1	3	49.6	0.84	>0.1
Fungal treatments								
Tween 0.01% ↔ Bip2, Bip5, ART 212	1	16.7	0.31	>0.5	1	31.1	45.27	**<0.001**
Bip2 ↔ Bip5 ↔ Art 212	2	15.6	0.55	>0.5	2	15.6	0.55	>0.5
Interval × Control, Tween 0.01% ↔ Bip2, Bip5, ART 212	27	178.2	0.94	>0.5	8	50.8	3.81	**<0.01**
Interval × Bip2 ↔ Bip5 ↔ Art 212	18	178.2	0.39	>0.5	5	51.0	1.76	>0.1
Experiment 2021	df	ddf	*F*	*P*	df	ddf	*F*	*P*
Time								
Interval	9	316.7	3.44	**<0.001**	3	83.5	27.03	**<0.001**
Control treatments								
Control ↔ H_2O injection ↔ Tween 0.01%	2	37.6	0.82	>0.1	2	92.6	0.16	>0.5
Interval x Control ↔ H_2O injection ↔ Tween 0.01%	18	315.7	1.04	>0.1	3	80.1	0.18	>0.5
Spray treatments								
Tween 0.01% ↔ Blastospores[1]	2	38.2	0.95	>0.1	2	34.9	30.12	**<0.001**
Time interval × Tween 0.01% ↔ Blastospores[1]	18	315.7	0.83	>0.5	6	81.4	4.24	**<0.001**
Tween 0.01% ↔ Condiospores[1]	2	41.7	0.53	>0.5	2	34.9	27.82	**<0.001**
Interval × Tween 0.01% ↔ Condiospores[1]	18	315.5	0.33	>0.5	6	81.3	3.59	**<0.01**
Blastospores[1] ↔ Conidiospores[1]	3	40.1	0.56	>0.5	3	23.9	0.08	>0.5
Interval x Blastospores[1] ↔ Conidiospores[1]	27	315.7	0.40	>0.5	9	82.3	4.10	**<0.001**
Injection treatments								
H_2O ↔ Blastospores[1]	2	26.4	10.13	**<0.001**	2	44.5	45.01	**<0.001**
Interval × H_2O ↔ Blastospores[1]	18	316.6	0.86	>0.5	5	80.8	0.14	>0.5
Blastospore application method								
Injection[1] ↔ Spray[1]	3	29.6	10.87	**<0.001**	3	28.4	4.36	**<0.05**
Interval × Injection ↔ Spray	27	316.2	0.94	>0.5	8	81.9	6.46	**<0.001**

[1] These terms include data from Bip2 and Bip5 of the respective application method or spore type. We did not perform separate analyses of the two fungal strains since their effects were statistically indistinguishable. In bold are the *P*-values showing significant differences.
We applied different contrasts to disentangle the effects of application methods, spore types, fungal strains, and time interval.

insects, which suggests that the cuticle is an important factor in determining the difference in susceptibility. We did not detect any differences in the virulence of the different fungal strains, species or spore types which shows the robustness of our findings. Our results suggest that larvae are in general resistant to the three commercially available fungal strains tested here. Thus, we do not see great potential in attempting to adapt to Japanese beetle larvae the state-of-the-art control of native scarabs. However, adults are susceptible to all three fungal strains. Hence, the control of adults with entomopathogenic fungi appears to be more promising.

In our meadow field experiments, we found evidence for good establishment and persistence of Bip5 in the soil, but only a marginal effect of Bip5 FCBK treatment on the abundance of *P. japonica* larvae. This is in contrast with results of Ramoutar et al. (52) and Behle et al. (21), who found clear, albeit variable, control effects when applying Bip5 to turf in well-controlled small-scale field trials. In addition, FCBK application at even lower doses has proven effective in the control of native grubs, and the method is well established (34). Two main factors are probably crucial for the success of the studies cited above. First, clear control effects on white grubs of either Japanese beetle, cockchafer (*Melolontha* spp.), June beetle (*Amphimallon* spp.) or garden chafer (*P. horticola*) were usually obtained when experiments were conducted in moist environments. Additionally, high soil temperature in summer promotes fungal growth and larval infection (53). Furthermore, an intermediate level of moisture and high temperatures have been shown to be most favorable for the infection of *P. japonica* larvae by *Metarhizium anisopliae* (41), while infectiveness is reduced under dry conditions (54). Second, the effectiveness of the fungal treatments is affected by the larval stage targeted (55) with greatest success reported for measures targeting first or second instar larvae (21). We conclude that the optimal setting for the control of *P. japonica* larvae with entomopathogenic fungi of the genera *Metarhizium* are moist soils during summer months, when early larval instars are present, and larvae are actively feeding directly below the sod.

These conditions were not met in our studies. In our first field experiment, Bip5 FCBK were applied in autumn and the soil was moist during the winter season due to frequent rainfall. However, most larvae were in their third instar and the activity of both *P. japonica* larvae and Bip5, was limited by low soil temperatures. In our second field experiment, we applied Bip5 FCBK in May to target eggs and young larval instars. The experimental site received very limited summer precipitation, and the resulting dry soils diminished the efficacy of Bip5. We therefore argue that in the actual infested zone in continental Europe, optimal conditions for entomopathogenic fungi to infect *P. japonica* larvae are rarely met, since moist soils, high temperatures and susceptible larval stages do not coincide.

The control of Japanese beetle adults in the infested zone in northern Italy relies upon insecticide spraying, leaving organic farmers without effective control measures against the invasive pest (phytosanitary service Piedmont, pers. comm.). The spraying of entomopathogenic fungi would offer an environmentally friendly option for organic farmers to protect their crops. Our study shows that Bip5 conidiospores can be applied effectively to soybean plants and that infective propagules persist on leaves over the entire flight period of the Japanese beetle, despite exposure to high temperatures and strong solar radiation. However, Bip5 conidiospore treatment was not more effective than the spore-free surfactant control. The effectiveness of these two treatments averaged around 20–30% (compared to around 70% for the Karate Zeon insecticide treatment; effectiveness calculated according to Abbott; 56), and likely is due to the surfactant in the formulation (57). The lack of an effect of Bip5 conidiospore suspension on the abundance of beetles in the plots was in stark contrast to the difference in the mortality of beetles collected from Bip5 conidiospore treated and surfactant control plots (Figure 2D). One explanation may be that a stronger or more rapid effect of the surfactant masked the effect Bip5. The relatively slow action of Bip5 also comes as a disadvantage in comparison to the insecticide Karate Zeon, which caused an immediate knock down of the beetles, with effects that lasted throughout the entire flight period. To accelerate the speed of kill of Bip5 conidiospore treatments, more spores need to come in contact with the beetles, since time-to-death is directly correlated with spore dose (unpublished data; 28). One solution might therefore be to increase the spore concentration of the applied suspension, or to spray repeatedly at shorter time intervals.

Soybean infestation levels in our experimental field were generally low, with an average of less than three beetles per plant. Leaf damage did not exceed one fully skeletonized leaf per plant, even in control plots. This damage level would not have justified the application of an insecticide against Japanese beetles (14), and it may be that effects of the Bip5 conidiospore treatment would have been more readily detectable under a higher insect infestation level.

Our laboratory experiments showed that spray applications of blasto- or conidiospores caused high mortality in adults but not in larvae, independent of the fungal strain. This contrasts previous studies (40, 41, 58); for example, Giroux et al. (40) found no difference in mortality between adults and larvae of Japanese beetle. However, that work used extremely high spore doses (40), which were 8000 times higher than those used here (10^7 spores mL^{-1}, which is commonly used in bioassays). In other laboratory studies, mycelial particles were added to soil as source of infection, resulting in elevated mortality of larvae caused by the applied fungi (41, 58), which contrasts with our findings. To the best of our knowledge, our study is thus the first that reports striking differences in the susceptibility of Japanese beetle adults and larvae to *M. brunneum* and *B. brongniartii*. There is, however, evidence from studies on other insect species that susceptibility to fungal infection differs between developmental stages, especially when larvae and adults do not share the same habitat and are consequently not equally exposed to entomopathogenic fungi (59, 60). Based on these findings, we hypothesize that developmental stages with long periods of exposure to soil-born fungal pathogens show higher resistance to infection than stages that are exposed for only a short time. In Japanese beetles, adults are exposed to attack by soil-borne pathogens during only a very short time at emergence, and in females during oviposition. In contrast, larval stages are exposed for more than half a year, between egg hatch and pupation. It follows that defense mechanisms in the latter should be stronger than in short-lived adults with limited exposure to soil-borne pathogens.

Results from our injection experiments provide evidence that this may be true at least for cuticular defense mechanisms in *P. japonica*. When we injected blastospores of Bip5 and Bip2 directly into the insects to override cuticular defense, we were able to infect both adults and larvae. This indicates that larvae possess a cuticle that protects them efficiently against fungal attack, while cuticular defense mechanisms appear to be negligible in adults. This may seem unexpected, since the heavily sclerotized cuticle of adult beetles appears very robust when compared to the soft-bodied larvae. Several studies have shown that *M. anisopliae* and other entomopathogenic fungi have difficulties penetrating thick and highly sclerotized areas of the integument of other beetle species and cicadas (22, 59, 61). However, spores of *M. anisopliae* preferably attach to the intersegmental membranes and around the setae of locusts (62), and *Beauveria bassiana* presumably penetrates *Tribolium castaneum* larvae through intersegmental membranes (59). Based on these findings, we hypothesize that the intersegmental membranes of adult Japanese beetles may be more prone to fungal attack than the less sclerotized but generally more robust cuticle of their larvae.

However, our results (Figure 3) suggest that cuticular defense mechanisms only partially explain the difference in susceptibility of adults and larvae. Larvae were still more resistant to Bip5 and Bip2 than adults, even when blastospores were injected. Adults reached more than 90% mortality after 7 days. In contrast, only around 30% of the larvae were dead after this period, and mortality averaged around 90% after 10 weeks only. This suggests that larvae are not only better protected by their cuticle but also possess more efficient internal defense mechanisms than adults.

Overall, we were able to successfully establish high numbers of *Metarhizium* sp. CFU in the soil by applying Bip5 FCBK. However, these did not effectively control Japanese beetle larvae in the field. Our results were similar in two independent field sites at two different dates, thus encompassing potential differences in susceptibility of *P. japonica* according to the larval stage. We are therefore confident that this finding is robust. We thus cannot recommend Bip5 FCBK application against Japanese beetle larvae in the infested zone in northern Italy as it is used against its native relatives in Europe. While there clearly is room for improvement by adopting more virulent and better-adapted fungal strains (63), and by optimizing their field application, our laboratory experiments indicate that Japanese beetle larvae are generally resistant to entomopathogenic fungi. This conclusion is in line with a literature review of laboratory and field experiments that found that the control of Japanese beetle larvae with entomopathogenic fungi is erratic and thus not recommended (13). Other environmentally friendly alternatives such as the application of entomopathogenic nematodes may be more promising for the control of *P. japonica* larvae (20).

In contrast, the control of adult Japanese beetles with entomopathogenic fungi appears more promising. We found high susceptibility of adult beetles in laboratory settings to Bip2, Bip5 and ART 212. Furthermore, we found that Bip5 can be used in foliar sprays on crops with conidiospores persisting under high UV radiation and heat. Although we did not find clear effects of the Bip5 conidiospore suspension on Japanese beetle abundance or crop damage in a soybean field, we were able to prove Bip5 infections in field-collected adults after application. More efficient spraying techniques or alternative spore dissemination strategies, such as attract and infest approaches (64, 65), may lead to a greater impact on adult *P. japonica* populations. In field experiments in which *M. anisopliae* treated and untreated adult Japanese beetles were released, a significantly lower number of treated individuals were recaptured compared to the control group (66). Furthermore, adults that are attracted to traps equipped with *Metarhizium* spp. conidiospores as inoculum are contaminated with a sufficient spore dose to cause and increase mortality (64, 65). Those contaminated beetles spread the spores to non-infected conspecifics (64, 65), further increasing fungal disease within the population. We suggest that it would be valuable to further test these attract and infest methods in regions with low Japanese beetle population densities to assess whether it may serve as a tool to reduce the flying population and, consequently, further spread of this invasive pest. We conclude that the application of entomopathogenic fungi can be an important tool in an integrated pest management strategy targeted against *P. japonica* adults but not against larvae.

Data availability statement

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

Author contributions

TG: conceptualization, methodology, investigation, statistical analysis and writing original draft. FS: investigation and formal analysis. PN: statistical analysis and writing. GG: funding acquisition, project administration, conceptualization, and writing. All authors contributed to the article and approved the submitted version.

Funding

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 861852, and an innovative research activity grant of Agroscope Open access funding by Agroscope.

Acknowledgments

Special thanks to Giovanni Bosio (phytosanitary service of Piedmont) for enabling the field experiments by providing us with field sites, sharing his knowledge, assisting with data collection and technical support. We thank Christian Schweizer (technician Extension Arable Crops, Agroscope) for technical support and valuable advice. Furthermore, we thank the entomology team of the Extension Arable Crops group for assisting in labor-intensive sampling.

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.

Since GG is topic editor for the P. japonica research topic, an independent associated editor of FIS was selected to manage the review process.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/finsc.2023.1138427/full#supplementary-material

References

1. European Commission. Commission delegated Regulation (EU) 2019/1702 *Off J Eur Union L* (2019) 260(8):8-10.

2. Eppo. *Eppo global database, popillia japonica* (2002). Available at: https://gd.eppo.int/taxon/POPIJA (Accessed May 19, 2022).

3. eCFR. *7 CFR part 301 subpart f, Japanese beetle* (2019). Available at: https://www.ecfr.gov/current/title-7/subtitle-B/chapter-III/part-301/subpart-F (Accessed May 19, 2022).

4. Fleming WE. *Biology of the Japanese beetle*. D.C., Washington: USDA tech (1972). p. 1449.

5. Kistner-Thomas EJ. The potential distribution and voltinism of the Japanese beetle (Coleoptera: scarabaeidae) under current and future climates. *J Insect Sci* (2019) 9(2):1–13. doi: 10.1093/jisesa/iez023

6. Pavesi M. *Popillia japonica* specie aliena invasiva segnalata in lombardia. *L'Informatore Agrario* (2014) 32:53–5.

7. Servizio fitosanitario Lombardia. *Popillia o scarabeo giapponese (Popillia japonica)* (2022). Available at: https://fitosanitario.regione.lombardia.it/wps/portal/site/sfr/DettaglioRedazionale/organismi-nocivi/insetti-e-acari/popillia-japonica (Accessed 10.10.2022).

8. Servizio fitosanitario Piemonte. *Lotte obbligatorie- coleottero scarabeide del giappone (Popillia japonica Newman)* (2022). Available at: https://www.regione.piemonte.it/web/temi/agricoltura/servizi-fitosanitari-pan/lotte-obbligatorie-coleottero-scarabeide-giappone-popillia-japonica-newman (Accessed 10.10.2022).

9. Servizio fitosanitario Ticino. *Coleottero giapponese (Popillia japonica)* (2022). Available at: https://www4.ti.ch/dfe/de/servizio-fitosanitario/organismi/coleottero-giapponese-popillia-japonica/.

10. Potter DA, Braman SK. Ecology and management of turfgrass insects. *Annu Rev Entomol* (1991) 36:383–406. doi: 10.1146/annurev.en.36.010191.002123

11. Potter DA. *Destructive turfgrass insects: biology, diagnosis, and control*. Chelsea MI: Ann Arbor Press (1998).

12. Redmond CT, Potter DA. Incidence of turf-damaging white grubs (Coleoptera: scarabaeidae) and associated pathogens and parasitoids on Kentucky golf courses. *Environ Entomol* (2010) 39(6):1838–47. doi: 10.1603/EN10172

13. Potter DA, Held DW. Biology and management of the Japanese beetle. *Annu Rev Entomol* (2002) 47:175–205. doi: 10.1146/annurev.ento.47.091201.145153

14. Shanovich HN, Dean AN, Koch RL, Hodgson EW. Biology and management of Japanese beetle (Coleoptera: scarabaeidae) in corn and soybean. *J Integr Pest Manag* (2019) 10(1):1–14. doi: 10.1093/jipm/pmz009

15. USDA APHIS. *Managing the Japanese beetle: a homeowner's handbook*. United States Department of Agriculture (USDA) (2015). doi: 10.1111/j.1365-2338.2006.01039.x.

16. Main AR, Webb EB, Goyne KW, Mengel D. Neonicotinoid insecticides negatively affect performance measures of non-target terrestrial arthropods: a meta-analysis. *Ecol Appl* (2018), 28(5)1232–44. doi: 10.1002/eap.1723

17. Chagnon M, Kreutzweiser D, Mitchell EA, Christy A. Morrissey CA, Noome DA, van der Sluijs JP. Risks of large-scale use of systemic insecticides to ecosystem functioning and services. *Environ Sci Pollut Res* (2015) 22:119–34. doi: 10.1007/s11356-014-3277-x

18. Siviter H, Muth F. Do novel insecticides pose a threat to beneficial insects? *Proc R Soc B* (2020) 287:20201265. doi: 10.1098/rspb.2020.1265

19. Felming WE. Integrating control of the Japanese beetle – a historical review. *USDA tech* (1976), 1545.

20. Paoli F, Marianelli L, Binazzi F, Mazza G, Benvenuti C, Peverieri G, et al. Effectiveness of different doses of *Heterorhabditis bacteriophora* against *Popillia japonica* 3rd instars: laboratory evaluation and field application. *Redia* (2017) 100:135–8. doi: 10.19263/REDIA-100.17.17

21. Behle RW, Richmond DS, Jackson MA, Dunlap CA. Evaluation of *Metarhizium brunneum* F52 (Hypocreales: clavicipitaceae) for control of Japanese beetle larvae in turfgrass. *J Econ Entomol* (2015) 108(4):1587–95. doi: 10.1093/jee/tov176

22. Hajek AE, Leger RJ. Interaction between fungal pathogens and insect hosts. *Annu Rev Entomol* (1994) 39:293–322. doi: 10.1146/annurev.en.39.010194.001453

23. Kabaluk JT, Svircev AM, Goettel MS, Woo SG. *The use and regulation of microbial pesticides in representative jurisdictions worldwide* (2010). IOBC Global. Available at: www.iobc-global.org (Accessed May 19, 2022).

24. Lacey LA, Grzywacz D, Shapiro-Ilan DI, Frutos R, Brownbridge M, Goettel MS. Insect pathogens as biological control agents: back to the future. *J Invert. Path* (2015) 132:1–41. doi: 10.1016/j.jip.2015.07.009

25. de Faria MR, Wraight SP. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. *Biol Contl.* (2007) 43:237–56. doi: 10.1016/j.biocontrol.2007.08.001

26. Thakur N, Kaur S, Tomar P, Thakur S, Yadav AN. Microbial biopesticides: current status and advancement for sustainable agriculture and environment. In: Rastegari AA, Yadav AN, Yadav N, editors. *New and future developments in microbial biotechnology and bioengineering*. Elsevier (2020). p. 243–82.

27. Ortiz-Urquiza A, Keyhani NO. Action on the surface: entomopathogenic fungi versus the insect cuticle. *Insects* (2013) 4(3):357–74. doi: 10.3390/insects4030357

28. Ansari MA, Vestergaard S, Tirry L, Moens M. Selection of a highly virulent fungal isolate, *Metarhizium anisopliae* CLO 53, for controlling *Hoplia philanthus*. *J Invertebr. Pathol* (2004) 85(2):89–96. doi: 10.1016/j.jip.2004.01.003

29. Nong X, Liu C, Lu X, Wang Q, Wang G, Zhang Z. Laboratory evaluation of entomopathogenic fungi against the white grubs, *Holotrichia oblita* and *Anomala corpulenta* (Coleoptera: scarabaeidae) from the field of peanut, arachis hypogaea. *Biocontrol Sci Technol* (2011) 21(5):593–603. doi: 10.1080/09583157.2011.566324

30. Zhang C, Xia Y. Identification of genes differentially expressed *in vivo* by *Metarhizium anisopliae* in the hemolymph of *Locusta migratoria* using suppression-subtractive hybridization. *Curr Genet* (2009) 55:399–407. doi: 10.1007/s00294-009-0254-x

31. Schrank A, Vainstein MH. *Metarhizium anisopliae* enzymes and toxins. *Toxicon* (2010) 56(7):1267–74. doi: 10.1016/j.toxicon.2010.03.008

32. Moino AJ, Alves SB, Lopes RB, Neves PMOJ, Pereira RM, Vieira SA. External development of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in the subterranean termite *Heterotermes tenuis*. *Sci Agric* (2002) 59(2):267–73. doi: 10.1590/s0103-90162002000200010

33. Keller S, Schweizer C, Keller E, Brenner H. Control of white grubs (*Melolontha melolontha* l.) by treating adults with the fungus *Beauveria brongniartii*. biocontrol sci. *Technol* (1997) 7(1):105–16. doi: 10.1080/09583159731090

34. Keller S, Schweizer C. Engelingsbekämpfung mit pilzen. *Mitt. Dtsch Ges. allg. angew. Entomol* (2008) 16:361–4.

35. Fornallaz C. Demökologische grundlagen und praktische durchführung der mikrobiellen bekämpfung des maikäfers *Melolontha melolontha* l. mit dem pilz *Beauveria brongniartii* (Sacc.) petch. *Diss. ETH* (1992), 9736. doi: 10.3929/ethz-a-000643446

36. Aregger-Zavadil E. Grundlagen zur autökologie und artspezifität des pilzes *Beauveria brongniartii* (SACC.) petch als pathogen des maikäfers (*Melolontha melolontha* l.). *Diss. ETH* (1992), 9735. doi: 10.3929/ethz-a-000643444

37. Kessler P, Matzke H, Keller S. The effect of application and soil factors on the occurrence of *Beauveria brongniartii* applied as a biological control agent in soil. *J Invertebr. Pathol* (2003) 84:15–23. doi: 10.1016/j.jip.2003.08.003

38. Kessler P, Enkerli J, Schweizer C, Keller S. Survival of *Beauveria brongniartii* in the soil after application as a biocontrol agent against the European cockchafer *Melolontha melolontha*. *BioControl* (2004) 49:563–81. doi: 10.1023/B:BICO.0000036441.40227.ed

39. Behle RW, Goett EJ. Dosage response mortality of Japanese beetle, masked chafer, and June beetle (Coleoptera: scarabaeidae) adults when exposed to experimental and commercially available granules containing *Metarhizium brunneum*. *J Econ Entomol* (2016) 109(3):1109–15. doi: 10.1093/jee/tow080

40. Giroux F, Lavallee R, Bauce E, Guertin C. Susceptibility of the Japanese beetle, *Popillia japonica* (Newman) (Coleoptera: scarabaeidae), to entomopathogenic hypocreales fungi. *Phytoprot* (2015) 95:1–6. doi: 10.7202/1028399ar

41. Villani MG, Krueger SR, Schroeder PC, Consolie F, Consolie NH, Preston-Wilsey LM, et al. Soil application effects of *Metarhizium anisopliae* on Japanese beetle (Coleoptera: scarabaeidae) behavior and survival in turfgrass microcosms. *Environm. Entomol* (1994) 23(2):502–13. doi: 10.1093/ee/23.2.502

42. Lacey LA, Martins A, Ribeiro C. The pathogenicity of *Metarhizium anisopliae* and *Beauveria bassiana* for adults of Japanese beetle, *Popillia japonica* (Coleoptera: scarabaeidae). *Eur J Entomol* (1994) 91:313–9.

43. Strasser H, Pernfuss B, Abendstein D, Stuppner H. *Pathogenicity of hyphomycetes against melolontha spp.* Innsbruck: BIPESCO Project, Final Report (2003) p. 1–10.

44. European Food Safety Authority. Conclusion on the peer review of the pesticide risk assessment of the active substance *Metarhizium anisopliae* var. *anisopliae* BIPESCO 5/F52. *EFSA J* (2012) 10(1):2498. doi: 10.2903/j.efsa.2012.2498

45. Strasser H, Forer A, Schinner F. Development of media for the selective isolation and maintenance of virulence of beauveria brongniartii. In: Jackson TA, Glare TR, editors. *Proc. 3rd international workshop on microbial control of soil dwelling pests* (Lincoln, New Zealand) (1996). p. 125–30.

46. Riba G, Ravelojoana AM. The parasexual cycle in the entomopathogenic fungus *Paecilomyces fumosoroseus* (Wize) brown and smith. *Can J Microbiol* (1984) 30(7):922–6. doi: 10.1139/m84-144

47. Enkerli J, Widmer F, Gessler C, Keller C. Strain-specific microsatellite markers in the entomopathogenic fungus *Beauveria brongniartii*. *Mycol Res* (2001) 105 (9):1079–87. doi: 10.1016/S0953-7562(08)61970-X

48. Mayerhofer J, Lutz A, Widmer F, Rehner SA, Leuchtmann A, Enkerli J. Multiplexed microsatellite markers for seven *Metarhizium* species. *J Invertebr. Pathol* (2015) 132:132–4. doi: 10.1016/j.jip.2015.09.006

49. Oulevey C, Widmer F, Kölliker R, Enkerli J. An optimized microsatellite marker set for detection of *Metarhizium anisopliae* genotype diversity on field and regional scales. *Mycol Res* (2009) 113(9):1016–24. doi: 10.1016/j.mycres.2009.06.005

50. Mayerhofer J, Enkerli J, Zelger R, Strasser H. Biological control of the European cockchafer: persistence of *Beauveria brongniartii* after long-term applications in the euroregion tyrol. *BioControl* (2015) 60(5):617–29. doi: 10.1007/s10526-015-9671-6

51. Fernández-Bravo M, Gschwend F, Mayerhofer J, Hug A, Widmer F, Enkerli J. Land-use type drives soil population structures of the entomopathogenic fungal genus *Metarhizium*. *Microorganisms* (2021) 9:1380. doi: 10.3390/microorganisms9071380

52. Ramoutar D, Legrand AI, Aim SR. Field performance of *Metarhizium anisopliae* against *Popillia japonica* (Coleoptera: scarabaeidae) and *Listronotus maculicollis* (Coleoptera: curculionidae) larvae in turfgrass. *J Entomol Sci* (2009) 45(1):20–6. doi: 10.18474/0749-8004-45.1.20

53. Strasser H, Erschbamer M. Effect of temperature on conidia germination and vegetative growth of *Metarhizium anisopliae*. *IOBC wprs Bull* (2003) 26(1):117–20.

54. Jaronski ST. Soil ecology of the entomopathogenic ascomycetes: a critical examination of what we (think) we know. In: Ekesi S, Maniania NK, editors. *Use of entomopathogenic fungi in biological pest management* (Kerala, India:Research Signpost) (2007). p. 1–53.

55. Keller S, Lomer CJ. "The *BeauveriaMelolontha* project: experiences with regard to locust and grasshopper control". *Biol Control Locusts Grasshoppers ed* (Wallingford. UK: CAB International) (1992) 279-286.

56. Abbott WS. A method of computing the effectiveness of an insecticide. *J Econ Entomol* (1925) 18:265–7. doi: 10.1093/jee/18.2.265a

57. Kaiser D, Handschin S, Rohr RP, Bacher S, Grabenweger G. Co-Formulation of *Beauveria bassiana* with natural substances to control pollen beetles – synergy between fungal spores and colza oil. *Biol Contr* (2019) 140:104106. doi: 10.1016/j.biocontrol.2019.104106

58. Krueger SR, Villani MG, Nyrop JP, Roberts DW. Effect of soil environment on the efficacy of fungal pathogens against scarab grubs in laboratory bioassays. *Biol Contr* (1991) 1:203–9. doi: 10.1016/1049-9644(91)90068-B

59. Baek S, Noh M, Mun S, Lee S, Arakane Y, Kim J. Ultrastructural analysis of beetle larva cuticles during infection with the entomopathogenic fungus, *Beauveria bassiana*. *Pest Manage Sci* (2022) 78:3356–64. doi: 10.1002/PS.6962

60. Kim H, Jeong S, Choi I, Yang J, Lee H, Kim J, et al. Mechanisms of insecticidal action of *Metarhizium anisopliae* on adult Japanese pine sawyer beetles (*Monochamus alternatus*). *Amer. Chem Soci* (2020) 5:25312–8. doi: 10.1021/acsomega.0c03585

61. Hänel H. The life cycle of the insect pathogenic fungus *Metarhizium anisopliae* in the termite nasutitermes exitiosus. *Mycopathologia* (1982) 80:137–45. doi: 10.1007/BF00437576

62. Zhang X, Yang Y, Zhan L. Ultrastructure of the entomopathogenic fungus *Metarhizium anisopliae* during cuticle penetration in the locust, *Locusta migratoria* (Insecto: acridida). *J Orth. Res* (2010) 19(1):115–9. doi: 10.1665/034.019.0117

63. Barzanti GP, Enkerli J, Benvenuti C, Strangi A, Mazza G, Torrini G, et al. Genetic variability of *Metarhizium* isolates from northern Italy as a possible resource for *Popillia japonica* natural control. *J Invertebrate Pathol* (2023) :107891. doi: 10.2139/ssrn.4220991

64. Klein MG, Lacey LA. An attractant trap for autodissemination of entomopathogenic fungi into populations of the Japanese beetle *Popillia japonica* (Coleoptera: scarabaeidae). *Biocontr. Sci Techn* (1999) 9(2):151–8. doi: 10.1080/09583159929730

65. Benvenuti C, Barzanti GP, Marianelli L, Sabbatini Peverieri G, Paoli F, Bosio G, et al. A new device for auto-disseminating entomopathogenic fungi against popillia japonica: a study case. *Bull Insectol* (2019) 72(2):219–25.

66. Lacey LA, Amaral JJ, Coupland J, Klein MG, Simoes AM. Flight activity of popillia japonica (Coleoptera: scarabaeidae) after treatment with metarhizium anisopliae. *Biol Contr* (1995) 5:157–72. doi: 10.1006/bcon.1995.1020

Frontiers in Insect Science

TYPE Original Research
PUBLISHED 12 May 2023
DOI 10.3389/finsc.2023.1151789

OPEN ACCESS

EDITED BY
Deguang Liu,
Northwest A&F University, China

REVIEWED BY
Kanakachari Mogilicherla,
Czech University of Life Sciences Prague,
Czechia
Dong Wei,
Southwest University, China

*CORRESPONDENCE
L. Adriana Avila
adriana.avila@auburn.edu

[†] These authors have contributed equally to this work and share first authorship

RECEIVED 26 January 2023
ACCEPTED 28 April 2023
PUBLISHED 12 May 2023

CITATION
Carroll E, Kunte N, McGraw E, Gautam S, Range R, Noveron-Nunez JA, Held DW and Avila LA (2023) Gene silencing in adult *Popillia japonica* through feeding of double-stranded RNA (dsRNA) complexed with branched amphiphilic peptide capsules (BAPCs).
Front. Insect Sci. 3:1151789.
doi: 10.3389/finsc.2023.1151789

Gene silencing in adult *Popillia japonica* through feeding of double-stranded RNA (dsRNA) complexed with branched amphiphilic peptide capsules (BAPCs)

Elijah Carroll[1†], Nitish Kunte[2†], Erin McGraw[2], Sujan Gautam[2], Ryan Range[2], Jose A. Noveron-Nunez[2], David W. Held[2] and L. Adriana Avila[1*]

[1]Department of Entomology and Plant Pathology, Auburn University, Auburn, AL, United States,
[2]Department of Biological Sciences, Auburn University, Auburn, AL, United States

Gene silencing by feeding double-stranded (dsRNA) holds promise as a novel pest management strategy. Nonetheless, degradation of dsRNA in the environment and within the insect gut, as well as inefficient systemic delivery are major limitations to applying this strategy. Branched amphiphilic peptide capsules (BAPCs) complexed with dsRNA have been used to successfully target genes outside and inside the gut epithelium upon ingestion. This suggests that BAPCs can protect dsRNA from degradation in the gut environment and successfully shuttle it across gut epithelium. In this study, our objectives were to 1) Determine whether feeding on BAPC-dsRNA complexes targeting a putative peritrophin gene of *P. japonica* would result in the suppression of gut peritrophin synthesis, and 2) gain insight into the cellular uptake mechanisms and transport of BAPC-dsRNA complexes across the larval midgut of *P. japonica*. Our results suggest that BAPC-dsRNA complexes are readily taken up by the midgut epithelium, and treatment of the tissue with endocytosis inhibitors effectively suppresses intracellular transport. Further, assessment of gene expression in BAPC- peritrophin dsRNA fed beetles demonstrated significant downregulation in mRNA levels relative to control and/or dsRNA alone. Our results demonstrated that BAPCs increase the efficacy of gene knockdown relative to dsRNA alone in *P. japonica* adults. To our knowledge, this is the first report on nanoparticle-mediated dsRNA delivery through feeding in *P. japonica*.

KEYWORDS

Japanese beetle, *Scarabaeidae*, dsRNA (double-stranded RNA), pest management, nanoparticles, oral delivery

1 Introduction

Invasive species account for significant ecological and economic impacts (1, 2). In 1916, a small, metallic-colored beetle from Japan, *Popillia japonica* Newman, was first detected near Riverton, NJ, USA. *P. japonica* is currently established in 28 states in the US, and continues to expand its range west and north in North America into previously non-infested states, territories, and provinces, likely through human-mediated transport (3, 4). The polyphagous nature, feeding on >300 plant species, and capable of forming large feeding aggregations on host plants (5, 6) are key factors in the success of *P. japonica* adults as pests in the extended geographic range. This species is a target for substantial insecticide usage in both larval and adult life stages, especially in areas with large monocultures of turfgrass such as roadsides, golf courses, and urban landscapes (6). Insecticide use targeting *P. japonica* adults and larvae are associated with secondary pest outbreaks (7) and interference with host finding by introduced natural enemies (8).

Targeting insect pests, especially beetles, with double-stranded ribonucleic acid (dsRNA) molecules has shown great promise as an alternative to chemical insecticides (9–11). Exogenous dsRNA activates the RNA interference (RNAi) pathway, which is a conserved and innate biological defense mechanism in eukaryotic organisms against viruses and transposons *via* post-transcriptional gene silencing (12). Unlike common chemical insecticides, dsRNAs are designed to target mRNA sequences unique to the target pest because of the necessity to have high sequence fidelity for gene silencing to occur (9). Furthermore, dsRNA has a low potential for persistence in the environment, including soil, sediment, and surface water compartments, because of its instability in environmental conditions and rapid microbial degradation (9, 13, 14).

The most field-applicable route of dsRNA delivery is *via* ingestion by the target insect (15). So far, the only commercially available dsRNA product for insect control is facilitated by genetically modified plants (10). However, these products involve plant transformation, which is not feasible for all plants/crops due to the cost and time of production, and extensive regulatory processes to evaluate environmental risk (16). Thus, exogenously applied products in the form of bio-pesticides may be a more feasible and cost-effective method for pest attacking multiple plant/cropping systems. The general use of dsRNA as an insecticide has been forestalled by the variability in efficacy of RNAi among species, life stage, dosage, delivery method, and target gene (15, 17–19). The observed variability in efficacy may be contributed to degradation of dsRNA in the environment and in the insect gut, inefficient uptake by the gut epithelium, defective RISC complex, and impaired systemic delivery (15, 20–22).The enzymes present in body fluids of *P. japonica* are highly efficient at degradation of dsRNA relative to other beetles that have been successfully targeted (23). Hence, it is imperative to provide a protectant to dsRNA for silencing effects to occur.

Nanoparticles can help to overcome the technical challenges associated with the oral delivery and efficiency of dsRNA. Nanoparticles are typically defined as particles ranging between 1 and 100 nm in size made of a variety of materials (i.e., lipids, peptides, polymers and metals) (15, 24). In most cases, nanoparticle/dsRNA complexes are formed by electrostatic interactions between the cationic groups of the nanoparticles and the negatively charged phosphate groups of dsRNA (25). Nanoparticles can prevent degradation of the dsRNA nucleotides by nucleases in the salivary glands and in the gut by blocking target sites for RNases (26, 27). Furthermore, the overall net charge of the complexes are typically positive, which is suitable for uptake by cell membranes (25, 28). Our research team developed branched amphiphilic peptide capsules (BAPCs) (25). BAPCs are formed through the spontaneous assembly of two branched amphiphilic peptides, bis(Ac-FLIVI)–K–K_4–$CONH_2$ and bis(Ac-FLIVIGSII)–K–K_4–$CONH_2$in water (29). Ingestion of BAPC-dsRNA complexes targeting a major genes associated with the unfolded protein response resulted in significant knockdown of gene expression levels and mortality rates in the red flour beetle (*Tribolium castaneum*) and in the pea aphid (*Acyrthosiphon pisum*) (25). The properties of BAPCs also make the synthesis scalable to large scale production as they can be stored for extended periods, self-assemble in pure water, and are effective at low μM concentrations.

In this study, BAPC-dsRNA complexes were evaluated for their efficiency in 1) knockdown of the peritrophin expression and subsequent mortality post-ingestion in adult *P. japonica*, and 2) uptake and transport across the larval midgut epithelial cells. Silencing of peritrophin can make insect gut more susceptible to insecticides, phytochemicals and pathogens affecting their metabolism, growth, development, and survival (30). We also analyzed BAPCs and BAPC-dsRNA size in a buffered solution with pH ~7.4 (similar to *P. japonica* midgut). Finally, we explored the cellular mechanism for uptake of BAPC-dsRNA complexes and transport across larval midgut (30). According with the literature review, this is the first report on gene knockdown in adult *P. japonica* using nanoparticle-mediated dsRNA delivery.

2 Methods

2.1 Specimens

Larvae of *P. japonica* were sourced from a commercial sod farm near Murfreesboro, TN and collected in April 2022. Larvae used for midgut assays were maintained by placing them in individual cells of ice cube trays and fed with carrot strips. In July 2022, field collected, adult female *P. japonica* used in the dsRNA feeding trials were shipped overnight from Michigan State University. The beetles were maintained in a container with sifted soil and fed on a diet of crape myrtle (*Lagerstroemia indica*) and rose (*Rosa* spp.) flowers. Female and male beetles were separated based on the morphology of the tibial spur and only insects that were free of obvious morphological defects or injuries were used in experiments (5).

2.2 Preparation of BAPCs and BAPC-dsRNA complexes

To form the BAPCs, two monomeric peptides, bis(Ac-FLIVI)-K-K_4-$CONH_2$ and bis(Ac-FLIVIGSII)-K-K_4-$CONH_2$, were synthesized using solid phase peptide synthesis as previously described by Avila et al. (2018) (25). These peptides are referred to as H5 and H9, respectively, after the number of residues in the hydrophobic branches. After synthesis, dried peptide was dissolved in trifluoroethanol (TFE). Concentration of each peptide was determined by measuring the absorbance of phenylalanine and subsequently dividing that value by two to account for the two phenylalanine residues per peptide. The two peptides were then mixed in equimolar ratios to create a 1mM final stock. TFE was then evaporated off using a FreeZone2.5 and refrigerated Centrivap Concentrator vacuum system (LabConco). BAPCs were assembled by adding 1 mL nuclease-free water and allowing the solution to sit at room temperature for 5 min, followed by an incubation at 4°C for at least 1 hr. At room temperature, the peptides spontaneously assemble into a bilayer and fuse, and the shift to 4°C slows the fusing of complexes and locks the BAPCs in a size range of 50-250 nm. At room temperature, the peptides spontaneously assemble into a bilayer and fuse, and the shift to 4°C slows the fusing of complexes and locks the BAPCs in a size range of 50-250 nm. BAPC-dsRNA complexes were formed by mixing the appropriate concentration of BAPCs with 1μg dsRNA and allowing the mixture to sit for 15 min at room temperature.

To form rhodamine-labelled BAPCs (Rh-BAPCs), half of the bis (Ac-FLIVI)-K-K_4-$CONH_2$ component was substituted with the same peptide labeled with the N-hydroxysuccinimide ester of rhodamine B covalently attached to the ϵ-amino group of the C-terminus lysine (bis (Ac-FLIVI)-K-K_3-K(Rh)-$CONH_2$). This resulted in a final molar ratio of 1 H9: 0.5 H5: 0.5 Rh-H5. Rh-BAPC-dsRNA complex were formed as described for unlabeled BAPC-dsRNA complexes.

2.3 Dynamic light scattering and electrophoretic retardation assay of BAPCs and BAPC-dsRNA complexes

BAPCs and BAPC–dsRNA complexes were suspended in a buffer simulating midgut pH, then size was measured *via* DLS using the Zetasizer Nano ZS (Malvern Instruments Ltd., Westborough, MA). A 500μM stock of BAPCs was prepared following the protocol previously described. BAPCs were then complexed with 1 μg of dsRNA if needed, and the BAPCs or BAPC-dsRNA complexes were transferred to phosphate buffered saline solution (pH = 7.4). Samples were incubated at room temperature for 5-10 minutes prior to analysis, and all DLS measurements were performed in triplicates. For the gel retardation assay, BAPC-dsRNA complexes of 20μM and 60μM BAPCs complexed with 1μg dsRNA were assembled and incubated in pH 7.4 buffer as described above. Following, complexes were mixed with RNA gel loading dye (Invitrogen, Waltham, MA) at a 1:1 ratio. Samples were then resolved onto a 2% agarose gel composed of 1× MOPS buffer and SYBR green stain, then electrophoresed at 100 V for 30 min. Control wells containing 1 μg of dsRNA only and BAPC concentrations of 20μM and 60 μM without dsRNA were included. The gel was imaged using ImageQuant LAS 4000 (GE Healthcare, Pittsburgh, PA, USA).

2.4 Selection of target gene and dsRNA synthesis

Due to availability of only five known mRNA sequences of *P. japonica* in NCBI database, we chose peritrophin, one of the available sequences, as a target for RNAi. Peritrophins play key protective roles during food processing in feeding life stages, growth, and development of larvae. To synthesize dsRNA, first total RNA was extracted from the gut tissue of *P. japonica* using a commercially available TRIzol reagent. After purification of total RNA, the RNA was reverse transcribed to cDNA using SuperScript II First-Strand Synthesis System, and the genomic DNA was removed by DNase I treatment (Invitrogen). The synthesized cDNA was then used as a template for the amplification of the peritrophin gene segment using following primers; forward primer: GCTGGTACCTACTTCAATCC, reverse primer: CATACAACCTGCATCTTCGG. Both primers were designed manually to amplify the peritrophin gene segment of ~300 bps with T7 promoter sequence flanking at 5' end of both primer sequences. Upon amplification and purification of T7 flanked peritrophin DNA, sense and antisense RNA strands were synthesized separately as per manufacturer protocol using TranscriptAid T7 High Yield Transcription Kit (Thermo Scientific™, Carlsbad, CA, USA). After transcription, the sense and antisense single-stranded RNA (ssRNA) were purified using LiCl precipitation, quantified using nanodrop and resuspended in duplex buffer (Integrated DNA Technologies Inc., USA). For annealing, both RNA strands were mixed in 1:1 molar ratio and annealed as recommended by the supplier. The quality of dsRNA was verified by 1% agarose gel electrophoresis and using a Nanodrop technique. In addition, we synthesized dsRNA sequence non-specific to *P. japonica* and used as a non-specific control.

2.5 Adult feeding and survivorship assay

One day prior to the experiment, 120 adult female beetles were randomly selected from a container and transferred to a plastic cup (Dart, Mason, MI) and deprived of food for 24 hr prior to feeding. Whatman GF/A filter papers (25 mm diam., Cytivia life sciences, Marlborough, MA) were cut into quarters and pinned between two 5 x 5 mm pieces of transparency film (Tri-state Visual Products, Highland Heights, KY) using stainless steel insect pins. Each filter paper quarter was treated with 40 μL of a 1 M sucrose solution and allowed to dry for 12 hr prior to applying the treatment to promote adult beetles feeding. Adult *P. japonica* will feed on filter papers amended with 1 M sucrose (31). Upon drying, filter papers for seven different treatment groups and 15 biological replicates were prepared by applying 40 μL of BAPC-dsRNA complexes.

On day 0, 100 food-deprived beetles were selected for the survivorship assay. Individuals were tested for vigor by flipping them on their dorsum and used only if they could right themselves within 5 min. Selected beetles were then transferred into an individual wax-bottomed plastic cup and randomly assigned to one of the seven treatments. Three extra beetles were randomly selected, deprived of food for 24 hr and their midgut tissues were isolated and preserved in TRIzol to assess effect of starvation on peritrophin gene expression. Once the beetles were transferred to wax-lined cups, filter paper quarters with BAPC complexes were placed inside and all beetles were transferred into a growth chamber at 25 ± 0.5° C. Beetles were allowed to feed until a treatment group had consumed either an average of 2/3 (66%) of the filter paper or 24 hr whichever occurred first. Post feeding, all filter papers were replaced with leaf disks (20 mm diam.) taken from freshly collected Virginia creeper (*Parthenocissus quinquefolia*) foliage, followed by replacement of these leaf disks daily until day 6. Until the end of experimental protocol or observed mortality, beetles were tested daily for vigor as previously described. Beetles that failed to vigor response were considered dead and eliminated from the study. Three beetles from each group were selected at random and dissected to isolate gut tissue for RNA extraction and analysis.

On day 7, a second dose of respective treatments was administered to surviving beetles through filter papers using the same methodology as described above. Data on survivorship was collected every 24 hr until day 14. Filter paper consumption was calculated using Image-J (32) by collecting the filter papers quarters after both doses. The area of filter paper after the assays was measured then a percentage of area consumed was calculated based on the initial area. The initial area was the average of six filter paper quarters not provided to beetles.

2.6 Quantitative polymerase chain reaction

Total RNA was isolated from adult *P. japonica* gut tissue with TRIzol Reagent (TRIzol (Ambion, Inc., Carlsbad, CA, USA). RNA concentration was measured using nanodrop and quality was evaluated using 260/280 and 260/230 ratio. cDNA was synthesized using SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA) according to manufacturer instructions and used as a template for the RT-qPCR. Each RT-qPCR sample contained 10 µL of synthesized cDNA, 0.8 µL of each primer (10 µM forward and reverse), 0.9 µL of nuclease free ddH2O, and 12.5 µL of Perfecta Sybr Green Supermix (Quanta Biosciences, Gaithersburg, MD, USA); totaling 25 µL. All reactions were performed using SYBR Green Master Mix and amplified under the following cycling conditions: beginning cycle at 95°C, 40 cycles at 95°C for denaturation, followed with 30 s at 65°C for annealing and extension, and ending with generation of a melting curve consisting of a single peak to rule out non-specific product and primer dimer formations. Each treatment group contained three biological replicates and two technical replicates. The expression levels of peritrophin and the number of transcripts per sample was estimated based on the Ct value. Due to unavailability of housekeeping gene sequences in *P. japonica*, we used β-actin gene sequence from the closely related species *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) to design primers and used as an internal loading control. Generated cDNA was then used to quantify changes in gene expression levels among different treatment groups by RT-qPCR. The expression levels of the genes were determined by 2−ΔΔCt* proportional calculation method. (The fold changes in peritrophin transcript levels relative to the β-actin). For statistical analysis, we performed a one-way ANOVA with Tukey's post-test to evaluate differences between treatment group $P < 0.05$.

2.7 Larval midgut transcytosis assay

To elucidate if transcytosis was involved in the translocation of BAPCs through midgut epithelium cells, live third instar *P. japonica* larvae were dissected to isolate the midgut tissues. The preparation for assays in the Ussing chamber have been described in our previous work (29). Larvae used in this assay were stored in a fridge at 4°C for 48 hr prior to dissections to stabilize the gut tissues. Incisions were made along the lateral-medial line of the larvae from the anterior to the posterior using corneal scissors. Insect pins were then used to secure the integument onto the dissection tray and expose the digestive tract. Incisions were performed on the midgut by making lateral-medial incisions anteriorly from the third gastric caecum. The tissues were then isolated from the body, rinsed with insect physiological solution described previously (29), and immediately mounted onto a modified 0.01 cm^2 slide. Dissected midgut tissue from larvae was inserted into a tissue holder slide which was placed inside of a Ussing chamber. This chamber creates an *ex vivo* gut environment through which transport of molecules across tissue may be studied. Buffer containing rhodamine dye labelled BAPCs was added to the luminal side of the tissue, and transcellular transport was determined by measuring rhodamine dye fluorescence on luminal as well as hemolymph side at discrete time points. Dissections were conducted with meticulous effort to avoid tissue punctures and to conserve orientation of the tissue relative to the lumen and the hemolymph.

After mounting, slides were slotted into the two-sided chamber, where tissues were then perfused with 3 mL of lumen or hemolymph buffer according to tissue orientation (29). To study the effect of inhibiting endocytosis on the transport of BAPC-dsRNA complexes, three replicate tissues were pre-incubated with 10µM chlorpromazine (CPZ) for 30 min before the addition of Rh-BAPC-dsRNA complexes. A final concentration of 50 µM Rh–BAPCs with or without 1 µg dsRNA was then added to the lumen side. Tissues were exposed for a period of 120 min, after which fluorescence was read using a BioTek Cytation 3 plate reader (λex = 544 nm; λem = 576 nm). Change in relative fluorescence over time was plotted to visualize the transport of Rh-BAPCs due to transcytosis. Fluorescence was measured in arbitrary fluorescence units (AFU's). To account for the variability of relative fluorescence between replicates, data were normalized using proportions.

2.8 Statistical analysis

Data analysis and plots were done using GraphPad Prism (version 8.0.0 for Windows, GraphPad Software, San Diego, California USA). To estimate the sample size in the survivorship assay, we employed the "resource equation method" (33). For survival curves we used the Log Rank Test. For the gene expression analysis, consumption of filter papers, and DLS experiments, we used one-way ANOVA using Tukey as post-test. Transcytosis experiments were analyzed using two-way ANOVA and Tukey as post-test. An alpha level of $P<0.05$ was used for all analyses.

3 Results

3.1 *P. japonica* artificial diets supplemented with BAPC-dsRNA complexes

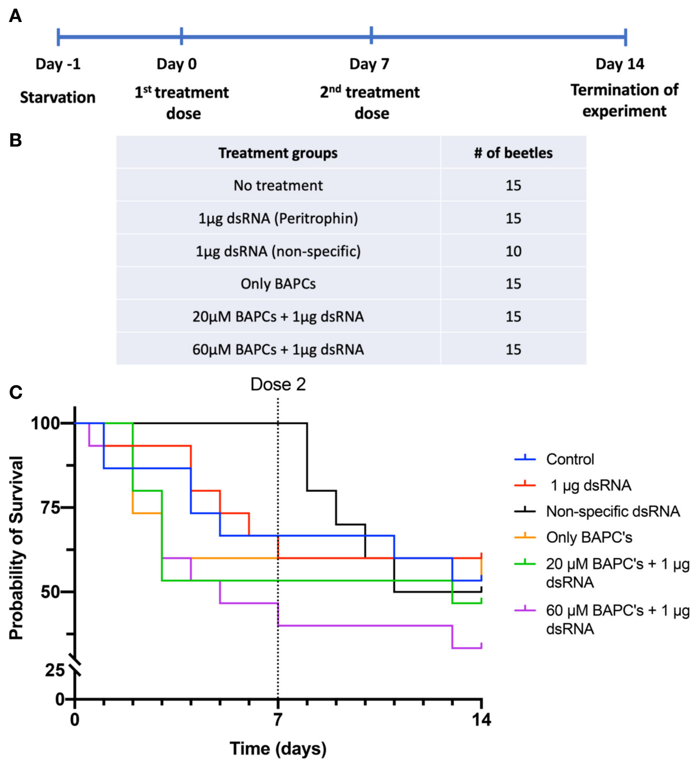
Males often perform less consistently in feeding assays relative to females because their behavior is more directed toward mating (31). Thus, we included adult females exclusively to evaluate the efficiency of the BAPCs to deliver dsRNA targeting peritrophin through feeding. As described in the methods section, the BAPC-dsRNA complexes were applied on filter-paper containing sucrose, a common phagostimulant used in *P. Japonica* assays (34, 35). The potential of BAPCs to deliver dsRNA in *P. japonica* was evaluated through survival assay and gene expression analysis.

We delivered two doses of BAPC-dsRNA complexes through feeding on day 0 and day 7, allowing them to feed on the treatment up to 24 hr (Figures 1A, B). Subsequently, we monitored all beetles daily for mortality up to 14 days (normal life span of adult *P. japonica* is 30-45 days). Survivorship by 14 days was only 33% in insects fed on diets containing 1 µg dsRNA+60 µM BAPCs ($t_{1/2} = 7_{\text{d}}$). On the other hand, 60% and 53% survivorship were observed at 14 days in beetles fed only dsRNA and untreated control groups, respectively. This difference in survivorship was not significant as determined by a log-rank test (Day 7: $P= 0.12$ & Day 14: $P = 0.6$, $df= 1$). Feeding of beetles on a diet of non-specific (non-peritrophin) dsRNA or with lower BAPCs concentration (1 µg dsRNA+ 20 µM BAPCs) also had no effect on survival ($t_{1/2} = 14_{\text{d}}$) (Figure 1C).

Consumption of filter papers vary greatly among individuals within same treatment group, therefore the average dosage delivered would be lower than provided. We calculated surface area of filter paper consumed as a proxy for dose ingested within 24 hr before being replaced with a diet of Virginia creeper leaf discs. On average, 38% of the filters were consumed across treatments, with an average of 22% of the filter paper consumed in the control dsRNA treatment (Supplementary Figure 1) ($P<0.01$, $F = 3.087$, $Dfn =6$, $Dfd=139$). Thus, less than 1 µg of dsRNA dose is sufficient to induce significant knockdown effects when delivered with the aid of an effective concentration of BAPCs.

3.2 Assessment of peritrophin-mRNA levels isolated from *P. japonica* midgut

We quantified the peritrophin transcript levels by RT-qPCR analysis to confirm that dsRNA induced gene silencing in the



Treatment groups	# of beetles
No treatment	15
1µg dsRNA (Peritrophin)	15
1µg dsRNA (non-specific)	10
Only BAPCs	15
20µM BAPCs + 1µg dsRNA	15
60µM BAPCs + 1µg dsRNA	15

FIGURE 1
Survival curves of *P. Japonica* post-ingestion of BAPCs formulations: Experimental outline of dsRNA feeding assay in *P. japonica* adult females **(A)**. Treatment groups included in the feeding assay **(B)**. The survival curve of *P. japonica* females upon feeding on BAPCs complexed with peritrophin-dsRNA (n = 15) **(C)**. The data were analyzed using a log rank test. There were no significant differences between treatment groups ($P >0.05$).

targeted gene. Ingestion of 20 µM BAPCs+ 1 µg dsRNA resulted in a 30-fold decrease in peritrophin gene expression, which was significantly different *(P<0.05, F= 6.840, Dfn= 4, Dfd= 10)* from the dsRNA alone group. Similarly, 60 µM BAPCs+ 1 µg dsRNA had the greatest gene silencing rate, with knockdown of expression by approximately 34-fold relative to non-treated control group (Figure 2). Although, quantification of the mRNA transcripts is congruent with the trends observed in the survivorship study, our results support the concept of BAPCs nanoparticles acting as dsRNA stabilizer, and cellular uptake enhancer. Furthermore, we also analyzed the integrity of the BAPCs formulations by measuring the size in a buffered solution with a pH similar to the *P. japonica* midgut (pH=7.4) (5).

3.3 Biophysical characterization of BAPCs and BAPC-dsRNA complexes

From a biophysical perspective, the stability or integrity of nanoparticles is used to describe the preservation of a particular nanostructure property (i.e., size). We assessed BAPCs stability by incubating in buffer of pH 7.4 using dynamic light scattering (DLS), According with Figure 3A, the BAPCs–dsRNA complexes displayed a size ranging between 250 to 350 nm, a size that is consistent with our previously reported DLS measurements performed in distilled water (29). BAPCs (60 µM) not associated with dsRNA exhibited a significant *(F = 10.45, P= 0.0002, Dfn=3, Dfd=20)* smaller hydrodynamic diameters than the BAPC-dsRNA complexes, confirming that the association of dsRNA increases the size of the BAPCs or causes BAPCs to cluster together. Furthermore, these results indicate that the complexes do not dissociate or aggregate in the buffered solution, proving structure stability in a pH environment consistent with the gut of *P. japonica* adults. Although multiple variables can play a role in nanoparticle stability inside the midgut, the pH is critical since it can lead to variation in nanoparticle charge and oxidation state. Regarding nuclease degradation of dsRNA, studies performed in mammalian cells support the notion that BAPCs protect dsRNA against nuclease degradation (29, 36). Target sites of RNAs might no longer be accessible to the catalytic core of RNases after the association with the BAPCs surface (37). It is important to mention that nucleases exclusively affect the dsRNA structure and not BAPCs. The downregulation of the peritrophin transcript levels also support the notion of nuclease protection conferred by BAPCs.

To elucidate the dsRNA binding capacity of BAPCs at concentrations used for the survivorship assay, we evaluated their electrophoretic mobility in a 2% RNA agarose gel. Our results indicated that association of dsRNA with the BAPCs surface led to a decreased migration of dsRNA that was dependent on BAPC concentration (Figure 3B). The formulation with the highest BAPCs concentration (60 µM) displayed a barely visible band, suggesting that all added dsRNA has firmly adhered to the BAPCs surface, which resulted in a poor interaction with the dye SYBR green. However, lower concentrations of BAPCs yielded a more visible dsRNA band, indicating more availability for SYBR green binding due to a weaker interaction of BAPCs and dsRNA. As expected, BAPCs not complexed with dsRNA showed no signal in the well.

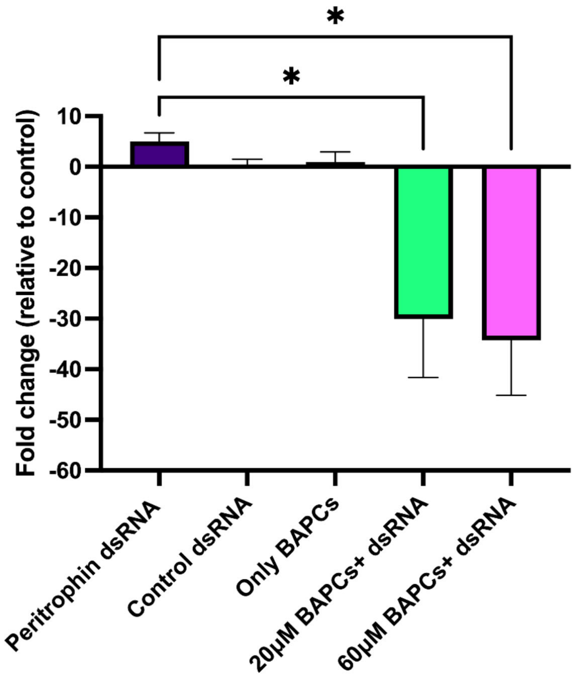


FIGURE 2
Analysis of gene expression upon dsRNA mediated gene silencing: peritrophin-mRNA transcript levels in the midgut of *P. japonica* upon feeding on BAPCs complexed with and without dsRNA analyzed using RT-qPCR. Fold change in peritrophin expression is normalized using β-actin as an internal control. Differences between values were compared by one way ANOVA using Tukey as post-test. Statistical significance: (*) *P < 0.05*; (**) *P < 0.01*. Non-statistical significance (*ns*) was considered when *P > 0.05*.

3.4 Midgut cellular uptake mechanisms of BAPC–dsRNA complexes

The alimentary tract of adult and 3rd instar larval stages of *P. japonica* have only been described in separate publications with supporting hand illustrations (38, 39) respectively. More recent photo images of the digestive tract of neonate and 3rd instar grubs have been published (40). Here, for the first time we present comparative images of the 3rd instar larval and adult alimentary tracts juxtaposed to highlight the morphological differences (Gryphax® Series Avior microscope camera, Jenoptic, Jena, Germany). These images confirm previous descriptions (38, 41) stating the adult midgut is narrower in width relative to the larval midgut. Through the many dissections for the reported experiments, we noted less tissue strength of the adult gut leading to those tissues tearing and shearing more easily than the larval midgut tissues (Figures 4A, B). The width and sensitivity of the adult midgut tissue was an obstacle to study BAPC-dsRNA complexes cellular uptake and transport across gut tissue. Consequently, only the larval midgut tissues were used to study these mechanisms in *P. japonica*. For larval dissections, we used the

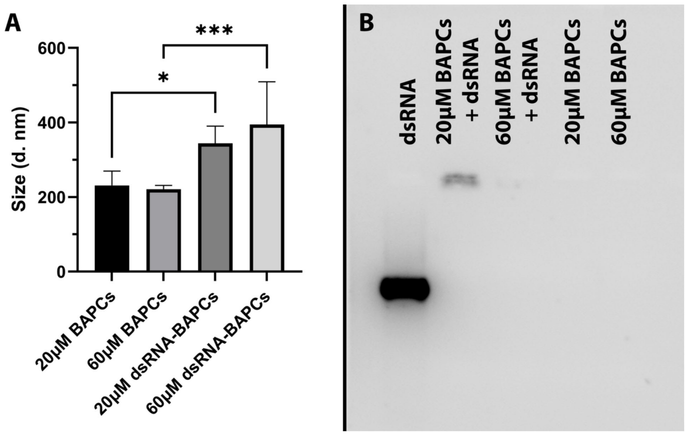


FIGURE 3
(A) Stability assessment of BAPCs and BAPC-dsRNA complexes in a buffer mimicking *P. japonica* gut (pH 7.4). **(B)** BAPC loading capacity assessed by the electrophoretic mobility shift assay. BAPC-dsRNA complexes were formed by mixing 20µM and 60µM BAPCs with 1µg dsRNA. Controls containing only 20µM or 60µM BAPCs without dsRNA were also run to show they did not produce background signal. Differences between values were compared by one way ANOVA using Tukey as post-test. Statistical significance: (*) $P < 0.05$; (***) $P < 0.001$. Non-statistical significance (ns) was considered when $P > 0.05$.

third gastric caecum to delineate between midgut and hindgut tissues (Figure 4A).

BAPCs and BAPC-dsRNA complexes transport across the gut tissue was assessed with the help of Ussing chamber (Figures 5A, B). Both formulations are actively transported across the gut tissue, with around 50% reduction in rhodamine fluorescence on luminal compartment (Figure 5C). However, BAPCs complexed with dsRNA slows significantly$(P<0.05,\ F= 5.841,\ Dfn=2,\ Dfd=6)$. the rate of transcellular transport compared to only BAPCs (Figure 5D). A plausible reason for the diminished transport can be related to the binding of the negatively charged dsRNA to the surface of BAPCs. The dsRNA association blocks a portion of the positively charged lysine residues exposed on the BAPCs surface thus becoming less cationic, and reducing cellular uptake by epithelial cells (42) (Figure 5).

To assess the role of endocytosis on the uptake of BAPC-dsRNA complexes, gut tissue was pre-incubated for 30 min with chlorpromazine, an inhibitor of clathrin-mediated endocytosis. Clathrin-mediated endocytosis is one of the major pathways by which cells transport extracellular cargo from outside the cell membrane to the interior *via* the formation of clathrin-coated endocytic vesicles. Following CPZ addition, Rh-BAPC-dsRNA complexes were added to luminal side, and the relative change in rhodamine fluorescence was measured as described earlier. As Rh-BAPCs are taken up from the luminal side, it is expected that fluorescence of this compartment will decrease. Subsequently, movement *via* transcytosis will deposit endocytosed Rh-BAPCs to the hemolymph compartment, increasing its fluorescence over time (29). Treatment of gut tissue with endocytic inhibitor abrogated BAPC-dsRNA uptake from luminal side with no change in rhodamine fluorescence up to 2h (Figure 5C). Similarly, there was no significant increase in rhodamine fluorescence was observed on hemolymph side (Figure 5D). It is also expected that a fraction of BAPCs or BAPCs-dsRNA remain trapped within the *P. japonica* gut cells, particularly in endosomal vesicles such as endosomes and lysosomes (43–45). Retrograde transport is also possible and has been documented for other nanoparticles (45). In other words, only some of the material that enters cells will undergo transcytosis.

Overall, these results indicate that cellular uptake of BAPCs and BAPC-dsRNA complexes is mediated by clathrin coated endocytic vesicle. Nonetheless, cellular uptake is a complex process and potentially other mechanisms can also be involved in the uptake of BAPCs formulations.

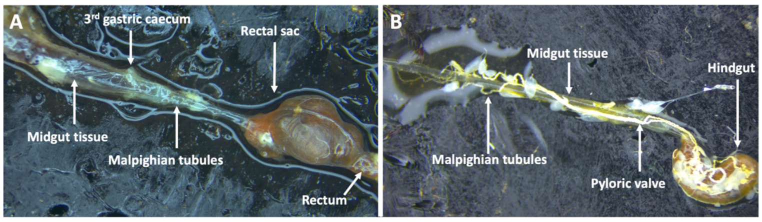


FIGURE 4
Digestive tract of *P. japonica*. **(A)** Larval digestive tract including midgut tissue, 3rd gastric caecum, Malpighian tubules, rectal sac, and the rectum. **(B)** Adult digestive tract including midgut tissue, hindgut, the pyloric valve, and Malpighian tubules (White, string-like organ).

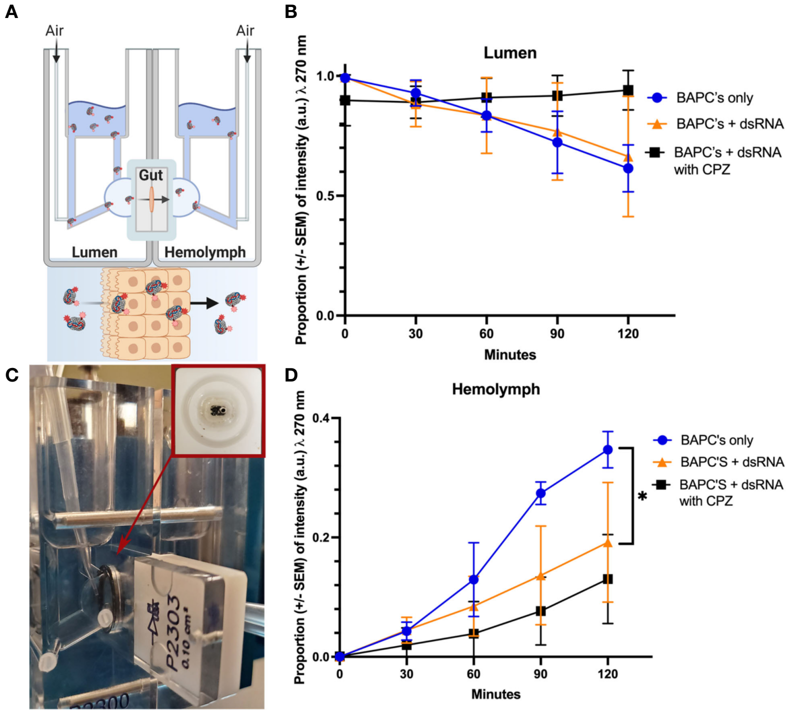


FIGURE 5
Cellular uptake study. Mechanism of Rh–BAPCs and Rh–BAPC-dsRNA cellular uptake by *P. japonica* midgut cells. **(A, B)** graphical representation and actual set up of Ussing chamber used for *ex vivo* analysis of BAPC-dsRNA complexes uptake and transport across *P. japonica* midgut tissue. **(C)** Mean relative fluorescence of Rh-BAPCs complexes on luminal side buffer and **(D)** Mean relative fluorescence of Rh-BAPCs complexes on hemolymph side buffer over 2 hr. Differences between values were compared by two-way ANOVA using Tukey as post-test. Statistical significance: (*) *P< 0.05*.

4 Discussion

In summary, we reported the first gene knockdown study in adult *P. japonica* by feeding of BAPC-dsRNA complexes. Although there was a numerical (20%) difference in survivorship between the 60 µM BAPCs +1 µg of peritrophin-dsRNA treatment and the non-treated control group, no statistical differences were observed between treatment groups. Here, we discuss the potential reasons for the lack of observed mortality in groups that ingested dsRNA. Peritrophic matrix proteins (PMP's) and analogs are diverse in Coleoptera, and few have been experimentally demonstrated to have significant effects on the structure and function of the peritrophic matrix. A total of 11 genes encoding PMP's have been identified and screened for phenotypic and mortality effects in *Tribolium castaneum*, of which only two resulted in lethal phenotypes during early and late pupal stages post-injection (46, 47). Thus, targeting of peritrophin genes alone may not be sufficient to achieve high mortality rates, but could be involved in important roles including protection, detoxification, absorption of nutrients, and increasing RNAi efficiency (46, 48, 49). Despite peritrophin silencing making the insect gut more susceptible to chemicals and pathogens affecting their metabolism, growth, and development, these effects might not be as lethal as other genes such as vATPAse, tubulin, or inhibitor of apoptosis (sequence not available). The future publication of a fully annotated *P. japonica* genome will provide better target genes for pest management purposes.

The concentration of body fluid required to degrade 50% of dsRNA (CB50) within one hour is between 45-94 fold lower relative to the CB50's of *Tribolium castaneum* and *Leptinotarsa decemlineata* (23). These are two model coleopterans that account for a majority of knowledge on how dsRNA impacts beetle species. *P. japonica* has a broad ecological host range, and utilizes a suite of detoxification enzymes induced by feeding to detoxify phytochemicals (35). For these reasons, ingested dsRNA faces a complex biochemical environment in the gut of *P. japonica*. When verifying gene expression, naked dsRNA resulted in no significant difference in expression relative to the non-treated control group. Although ingestion of naked dsRNA did not lead to gene silencing, we observed that the fold change in peritrophin expression in both BAPC-dsRNA treatment groups was significantly lower ($P < 0.05$) compared to the control groups (non-treated control and dsRNA only). These results suggest that increasing molar concentrations of BAPCs (>20 µM) improves efficiency of dsRNA delivery, resulting in the desired biological response. Nonetheless, our previous work with BAPCs in different organisms indicate that BAPCs concentrations >60 µM may trigger cytotoxicity (29, 36). Thus, higher BAPCs concentrations were not tested. While the presence of cationic moieties facilitate binding with the cell membrane, excessive cationic charge can also disrupt cell membrane's potential and lead to cell death (50–52). Therefore, the optimal dose of BAPCs and any other cationic nanoparticle must be carefully optimized for each nanoparticle to avoid undesirable outcomes. Our results support that BAPC nanoparticles are effective protectants of dsRNA in insect midgut environments and suggest protectants may be required for efficient RNAi in *P. japonica*.

After ingestion, dsRNA passes into the *P. japonica* midgut (15). The midgut is composed of three types of epithelial cells: columnar cells, endocrine cells, and stem cells. Presumably, it is in these cells where dsRNA uptake and processing take place. It has been reported in insects that two main mechanisms are involved in the internalization of dsRNA: receptor mediated uptake or endocytosis. The best documented endocytic route in insects is the clathrin-dependent pathway. In the experiment using 3rd instar larval *P. japonica* midguts in an Ussing chamber, it was observed that fluorescence decreased in the lumen compartment and increased in the hemolymph in a time-dependent manner consistent with our previous reports on transcytosis (29). However, in the presence of CPZ, a clathrin-mediated endocytosis inhibitor, there was a noticeable lack of change in the relative fluorescence in the lumen and smaller increase in hemolymph fluorescence relative to BAPC's alone. Formation of a clathrin-coated pit is initiated by the rearrangement of various accessory and cytoskeletal proteins along with the creation of a clathrin-coated pit at the inner surface of the cell membrane. CPZ inhibits the anchoring of clathrin and adaptor protein 2 (AP2) complex to endosomes, thereby preventing the assembly of these coated pits (53). This suggests that clathrin-mediated endocytosis may play a significant role in the uptake of NP in the gut, but other pathways are also likely present as some Rh-BAPCs movement was still observed. Despite the increased number of articles demonstrating nanoparticles-dsRNA mediated gene silencing, fundamental mechanisms such as uptake midgut cells or transport to the hemolymph are not widely reported. Thus, our findings are particularly relevant as they suggest mechanisms that could potentially enable systemic delivery or can lead to a more tailored nanoparticle design for gene silencing.

Overall, BAPCs provide a means of reliably protecting dsRNA through oral delivery to *P. japonica*. BAPCs are a new class of biomaterial developed by our research group that stands out in the crowded field of nanoparticle delivery systems due to two crucial features:1) they are assembled exclusively in water, and 2) they contain four free lysine Ɛ-amino groups with pKa values between 9 and 10.5, which makes them stable in neutral and alkaline insect guts. According with DLS, the BAPCs-dsRNA complexes form compact clusters with size ranging from 250 – 350 nm in a pH environment consistent with the gut of *P. japonica* adults. Association of BAPCs with dsRNA confers protection to dsRNA by hidden target sites for RNases, resulting in the stabilization of dsRNA. The use of dsRNA and nanoparticles currently appears expensive when compared with relatively low cost of common insecticides. It is unlikely that dsRNA technology will replace the use of conventional insecticides for the management of *P. japonica*. However, providing targeted control will reduce the negative impacts on non-target arthropods associated with the use of insecticides to control both economically important life stages of *P. japonica*.

Data availability statement

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

Author contributions

EC and NK: These authors have contributed equally to this work and share first authorship. Both authors performed survivorship assay, gut dissection for transcytosis experiment and gene expression analysis. In addition, both have equally contributed in preparation of this manuscript. EM: Performed transcytosis experiment on insect midgut tissue. SG: Extracted total RNA from insect gut tissue to synthesize dsRNA for survivorship assay. RR: Provided guidance on primer design, gene expression analysis and data interpretation. JN-N: Helped in synthesis of BAPCs and BAPC-dsRNA formulation as well as their characterization. DH: Provided guidance on planning of insect feeding assay and data collection and interpretation. LA: Supervise the project, provide funds and conceived the original idea of BAPCs use for gene silencing through feeding. All authors contributed to the article and approved the submitted version.

Funding

This project was supported by the Alabama Agricultural Experiment Station and the Hatch program of the National Institute of Food and Agriculture, U.S. Department of Agriculture.

Conflict of interest

The authors LAA and DH declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/finsc.2023.1151789/full#supplementary-material

References

1. Diagne C, Leroy B, Vaissière AC, Gozlan RE, Roiz D, Jarić I, et al. High and rising economic costs of biological invasions worldwide. *Nature* (2021) 592(7855):571–6. doi: 10.1038/s41586-021-03405-6

2. Venette RC, Hutchison WD. Invasive insect species: global challenges, strategies & opportunities. *Front Insect Sci* (2021) 1:650520. doi: 10.3389/finsc.2021.650520

3. Allsopp PG. Japanese Beetle, popillia japonica Newman (Coleoptera: scarabaeidae): rate of movement and potential distribution of an immigrant species. *Coleopt Bull* (1996) 50:81–95.

4. Althoff ER, Rice KB. Japanese Beetle (Coleoptera: scarabaeidae) invasion of north America: history, ecology, and management. *J Integr Pest Manag*, (2022) 13(1): 2; 1–11. doi: 10.1093/jipm/pmab043

5. Fleming WE. Biology of the Japanese beetle. *USDA Tech Bull* (1972) 1449:1–129.

6. Potter DA, Held DW. *Biology and management of the Japanese beetle* .doi: 10.1146/annurev.ento.47.091201.145153.

7. DeBach P, Rose M. Environmental upsets caused by chemical eradication. *California Agriculture* 31(7). Agric (1977) p. 8–10.

8. Rogers ME, Potter DA. Effects of spring imidacloprid application for white grub control on parasitism of Japanese beetle (Coleoptera: scarabaeidae) by *Tiphia vernalis* (Hymenoptera: tiphiidae). *J Econ Entomol* (2003) 96(5):1412–9. doi: 10.1603/0022-0493-96.5.1412

9. Rodrigues T, Sridharan K, Manley B, Cunningham D, Narva K. Development of dsRNA as a sustainable bioinsecticide: from laboratory to field. In: *Crop protection products for sustainable agriculture*. Washington D.C.: American Chemical Society (2021). p. 65–82. doi: 10.1021/bk-2021-1390.ch005

10. Head GP, Carroll MW, Evans SP, Rule DM, Willse AR, Clark TL, et al. Evaluation of SmartStax and SmartStax PRO maize against western corn rootworm and northern corn rootworm: efficacy and resistance management. *Pest Manag Sci* (2017) 73(9):1883–99. doi: 10.1002/ps.4554

11. Palli SR. RNA Interference in Colorado potato beetle: steps toward development of dsRNA as a commercial insecticide. *Curr Opin Insect Sci* (2014) 6:8. doi: 10.1016/j.cois.2014.09.011

12. Obbard DJ, Gordon KHJ, Buck AH, Jiggins FM. The evolution of RNAi as a defence against viruses and transposable elements. *Philos Trans R Soc B Biol Sci* (2009) 364(1513):99–115. doi: 10.1098/rstb.2008.0168

13. Bachman PM, Huizinga KM, Jensen PD, Mueller G, Tan J, Uffman JP, et al. Ecological risk assessment for DvSnf7 RNA: a plant-incorporated protectant with targeted activity against western corn rootworm. *Regul Toxicol Pharmacol* (2016) 81:77–88. doi: 10.1016/j.yrtph.2016.08.001

14. Bachman P, Fischer J, Song Z, Urbanczyk-Wochniak E, Watson G. Environmental fate and dissipation of applied dsRNA in soil, aquatic systems, and plants. *Front Plant Sci* (2020) 11:21. doi: 10.3389/fpls.2020.00021

15. Kunte N, McGraw E, Bell S, Held D, Avila LA. Prospects, challenges and current status of RNAi through insect feeding. *Pest Manag Sci* (2020) 76(1):26–41. doi: 10.1002/ps.5588

16. Cagliari D, Dias NP, Galdeano DM, dos Santos EÁ, Smagghe G, Zotti MJ. Management of pest insects and plant diseases by non-transformative RNAi. *Front Plant Sci* (2019) 10:1319. doi: 10.3389/fpls.2019.01319

17. Cooper AM, Silver K, Zhang J, Park Y, Zhu Y. Molecular mechanisms influencing efficiency of RNA interference in insects. *Pest Manag Sci* (2018) 75:18–28. doi: 10.1002/ps.5126

18. Powell ME, Bradish HM, Gatehouse JA, Fitches EC. Systemic RNAi in the small hive beetle aethina tumida Murray (Coleoptera: nitidulidae), a serious pest of the European honey bee apis mellifera. *Pest Manag Sci* (2017) 73(1):53–63. doi: 10.1002/ps.4365

19. Haller S, Widmer F, Siegfried BD, Zhuo X, Romeis J. Responses of two ladybird beetle species (Coleoptera: coccinellidae) to dietary RNAi. *Pest Manag Sci* (2019) 75 (10):2652–62. doi: 10.1002/ps.5370

20. Silver K, Cooper AMW, Zhu KY. Strategies for enhancing the efficiency of RNA interference in insects. *Pest Manag Sci* (2021) 77(6):2645–58. doi: 10.1002/ps.6277

21. Cooper AMW, Yu Z, Biondi M, Song H, Silver K, Zhang J, et al. Stability of double-stranded RNA in gut contents and hemolymph of ostrinia nubilalis larvae. *Pestic Biochem Physiol* (2020) 169:104672. doi: 10.1016/j.pestbp.2020.104672

22. You L, Zhang F, Huang S, Merchant A, Zhou X, Li Z. Over-expression of RNA interference (RNAi) core machinery improves susceptibility to RNAi in silkworm larvae. *Insect Mol Biol* (2020) 29:353–62. doi: 10.1111/imb.12639

23. Singh IK, Singh S, Mogilicherla K, Shukla JN, Palli SR. Comparative analysis of double-stranded RNA degradation and processing in insects. *Sci Rep* (2017) 7(1):1–12. doi: 10.1038/s41598-017-17134-2

24. Miele E, Spinelli GP, Miele E, Di FE, Ferretti E, Tomao S, et al. Nanoparticle-based delivery of small interfering RNA: challenges for cancer therapy. *Int J Nanomedicine* (2012) 7:3657. doi: 10.2147/IJN.S23696

25. Avila LA, Chandrasekar R, Wilkinson KE, Balthazor J, Heerman M, Bechard J, et al. Delivery of lethal dsRNAs in insect diets by branched amphiphilic peptide capsules. *J Control Release* (2018) 273:139–46. doi: 10.1016/j.jconrel.2018.01.010

26. Christiaens O, Tardajos MG, Reyna ZLM, Dash M, Dubruel P, Smagghe G. Increased RNAi efficacy in spodoptera exigua *via* the formulation of dsRNA with guanylated polymers. *Front Physiol* (2018) 9:316. doi: 10.3389/fphys.2018.00316

27. Lomate PR, Bonning BC. Distinct properties of proteases and nucleases in the gut, salivary gland and saliva of southern green stink bug, nezara viridula. *Sci Rep* (2016) 6:1–10. doi: 10.1038/srep27587

28. Das S, Debnath N, Cui Y, Unrine J, Palli SR. Chitosan, carbon quantum dot, and silica nanoparticle mediated dsRNA delivery for gene silencing in aedes aegypti: a comparative analysis. *ACS Appl Mater Interfaces* (2015) 7(35):19530–5. doi: 10.1021/acsami.5b05232

29. McGraw E, Roberts JD, Kunte N, Westerfield M, Streety X, Held D, et al. Insight into cellular uptake and transcytosis of peptide nanoparticles in spodoptera frugiperda cells and isolated midgut. *ACS Omega* (2022) 7(13):10933–43. doi: 10.1021/acsomega.1c06638

30. Hegedus D, Erlandson M, Gillott C, Toprak U. New insights into peritrophic matrix synthesis, architecture, and function. *Annu Rev Entomol* (2009) 54:285–302. doi: 10.1146/annurev.ento.54.110807.090559

31. Keathley CP, Potter DA, Houtz RL. Freezing-altered palatability of Bradford pear to Japanese beetle: evidence for decompartmentalization and enzymatic degradation of feeding deterrents. *Entomol Exp Appl* (1999) 90(1):49–59. doi: 10.1046/j.1570-7458.1999.00422.x

32. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: An open-source platform for biological-image analysis. *Nat Methods* (2012) 9 (7):676–82. doi: 10.1038/nmeth.2019

33. Arifin WN, Zahiruddin WM. Sample size calculation in animal studies using resource equation approach. *Malaysian J Med Sci* (2017) 24(5):105. doi: 10.21315/mjms2017.24.5.11

34. Ladd TL. Influence of sugars on the feeding response of Japanese beetles (Coleoptera: scarabaeidae). *J Econ Entomol* (1986) 79(3):668–71. doi: 10.1093/jee/79.3.668

35. Adesanya A, Liu N, Held DW. Host suitability and diet mixing influence activities of detoxification enzymes in adult Japanese beetles. *J Insect Physiol* (2016) 88:55–62. doi: 10.1016/j.jinsphys.2016.03.002

36. Kunte N, Westerfield M, Mcgraw E, Choi J, Akinsipe T, Whitaker SK, et al. Evaluation of transfection efficacy, biodistribution, and toxicity of branched amphiphilic peptide capsules (BAPCs) associated with mRNA. *Biomater Sci* (2022) 10:6980–91. doi: 10.1039/D2BM01314B

37. Katoch R, Thakur N. Insect gut nucleases: a challenge for RNA interference mediated insect control strategies. *Int J Biochem Biotechnol* (2012) 1(8):198–203.

38. Swingle MC. Anatomy and physiology of the digestive tract of the Japanese beetle. *J Agric Res* (1930) 41:181–96.

39. Beard RL. *Studies on the milky disease of Japanese beetle larvae*. New Haven: Connecticut Agricultural Experiment Station (1945).

40. Avila-Arias H, Scharf ME, Turco RF, Richmond DS. Soil environments influence gut prokaryotic communities in the larvae of the invasive Japanese beetle popillia japonica Newman. *Front Microbiol* (2022) 13:854513–3. doi: 10.3389/fmicb.2022.854513

41. Beard RL. Physiological effects of hemorrhage in Japanese beetle larvae. *J New York Entomol Soc* (1949) 57:79–91.

42. Avila LA, Aps LRMM, Ploscariu N, Sukthankar P, Guo R, Wilkinson KE, et al. Gene delivery and immunomodulatory effects of plasmid DNA associated with branched amphiphilic peptide capsules. *J Control Release.* (2016) 241:15–24. doi: 10.1016/j.jconrel.2016.08.042

43. Cui Y, Shan W, Zhou R, Liu M, Wu L, Guo Q, et al. The combination of endolysosomal escape and basolateral stimulation to overcome the difficulties of "easy uptake hard transcytosis" of ligand-modified nanoparticles in oral drug delivery. *Nanoscale* (2018) 10(3):1494–507. doi: 10.1039/C7NR06063G

44. Lu W, Sun Q, Wan J, She Z, Jiang XG. Cationic albumin–conjugated pegylated nanoparticles allow gene delivery into brain tumors via intravenous administration. *Cancer Res* (2006) 66(24):11878–87. doi: 10.1158/0008-5472.CAN-06-2354

45. Yang D, Liu D, Qin M, Chen B, Song S, Dai W, et al. Intestinal mucin induces more endocytosis but less transcytosis of nanoparticles across enterocytes by triggering nanoclustering and strengthening the retrograde pathway. *ACS Appl Mater Interfaces* (2018) 10(14):11443–56. doi: 10.1021/acsami.7b19153

46. Jasrapuria S, Arakane Y, Osman G, Kramer KJ, Beeman RW, Muthukrishnan S. Genes encoding proteins with peritrophin a-type chitin-binding domains in tribolium castaneum are grouped into three distinct families based on phylogeny, expression and function. *Insect Biochem Mol Biol* (2010) 40(3):214–27. doi: 10.1016/j.ibmb.2010.01.011

47. Agrawal S, Kelkenberg M, Begum K, Steinfeld L, Williams CE, Kramer KJ, et al. Two essential peritrophic matrix proteins mediate matrix barrier functions in the insect midgut. *Insect Biochem Mol Biol* (2014) 49(1):24–34. doi: 10.1016/j.ibmb.2014.03.009

48. Yoon JS, Tian Hg, JG M, SH C, Douglas AE. Candidate genetic determinants of intraspecific variation in pea aphid susceptibility to RNA interference. *Insect Biochem Mol Biol* (2020) 123:103408. doi: 10.1016/j.ibmb.2020.103408

49. Davenport M, Alvarenga PH, Shao L, Fujioka H, Bianconi ML, Oliveira PL, et al. Identification of the aedes aegypti peritrophic matrix protein AeIMUCI as a heme-binding protein. *Biochemistry* (2006) 45(31):9540–9. doi: 10.1021/bi0605991

50. McConnell KI, Shamsudeen S, Meraz IM, Mahadevan TS, Ziemys A, Rees P, et al. Reduced cationic nanoparticle cytotoxicity based on serum masking of surface potential. *J BioMed Nanotechnol* (2016) 12(1):164. doi: 10.1166/jbn.2016.2134

51. Leroueil PR, Berry SA, Duthie K, Han G, Rotello VM, McNerny DQ, et al. Wide varieties of cationic nanoparticles induce defects in supported lipid bilayers. *Nano Lett* (2008) 8(2):420–4. doi: 10.1021/nl0722929

52. Li Y, Ju D. The role of autophagy in nanoparticles-induced toxicity and its related cellular and molecular mechanisms. In: *Advances in experimental medicine and biology*. New York: Springer New York LLC (2018). p. 71–84. doi: 10.1007/978-3-319-72041-8_5

53. dos Santos T, Varela J, Lynch I, Salvati A, Dawson KA. Effects of transport inhibitors on the cellular uptake of carboxylated polystyrene nanoparticles in different cell lines. *PloS One* (2011) 6(9):e24438. doi: 10.1371/journal.pone.0024438

frontiers | Frontiers in Insect Science

TYPE Review
PUBLISHED 18 May 2023
DOI 10.3389/finsc.2023.1175138

OPEN ACCESS

EDITED BY
Mohannad Ismail,
University of Florida, United States

REVIEWED BY
Arthur Vieira Ribeiro,
University of Minnesota Twin Cities,
United States
Jean-Claude Grégoire,
Université libre de Bruxelles, Belgium

*CORRESPONDENCE
Leonardo Marianelli
✉ leonardo.marianelli@crea.gov.it

†These authors have contributed equally to this work

RECEIVED 27 February 2023
ACCEPTED 28 April 2023
PUBLISHED 18 May 2023

CITATION
Gotta P, Ciampitti M, Cavagna B, Bosio G, Gilioli G, Alma A, Battisti A, Mori N, Mazza G, Torrini G, Paoli F, Santoiemma G, Simonetto A, Lessio F, Sperandio G, Giacometto E, Bianchi A, Roversi PF and Marianelli L (2023) *Popillia japonica* – Italian outbreak management. *Front. Insect Sci.* 3:1175138. doi: 10.3389/finsc.2023.1175138

Popillia japonica – Italian outbreak management

Paola Gotta[1†], Mariangela Ciampitti[2†], Beniamino Cavagna[2], Giovanni Bosio[1], Gianni Gilioli[3], Alberto Alma[4], Andrea Battisti[5], Nicola Mori[6], Giuseppe Mazza[7], Giulia Torrini[7], Francesco Paoli[7], Giacomo Santoiemma[5], Anna Simonetto[3], Federico Lessio[1], Giorgio Sperandio[3], Emanuela Giacometto[1], Alessandro Bianchi[2], Pio Federico Roversi[7] and Leonardo Marianelli[7*]

[1]Settore Fitosanitario e servizi tecnico-scientifici – Piedmont Region, Turin, Italy, [2]Directorate General (DG) Agricoltura Servizio Fitosanitario Regionale, Lombardy Region, Milan, Italy, [3]Dipartimento di Ingegneria Civile Ambiente Territorio Architettura e Matematica (DICATAM), University of Brescia, Brescia, Italy, [4]Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), University of Turin, Turin, Italy, [5]Department of Agronomy, Food, Natural Resources, Animals and the Environment (DAFNAE), University of Padua, Padua, Italy, [6]Department of Biotechnology, University of Verona, Verona, Italy, [7]Council for Agricultural Research and Economics (CREA) - Research Centre for Plant Protection and Certification, Florence, Italy

Popillia japonica, a priority pest for the EU, was first detected in Northern Italy in 2014. Since its discovery, the outbreak extended over an area of more than 16,000 square kilometers in Northern Italy and Southern Switzerland. In this review, we summarize the state-of-the-art of research conducted in Italy on both the spreading capacity and control measures of *P. japonica*. Chemical, physical, and biological control measures deployed since its detection are presented, by highlighting their strengths and weaknesses. An in-depth study of the ecosystems invaded by *P. japonica* disclosed the presence and pathogenicity of natural strains of entomopathogenic fungi and nematodes, some of which have shown to be particularly aggressive towards the larvae of this pest under laboratory conditions. The Plant Health authorities of the Lombardy and Piedmont regions, with the support of several research institutions, played a crucial role in the initial eradication attempt and subsequently in containing the spread of *P. japonica*. Control measures were performed in the infested area to suppress adult populations of *P. japonica* by installing several traps (e.g., for mass trapping, for auto-dissemination of the fungus *Metarhizium anisopliae*, and "attract & kill"). For larval control, the infested fields were treated with commercial strains of the entomopathogenic fungus *M. anisopliae* and nematode *Heterorhabditis bacteriophora*. Future studies will aim at integrating phenological and spread models developed with the most effective control measures, within an ecologically sustainable approach.

KEYWORDS

biological control agents, biological invasion, chemical control, EU priority pest, Japanese beetle, pest management, spatial analysis

1 Introduction

The Japanese beetle, *Popillia japonica* Newman, 1841 (Coleoptera: Scarabaeidae) is a scarab beetle native to Japan and known to be a pest of agricultural crops, turfs, ornamental and forest plants in the introduction areas. The beetle has more than 300 host plants and is a strong flier, in addition, it has a great ecological plasticity that allows it to invade large areas in a short time (1–4).

Popillia japonica was introduced in 1916 in the US (1) and, within 100 years, it colonized most of the eastern and central US territories along with a part of eastern and western Canada (5). In Europe, *P. japonica* was detected for the first time in the early 1970s on Terceira Island (Azores archipelago). Since its first discovery, phytosanitary measures have been applied to manage *P. japonica*, such as chemicals, mass trapping, and biological control agents (6–10). Nevertheless, over the past 50 years, the infestation has reached other Azores islands: Faial, Flores, Pico, São Jorge, São Miguel, and Graciosa (11, 12). Due to its spread capacity and the potential impact on crops, *P. japonica* has been included in the European Plant Protection Organization (EPPO) list A2 as a quarantine pest recommended for regulation (https://www.eppo.int/ACTIVITIES/plant_quarantine/A2_list). In 2019, *P. japonica* was listed as a priority pest (13) and classified as the second most crucial potential priority pest in Europe (14, 15). In July 2014, a wildlife photographer posted a photo of *P. japonica* on the naturalist forum “Natura Mediterraneo” (https://www.naturamediterraneo.com/forum/). This was the first report of this pest in north-western Italy and in mainland Europe (16). The Ticino River valley, a Natural Park between the Lombardy and Piedmont regions, located in Northern Italy, was soon identified as the outbreak area as Plant Health authorities of the Lombardy and Piedmont regions (parts of the National Plant Protection Organization) detected a high-density of larvae, which locally exceeded 300 individuals per square meter. This high level of infestation attracted many vertebrate larval predators such as birds, moles, and wild boars, which resulted in further damage to the turf. However, the major impacts on crops, such as vineyards (Figure 1), soybean, and corn, were caused by adults feeding on leaves and fruits. Furthermore, the damage was also observed in private orchards of peach, plum, apple, persimmon, and other types of fruit plants present in the infested areas. Defoliation also involved vegetables and ornamental plants (17). The Plant Health authorities of the Lombardy and Piedmont regions reacted promptly, and several phytosanitary measures were adopted to monitor the area and to contain the *P. japonica* populations. Visual inspections and control measures were focused on all high-risk sites, e.g., plant nurseries and sites suitable for passive dispersal, such as industrial areas, trucking companies, railway stations, sports fields, playgrounds, boulevards, waste collection areas, petrol stations, car and truck parking areas, swimming pools, and shopping centers, considering the hitchhiking behavior of *P. japonica* adults (5, 18). Control measures were performed in the infested area to suppress adult populations of *P. japonica* by installing several double-baited traps that attract both sexes using a floral attractant and a synthetic pheromone: i) funnel traps for mass trapping, ii) traps for auto-dissemination of the fungus *Metarhizium anisopliae* (Metch.) Sorok, 1883, and iii) “attract & kill” devices containing alpha-cypermethrin or deltamethrin (19, 20). For larval control, 2,200 ha were treated with biological control agents such as the entomopathogenic nematode *Heterorhabditis bacteriophora* (Poinar, 1975) (Nematoda: Rhabditidae) and the entomopathogenic fungus *M. anisopliae* (21). Specific risk-based plans and peculiar prescriptions were provided in the airport and cargo areas of Malpensa and Cameri (respectively in the Lombardy and Piedmont regions), located within the infested area, to avoid the spread of *P. japonica* adults from an infested to pest-free area.

The huge efforts made to cope with *P. japonica* limited the spread of this pest in Italy, which reached 16,232 km^2 (Europhyt Outbreak No. 574, Update 07/2022-12-05) (Figure 2). Nevertheless, the pest was established also in the contiguous area of Ticino in Southern Switzerland (22), while interceptions outside the current area of infestation were reported in the Netherlands (23), Germany (24), and Italy (25, 26).

Since the first year of discovery, several research activities were conducted by the Plant Health authorities of the Lombardy and Piedmont regions and national research institutions (University of Brescia, Turin, Padua and Verona, and CREA-Research Centre for Plant Protection and Certification of Florence), to understand the ecology of the pest in the new introduction range and to contain larval and adult populations.

Here, we reviewed and summarized the knowledge on the processes determining the potential spread of *P. japonica* in recently invaded areas in Italy. In addition, we reviewed the chemical, physical, and biological control methods adopted against this pest, by considering their pros and cons. Finally, we pinpoint management techniques to be considered for the development of integrated control strategies.

2 Potential spread of *Popillia japonica* on the Italian territory

2.1 Prevention of the spread of *Popillia japonica* in Italy

Nurseries are one of the highest risk sites and therefore deserve a system of prescription and prevention measures to exclude the presence of *P. japonica*. Notwithstanding the movement of plants with soil, from infested to pest-free areas is banned, the Plant Health authorities of the Lombardy and Piedmont regions may authorize the movement if the plants have been grown in a pest-free production site of a registered operator, as defined by the Regulation (EU) 2016/2031 (art. 65) (27), subjected to official inspections in compliance with the Commission Implementing Regulation (EU) 2019/66 (28) and the Commission Implementing Regulation (EU) 2021/2285 (29) for the detection of *P. japonica*. However, one of the following requirements must be met to avoid the passive transport of *P. japonica*: i) the plants must be grown in sites with complete physical protection; ii) any soil residuals that could harbor juvenile stages have to be removed; iii) only commercial potting soil subjected to an insecticide soil treatment is used. The reuse of potting soil is possible only after a heat

FIGURE 1
Damage caused by adults of *Popillia japonica* on vineyards.

treatment at 49°C for at least 15 minutes. Concerning the field-grown plants, some precautions should be applied such as: i) milling the soil at least four times, at a depth of 15 cm, ii) treating the soil with insecticides, and iii) removing, for large plants, the first 20 cm of soil.

2.2 Spread of *Popillia japonica*

The study of the invasion process is essential to ensure the implementation of proper management plans for both the eradication and the containment of invasive species. The development and use of different spatial approaches to study the spread and support the management of invasive species have been well covered over the past 15 years (30–32). Surprisingly, the spatial dynamics of *P. japonica* have received little attention. Some authors have focused on investigating the local patchy distribution of both larvae and adults (33–36) or the flight activity of the species (4, 37). However, the assessment of the population spread of *P. japonica* at the landscape scale is less represented (38, 39).

This chapter explores and summarizes the available knowledge on the processes determining the potential spread of *P. japonica* in newly invaded areas, particularly the results of the data analysis and modeling tools proposed for describing pest spread in Northern

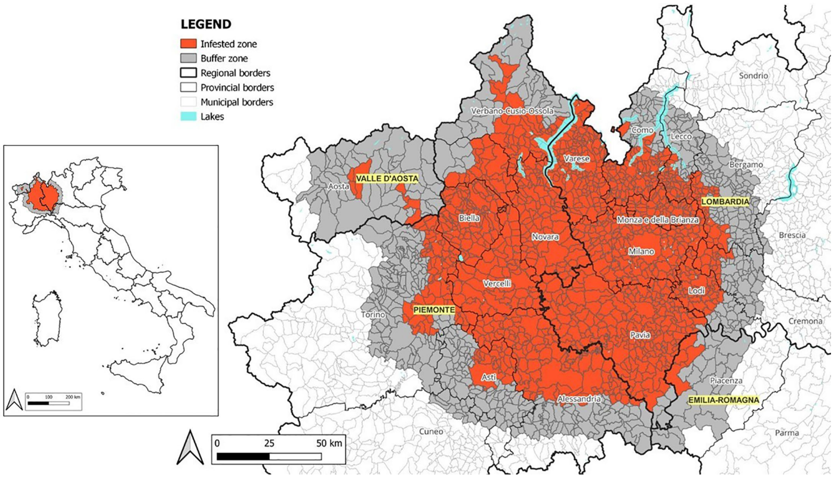


FIGURE 2
Italian demarcated area of *Popillia japonica*.

Italy. We considered three fundamental steps in the spatial spread of *P. japonica*: i) the dispersal process, ii) the pattern of population growth, and iii) the rate of population spread.

2.2.1 Dispersal

The pattern of dispersal of *P. japonica* can be investigated by considering two main components: i) individual short-distance dispersal based on random movement or some sort of guided flight based on cues related both to food resources (for both males and females) and to mate finding (only for males), and ii) the occurrence of a discrete event of long-distance dispersal based on natural means or due to human-assisted transportation.

The individual dispersal capacity of *P. japonica* is highly influenced by several parameters. Cloud cover, strong winds (above 20 km/h), or suboptimal temperatures (above or below 25.5°C) (37), in addition to land management practices such as intercropping systems (40, 41) or the application of pesticide treatments (42), significantly reduce the flight activity. In Italy, the main flight activity of *P. japonica* is known to occur between 12:00 pm and 3:00 pm in summer with low levels of relative humidity (43). This pest can fly an average distance of about 2.3 km in 24 hours, with up to 12 km in some cases, as demonstrated by means of a mark-capture technique (4). *Popillia japonica* adults show strong gregarious behavior, which facilitates finding food sources and/or mates. Mated females commonly represent the pioneers that colonize novel areas. Then, both sexual and feeding-induced odor attractants cause aggregation by the joiner individuals, providing further mating opportunities to the females, which have to mate more than once in their lifetime (2, 35).

Long-distance dispersal through the movement of infested soil and/or plants for planting or hitchhiking is also possible for *P. japonica*. The occurrence of long-distance spread events has also been documented outside the global infestation areas (Germany and the Netherlands) (23, 24). Between 2021 and 2022, new incursions of *P. japonica* adults were also reported in other parts of Italy such as Sardinia and Friuli Venezia Giulia regions (25, 26). These events could start new infestation *foci* from which a continuous spread process can originate. The consequence of stratified dispersal (44), summed to the continuous spread with discrete and long jumps events is poorly investigated for *P. japonica* but, as for other species, we expect it will be responsible for the establishment of an exponential pattern of growth in the infested area (45). To manage the risk associated with long-distance dispersal, various measures (including monitoring, removal of host plants, and treatments) are applied in high-risk areas such as big parking areas, loading and unloading docks, refueling stations, ports, and airports (46).

2.2.2 Population growth

The continuous spread of a pest can be considered the result of both individual dispersal and population growth (44). Therefore, knowledge about the population growth of *P. japonica* is fundamental for better understanding the invasion process occurring in Northern Italy and for planning and implementing appropriate control measures (47, 48). The population growth pattern of *P. japonica* was investigated during the ongoing invasion process occurring in Northern Italy. Monitoring data on larvae (through soil cores) and adults (using Trécé$^{\text{TM}}$ traps baited with dual semiochemical lure that attracts both sexes) collected since 2015 in the Lombardy region were analyzed to predict both the phenological patterns of the species in the infested area (49) (Figure 3) and its population growth pattern over the years of infestation. The latter was estimated using a time-discrete logistic model (the Beverton-Holt model, see 50) on the available adult population abundance data collected through Trécé$^{\text{TM}}$ traps. The results showed that, during the first years of infestation in a novel area, *P. japonica* population abundance is rather low, making the early detection of the pest relatively difficult. After this initial phase, in case the area is suitable for the presence of *P. japonica*, population build-up is rather prominent following a logistic-type (i.e., sigmoid) pattern that leads to high adult population density (on average, the daily adult population abundance reaches 200-300

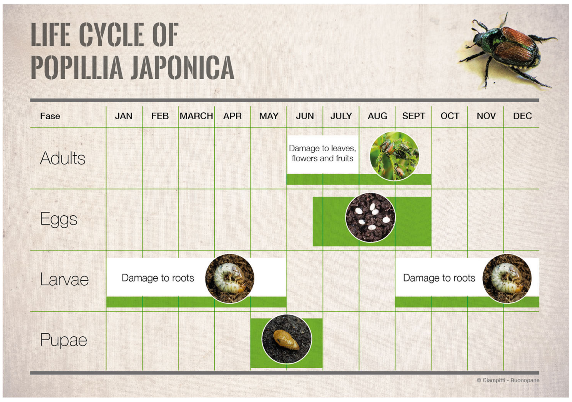


FIGURE 3
Life cycle of *Popillia japonica* in the Italian outbreak.

individuals per trap per day after 4-5 years since the first infestation). Model predictions show that *P. japonica* reaches the maximum population abundance after 7-8 years since the first outbreak.

2.2.3 Population spread

Most of the available knowledge on *P. japonica* spread comes from studies conducted in the US. These studies report varying results, depending on the time elapsed since the first infestation and the overall suitability of the investigated area. In Smith and Hadley (51), a spread rate ranging from 16 to 24 km/year was reported a decade after the first infestation of *P. japonica*. Fox (52) later reported a spread rate ranging from 3 to 24 km/year. Allsopp (38) reported that the spread rate of *P. japonica* was increasing over time, being 7.7 km/year between 1927 and 1938, and 11.9 km/year between 1939 to 1951.

Different approaches and modeling tools were applied to estimate and predict the spread rate of *P. japonica* populations, and to explain the role of relevant variables influencing the spreading process. Mondino et al. (39) proposed an iterative spatially-based model to interpret and forecast the spreading dynamics of *P. japonica*. Five years of trapping data were used for model parametrization (2015-2016) and validation (2017–2019). Variograms from both trapping data and model simulations suggested a range of spatial autocorrelation ranging from 7.5 to 15 km (with a determination coefficient (R^2) ranging between 0.39 and 0.87). The model proposed by Mondino et al. (39) includes a source dilution factor describing the rate of *P. japonica* moving away from a given position, based upon variograms, a parameter quantifying the increase in *P. japonica* population at the same location over years, and a mortality factor estimated based on literature data. Mondino et al. (39) also considered the role of land cover in determining the probability of colonization of new areas. Prediction maps indicate a potential spread of *P. japonica* mainly southwards and southeastwards, matching the distribution of suitable land covers (e.g., meadows, croplands, woods).

With the comprehensive monitoring dataset (from 2015 to 2021) on the time-series adult catches of the infested areas in the Lombardy region, the spatial and temporal dynamics of *P. japonica* population abundance were investigated using a discrete-time continuous-space reaction-diffusion model (50, 53). This analysis allowed the confirmation that the speed of invasion varies over different spatial directions starting from the area of the establishment. To better understand the spreading process and the factors affecting the speed of invasion, 14 different spreading trajectories were analyzed in that area. The estimated speed of the traveling fronts showed a fair degree of variability (coefficient of variation = 34%) with a maximum speed higher than 13 km/year and a minimum speed lower than 5.5 km/year, 2.5 times less than the maximum. The analysis showed that the suitability of the habitats plays an important role in determining the speed of the traveling fronts. In the fastest-moving directions, more than 80% of the habitats are suitable for the presence of pest (e.g., arable land, perennial meadows, broadleaf forests, urban green areas, rice fields, or agricultural woodlands). In the slower speed trajectories, over 40% of the habitats are not suitable for the pest (e.g., coniferous or mixed forests, or non-vegetated urban areas). From preliminary estimates, it appears that the traveling front speed increases by about 1.5 km/year for every 10% increase in areas suitable for *P. japonica*. The data analysis on larval abundance from soil samples (from 2015 to 2021 of the infested areas in the Lombardy region) showed that *P. japonica* prefers soils that are not loamy sand or acidic. In particular, the relation of pH on species' suitability shows a non-linear trend and it seems strongly influenced by soil particle size (54–56). Soils with a medium organic matter content are preferred by *P. japonica*, rather than soils with high or low organic matter content (33, 56, 57). Results also showed a non-linear trend between habitat suitability and soil humidity, with extremely dry (58, 59) and relatively wet soils (56) being not suitable for the pest. Similarly, extremely low (below 13°C) or high (above 34°C) temperatures are not suitable for the pest (2, 56, 58, 59). The role of climate and climate change on the potential distribution of *P. japonica* has been studied by Zhu et al. (60) and Kistner-Thomas (59). Changing temperatures are expected to favor the occurrence of the pest in areas above 37° N latitude. Global warming is also expected to increase the area where the species is able to complete a single generation per year, especially in areas above 37° N latitude (59). These effects could lead to an increase in the population abundance of *P. japonica* in the northernmost areas of its range, which could have a positive effect on the population growth and spread rate of the pest (44). In contrast, areas below 8° N latitude are expected to become less suitable for the pest due to an increase in temperature above the optimal range (59).

3 *Popillia japonica* outbreak management

3.1 Chemical control

In the early stages of the spread of an invasive pest, the use of insecticides is critical to meet immediate pest management needs. The use of broad-spectrum insecticides to control growing *P.*

japonica populations was widely used in the early years of the last century in the US due to their effectiveness and relatively low cost (2, 61, 62). Problems caused to non-target insect populations, as well as to human health, and other warm-blooded animals, have curtailed their use and changed spraying methods (5).

Extensive EU legislation regulates the marketing (Regulation (CE) 2009/1107) (63), and use (Directive 2009/128/EC) (64) of plant protection products and their residues in foodstuffs. While this regulation reduces the environmental risks of the pesticide, it negatively affects the availability of active ingredients (AIs) for *P. japonica* containment. The current limitation of products registered against this species required experimental tests to evaluate the effectiveness of the AIs available in European countries.

In 2017-2019, field trials were carried out in vineyards in Novara (North-eastern Piedmont) testing chemical insecticides and organic products with repellent and/or phago-deterrent effects against *P. japonica* adults. The effectiveness of the different substances was evaluated by counting the adults before and after treatments and by estimating the defoliation rates in the different plots. Deltamethrin, etofenprox, lambda-cyhalothrin, acetamiprid, and chlorantraniliprole showed a high-medium efficacy in reducing adult infestations and defoliation rates, while tau-fluvalinate, chlorpyrifos-methyl, and organic pyrethrins had lower activity. Among the repellent/phago-deterrent substances, neem oil and zeolite (chabazite) were quite ineffective, while kaolin clay reduced the number of adults feeding on the vines (65). Additionally, the side effects on phytoseiid mites were evaluated by inspecting leaf samples in the laboratory under a stereomicroscope, but only pyrethroids negatively affected the predatory mite populations (65).

In 2019 and 2020, 20 AIs representative of chemical and organic insecticides registered in Europe for the management of adult beetles were tested. The trials were carried out at five sites located in the infested area (Milano and Varese provinces). The target plant species were three high-value crops (grapevine *Vitis vinifera* L., peach *Prunus persica* (L.) Batsch, and corn *Zea mays* L.) and two landscape plants (goat willow *Salix caprea* L. and Virginia creeper *Parthenocissus quinquefolia* (L.) Planch.). For each site, branches were covered with a protective net (70 × 100 cm, mesh 1 × 1 mm) and 25 adult beetles were introduced. Three experimental conditions were tested for each insecticide: contact, short-term, and long-term (residual) effects. Beetles were introduced before the spraying (contact), right after (short-term), and one week after (long-term) (66). Four replicates in each experimental condition and site were used and insect mortality was assessed until three weeks after the treatment. Acetamiprid, deltamethrin, lambda-cyhalothrin, and phosmet, which are broad-spectrum insecticides, showed to be effective in killing beetles under all experimental conditions. Most of the other AIs were effective only by contact and short-term residual. The organic AIs were not effective under any condition; a mixture of an organic ingredient, paraffinic mineral oil, with cypermethrin showed good efficacy on contact (66, 67).

The lack of long-term efficacy of the insecticides registered in Europe for adult management led to applications repeated many times during *P. japonica* flight period, starting from adult emergence (65). Moreover, the low selectivity of the effective AIs suggests integrating these chemicals into a general management plan that envisages the use of these pesticides only where necessary. To limit the adoption of chemical products and preserve non-target species in the environment, the use of Long-Lasting Insecticide-treated Nets (LLINs) activated with pyrethroids has been tested since 2017 (68). This technology was originally conceived to protect people from vector-borne diseases, such as malaria or yellow fever, and then its use has been extended to agricultural management (69–75). To protect crops and stored products from *P. japonica* attract-and-kill devices (frames containing floral attractant and synthetic pheromone lures), covered with LLINs (usually made of polyester or polyethylene fibers) impregnated with or coated by insecticides such as alfa-cypermethrin or deltamethrin at concentrations ranging from 1 to 4 mg AI/g fiber, are being used (Figure 4A). In this way, *P. japonica* adults are attracted by the lure, get in touch with the LLIN and eventually die. To evaluate the effectiveness of LLINs against *P. japonica*, laboratory tests were performed with adult *P. japonica*. The insects (both males and females) were allowed to walk on the LLIN at different exposure times. At the end of the experiment, mortality ranged from 89% to 100% for alfa-cypermethrin, while 100% mortality was observed for deltamethrin irrespective of the exposure time (68). An evaluation of how long alfa-cypermethrin LLIN can be effective during the flight season was also carried out. As a result, the mortality of *P. japonica* decreased by about 30% after one month of field exposure, although still significantly different from the control. After two and three months of field exposure, the mortality was similar to that of the control (43). This is not unexpected because pyrethroids are known to decay under sunlight exposure (76), and so does their killing effectiveness. LLINs were deployed on a large scale by the Plant Health authorities of the Lombardy and Piedmont regions to control this pest in the infested area.

3.1.1 Experimental soil-injection machine

To control *P. japonica* larvae in the soil, an experimental soil-injection machine ("Eco Defender 25"), was conceived and produced in collaboration with the company "MA/AG" and tested in two highly infested perennial meadows in the Lombardy region. The machine injects liquid solutions into the soil with minimum turf damage and soil agronomic characteristics perturbation. At the end of the summer of 2020 and 2021, biological control agents (entomopathogenic nematodes-EPNs, and fungi-EPF) and the insecticide Acelepryn (AI chlorantraniliprole at 20%), authorized for turf application by derogation in accordance with Regulation (CE) 2009/1107 (art. 53) (63), were applied. The effects of the products were evaluated by counting the number of live larvae in the soil after 40 days of the application. There was a general reduction of larval density in the treated plots, with the Acelepryn formulation performing better in the short term (67).

3.2 Physical control

3.2.1 Weed mulching products and humidity control on potted plants

Due to the restrictions on transporting plants/soil from areas infested with *P. japonica*, a new approach was used to prevent beetle oviposition in grapevine potted plants. In 2020, in a nursery located

FIGURE 4
(A) Attract & kill device with long-lasting insecticide-treated net. (LLIN) The LLIN is mounted on a tripod frame made of telescopic tubes extendable up to 2 m in height **(B)** Traps (H: 35 cm, L: 60 cm, W: 18 cm) for auto-dissemination of the fungus *Metarhizium anisopliae*.

in the infested area of the Lombardy region, three commercial weed mulching products for potted plants were used to assess the oviposition ability of female *P. japonica*. The tested weed mulching products were coconut fiber mulching discs (two densities), jute fabric, and wood chips (Figure 5). Results showed that coconut mulching was able to strongly reduce oviposition and further larval development (67, 77).

In 2021, in the same nursery, the survival of *P. japonica* larvae was challenged by applying a mild water stress treatment to potted plants. In the pots subjected to daily irrigation, the average number of larvae found was about five times higher than that in water-stressed pots. At the end of the experiment, both daily irrigated and mildly stressed plants returned to normal conditions (78).

FIGURE 5
Trial of physical barriers deployed above the soil in containers for ornamental plants in a nursery.

3.2.2 Nets barrier

To protect nursery plants from adult insect damage, in a heavily infested nursery located in the Lombardy region, three net types were tested as physical barriers to protect grapevine potted plants from *P. japonica* adults. The types of nets were anti-hail nets, anti-hail nets treated with permethrin, and insect-proof nets. All tested nets were equally able to protect the plants from adult beetles for the entire duration of the trial compared to those with no protection. The use of an anti-hail net could be the best option to protect nursery plants given the lower costs compared with an insect-proof net (79).

4 Biological control

Several methods have been developed to control *P. japonica* and environmentally friendly strategies, based on the use of natural enemies, have been reported since 1920 (e.g., 80, 81), and tested also in Italy. Many EPF are relatively common worldwide, often trigger epizootics, and can therefore be considered an important factor in controlling insect populations. Several EPF (*Metarhizium* spp. Sorok. and *Beauveria* sp. Vuill.) have also been tested against larvae, pupae, and adults of *P. japonica* in laboratory, semi-field, and field trials worldwide but with contrasting results (2). At the beginning of the Italian invasion, different species of commercial EPF were tested in both controlled and open-field trials. *Beauveria bassiana* (Bals.-Criv.) Vuill. (1912) (commercial strain ATCC 74040) was tested against adults in an ornamental plant nursery in Northern Italy, but the treatment was found to be ineffective (67). The genus *Metarhizium*, on the other hand, has been evaluated several times. Benvenuti et al. (82) tested the commercial *Metarhizium brunneum* Petch, 1939 strain BIPESCO5 against pupae in semi-field applications and found an overall adult mortality of about 40% in 12 days. Benvenuti et al. (83) tested an experimental auto-disseminating device (Figure 4B) that attracts, infects, and releases adults into the environment to spread the EPF to healthy populations. The device was activated with the two commercial products available in Italy (GranMet® and Met52®) containing *M. brunneum*. In the horizontal transmission trials with GranMet®, healthy *P. japonica* adults were in contact with a single infected individual. In this case, a mortality of 100% was observed after 19 days, while in the trial with Met52®, the mortality was 30-65%. This result appeared promising and, if confirmed in extensive field trials, could represent a new tool for biological control strategies against the Japanese beetle. By contrast, Bosio et al. (65) found that Met52® applied as a foliar spray against the adults did not differ from the control. To cope with *P. japonica* populations, Barzanti et al. (84) investigated the possibility of exploiting the presence of native strains of *Metarhizium* spp. in natural environments. For this purpose, the presence of *Metarhizium* species was analyzed in the soils of *P. japonica* infested areas. Four *Metarhizium* species were identified (*M. robertsii* J. F. Bisch., Rehner and Humber, 2009; *M. brunneum*; *M. guizhouense* Q.T. Chen and H. L. Guo, 1986, *M. lepidiotae* J. F. Bisch., Rehner and Humber, 2009) and used in virulence laboratory tests, with *M. robertsii* showing the best performance (84). The study confirmed the presence of native *Metarhizium* strains that can attack this invasive beetle and launched a debate on their future use in IPM programs. Entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) have shown greater potential for the biological control of *P. japonica* than other natural biological agents (2). Therefore, EPNs, in association with other biological control agents, have been an important component of integrated pest management strategies in the US (2). The virulence of nematode species and strains against *P. japonica* grubs differed substantially (e.g., 85, 86) and contradicting results regarding the role of the larval stage on the susceptibility of *P. japonica* to EPNs, in laboratory studies, were reported. In Italy, for example, Paoli et al. (87) found that third-instar susceptibility to *Heterorhabditis bacteriophora* was higher in pre-overwintering than in overwintered larvae. Concerning EPNs at the beginning of the invasion in Italy, laboratory and field experiments were conducted with several native and commercial strains of *H. bacteriophora* and *Steinernema carpocapsae* Weiser, 1955 (Nematoda: Rhabditidae), in order to develop baseline data for a biological control approach for this outbreak (88). In the laboratory, *H. bacteriophora* strains caused higher mortality than *S. carpocapsae*, and the same results were obtained in micro-plot (2 × 6 m) field trials with an autochthonous strain of *H. bacteriophora*. Finally, in a large-plot (20 × 5 m) field trial, the commercial *H. bacteriophora* product (Larvanem) provided 46% larval mortality (88, 89) (Figure 6). This study highlighted that *H. bacteriophora* strains have good potential as biological control agents for the larvae of the invasive *P. japonica* in Northern Italy. This work, together with the restriction that only native EPN species can be field-released in Italy (EU Habitats Directive, art. 12, DPR 120/2003), has encouraged the search for local and better adapted EPN strains, especially in the two regions mostly infested by *P. japonica*: Piedmont (90) and Lombardy regions (91). Torrini et al. (90) reported that soils in the Ticino valley are rich in EPNs. The evaluation of all these EPN natural strains in laboratory assays confirmed that larval mortality was higher for pre-wintering than for post-wintering larvae, as already reported by Paoli et al. (87), and that *H. bacteriophora* natural strains are more efficient in controlling *P. japonica* larvae (88). Since native EPN isolates possess physiological traits that are adapted to local ecological conditions, the idea of supporting the ecosystem by propagating EPNs that thrive in their native soil could be considered as a basis for an eco-friendly approach to control this pest. In addition, a density-dependent response of EPNs to *P. japonica* has been shown since its introduction, indicating the great potential of these organisms as natural regulators of *P. japonica* populations (91). However, a decline in native scarab beetle populations has also been observed, indicating the generalist nature of soil EPNs (91). In addition to classic EPNs, other parasitic nematodes of *P. japonica* have been reported in Italy, such as the new species *Hexamermis popilliae* Poinar, 2017 (Nematoda: Mermithidae) (92) (Figures 7A, B) and *Oscheius myriophilus* (Poinar, 1986) (Nematoda: Rhabditidae) (93), both isolated from *P. japonica*. However, further studies are needed to assess the specificity of these nematodes and their possible use as biological control agents of *P. japonica*. In Italy, no parasitoids have been identified so far. However, it is noteworthy that the allochthonous parasitoid wasps *Tiphia vernalis* Rohwer, 1924, and *T. popilliavora* Rohwer, 1920 (Hymenoptera: Tiphiidae), which attack larvae, have established at low density in a wide range over the US. The same situation was observed for the fly *Istocheta aldrichi* (Mesnil, 1953) (Diptera: Tachinidae), which parasitizes adults (2) in the US. In Italy, generalist invertebrates (e.g., spiders) and vertebrate predators, (e.g., moles, birds) are under evaluation as potential natural enemies of *P. japonica*. Predators, especially ants, carabids, and spiders have caused high rates of mortality of *P. japonica* eggs and young larvae in the US (94, 95). The microsporidian *Ovavesicula popilliae* (Andreadis and Hanula, 1987) and the pathogenic bacteria, particularly *Bacillus* spp. and *Paenibacillus popilliae* (Dutky) Pattersson et al., 1999, causal agents of the milky disease, are present in the US (2, 96), but there are no data on their occurrence in Italy. In conclusion, considering that several EPN natural strains have been isolated in Italy and that an

FIGURE 6
Treatments with entomopathogenic nematodes (EPN) in fields infested with *Popillia japonica* larvae.

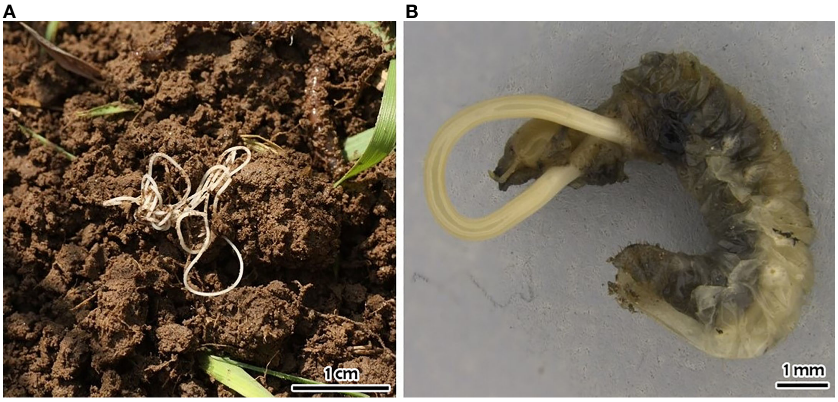


FIGURE 7
Hexamermis popilliae: **(A)** free living specimen *sensu* Mazza et al. (92); **(B)** third-stage postparasitic juvenile emerging from *Popillia japonica* larva.

indigenous *H. bacteriophora* caused high mortality, EPNs seem to be particularly relevant for the control of this insect in Italy. EPNs can be combined with other methods such as insecticides (2) and EPF (97) to increase the pest control rate. However, several factors still limit the adoption of EPNs, such as the high cost, limited availability, short shelf-life, and formulations (2).

5 Conclusions

The accidental introduction of *P. japonica* into mainland Europe has led the Italian Plant Protection Organization to promote the development of multiple strategies to control the pest population and to deploy phytosanitary measures to prevent its spread. Previous knowledge from the US guided the first responses to the *P. japonica* invasion in Italy but, given the peculiarities of the local landscapes/crops and legislation restrictions on the use of chemicals and classical biological control agents, in-depth studies were needed for the development of management strategies to mitigate the impacts of *P. japonica* in newly invaded areas. Different control methods (i.e., chemical, physical, and biological control) have been developed and effective phytosanitary measures were taken to prevent the spread of the pest through the movement of plants, commodities, and vehicles from infested to pest-free areas. Despite ongoing regulatory efforts, *P. japonica* remains a threat and new areas have been colonized. As it was not possible to eradicate the pest and completely stop its natural spread, it is necessary to continue working on more effective and sustainable solutions in the context of an integrated management perspective.

In this context, the models developed to predict the phenology and spread of *P. japonica* can support the regulatory authorities in guiding decision-making toward rational and sustainable management of the pest.

As the use of insecticides in landscapes is increasingly restricted and registrations of some chemicals declined in the meanwhile, a higher level of protection could be achieved through an integrated approach that takes into account the specific landscape of an area and the occurrence of the main hosts of the pest. In addition to the use of spraying chemicals, which are effective under most conditions, the use of EPNs showed to be promising in lowering the larval population of *P. japonica* in treated areas and therefore can be a more eco-friendly or low-impact approach to control this pest. Moreover, the use of attract-and-kill devices turned out to be an effective strategy requiring minimal management effort with a high impact on adult populations. This strategy could be useful to slow down the spread rate of *P. japonica*. So far, EPF against larvae have only shown a significant effect in laboratory experiments, while results in the field seem to be limited. As far as parasitoids are concerned, there are no cases of natural and effective parasitism in Italy, and the possibility of importing parasitoids and other biological control agents from the native area of *P. japonica* is currently under investigation.

In conclusion, the knowledge of *Popillia japonica* acquired in Italy in recent years made it possible to better assess the population abundance, phenology, and spread. Moreover, these in-depth studies provided the basis for validating and directing control measures toward effective, sustainable, and environmentally sound management, as well as for developing new legislation for the control of this alien pest in the threatened European territory.

Author contributions

This review was coordinated by the expert working group "Tavolo Tecnico *Popillia japonica*" MC, PG, GB, BC, EG, AA, NM, PR, and LM. All working group members contributed to the concept and structuring of the manuscript. MC, PG, GB, AA, NM, LM, GG, GM, GT, FP, and ABa wrote the first draft and all other authors (BC, AS, FL, GSa, GSp, EG, PR, and ABi) provided significant inputs into the text. All authors contributed to the article and approved the submitted version.

Funding

This work has received funding from the Piedmont region Grant/Award number D. n. 1161—29/11/2016 (COBIPO Project), from the

Lombardy region: New methodologies for the integrated management of *Popillia japonica* (GESPO Project) and Prevention and control of new phytosanitary emergencies in Lombardy region: *Popillia japonica* and *Xylella fastidiosa* (PRECONFITOLOMB Project) Grant/Award number D.d.s. n. 19442 – 21/12/2018; and from the European Union's Horizon 2020 research and innovation programme "IPM POPILLIA" Integrated pest Management of the invasive Japanese Beetle, *Popillia japonica*, under grant agreement No. 861852.

Conflict of interest

The authors GT and LM declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

The remaining 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.

The reviewer J-CG declared a past co-authorship with the authors AB and GG to the handling Editor.

Publisher's note

References

1. Fleming WE. *Biology of the Japanese beetle (Technical bulletin no. 1449)*. Washington DC: US Department of Agriculture (1972).

2. Potter DA, Held DW. Biology and management of the Japanese beetle. *Annu Rev Entomol* (2002) 47:175–205. doi: 10.1146/annurev.ento.47.091201.145153

3. EFSA Panel on Plant Health (PLH), Bragard C, Dehnen-Schmutz K, Di Serio F, Gonthier P, Jacques MA, et al. Pest categorisation of *Popillia japonica*. *EFSA J* (2018) 16 (11):e05438. doi: 10.2903/j.efsa.2018.5438

4. Lessio F, Pisa CG, Picciau L, Ciampitti M, Cavagna B, Alma A. An immunomarking method to investigate the flight distance of the Japanese beetle. *Entomol Gen* (2022) 42(1):45–56. doi: 10.1127/entomologia/2021/1117

5. Althoff ER, Rice KB. Japanese Beetle (Coleoptera: scarabaeidae) invasion of north America: history, ecology, and management. *J Integr Pest Manage* (2022) 13(1):2. doi: 10.1093/jipm/pmab043

6. Martinus A, Simoes N. Suppression of the Japanese beetle in the Azores: an ecological approach. *Ecol Bull* (1988) 39:99–100.

7. Simões N, Laumond C, Bonifassi E. Effectiveness of *Steinernema* spp. and *Heterorhabditis bacteriophora* against *Popillia japonica* in the Azores. *J Nematol* (1993) 25(3):480–5.

8. Mendes C, Lacey L, Amaral J, Klein M. Biological control of *Popillia japonica* on terceira island (Azores, portugal): potential of *Bacillus popillae*. *Bull OILB/SROP* (1994) 17(3):31–4.

9. Lacey LA, Martins A, Ribeiro C. The pathogenicity of metarhizium anisopliae and *Beauveria bassiana* for adults of the Japanese beetle, *Popillia japonica* (Coleoptera: scarabaeidae). *Eur J Entomol* (1994) 91(3):313–9.

10. Lawrence A, Rosa JS, Nelson O, Es J, Harry K. Comparative dispersal and larvicidal activity of exotic and Azorean isolates of entomopathogenic nematodes against *Popillia japonica* (Coleoptera: Scarabaeidae). *Eur J Entomol* (2001) 98:439–44.

11. Vieira V. The Japanese beetle *Popillia japonica* Newman 1838 (Coleoptera: scarabaeidae) in the Azores islands. *Boletin Sociedad Entomologica Aragonesa* (2008) 43:450–1.

12. EPPO. *RS. EPPO reporting service no. 08. num. article 2019/158 update of the situation of Popillia japonica in Portugal (Azores)*. (2019).

13. European Commission. Regulation (EU) 2019/1702 on supplementing regulation (EU) 2016/2031 of the European parliament and of the council by establishing the list of priority pests. *Off J Eur Union* (2019).

14. EFSA, Baker R, Gilioli G, Behring C, Candiani D, Gogin A, et al. Report on the methodology applied by EFSA to provide a quantitative assessment of pest-related criteria required to rank candidate priority pests as defined by regulation (EU) 2016/2031. *EFSA J* (2019) 17(6):e05731. doi: 10.2903/j.efsa.2019.5731

15. Sánchez B, Barreiro-Hurle J, Soto-Embodas I, Rodriguez-Cerezo E. *The impact indicator for priority pests (I2P2): a tool for ranking pests according to regulation (EU) 2016/2031* Vol. 10. . Maastricht: Europen Union (2019). p. 585182.

16. Pavesi M. *Popillia japonica* specie aliena invasiva segnalata in lombardia. *L'Informatore Agrario* (2014) 32:53–5.

17. Bosio G, Piazza E, Giacometto E. *Popillia japonica*, una specie in progressiva diffusione. *L'Informatore Agrario* (2022) 21:53–9.

18. Poggi S, Desneux N, Jactel H, Tayeh C, Verheggen F. A nationwide pest risk analysis in the context of the ongoing Japanese beetle invasion in continental Europe: the case of metropolitan France. *Front Insect Sci* (2022) 2:1079756. doi: 10.3389/finsc.2022.1079756

19. Regione Piemonte. *Popillia japonica, risultati piano azione 2017* (2017). Available at: http://www.regione.piemonte.it/web/sites/default/files/media/documenti/2018-12/risultati_piano_azione_2017.pdf (Accessed 04 April 2023).

20. Regione Lombardia. *Popillia: al via il piano di controllo 2022* (2022). Available at: http://www.ersaf.lombardia.it/it/b/11825/popilliaalviailpianodicontrollo (Accessed 04 April 2023).

21. Bosio G, Cavagna B, Marianelli L. *Popillia japonica* (2018). Available at: https://www.ersaf.lombardia.it/it/file/2552/2cb99a45/Popillia+japonica+Presentazione+Giornate+Fito_7_3_2018_784_36235.pdf (Accessed 15/10/2022). Presented during the congress "Giornate fitopatologiche" – Chianciano Terme.

22. EPPO. *RS. EPPO reporting service no. 05. num. article: 2021/104 update on the situation of popillia japonica in Switzerland*. (2021).

23. EPPO. *RS. EPPO reporting service no. 02. num. article: 2019/034 dead beetle of Popillia japonica found in trap at schiphol airport (NL)*. (2019).

24. EPPO. *RS. EPPO reporting service no. 08. num. article:2022/165 new finding of Popillia japonica in Germany*. (2022).

25. Bassi C, Benvenuto L, Bernardinelli I, Drosghig A, Malossini G. *Popillia japonica*: primi rinvenimenti in friuli-venezia giulia. *Notiziario ERSA* (2022) 3:43–7.

26. EPPO. *RS. EPPO reporting service no. 04. num. article: 2022/081 update of the situation of Popillia japonica in Italy*. (2022).

27. European Commission. Regulation (EU) 2016/2031 on protective measures against pests of plants, amending regulations (EU) no 228/2013, (EU) no 652/2014 and (EU) no 1143/2014 of the European parliament and of the council and repealing council directives 69/464/EEC, 74/647/EEC, 93/85/EEC, 98/57/EC 2000/29/EC 2006/91/EC and 2007/33/EC. *Off J Eur Union* (2016).

28. European Commission. Commission implementing regulation (EU) 2019/66 of 16 January 2019 on rules on uniform practical arrangements for the performance of official controls on plants, plant products and other objects in order to verify compliance with union rules on protective measures against pests of plants applicable to those goods. *Off J Eur Union* (2019).

29. European Commission. Commission implementing regulation (EU) 2021/2285 of 14 December 2021 amending implementing regulation (EU) 2019/2072 as regards the listing of pests, prohibitions and requirements for the introduction into, and movement within, the union of plants, plant products and other objects, and repealing decisions 98/109/EC and 2002/757/EC and implementing regulations (EU) 2020/885 and (EU) 2020/1292. *Off J Eur Union* (2021).

30. Lessio F, Mondino EB, Alma A. Spatial patterns of *Scaphoideus titanus* (Hemiptera: cicadellidae): a geostatistical and neural network approach. *Int J Pest Manage* (2011) 57:205–16. doi: 10.1080/09670874.2011.566642

31. Gilioli G, Pasquali S, Tramontini S, Riolo F. Modelling local and long-distance dispersal of invasive chestnut gall wasp in Europe. *Ecol Modell* (2013) 263:281–90. doi: 10.1016/j.ecolmodel.2013.05.011

32. Hudgins EJ, Liebhold AM, Leung B. Predicting the spread of all invasive forest pests in the united states. *Ecol Lett* (2017) 20:426–35. doi: 10.1111/ele.12741

33. Dalthorp D, Nyrop J, Villani MG. Spatial ecology of the Japanese beetle, *Popillia japonica*. *Entomol Exp Appl* (2000) 96:129–39. doi: 10.1046/j.1570-7458.2000.00688.x

34. Hamilton RM, Foster RE, Gibb TJ, Sadof CS, Holland JD, Engel BA. Distribution and dynamics of Japanese beetles along the Indianapolis airport perimeter and the influence of land use on trap catch. *Environ Entomol* (2007) 36:287–96. doi: 10.1093/ee/36.2.287

35. Kowles KA, Switzer PV. Dynamics of aggregation formation in Japanese beetles, *Popillia japonica*. *J Insect Behav* (2012) 25:207–21. doi: 10.1007/s10905-011-9291-7

36. Sara SA, McCallen EB, Switzer PV. The spatial distribution of the Japanese beetle, *Popillia japonica*, in soybean fields. *J Insect Sci* (2013) 13:36. doi: 10.1673/031.013.3601

37. Lacey LA, Amaral JJ, Coupland J, Klein MG. The influence of climatic factors on the flight activity of the Japanese-beetle (Coleoptera:Scarabaeidae): implications for use of a microbial control agent. *Biol Control* (1994) 4:298–303. doi: 10.1006/bcon.1994.1038

38. Allsopp PG. Japanese Beetle, *Popillia japonica* Newman (Coleoptera: scarabaeidae): rate of movement and potential distribution of an immigrant species. *Coleopt Bull* (1996) 50:81–95.

39. Mondino EB, Lessio F, Bianchi A, Ciampitti M, Cavagna B, Alma A. Modelling the spread of *Popillia japonica* Newman (Coleoptera: scarabaeidae) from a recently infested area. *Entomol Gen* (2022) 42(5):713–21. doi: 10.1127/entomologia/2022/1370

40. Bohlen PJ, Barrett GW. Dispersal of the Japanese beetle (Coleoptera: scarabaeidae) in strip-cropped soybean agroecosystems. *Environ Entomol* (1990) 19:955–60. doi: 10.1093/ee/19.4.955

41. Holmes DM, Barrett GW. Japanese Beetle (*Popillia japonica*) dispersal behavior in intercropped vs. monoculture soybean agroecosystems. *Am Midl Nat* (1997) 137 (2):312–9. doi: 10.2307/2426850

42. Lacey LA, Amaral JJ, Coupland J, Klein MG, Simoes AM. Flight activity of *Popillia japonica* (Coleoptera: scarabaeidae) after treatment with *Metarhizium anisopliae*. *Biol Control* (1995) 5:167–72. doi: 10.1006/bcon.1995.1020

43. Paoli F, Iovinella I, Barbieri F, Sciandra C, Sabbatini Peverieri G, Mazza G, et al. Effectiveness of field-exposed attract-and-kill devices against the adults of *Popillia japonica* (Coleoptera: scarabaeidae): a study on duration, form and storage. *Pest Manage Sci* (2023). doi: 10.1002/ps.7504

44. Liebhold AM, Tobin PC. Population ecology of insect invasions and their management. *Annu Rev Entomol* (2008) 53:387–408. doi: 10.1146/annurev.ento.52.110405.091401

45. Shigesada N, Kawasaki K. Invasion and the range expansion of species: effects of long-distance dispersal. In: Bullock J, Kenward R, Hails R, editors. *Dispersal Ecol* Oxford, UK: Blackwell Science (2002). p. 350–73.

46. EPPO. *PM 9/21 (1) Popillia japonica: procedures for official control* Vol. 46. EPPO Bull (2016) p. 543–55. doi: 10.1111/epp.12345

47. Hulme EJ. An introduction to plant biosecurity: past, present and future. In: Gordh G, McKirdy S, editors. *The handbook of plant biosecurity*. Dordrecht: Springer Netherlands (2014). p. 1–25.

48. Hall AA, Gherlenda AN, Hasegawa S, Johnson SN, Cook JM, Riegler M. Anatomy of an outbreak: the biology and population dynamics of a c ardiaspina psyllid species in an endangered woodland ecosystem: population dynamics of a psyllid outbreak. *Agr For Entomol* (2015) 17:292–301. doi: 10.1111/afe.12106

49. Gilioli G, Sperandio G, Simonetto A, Colturato M, Battisti A, Mori N, et al. Modelling diapause termination and phenology of the Japanese beetle, *Popillia japonica*. *J Pest Sci* (2021) 95:869–80. doi: 10.1007/s10340-021-01434-8

50. Lutscher F. Modeling with integrodifference equations. In: *Integrodifference equations in spatial ecology. interdisciplinary applied mathematics*, vol. 49. Cham: Springer (2019). p. 9–22. doi: 10.1007/978-3-030-29294-2_2

51. Smith LB, Hadley CH. *The Japanese beetle*. Washington DC: United States Department of Agriculture (1926) p. 1–67.

52. Fox H. The distribution of the Japanese beetle in 1930 and 1931, with special reference to the area of continuous infestation. *J Econ Entomol* (1932) 25:396–407. doi: 10.1093/jee/25.2.396

53. Kot M, Lewis MA, van den Driessche P. Dispersal data and the spread of invading organisms. *Ecology* (1996) 77:2027–42. doi: 10.2307/2265698

54. Hawley IM, Dobbins TN. The distribution and abundance of the Japanese beetle from 1935 through 1943, with a discussion of some of the known factors that influence its behavior. *J.N.Y. Entomol Soc* (1945) 53:1–20.

55. Régnière J, Rabb RL, Stinner RE. *Popillia japonica* (Coleoptera: scarabaeidae): a mathematical model of oviposition in heterogeneous agroecosystems. *Can Entomol* (1979) 111:1271–80. doi: 10.4039/Ent1111271-11

56. Simonetto A, Sperandio G, Battisti A, Mori N, Ciampitti M, Cavagna B, et al. Exploring the main factors influencing habitat preference of *Popillia japonica* in an area of recent introduction. *Ecol Inform* (2022) 70:101749. doi: 10.1016/j.ecoinf.2022.101749

57. Szendrei Z, Mallampalli N, Isaacs R. Effect of tillage on abundance of Japanese beetle, *Popillia japonica* Newman (Col., scarabaeidae), larvae and adults in highbush blueberry fields. *J Appl Entomol* (2005) 129:258–64. doi: 10.1111/j.1439-0418.2005.00961.x

58. Allsopp PG, Klein MG, McCoy EL. Effect of soil moisture and soil texture on oviposition by Japanese beetle and rose chafer (Coleoptera: scarabaeidae). *J Econ Entomol* (1992) 85:2194–200. doi: 10.1093/jee/85.6.2194

59. Kistner-Thomas EJ. The potential global distribution and voltinism of the Japanese beetle (Coleoptera: scarabaeidae) under current and future climates. *J Insect Sci* (2019) 19:16. doi: 10.1093/jisesa/iez023

60. Zhu G, Li H, Zhao L. Incorporating anthropogenic variables into ecological niche modeling to predict areas of invasion of *Popillia japonica*. *J Pest Sci* (2017) 90:151–60. doi: 10.1007/s10340-016-0780-5

61. Bethke JA, Cloyd RA. Pesticide use in ornamental production: what are the benefits? *Pest Manage Sci* (2009) 65:345–50. doi: 10.1002/ps.1695

62. Shanovich HN, Dean AN, Koch RL, Hodgson EW. Biology and management of Japanese beetle (Coleoptera: scarabaeidae) in corn and soybean. *J Integr Pest Manage* (2019) 10:9. doi: 10.1093/jipm/pmz009

63. European Commission. Regulation (CE) 1107/2009 concerning the placing of plant protection products on the market and repealing council directives 79/117/EEC and 91/414/EEC. *Off J Eur Union* (2009).

64. European Commission. Directive 2009/128/EC establishing a framework for community action to achieve the sustainable use of pesticides. *Off J Eur Union* (2009).

65. Bosio G, Giacometto E, Vigasio M, Ferrari D, Viglione P, Renolfi F, et al. Prove di lotta contro *Popillia japonica* in vigneto nel nord piemonte. *ATTI Giornate Fitopatologiche* (2020) 1:283–92.

66. Santoiemma G, Battisti A, Gusella G, Cortese G, Tosi L, Gilioli G, et al. Chemical control of *Popillia japonica* adults on high-value crops and landscape plants of northern Italy. *Crop Protect* (2021) 150:105808. doi: 10.1016/j.cropro.2021.105808

67. Santoiemma G, Mori N, Battisti A, Sperandio G, Glazer I, Bianchi A, et al. Gestione integrata di *Popillia japonica* nel nord italia (Integrated management of *Popillia japonica* in northern Italy). *Arbor* (2021) 2:7–15.

68. Marianelli L, Paoli F, Sabbatini Peverieri G, Benvenuti C, Barzanti GP, Bosio G, et al. Long-lasting insecticide-treated nets: a new integrated pest management approach for *Popillia japonica* (Coleoptera: scarabaeidae). *Integr Environ Assess* (2019) 15:259–65. doi: 10.1002/ieam.4107

69. Skrzecz I, Grodzki W, Kosibowicz M, Tumialis D. The alpha-cypermethrin coated net for protecting Norway spruce wood against bark beetles (Curculionidae, scolytinae). *J Plant Prot Res* (2015) 55:156–61. doi: 10.1515/jppr-2015-0020

70. Kuhar TP, Short DB, Krawczyk G, Leskey TC. Deltamethrin-incorporated nets as an integrated pest management tool for the invasive *Halyomorpha halys* (Hemiptera: pentatomidae). *J Econ Entomol* (2017) 110:543–5. doi: 10.1093/jee/tow321

71. Gökçe A, Bingham GV, Whalon ME. Impact of long-lasting insecticide incorporated screens on Colorado potato beetle and plum curculio. *Turk J Agric For* (2018) 42:38–44. doi: 10.3906/tar-1709-55

72. Lindsay SW, Thomas MB, Kleinschmidt I. Threats to the effectiveness of insecticide-treated bednets for malaria control: thinking beyond insecticide resistance. *Lancet Glob Health* (2021) 9:e1325–31. doi: 10.1016/S2214-109X(21)00216-3

73. Sabbatini Peverieri G, Binazzi F, Marianelli L, Roversi PF. Lethal and sublethal effects of long-lasting insecticide-treated nets on the invasive bug halyomorpha halys. *J Appl Entomol* (2018) 142:141–8. doi: 10.1111/jen.12428

74. Ranger CM, Werle CT, Scultz PB, Addesso KM, Oliver JB and Reding ME. Long-lasting insecticide netting for protecting tree stems from attack by ambrosia beetles (Coleoptera: curculionidae: scolytinae). *Insects* (2020) 11(1):8. doi: 10.3390/insects11010008

75. Wilkins RV, Campbell JF, Zhu KY, Starkus LA, McKay T and Morrison WR III. Long-lasting insecticide-incorporated netting and interception traps at pilot-scale warehouses and commercial facilities prevents infestation by stored product beetles. *Front Sustain Food Syst* (2021) 4:561820. doi: 10.3389/fsufs.2020.561820

76. Dave RS, Patel AR. Photochemical and photocatalytic of cypermethrin under UV radiation. *Der pharma Chem* (2010) 2:152–8.

77. Mori N, Santoiemma G, Glazer I, Gilioli G, Ciampitti M, Cavagna B, et al. Management of *Popillia japonica* in container-grown nursery stock in Italy. *Phytoparasitica* (2022) 50:83–9. doi: 10.1007/s12600-021-00948-2

78. Pavasini M. *Gestione integrata di Popillia japonica nella filiera vivaistica*. Verona, IT: University of Verona – Department of Biotechnology, Bachelor's degree thesis in Viticultural and Oenological Science and Technology (2021). p. 33.

79. Anselmi L. *Indagini sui mezzi di contenimento fisici per il controllo di Popillia japonica nella filiera vivaistica*. Verona, IT: University of Verona – Department of Biotechnology, Bachelor's degree thesis in Viticultural and Oenological Science and Technology (2022). p. 31.

80. Fleming WE. Biological control of the Japanese beetle. *USDA Tech. Bull* (1968) 1383:383–91.

81. Potter DA. *Destructive turfgrass insects. biology, diagnosis, and control*. Chelsea, MI: Ann Arbor Press (1998). p. 344.

82. Benvenuti C, Marianelli L, Paoli F, Mazza G, Roversi PF. *Infecting the Japanese beetle emerging adults in soils treated with metarhizium anisopliae Met52®: a preliminary semi-field study*. Napoli, Italy: XI European Congress of Entomology (2018). p. 204. doi: 10.19263/REDIA-100.17.17

83. Benvenuti C, Barzanti GP, Marianelli L, Sabbatini Peverieri G, Paoli F, Bosio G, et al. A new device for auto-disseminating entomopathogenic fungi against *Popillia japonica*: a study case. *Bull Insectol* (2019) 72:219–25.

84. Barzanti GP, Enkerli J, Benvenuti C, Strangi A, Mazza G, Torrini G, et al. Genetic variability of *Metarhizium* isolates from the ticino valley natural park (Northern Italy) as a possible microbiological resource for the management of *Popillia japonica*. *J Invertebr Pathol* (2023) 197:107891. doi: 10.1016/j.jip.2023.107891

85. Grewal PS, Grewal SK, Malik VS, Klein MG. Differences in susceptibility of introduced and native white grub species to entomopathogenic nematodes from various geographic localities. *Biol Control* (2002) 24(3):230–7. doi: 10.1016/S1049-9644(02)00025-7

86. Koppenhöfer AM, Grewal PS, Fuzy EM. Virulence of the entomopathogenic nematodes *Heterorhabditis bacteriophora*, *Heterorhabditis zealandica*, and *Steinernema scarabaei* against five white grub species (Coleoptera: scarabaeidae) of economic importance in turfgrass in north America. *Biol Control* (2006) 38(3):397–404. doi: 10.1016/j.biocontrol.2005.12.013

87. Paoli F, Marianelli L, Torrini G, Mazza G, Benvenuti C, Bosio G, et al. Differential susceptibility of *Popillia japonica* 3rd instars to *Heterorhabditis bacteriophora* (Italian strain) at three different seasons. *Biocontrol Sci Technol* (2017b) 27(3):439–44. doi: 10.1080/09583157.2017.1285866

88. Marianelli L, Paoli F, Torrini G, Mazza G, Benvenuti C, Binazzi F, et al. Entomopathogenic nematodes as potential biological control agents of *Popillia japonica* (Coleoptera, scarabaeidae) in piedmont region (Italy). *J Appl Entom* (2018) 142(3):311–8. doi: 10.1111/jen.12470

89. Paoli F, Marianelli L, Binazzi F, Mazza G, Benvenuti C, Sabbatini Peverieri G, et al. Effectiveness of different doses of *Heterorhabditis bacteriophora* against *Popillia japonica* 3rd instars: laboratory evaluation and field application. *Redia* (2017a) 100:135–8. doi: 10.19263/REDIA-100.17.17

90. Torrini G, Paoli F, Mazza G, Simoncini S, Benvenuti C, Strangi A, et al. Evaluation of indigenous entomopathogenic nematodes as potential biocontrol agents against *Popillia japonica* (Coleoptera: scarabaeidae) in northern Italy. *Insects* (2020) 11(11):804. doi: 10.3390/insects11110804

91. Glazer I, Santoiemma G, Battisti A, De Luca F, Fanelli E, Troccoli A, et al. Invasion of *Popillia japonica* in Lombardy, Italy: interactions with soil entomopathogenic nematodes and native grubs. *Agr For Entomol* (2022) 24(4):600–8. doi: 10.1111/afe.12524

92. Mazza G, Paoli F, Strangi A, Torrini G, Marianelli L, Sabbatini Peverieri G, et al. *Hexamermis popilliae* n. sp. (Nematoda:Mermithidae) parasitizing the Japanese beetle *Popillia japonica* Newman (Coleoptera: scarabaeidae) in Italy. *Syst Parasitol* (2017) 94:915–26. doi: 10.1007/s11230-017-9746-0

93. Goda N, Mirzaei M, Brunetti M. Potentially entomopathogenic nematode isolated from *Popillia japonica*: bioassay, molecular characterization and the associated microbiota. *Bull Insectol* (2020) 73(2):295–301.

94. López R, Potter DA. Ant predation on eggs and larvae of the black cutworm (Lepidoptera: noctuidae) and Japanese beetle (Coleoptera:Scarabaeidae) in turfgrass. *Environ Entomol* (2000) 29:116–25. doi: 10.1603/0046-225X-29.1.116

95. Zenger JT, Gibb TJ. Impact of four insecticides on Japanese beetle egg predators and white grubs in turfgrass. *J Econ Entomol* (2001) 94:145–49. doi: 10.1603/0022-0493-94.1.145

96. Smitley D, Hotchkiss E, Buckley K, Piombiono M, Lewis P, Studyvin J. Gradual decline of Japanese beetle (Coleoptera: scarabaeidae) populations in Michigan follows establishment of *Ovavesicula popilliae* (Microsporidia). *J Econ Entomol* (2022) 115 (5):1432–41. doi: 10.1093/jee/toac085

97. Bertoglio M, Ceriani F, Abelli A, Kron Morelli R, Cassani S, Suss L. *Popillia japonica*: valutazione dell'efficacia di due strategie di controllo biologico in condizioni controllate. *ATTI Giornate Fitopatologiche* (2018) 1:439–44.

frontiers | Frontiers in Insect Science

TYPE Original Research
PUBLISHED 14 June 2023
DOI 10.3389/finsc.2023.1176405

OPEN ACCESS

EDITED BY
William D. Hutchison,
University of Minnesota Twin Cities,
United States

REVIEWED BY
Gigi DiGiacomo,
University of Minnesota Twin Cities,
United States
Leonardo Marianelli,
Council for Agricultural and Economics
Research (CREA), Italy

*CORRESPONDENCE
Franziska B. Straubinger
franziska.straubinger@tum.de

RECEIVED 28 February 2023
ACCEPTED 29 May 2023
PUBLISHED 14 June 2023

CITATION
Straubinger FB, Venus TE, Benjamin EO and Sauer J (2023) Private management costs of *Popillia japonica*: a study of viticulture in Italy.
Front. Insect Sci. 3:1176405.
doi: 10.3389/finsc.2023.1176405

Private management costs of *Popillia japonica*: a study of viticulture in Italy

Franziska B. Straubinger[1]*, Terese E. Venus[1,2], Emmanuel O. Benjamin[1] and Johannes Sauer[1]

[1]Chair Group of Production and Resource Economics, Technical University of Munich (TUM), Munich, Germany, [2]Research Group of Bioeconomy Economics, University of Passau, Passau, Germany

The Japanese beetle (*Popillia japonica*) is classified as a high-priority pest in the European Union and is reported to have caused extensive damage to grapevine leaves in Italy. As there are few studies, which measure the beetle's socio-economic impact, we conduct a first descriptive assessment of grapevine farmers' perception of the beetle's impact and assess the pest's effect on private management costs using a partial budgeting approach. Our sample includes data from 65 producers and 118 vineyard plots. In terms of farmers' perception, we find that farmers anticipate increased management costs and believe a further spread of the beetle will lead to at least moderate yield and quality damages for the majority of plots (58-91%). While farmers do not expect to stop grapevine cultivation for the majority of vineyard plots, affected farmers they believe it is likely to very likely for 29% of plots. We also find that affected farmers rate their vines' resilience higher than unaffected farmers do. Using a partial budgeting approach, we find that a Japanese beetle infestation leads on average to a net income decrease of around €2727 per hectare. This decrease is due to an average increase in labor costs of around €1715. Additionally, an average yield reduction that results in a revenue loss of around €966 and additional control costs of around €47 per infested hectare, further contribute to the net income decrease. Even though the small number of observations does not allow us to make conclusions about the beetle's impact on the Italian viticulture sector as a whole, our findings provide first insights and demonstrate the need for environmentally friendly and effective control products that can replace labor-intensive manual control measures, which are currently applied in Japanese beetle infested vineyards.

KEYWORDS

Japanese beetle, invasive species, biological invasion, pest management, partial budget, grape production, socio-economic impact

1 Introduction

The beetle *Popillia japonica* (Japanese beetle), native to Japan, is an invasive insect pest in Europe and considered a major threat to European agriculture. It can feed on more than 300 different host plants (1) and there are many areas in Europe with suitable climatic conditions for its proliferation (2, 3). The beetle is considered a priority pest candidate in the EU (4) and ranked as the 2nd most dangerous pest for crops by the European Food and Safety Authority (5). In mainland Europe, the invasive insect was first detected in Italy in the Ticino Valley Natural Park in 2014 (6). It has since spread rapidly across the country. In 2020, the official infestation zone in Italy covered an area of 7,550 km2 (7) and only two years later in 2022, it increased to 16,232 km2 (Bosio, personal communication)[1]. In Italy, the infested zone covers parts of the Piedmont, Lombardy, and Emilia-Romagna regions, which include major wine-producing areas (8). The delimited area also includes part of the Valle d´Aosta region (9). In addition to Italy, the Japanese beetle has been detected in other European member states including Portugal (Azores) (10), Switzerland (11) and single beetle findings in Germany (12) (Figure 1).

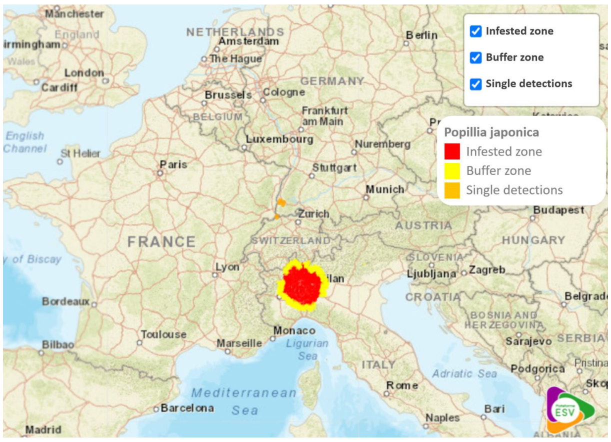


FIGURE 1
Japanese beetle infestation zone, buffer zone and single detections in mainland Europe. Source: adapted from ESV (13).

Although the Japanese beetle has been in mainland Europe for almost a decade, there are limited studies on its socioeconomic impact on agribusinesses and perception of affected farmers. Until now, the majority of studies have focused on projecting the beetle's future impact. For example, Straubinger et al. (14) estimate that without effective management of the beetle, a full infestation in the EU could lead to potential annual damage costs of €30 million up to €7.8 billion for the host crops of grain maize, soy, apple, peach, cherry, and grapes. For Italy, it has been estimated that a full Japanese beetle infestation could lead to annual damage costs of around €68 million with grapes accounting for 74% of the damage (14). Grapevine is one of the pests preferred host crops (15, 16) and large numbers of the adult beetle can be found between June and July in infested viticulture areas in Italy (7). Bosio et al. (17) report that vineyards in Piedmont frequently deal with infestations of 200-300 adult Japanese beetles per vine, with peaks of over 1,000 adults. This has prompted concerns about the beetle's impact on Italian viticulture.

To address these concerns, we assess grape farmers' perception of the infestation and evaluate the impact of the Japanese beetle on grape yield and private management costs using a partial budgeting approach. Our assessment is based on a survey of 65 Italian grape producers conducted in 2022 as well as secondary data from the literature. We present a descriptive assessment of the pest's economic damage on yield and use the partial budgeting approach to show the additional costs affected farmers may accrue. To the best of our knowledge, this is the first study assessing the private management costs of the Japanese beetle in Europe using primary data. Thus, despite the small sample size, this study provides an important case study for neighboring regions that may be affected by the beetle in the future.

2 Materials and methods

2.1 Data compilation

To assess farmer perception and management costs associated with a Japanese beetle infestation in viticulture, we collected quantitative data on production and control methods through an online survey using the survey tool Tivian and combined it with secondary data on labor (18) and material costs (19–22). For the survey, we used a structured questionnaire and voluntary response sampling (see Appendix A). A preliminary version of the questionnaire was designed based on surveys from the existing literature on grapevine cultivation and translated from English to Italian by an Italian native speaker. Before conducting the survey, it was tested by an Italian wine grower, a wine expert and a phytosanitary expert. We developed the final form of the survey incorporating their feedback on the questionnaire draft. The link to access the online survey was distributed through Italy's largest organization of wine producers (Vignaioli Piemontesi), the phytosanitary service of Piedmont region (Regione Piemonte) and the Council for Agricultural Research and Economics (CREA) from August until October 2022. A minimum of 160 grape producers were invited to participate *via* e-mail through the three institutes. Overall, according to the wine association (Vignaioli Piemontesi), their members produce about 30% of the wine in Piedmont with 325 members, which is made up of 291 individual wineries and 34 cooperative wineries, which represents about 6,000 individual wineries (23). According to the FADN's public database, in 2020, there were 6,672 wineries in Piedmont and 88,692 in Italy (24). To all participants of the survey, we guaranteed anonymity and the publication of results only in aggregated form. In total, 65 Italian farmers completed the survey and provided information on 119 vineyard plots.

The survey included questions about farm characteristics and management practices that could be affected by a Japanese beetle infestation in 2021 (see Table 1 for an overview of potential

---

[1] Notification from central phytosanitary service - Italy to the europhyt portal outbreak. (2023).

TABLE 1 Overview of additional private management costs potentially caused by a Japanese beetle infestation.

Type of additional costs	Reason
Labor	Visual monitoring
	Physical removal of Japanese beetles on vine plants
	Spraying of plant protection products
Insecticides	Reduction of Japanese beetle population on vine plants
Mass/Pheromone traps[1]	Reduction of Japanese beetle population on vine plants

1 Mass/pheromone traps might be applied by the farmer to reduce population, however it should be noted that generally they are not recommended by phytosanitary experts as they might attract more Japanese beetles than they are able to capture (17).

TABLE 2 Partial budgeting format. Based on the layout of Frem et al. (28) & Soliman et al. (26).

Costs		EUR	Benefits		EUR
A)	Additional costs		C)	Additional revenues	
	Control & protection costs				
	Labor costs				
B)	Reduced revenues		D)	Reduced costs	
	Yield and/or Quality losses				
Total costs: A + B			**Total benefits: C + D**		
Net change in profit: C + D - A - B					

additional management costs). The data were collected on the plot level to be able to compare affected and unaffected plots within the same farm. In addition, to understand the farmers' perception of the Japanese beetle's future impact on vineyards, we included questions about how a spread of the pest will affect the yield, quality, management costs and future cultivation of their vineyard plots. Moreover, the survey contained questions about how farmers perceive the leaf damage tolerance level of their vines. We considered this question important as the beetle damages grapevine by feeding on its leaves, thereby limiting the plant's ability to photosynthesize and, in turn, affecting grape quality.

2.2 Analysis

Our analysis was conducted in two parts. First, we provide a descriptive analysis of farmers' perceptions of the beetle's impact. Second, we use a partial budgeting approach to assess the beetle's impact on private management costs.

The partial budget (PB) analysis is a commonly used method to assess the direct economic consequence of a change at the farm business level like the introduction of a pest (25, 26). It is one of several main economic impact assessment techniques used in pest risk analysis (26, 27), which focuses on the direct economic impact on the farmer. Other methods like the partial equilibrium modelling, input-output analysis and computable general equilibrium model also take into account indirect effects caused through price effects and linkages to other agricultural markets or sectors (26). However, depending on the purpose of the analysis and the contextual framework these methods might not be suitable (26). In our case, where the Japanese beetle invasion in Europe is still at an early phase affecting mostly grapevine farmers in the Piedmont region, we can assume that it has resulted only in limited indirect effects. Hence, the PB technique is the optimal choice because it focuses on the producer. The method is based on the notion that changes will lead to adjustments in the farming system such as reductions or increases in some costs and revenues (14, 25, 28). Adding up the negative and/or positive cost changes will lead to the overall net change in profit (26) as presented in Table 2. A positive net economic effect of a change can be achieved if the sum of the positive economic effects exceeds that of the negative effects. Thus, PB is a basic but popular tool due to its simplicity and transparency (26). However, it is not designed to analyze the profit or loss of a business as a whole but instead focuses only on the specific costs or revenue components that could be affected by the change (25, 26).

This study assumes that a Japanese beetle infestation may potentially lead to a net decrease in farm income of grape producers. Our analysis focuses on comparing the economic consequences of a Japanese beetle infestation in a vineyard. To assess the per hectare net change in profit between affected and unaffected vineyard plots, the potential yield loss and associated costs per hectare of labor, insecticides and usage of other control methods like pheromone traps have been calculated. This calculation is based on primary and secondary data. To estimate the costs of labor, we used the wages established in the collective agreement for agriculture (18). The costs of control methods are based on average application rates of the farmers combined with recent market prices of the products (19–22).

3 Results

3.1 Sample characteristics

A total of 65 Italian grape producers completed the survey. The respondents provided information on 118 vineyards plots, of which 47 were affected by the Japanese beetle in 2021 (Figure 2). Most of the affected vineyards are located in the Piedmont region.

The average age of the surveyed farmers was between 44 and 49 years. Over 90% of respondents had completed an upper secondary school education. In total, 27 farmers reported that they were affected by the beetle and 38 were not affected.

Most of the farmers affected by the beetle had an income below 10000 € per year and managed their farm conventionally as a side-business with a viticulture area of less than 10 hectares. In comparison, most unaffected farmers identified farming as their main business with over 50% having an annual income above 10000 € per year. Furthermore, almost 50% of unaffected producers were

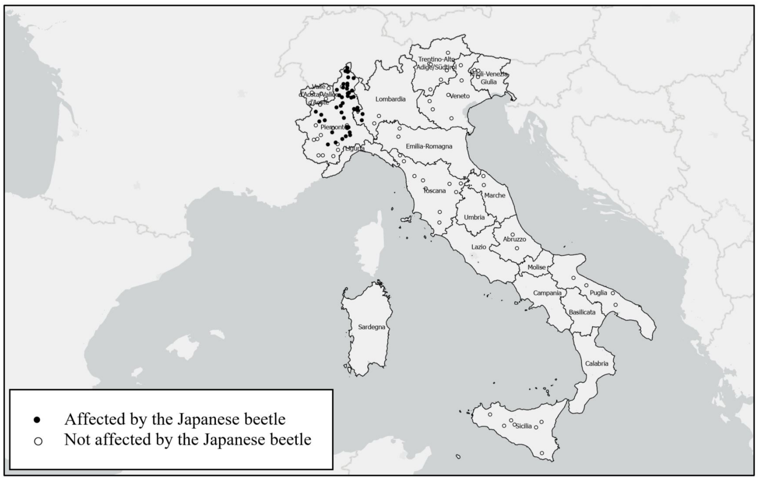


FIGURE 2
Overview of vineyard plot locations. Each dot represents a vineyard. The observations were randomly placed within the regions and do not represent the actual locations to preserve the anonymity of the survey participants.

using organic standards of production and even though the viticulture area of most of the farmers is below 10 hectares, the sample includes a few big grape producing businesses with a viticulture area of more than 50 hectares and an annual income over 120000 € (see Table 3).

3.2 Farmers' perception of the Japanese beetle infestation

The assessment of farmers' perception of the Japanese beetle infestation is based on the following four variables: the beetle's impact on yield damage, quality damage, management costs and the possibility of having to stop the cultivation of grapevine on their vineyard due to the pest. For the first three variables, farmers could choose if they perceive the damage or the potential increase in management costs on their vineyard as negligible, minor, moderate, major or severe. For the fourth question, they could indicate the likelihood of having to stop grapevine cultivation on their vineyard due to the beetle as very unlikely, unlikely, neutral, likely or very likely. In total, up to 56 farmers responded to these perception questions for 103 vineyard plots. Fisher's exact test was used to find out if there was a difference regarding perceptions of the Japanese beetle's impact between affected and unaffected vineyard plots. The test indicated no statistically significant difference between affected and unaffected plots regarding the perception on yield (p=0.069), quality damage (p=0.796), management costs (p=0.479) and the possibility to stop the cultivation of grapevine on their plot (p=0.581).

This study found that for around 75% of the unaffected plots (N=45), farmers expect moderate to severe yield damage in the case of a further spread of the Japanese beetle (see Figure 3). For affected plots on the other hand, farmers expect that the beetle will lead to such yield damage levels for around 58% of the plots (N=25). For the remaining 42% of affected plots (N=18), producers state that a future spread of the beetle could also result in minor or negligible damage to their yield. Farmers' perception of the beetle's impact on grape quality shows that for around 70% of affected (N=30) and 73% of unaffected plots (N=43), producers believe in moderate up to severe quality damage. Farmers also expect moderate to severe increases in management costs due to the Japanese beetle for around 91% of affected (N=41) and 89% of unaffected plots (N=50). Only for around 9% of affected (N=4) and 11% (N=6) of unaffected plots it was indicated that a minor or negligible increase in management costs could be possible. Even though most of the farmers expect the Japanese beetle to increase their management costs, they think it is unlikely to very unlikely for around 47% of affected (N=4) and 57% of unaffected plots (N=6) that they will have to stop the cultivation of grapevine on their vineyard plot because of the pest. For around 29% of affected (N=13) and 16% of unaffected plots (N=9) however, producers indicated that it is likely to very likely.

In addition to evaluating farmers' perception of the Japanese beetle impact on their grapevine production, we also wanted to find out how they assess the leaf damage tolerance of their vines. In order to do so, we asked the farmers for each of their plot(s) how much leaf damage they think that the vines can tolerate before experiencing significant negative impact on their grape yield and quality. In total 54 farmers gave an answer and provided estimates for 94 vineyard plots. Similarly as before, Fisher's exact test was performed to explore differences in answers between affected and unaffected plots. The test revealed a statistically significant difference between affected and unaffected plots on the perception of vines' leaf damage tolerance (p<0.001).

According to the results of the survey, for the majority of unaffected plots (55%, N=26) farmers believe that their plants can only tolerate leaf damage levels up to 10% before experiencing a significant negative impact on their grape yield and quality (see Figure 4). However, for most of the affected plots (79%, N=37) farmers indicated a higher tolerance level, which is between 20 to 30%.

TABLE 3 Socioeconomic characteristics of the farmers in the sample.

Characteristics	Categories	Affected Farmers (N=27)			SD	Unaffected Farmers (N=38)			SD
		Respondents				Respondents			
		No.	(%)	Mean		No.	(%)	Mean	
Age				48.67	13.9			43.68	10.8
	>= 55 years	9	33.3			7	18.9		
Education	>= upper secondary	25	92.6			38	100		
Farm as side-business		12	70.6			9	26.5		
Total farm size (ha)				4.66	6.11			41.91	97
	< 10 ha	21	77.8			17	44.7		
	10-49.9 ha	6	22.2			16	42.1		
	> 50 ha	0	0			5	13.2		
Viticulture area (ha)				2.64	5.4			22	57.65
	< 10 ha	25	92.6			24	63.2		
	10-49.9 ha	2	7.4			11	28.9		
	> 50 ha	0	0			3	7.9		
Labor (non-paid/own, family)				1.75	0.85			4.62	5.01
Labor (paid, all season)				0.11	0.32			2.94	4.76
Labor (paid, seasonal)				0.4	0.75			6.17	9.44
Organic		3	11.1			18	47.4		
Farm income (€)	< 10.000	17	85			15	48.4		
	10.001-120.000	3	15			12	38.7		
	>120.000	0	0			4	12.9		

Due to missing observations, the respective percentage of respondents for the farm income variable refers to a total of 20 affected and 31 unaffected farmers.

3.3 Partial budgeting analysis

To investigate the Japanese beetle's impact on the private management costs of grape producers, we have calculated the average difference in yield, labor and control measures between affected and unaffected plots (see Table 4). Since most of the affected plots were managed conventionally, we decided to exclude organically managed vineyards from the sample in order to control for potential differences in management practices. The sample for the PB analysis hence includes 42 affected and 38 unaffected plots.

We combined the average yield per hectare of affected and unaffected plots with the indicated average price of conventional farmers, which is 77 € per quintal. The price estimate seems to be realistic when compared to the latest available producer price of grapes in Italy from Eurostat, which is 66.2 € per quintal for the year 2018 (29). In addition, we asked the participants about the total amount of labor, which was necessary to manage their vineyard plot in 2021. While it took on average 116 days to manage one hectare when not affected by the Japanese beetle, this increases to around 148 days when affected by the pest. From Table 4 we can see that the difference in labor of affected plots can be explained through an increase in other control methods like visually inspecting the vineyard or manually picking the beetle, which are labor-intensive control measures. It should be noted that we did not include these two control measures in the PB analysis as those costs are already accounted for in the labor per hectare estimate and are shown only to provide additional information on the labor differences. The difference in costs of control measures per hectare have been calculated taking into account the average difference in application rates between affected and unaffected plots and multiplying it with the share of plots that use the specific product. This value is then combined with recent market prices of the products (19–22). One of the main differences in the use of control products is that a higher percentage of affected plots was treated with insecticides containing the active ingredient acetamiprid (80%) and deltamethryn (45%) (see Table 4). In addition, the average application rate for products with acetamiprid was higher for affected plots. This increase in application for insecticides containing acetamiprid and deltamethryn makes sense since they are among the active substances registered against adults of the Japanese beetle (3).

Table 5 shows the results of the PB analysis based on the above-mentioned cost estimates. We found that vineyards experience, on average, a reduction in net income of around 2727 € per infested hectare compared to unaffected ones. Among the different cost components, labor was the main additional expense affecting the

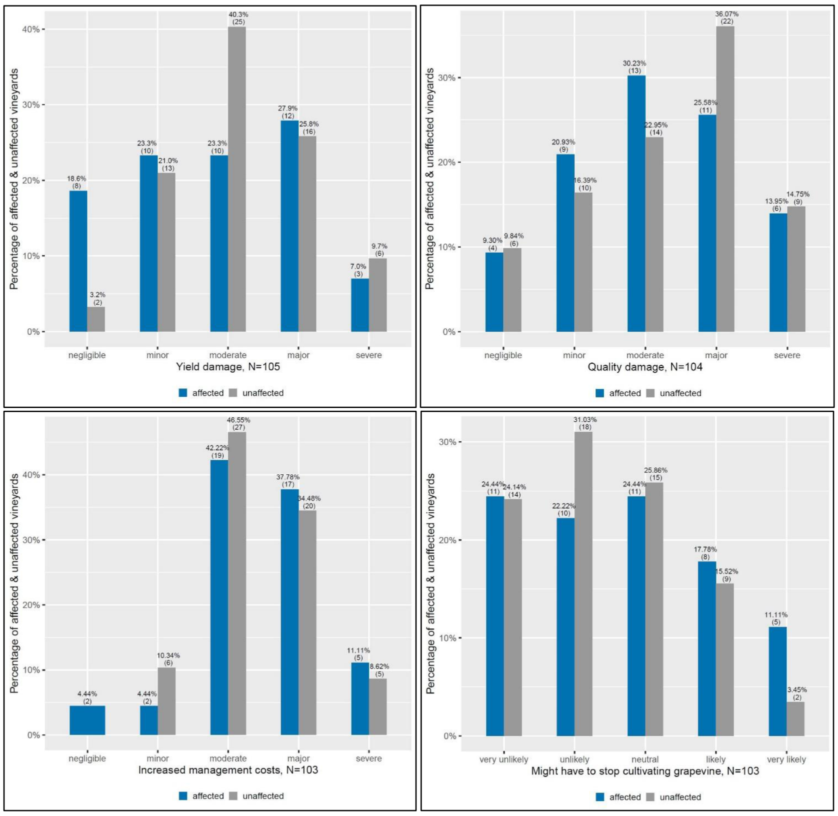


FIGURE 3
Farmer perceptions of how the spread of the Japanese beetle will affect future grapevine production.

net income loss followed by a revenue reduction of around 966 € due to yield loss. The cost difference in the usage of control methods is around 47 € per infested hectare.

4 Discussion

In this study, we assess two sides of the Japanese beetle infestation's impact in Italy through farmers' perceptions and the partial budget analysis.

In our investigation of farmers' perception of the effect of the Japanese beetle on grapevines, we find that farmers expect at least moderate yield and quality damages for the majority of plots (58-76%, N=25-47). While farmers consider the possibility that they might have to stop their grapevine cultivation as (very) unlikely or neutral (71-81%, N=32-47), at least moderately increased management costs are expected for the majority of vineyard plots (90-91%, N=41-52). However, even though not statistically different at the 95% level, there are some differences in opinion for affected and unaffected plots, which are worth mentioning. Although unaffected farmers are concerned about future yield damage, a large percentage of affected farmers (42%, N=18) are less afraid about the beetle's impact on yield. This could be due to the fact that in our sample most of the affected producers have smaller farms, which they manage conventionally as a side-business. The group of the unaffected farmers, on the other hand, may be more concerned about the future spread of the beetle as most of them run their farms full-time and almost 50% of them are producing with organic standards, for which only limited control options exist at the moment.

The responses of farmers regarding the leaf damage tolerance of vines also shows differences between affected and unaffected plots, which are statistically significant. While for 55% of the unaffected plots (N=26), farmers state that their plants can only tolerate leaf damage up to 10% before experiencing a significant negative impact on their yield and quality, they do so only for 6.5% of the affected plots (N=3). For around 90% of plots affected by the beetle (N=42) higher tolerance levels have been indicated with most of them stating that a defoliation between 20 to 30% is possible. As farmers with affected plots differ in terms of perceived leaf damage tolerance, farmers with affected plots might have more knowledge and experience with leaf damages and accurately anticipate the impact of defoliation on their plants. The expectations of these experienced farmers are in line with a recent study from the US, which found that with a defoliation higher than 30 to 35%, the feeding of the Japanese beetle starts to show negative effects on various quality parameters of the wine grape variety "Frontenac" (30).

In our partial budgeting analysis, we find that vineyards affected by the Japanese beetle experience, on average, a net loss of around 2727 € per infested hectare with labor being one of the key cost increases. Additional labor may be necessary due to increased

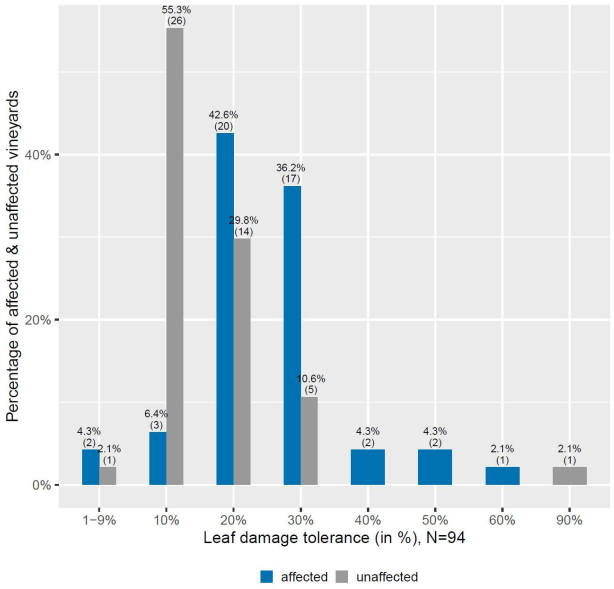


FIGURE 4
Farmer perception of the leaf damage tolerated until there is a significant negative impact on grape yield & quality.

TABLE 4 Overview of yield, labor and control measures of affected and unaffected vineyard plots.

Particulars	Affected Plot (N=42)		Mean application no. (if indicated)	Cost (€ per application & ha)	Unaffected Plot (N=38)		Mean application no. (if indicated)	Cost (€ per application & ha)
	(%)	Mean			(%)	Mean		
Yield per ha (in quintals)	71.4	50.4			71.1	62.9		
Price (€ per quintal)		77.3				77.3		
Labor per ha (in days)	90.5	147.5			55.3	116.4		
Labor costs (€/hour)		6.9				6.9		
Insecticides								
Acetamiprid	81.0		2.1	66.3	48.5		1.5	66.3
Clorantraniliprolo	4.8		*1.0*	59.7	0.0		0.0	59.7
Deltamethryn	45.2		2.1	25.6	3.0		2.0	25.6
Emamectina benzoato	0.0		0.0	52.8	6.1		1.0	52.8
Etofenprox	9.5		1.0	54.4	27.3		1.0	54.4
Flupyradifuron	4.8		2.0	38.8	24.2		1.0	38.8
Metossifenozide	0.0		0.0	39.2	6.1		1.0	39.2
Tau-fluvalinate	0.0		0.0	23.9	6.1		*1.0*	23.9
Bacillus thuringiensis	0.0		0.0	28.8	12.1		2.0	28.8
Beauveria bassiana	4.8		*1.0*	133.8	0.0		0.0	133.8
Insect soap	0.0		0.0	2.4	6.1		*1.0*	2.4
Orange oil	2.4		2.7	52.5	9.1		3.1	52.5

(Continued)

TABLE 4 Continued

Particulars	Affected Plot (N=42)		Mean application no. (if indicated)	Cost (€ per application & ha)	Unaffected Plot (N=38)		Mean application no. (if indicated)	Cost (€ per application & ha)
	(%)	Mean			(%)	Mean		
Paraffin oil	0.0		0.0	176.0	6.1		*1.0*	176.0
Pyrethrine	2.4		2.0	92.4	15.2		1.0	92.4
Rapeseed oil	2.4		2.0	103.2	3.0		*1.0*	103.2
Spinosad	0.0		0.0	95.4	15.2		1.0	95.4
Other control methods								
Mass/pheromone trap (trap per ha)	16.7		15.4	10.3	6.1		2.0	10.3
Insect picking by hand (h per ha)	35.7		38.7		0.0		NA	
Visual inspection (h per ha)	54.8		20.6		51.5		2.4	

Values written in italics indicate that the product was applied but the corresponding information on the number of applications is missing and therefore one application was assumed. The per hectare costs of insecticides and other control methods is based on the recommended dosage per hectare combined with mean market prices of the products containing the active ingredient sourced from online market shops (19–22). The costs of labor are based on the wages established in the collective agreement for agriculture (18). For the calculation of the mean yield, entries of 0 and more than 200 quintals per ha were removed. Similarly labour per ha of over 800 days and a price of less than five Euros per quintal were unlikely and were therefore removed from the dataset.

intensive monitoring, additional applications of insecticides or even manual picking of the adult beetles every morning during their flight period. Another important cost factor is the decrease in revenue due to yield loss. Hence, Japanese beetle affected vineyard plots have a net decrease in income per infested hectare due to yield loss, additional applications of insecticides and increased workload. However, the observed variations between affected and unaffected vineyards cannot be interpreted as causal. As weather conditions and other pest outbreaks could play a major role, future studies should control for these factors. In addition, the PB analysis relies on a small number of observations and the descriptive results can therefore not be seen as representative of Italy.

In summary, the Japanese beetle can negatively affect viticulture in Italy through a potential yield loss and increased labor and control costs. This is also reflected in the perception of the farmers. One of the main cost components identified in this study is the additional labor required to deal with the pest. Farmers may be forced to handpick the adult beetles from their vines because only few control methods against the beetle currently exist. While spraying of insecticides only helps in the short term, re-infestation of the vines is possible within a few days after treatment (17). Hence, additional insecticide applications are necessary but not unlimited due to their negative effects on beneficial organisms and the environment. Additionally, increased consumer concerns about products with pesticide residues as well as potential problems with pests developing resistance to synthetic chemical products (31) make future research and investment in the development of effective and environmentally friendly control measures necessary. Finally, our study tries to give a first picture on how the Japanese beetle affects grapevine producers in Italy. However, as the beetle is spreading, future studies at the farm level are essential, especially due to our small sample size and since the

TABLE 5 Partial budget analysis and net change in profits of Japanese beetle affected vineyard compared to unaffected vineyard.

Affected vs. Unaffected						
Costs		€ EUR/ha		Benefits		€ EUR/ha
A)	Additional costs			C)	Additional revenues	0
	Insecticides	21.62				
	Other Control methods	25.16				
	Labor	1,715.06				
B)	Reduced revenues			D)	Reduced costs	0
	Yield	965.60				
Total costs: A + B		2,727.44		**Total benefits: C + D**		0
Net change in profit: C + D - A – B			-2,727.44			

affected producers in our sample represent mostly small farms. In addition, also the impact of the beetle on specific grape varieties as well as other host crops like maize, blueberry or hazelnut, at different levels of infestation across Europe needs to be evaluated.

Data availability statement

The raw, anonymized data supporting the conclusions of this article are not available as the authors are not entitled to disclose the data to third parties or institutions outside the IPM Popillia research group. Requests to access the datasets should be directed to Franziska Straubinger, franziska.straubinger@tum.de..

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

Author contributions

FS and TV: conceptualization and methodology. FS: formal analysis and writing of original draft. TV and BE: reviewing. BE and JS: resources, project administration, and funding acquisition. All authors provided a final review and approved the submitted version.

Funding

This research was supported by the IPM-Popillia project, funded by the European Union´s Horizon 2020 research and innovation program under grant agreement No 861852.

Acknowledgments

We thank our project partners Vignaioli Piemontesi, Regione Piemonte, CREA and Simone Roverelli for their help with the field survey and data collection. A special thanks to Giovanni Bosio, Enzo Piazza (Regione Piemonte) and Michele Vigasio (Vignaioli Piemontesi) for providing us with expert knowledge on wine grape cultivation and the Japanese beetle infestation in Italian vineyards. We also would like to express our gratitude to all the Italian grape producers, who filled out the online questionnaire.

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.

The reviewer LM declared a shared consortium IPM POPILLIA with the authors FS, EB and JS to the handling editor.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/finsc.2023.1176405/full#supplementary-material

References

1. EPPO. *Popillia japonica. EPPO datasheets on pests recommended for regulation* (2022). Available at: https://gd.eppo.int/taxon/POPIJA/datasheet.
2. Kistner-Thomas EJ. The potential global distribution and voltinism of the Japanese beetle (Coleoptera: scarabaeidae) under current and future climates. *J Insect Sci* (2019) 19(2):1–13. doi: 10.1093/jisesa/iez023
3. Borner L, Martinetti D, Poggi S. A new chapter of the Japanese beetle invasion saga: predicting suitability from long-infested areas to inform surveillance strategies in Europe. *bioRxiv* (2022). doi: 10.1101/2022.11.14.515960
4. European Commission. *COMMISSION DELEGATED REGULATION (EU) 2019/1702 of 1 august 2019 supplementing regulation (EU) 2016/2031 of the European parliament and of the council by establishing the list of priority pests*. Official Journal of the European Communities (2019).
5. Sánchez B, Barreiro-Hurle J, Soto Embodas I, Rodriguez-Cerezo E. *The impact indicator for priority pests (I2P2): a tool for ranking pests according to regulation (EU)*. Luxembourg: Publications Office of the European Union (2019). doi: 10.2760/585182
6. EPPO. *First report of popillia japonica in Italy* (2014). Available at: https://gd.eppo.int/reporting/article-3272.
7. EPPO. *Update on the situation of popillia japonica in Italy* (2020). Available at: https://gd.eppo.int/reporting/article-6794.
8. Festa G, Shams SR, Metallo G, Cuomo MT. Opportunities and challenges in the contribution of wine routes to wine tourism in Italy – a stakeholders' perspective of development. *Tourism Manage Perspect* (2020) 33:100585. doi: 10.1016/j.tmp.2019.100585
9. EPPO. *Update of the situation of popillia japonica in Italy* (2022). Available at: https://gd.eppo.int/reporting/article-7312.
10. EPPO. *Update of the situation of popillia japonica in Portugal (Azores)* (2019). Available at: https://gd.eppo.int/reporting/article-6588.
11. EPPO. *Update on the situation of popillia japonica in Switzerland* (2021). Available at: https://gd.eppo.int/reporting/article-7043.
12. EPPO. *New finding of popillia japonica in Germany* (2022). Available at: https://gd.eppo.int/reporting/article-7397.
13. ESV P. *BULLETIN MENSUEL N°36: popillia japonica* (2021). Available at: https://plateforme-esv.fr/sites/default/files/2021-12/BMnovembre2021_S44_45_46_47_48v2.html.
14. Straubinger FB, Benjamin EO, Venus TE, Sauer J. The economic importance of early pest control: new insights from potential popillia japonica infestation in Europe. *agriRxiv* (2022) 20220316785. doi: 10.31220/agriRxiv.2022.00151
15. Klein M. *Popillia japonica (Japanese beetle). CABI compendium*. CABI International (2022). doi: 10.1079/cabicompendium.43599.

16. Bosio G, Giacometto E, Venanzio D. Popillia japonica, come contenere i danni. *Edizioni L´Informatore Agrario* (2018) 12:58–61.

17. Bosio G, Piazza E, Giacometto E. Popillia japonica, una specie in progressiva diffusione. *Edizioni L´Informatore Agrario* (2022) 21:53–60.

18. Confederazione Generale dell´Agricoltura Italiana. *Contratto collettivo nazionale di lavoro per gli operai agricoli e florovivaistie*. Rome (2018).

19. AgriPiù. *Vendita di prodotti per l´agricoltura, l´allevamento e il giardino dal* (1972). Available at: https://agripiu.macommerce.it/.

20. BayWa. *Für die landwirtschaft*. Available at: https://www.baywa.de/de/pflanzenbau/gemuese/pflanzenschutz/insektizide/biofa-micula-insektizid-gegen-saugende-insekten-und-milben/p-000000000001725585/.

21. Fitogarden. *Consulta l´ampio catalogo di fitosanitari*. Available at: https://fitogarden.com/.

22. Vendita Fitofarmaci. *Il tuo foritore di fitofarmaci online*. Available at: https://venditafitofarmaci.it/.

23. Vignaioli Piemontesi. *IL MONDO VIGNAIOLI PIEMONTESI*. Available at: https://www.vignaioli.it/chi-siamo/il-mondo-vignaioli-piemontesi/.

24. European Commission. *FADN public database (SO)* (2022). Available at: https://agridata.ec.europa.eu/extensions/FADNPublicDatabase/FADNPublicDatabase.html.

25. Del Fava E, Ioriatti C, Melegaro A. Cost-benefit analysis of controlling the spotted wing drosophila (Drosophila suzukii (Matsumura)) spread and infestation of soft fruits in trentino, northern Italy. *Pest Manag Sci* (2017) 73:2318–27. doi: 10.1002/ps.4618

26. Soliman T, Mourits M, Oude Lansink A, van der Werf W. Economic impact assessment in pest risk analysis. *Crop Prot* (2010) 29:517–24. doi: 10.1016/j.cropro.2009.12.014

27. DiGiacomo G, Gullickson MG, Rogers M, Peterson HH. Hutchison WD. partial budget analysis of exclusion netting and organic-certified insecticides for management of spotted-wing drosophila (Diptera: drosophilidae) on small farms in the upper Midwest. *J Econ Entomol* (2021) 114:1655–65. doi: 10.1093/jee/toab087

28. Frem M, Fucilli V, Nigro F, El Moujabber M, Abou Kubaa R, La Notte P, et al. The potential direct economic impact and private management costs of an invasive alien species: xylella fastidiosa on Lebanese wine grapes. *NB* (2021) 70:43–67. doi: 10.3897/neobiota.70.72280

29. Eurostat. *Unit values at producer prices - grapes* (2023). Available at: https://ec.europa.eu/eurostat/databrowser/product/page/AACT_UV02:custom_5828609.

30. Ebbenga DN, Burkness EC, Clark MD, Hutchison WD. Impact of adult popillia japonica (Coleoptera: scarabaeidae) foliar feeding injury on fruit yield and quality of a temperate, cold-hardy wine grape, ‘Frontenac’. *Front Insect Sci* (2022) 2. doi: 10.3389/finsc.2022.887659

31. Pertot I, Caffi T, Rossi V, Mugnai L, Hoffmann C, Grando MS, et al. A critical review of plant protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. *Crop Prot* (2017) 97:70–84. doi: 10.1016/j.cropro.2016.11.025

frontiers | Frontiers in Insect Science

TYPE Original Research
PUBLISHED 02 October 2024
DOI 10.3389/finsc.2024.1465829

OPEN ACCESS

EDITED BY
Deguang Liu,
Northwest A&F University, China

REVIEWED BY
Eustachio Tarasco,
University of Bari Aldo Moro, Italy
Dexian Li,
Northwest A&F University, China

*CORRESPONDENCE
William D. Hutchison
✉ hutch002@umn.edu

†PRESENT ADDRESS
Adam G. Toninato,
Mitsui Chemicals America, Rye Brook, NY,
United States
Eric C. Burkness,
Minnesota Department of Agriculture, St.
Paul, MN, United States

RECEIVED 16 July 2024
ACCEPTED 16 September 2024
PUBLISHED 02 October 2024

CITATION
Toninato AG, Burkness EC and Hutchison WD (2024) Spatial distribution and fixed-precision sequential sampling plans for *Popillia japonica* (Coleoptera: Scarabaeidae) adults in primocane raspberry: influence of foliar insecticides.
Front. Insect Sci. 4:1465829.
doi: 10.3389/finsc.2024.1465829

Spatial distribution and fixed-precision sequential sampling plans for *Popillia japonica* (Coleoptera: Scarabaeidae) adults in primocane raspberry: influence of foliar insecticides

Adam G. Toninato†, Eric C. Burkness† and William D. Hutchison*

Department of Entomology, University of Minnesota, St. Paul, MN, United States

The Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), an invasive species from northern Japan, was first detected in Minnesota in 1968. According to fruit growers and the Minnesota Department of Agriculture, population size and feeding damage has been an increasing concern since 2010. Based on trap-catch data, populations have recently exceeded 4,000 beetles/trap/week during July-August near raspberry fields, and can increase by an order of magnitude within 7-10 days. The primary goals of this study were to assess the spatial distribution of *P. japonica* adults in raspberry, and to develop and validate a practical fixed-precision sequential sampling plan for grower use. Taylor's Power Law (TPL) regression was used to characterize the beetle's spatial pattern in research plots and commercial fields, either with or without insecticide applications. We then used Green's plan to develop an enumerative sequential sampling plan to estimate *P. japonica* density in primocane raspberry. Beetle population data were collected at two locations in southern Minnesota, including the Rosemount Research and Outreach Center, and a commercial field near Forest Lake. The TPL results, via slope comparisons, indicated no significant differences in *P. japonica* spatial pattern between insecticide treated plots versus untreated plots, or among 4 different insecticides ($P>0.05$). Utilizing all spatial pattern data, we characterized the distribution of *P. japonica* beetles to be highly aggregated in raspberry, with TPL slopes ranging from $b = 1.38$ to 1.55; all slopes were found to be >1.0. Although the slopes were not significantly different, we accounted for variability in spatial pattern by using 33 independent data sets, and the Resampling for Validation of Sampling Plans (RVSP) model to validate a sampling plan with a final average precision level of 0.25 (SEM/mean), recommended for integrated pest management (IPM) purposes. The final sampling plan required an average sample number of only 15, 1-m-row samples, while providing high relative net precision (RNP), and thus a cost-effective, efficient sample plan for growers.

KEYWORDS

IPM decision-making, Japanese beetle, Taylor's Power Law, resampling, IPM, sequential sampling

Introduction

The Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), is native to northern Japan and highly invasive in the U.S. The beetle was first detected in New Jersey in 1916 (1, 2), and gradually spread west and southwest. *Popillia japonica* has now been documented in at least 31 states, and was first detected in Minnesota in 1968 (3). Since then, *P. japonica* populations remained relatively small and were primarily limited to urban areas within the 7-county metro area of Minneapolis & St. Paul (4). However, since 2010, *P. japonica* outbreaks have occurred more frequently in Minnesota, and have increasingly been a concern to growers of high-value fruit crops (5, 6). *Popillia japonica* adults are known to colonize over 300 wild and cultivated plant species across 79 families, resulting in a characteristic leaf "skeletonizing" feeding pattern (2). Adult feeding during July and August in the Midwest U.S. can quickly result in high defoliation rates on economically valuable hosts, particularly linden trees, roses, and numerous field and horticultural crops (2, 3, 6, 7). In addition to direct feeding damage, invasive arthropod species can often alter the feeding patterns of native pests (e.g., 8), and consequently disrupt existing Integrated Pest Management (IPM) programs (9, 10). Specifically, *P. japonica* has been shown to exacerbate feeding injury by a native insect, the Green June Beetle (*Cotinis nitida*), that is preferentially attracted to grapes damaged by *P. japonica.* In turn, yeast production and premature fermentation impacts fruit quality (8).

Among the most attractive fruit crops in the Midwest region, raspberries, apples and wine grapes are readily damaged (2, 5, 7, 11–13). In Minnesota, both summer (floricane) and fall (primocane) raspberries are significant sources of income to growers. DiGiacomo et al. (14) recently found that Driscoll Inc. (Watsonville, CA) documented high consumer demand for raspberries in Minnesota, where the Minneapolis-St. Paul market consumed 132% more fresh raspberries than the average U.S. household. High in vitamin C and K, with high antioxidant capacity, modern raspberry cultivars have been categorized as a "super food" (15). It was recently estimated that raspberry production in Minnesota yields 3.51 million pounds of marketable product ($8.42 lb/ac), valued annually at $70.6 million (14).

Because of the value of raspberries to the Minnesota fruit industry, many fruit growers utilize a "pick your own" production system, as the need for frequent harvests is labor intensive (14). However, scheduling both pest management activities, insecticide applications, as well as safe harvest dates can be challenging (6, 14). Raspberry is a perennial crop with one of two different harvest cycles; floricane is harvested throughout the summer (primarily July-August), and primocane harvested during autumn (August-October). Primocane raspberries are most commonly grown in Minnesota and are therefore at greater risk from insect pest feeding on foliage from the mid-late portion of the growing season (5, 7, 14). *Popillia japonica* prefer feeding on raspberry leaf tissue, even in the presence of other known host plants (16). Burkness et al. (11), in a 3-year study, found that the beetle attacked raspberry fruit ~20% of time. In addition to direct damage to fruit, feeding injury to foliage may also interfere with photosynthesis and late-season fruit production (17). Primocane raspberries also endure the most feeding pressure from *P. japonica* during the transition from vegetative growth to fruiting, as sucrose and nutrients are stored for berry production during July and August (17); this period also coincides with peak flights of *P. japonica* (7, 18).

Considerable Integrated Pest Management (IPM) research to date has focused on the *P. japonica* larval (white grub) stage of the insect's life cycle (1, 3, 19). However, relatively little applied research in the Midwest U.S. has been directed toward adult *P. japonica* ecology or an understanding of the impact on fruit crops, including yield or quality of raspberries. With concerns about the beetle's rapid colonization of fruit crops (e.g., 5, 6, 11), and that the highly visible impact of feeding damage may increase insecticide use, growers are currently in need of an effective monitoring tool to estimate the number of *P. japonica* adults present, and thus make objective IPM decisions. The primary control practice currently includes the use of foliar insecticides soon after finding a few *P. japonica* beetles, or high levels of defoliation. In addition, the process of sampling for beetles, or estimating defoliation is a challenge, depending on the experience of growers or consultants (e.g., 1). As part of our research to develop new IPM programs, monitoring systems are needed to track beetle population trends, such as the use of semiochemical-baited traps to understand regional pest pressure (5). In addition, for individual crops such as raspberries, statistically sound sampling methods are necessary for future use with economic or "action" thresholds, to better assess if and when insecticide applications are necessary (20).

Although time-saving, sequential sampling plans are available to assist with sampling plan design (21, 22), the challenge is that the "fixed precision level" proposed for enumerative plans (e.g., 23) is actually variable for any given sampling session (or bout) (24). The desired precision level is an expectation that assumes multiple sampling sessions. To overcome this concern and to assist with sampling plan validation, Naranjo and Hutchison (25) developed the Resampling for Validation of Sample Plans (RVSP) program to allow researchers to quantify how the variability in actual precision levels obtained based on actual sampling data sets. This approach has proven useful for a variety of arthropod species, and for the validation of both enumerative (25–27) and presence-absence (28–30) sampling plans.

Given the high value of raspberries in Minnesota (14), and the subsequent use of multiple insecticides for *P. japonica*, we were motivated to develop and validate a statistically sound sampling plan for grower use. To do so, we utilized the well-known Taylor's Power Law regression (31) to characterize the spatial pattern of *P. japonica* adults, and the RVSP resampling approach to validate a cost-effective sequential sampling plan (23, 25). To provide additional efficiency, we examined the beetle's spatial pattern within the raspberry canopy, to minimize the area sampled.

Materials and methods

Sample data

The primary data sets used for sampling *P. japonica* adults, without insecticide use, were collected during the summers of 2018, 2020, and 2021, using a primocane (fall bearing) variety, 'Heritage.' The primary research field site was located at the Rosemount Research & Outreach Center, University of Minnesota (ROC-North, 44.71520N, -93.09744W). Raspberries were maintained using standard production guidelines for fertility, and supplemental irrigation as needed (11, 32). This site (~ 0.1 ha) consisted of several 2-row research plots (4.6m long), with rows separated by 3.1 m of turf maintained between rows to minimize erosion. In 2020, 22 data sets were collected from 5 July to 3 September; in 2021, 34 data sets were collected from 5 July to 30 August. In addition, in 2018, four high-density samples were collected in commercial fields of primocane (fall bearing) raspberries, 'Prelude' and 'Nova,' at a site near Forest Lake, MN (45.22832N, -92.89175W). These samples were collected prior to insecticide use. The field size, including both varieties was ~0.2 ha, with row spacing at 3.1m, also separated by turf between rows. In total, 60 data sets from untreated research plots and the commercial field were available for analysis. For all samples, the same 1-m row, canopy sample unit was used. On each sample date, 16 to 60 randomly selected 1-m row samples were taken using a visual whole canopy inspection, to record adult *P. japonica* densities (e.g., 5). Each data set was then used to calculate the mean and variance for sampling plan development and validation.

In addition to the ROC-North site, a second site, ROC-South (44.69104N, -93.07326W), was also established using 'Heritage' raspberry, where an insecticide study was conducted to assess efficacy and spatial pattern of *P. japonica* adults; this was ~4.5km south of ROC-North. ROC-south included 8 eight quadrants running east to west, where each quadrant contained 11, 3.1m rows of 'Heritage' raspberries separated by 3.1m turf alleys as previously described. As with ROC-north, the site was maintained using standard production guidelines for fertility, and supplemental irrigation as needed. The plots were assigned to 5 insecticide treatments, with 4 replications each, in a randomized complete block design (RCBD). The insecticide study in 2020 was conducted with sprays applied every two weeks from July 10 to August 28. Carbaryl (Sevin®, Monsanto, St. Louis MO), and spinosad (Entrust®, Corteva, Wilmington DE) were applied at rates of 32.0 and 6.0 oz of product/ac, respectively in a randomized complete block design. The zeta-cypermethrin (Mustang Maxx®, FMC, Philadelphia PA.) treatment, at a rate of 4.0 oz of product/ac, was applied weekly with the intent to maintain a "beetle free" zone. In 2021, the same trial was conducted at ROC-south, however, the spinosad treatment was replaced with acetamiprid (Assail®, United Phosphorous Inc., Bandra, East Mumbai), at a rate of 4.5oz of product/ac. In 2021, carbaryl and zeta-cypermethrin were applied at the same rates and intervals as in 2020. As with all other sampling data, the 1-m-row sample unit was used. Total data sets available for spatial pattern analysis ranged from 22-27, depending on the insecticide. In addition, a total of 16 data sets (4 data sets for each of 4 insecticides) were set aside prior to analysis, for sampling plan validation (25). Adult beetles from each study were identified by external anatomy and coloration unique to *P. japonica* (3, 19) and validated by comparisons to specimens previously deposited to the Insect Museum in the Department of Entomology, University of Minnesota.

Spatial pattern

We examined the spatial distribution of *P. japonica* adults using Taylor's Power Law (TPL) which is based on a logarithmic relationship between the sample variance (s^2) and the sample mean (m) (31), such that:

$$s^2 = a(m)^b$$

In practice, a and b are estimated by linear regression of $\log(s^2)$ as a function of log(m), where b is the slope, and index of aggregation. In theory, the value of b is independent of mean density and is relatively "constant" for a species in a given environment (31). Among many aggregation indices, TPL has been found to provide robust estimates of spatial pattern (e.g., 22, 26, 28). During 2020-2021, a total of 60 data sets collected from raspberry plots not treated with insecticides were used to calculate the mean/variance estimates. The TPL regression was used to quantify the relationship between log variance and log mean, for 43 of the 60 population density samples. The remaining 17 samples were selected to reflect a density range from low-high, and set aside as independent data sets to be used for RVSP validation analysis (see below). The slope of the TPL regression line (*b)* is indicative of the spatial pattern of the population sampled; i.e., if $b < 1$, $b = 1$, or $b > 1$, the population can be characterized as having a uniform, random, or aggregated spatial distribution, respectively (22). In addition to the primary TPL regression (N=43), the spatial pattern for *P. japonica adults* was determined by comparing the slopes for the TPL regressions for the 4 insecticides mentioned previously, as well as the untreated plots. The total number of sample dates per insecticide ranged from 22-26, for each of the two years. All regressions were conducted using RStudio (33).

Enumerative sequential sampling

Development of the sampling plan was based on Green's (23) "stop-line" model to estimate population density, after a successive number of samples are taken, using the formula:

$$T_n \geq (an^{1-b}/SE/m^2)^{1/(2-b)}$$

where T_n is the cumulative number of individuals sampled for n samples, a is the antilog of a from the TPL regression, b is the slope from TPL, SE/m = D for precision level (e.g., D=0.10, 0.25). With this plan, one continues to take successive samples until the appropriate T_n is exceeded to estimate insect density, for the desired precision level.

Validation of Green's plan was conducted by selecting 17 of the 60 sample data sets taken over two years at ROC-North prior to calculating TPL values, for use as independent data sets for validation (Naranjo & Hutchison 2007). The 17 data sets for validation were selected to represent a range of population densities from low to high. In addition, because of the potential variation in spatial pattern for insecticide treated plots, 16 sample dates were also selected from the insecticide study (4 samples for each of 4 insecticides), for a total of 33 validation data sets; for all validation data sets, the observed mean density ranged from 0.75 to 28.1 beetles per 1-m row. All validation data were then used with the TPL parameter estimates (a, b values) and Green's plan to evaluate plan performance, via the Resampling for Validation of Sample Plans (RVSP) program (25). Using the RVSP package, during each sampling session (bout), the resampling method assumes that a unique set of random samples are taken to estimate density to mimic one taking samples at random in the field. Final plan performance is assessed after the sampling process has been repeated (simulated) 500 times for each data set. The outcomes for the actual precision levels achieved for each RVSP simulation, are compared to the desired precision, and subsequently changed, as an iterative process to achieve the final goals of D=0.10 and D=0.25, for research and IPM purposes, respectively (34). In addition, RVSP provides the predicted average population density, for comparison with actual population density, and average sample number (ASN) for each of the 500 simulation runs. Once the ASN was determined for each precision level, we also calculated Relative Net Precision (RNP), to compare the efficiency of each sampling plan based on the sampling cost and precision (27, 35),

$$\text{Relative net precision} = (1/(RV * c)) * 100,$$

where RV is relative variation (SE/mean) * 100 (34), and c is the total cost (in time) for collecting the selected sample, usually measured in person-hours.

Canopy strata

To further improve the efficiency of sampling, and reduce sampling cost via a more refined sample unit, we conducted a study in 2021 to determine how *P. japonica* beetles were distributed within the raspberry canopy. On 10 separate sample dates in 2021, untreated 'Heritage' raspberry plots at ROC-North were used to visually sample the top-third (0-15 cm), middle-third (16-30 cm), and bottom-third (31-45 cm) strata of the canopy, to assess the potential for differential beetle density among strata. Nondestructive random sampling, using the 1-m-row sample unit, was conducted on each sampling date (July-August), by selecting five plants at random, within the middle 2 rows/plot, in each of four quadrats. The insect strata data was transformed using a square-root transformation [$\sqrt{(x+0.5)}$], where x is the number of adults recorded per sample per date. The mean number of beetles per 1-m-row, and SEMs, and the proportion of beetles in each strata were calculated for analysis. Prior to analysis, the proportion data were arc-sine transformed. The strata density data were analyzed using ANOVA with RStudio (33), and Tukey's HSD test for means comparison (P=0.05).

Results

Spatial pattern

Taylor's Power Law regression analyses of log-variance as a function of the log-mean, for *P. japonica* adults indicated a strong positive relationship for beetle populations, as measured by the 1-m-row sample unit (Figure 1). The pattern was consistent regardless of the insecticide used or if the data were collected from untreated plots. For all treatment regressions, R^2 estimates ranged from 0.79-0.89. Importantly, the TPL slope (b) values for all data sets, either from untreated or insecticide treated plots, were statistically greater than 1, (P<0.05, Table 1), indicating an aggregated spatial pattern for *P. japonica* adults in raspberry. We also did not find significant differences in slopes between the insecticide treated and untreated plots, nor differences among the four insecticides (P>0.05, Table 1).

Enumerative sequential sampling and validation

The development and validation of Green's sequential sampling plan, via RVSP, suggested that to achieve an observed average precision level (D) of ~0.10, a high average sample size of 106 sample units are required (Table 2). By contrast, to achieve an average precision level (D) of ~0.25 for IPM decision-making, an average sample size of only 15, 1-m-row samples are required (Table 3). The initial 'desired precision' levels specified in the RVSP validation were higher than expected, and were therefore decreased to 0.21 and 0.08, to achieve the desired actual precision levels of 0.25 and 0.10, respectively. This option with RVSP is often necessary to adjust (fine-tune) the precision levels to determine optimum final sample size (26, 27). Based on RVSP analysis, average maximum and minimum sample sizes were 23 and 15 for a precision levels of D = 0.25. By contrast, average maximum and minimum sample sizes were 123 and 106 were necessary for a precision level of D = 0.10. The full range of expected, average sample size requirements are illustrated in Figure 2 (see also Supplementary Table S1). Finally, the results for sampling efficiency, based on RNP, which includes the time to take samples for a given ASN at each precision level are shown in Table 4. As expected, the RNP was much higher, and most cost-effective (21.40) for the IPM-based precision level of D = 0.25.

Canopy strata

The canopy strata distribution study, where the canopy was equally partitioned vertically across three equally spaced strata (15 cm intervals), indicated significant differences in *P. japonica* beetle density (P<0.05, Table 5). Over the course of 10 sample dates and using the 1-m-row sample unit, a significantly higher number of beetles were observed in the top 1/3 of the canopy (P<0.05); there were also significant differences between the mid- and bottom 1/3 strata. Likewise, the mean proportion of beetles found varied significantly by strata (P<0.05, Table 5), with 78.8% of the *P. japonica* adults found in the top 1/3 of the canopy. These results suggest that additional savings in sampling time could be reduced by focusing the sampling effort on the top 1/3 of the canopy.

Discussion

In this study we found that *P. japonica* adults were highly aggregated on primocane (fall-bearing) raspberries regardless of

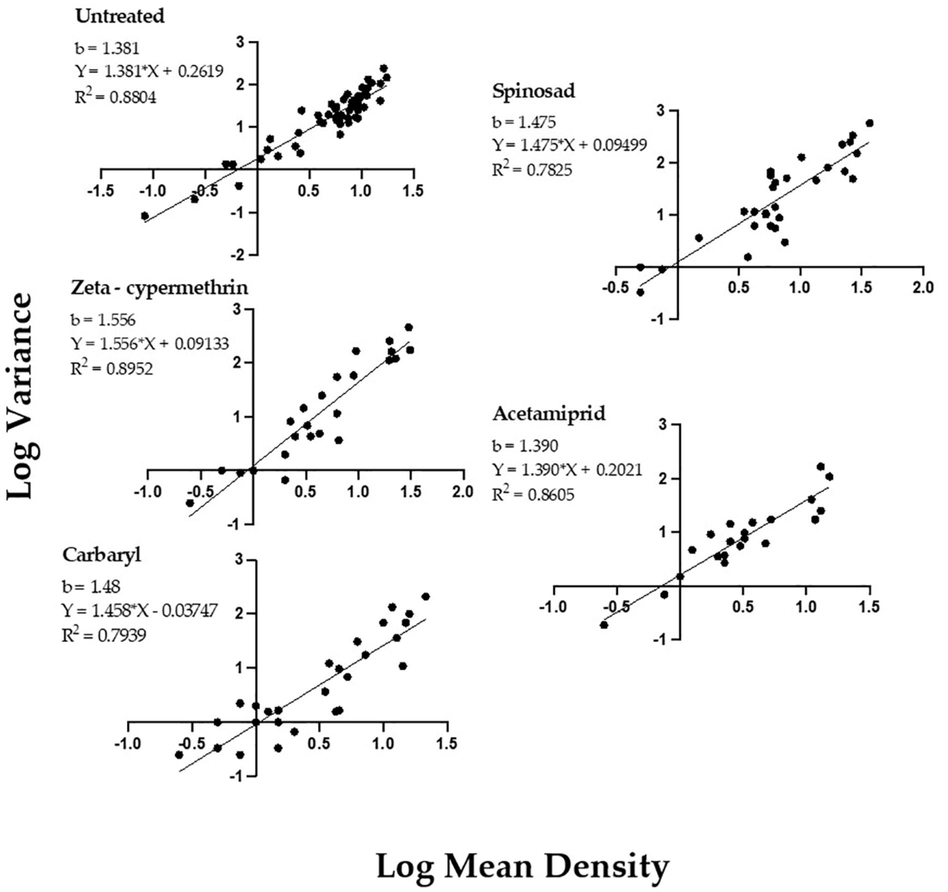


FIGURE 1
Taylor's Power Law for *P. japonica* adult density and variance (log transformed), collected on primocane ('Heritage') raspberry, Rosemount MN, Forest Lake MN 2020, and Rosemount MN 2021 (see also Table 1).

TABLE 1 Taylor's Power Law slope comparisons and mean density for *P. japonica* adults in raspberry, where sampling was conducted in untreated plots, and those treated with foliar insecticides, Rosemount, MN, 2021-2022.

Active Ingredient	a	*b* (± SE)[1,2]	P	R^2	N	mean[3] density/1-m
Zeta-cypermethrin	0.091	1.556 (± 1.49)	<0.01	0.89	24	3.45
Carbaryl	-0.037	1.450 (± 0.42)	<0.01	0.79	27	4.00
Spinosad	0.095	1.475 (± 1.13)	<0.01	0.78	27	12.88
Acetamiprid	0.202	1.390 (± 0.65)	<0.02	0.86	20	5.44
Untreated check	0.262	1.381 (± 0.28)	<0.02	0.88	43	9.55

[1] Multiple comparison test for slopes (b), with a Bonferroni correction, indicated no significant differences among slopes (P>0.75).
[2] All slopes were found to be significantly >1.0 (P<0.05), indicating an aggregated spatial distribution.
[3] Mean densities for beetles per 1-m row for both years, with the exception of spinosad data collected in 2021, and acetamiprid collected in 2022.

whether insecticides had been applied. Although the insecticides included in this study can reduce beetle densities differentially (36), our results indicate that the sampling plan presented is robust and applicable to commercial field situations for IPM decision-making. Aggregation behavior of *P. japonica* adults can be attributed to several factors related to the beetle's ecology. First, mated females usually oviposit in moist or irrigated soil supporting turf grass or nearby pastures (1, 19), where subsequent grub (larval) populations are also known to exhibit an aggregated spatial pattern (37, 38). Secondly, *P. japonica* adults emerging from these source populations each summer are often aggregated as well. Sara et al. (39) observed an aggregated distribution of *P. japonica* adults in soybean, where beetle density was most pronounced along field edges. More recently, a similar trend was observed for *P. japonica* adults in commercial vineyards (13). Thirdly, a primary mechanism responsible for initial beetle aggregations on several plant

TABLE 2 Resampling simulations used to validate a fixed precision, sequential sampling plan (23), for *P. japonica* adult density (1-m-row), by using a pre-set precision level of 0.08 (desired 0.10), via Taylor's Power Law ($a = 1.83$, and $b = 1.38$).

Validation Data Set	Observed Mean Density	Avg. statistics for 500 sequential sampling simulations[1]						
		Mean Density	Precision			Avg. sample no.		
			Mean	Min.	Max	Mean	Min.	Max
1	0.750	0.751	0.167	0.131	0.194	258	193	319
2	0.833	0.836	0.076	0.067	0.087	242	200	275
3	1.333	1.338	0.124	0.108	0.142	192	163	226
4	1.500	1.507	0.092	0.074	0.105	180	161	205
5	1.833	1.832	0.072	0.064	0.079	163	145	186
6	2.000	1.991	0.109	0.093	0.125	157	134	184
7	2.187	2.203	0.099	0.085	0.116	149	130	172
8	2.437	2.445	0.096	0.081	0.111	141	121	163
9	2.750	2.778	0.105	0.089	0.122	133	114	157
10	2.917	2.921	0.102	0.085	0.122	129	110	154
11	3.500	3.501	0.073	0.061	0.083	118	105	131
12	4.000	4.021	0.104	0.083	0.127	110	97	133
13	4.667	4.805	0.186	0.151	0.201	102	77	138
14	5.083	5.084	0.054	0.043	0.066	98	90	107
15	5.333	5.316	0.087	0.076	0.098	96	84	110
16	6.000	6.021	0.048	0.038	0.059	90	84	96
17	6.667	6.714	0.106	0.062	0.127	85	73	101
18	6.966	6.969	0.080	0.062	0.099	83	74	95
19	7.420	7.556	0.120	0.085	0.155	80	68	95
20	8.170	8.239	0.089	0.072	0.109	77	69	86
21	8.291	8.284	0.074	0.058	0.094	76	68	87
22	8.300	8.401	0.110	0.085	0.146	76	64	91
23	9.515	9.612	0.101	0.083	0.123	71	60	82
24	10.213	10.233	0.086	0.057	0.119	69	59	76
25	10.828	10.933	0.112	0.079	0.146	67	58	79
26	11.529	11.554	0.099	0.078	0.122	65	57	74
27	12.430	12.421	0.063	0.047	0.084	62	55	69
28	13.333	13.417	0.095	0.072	0.112	60	53	70
29	13.667	13.761	0.070	0.054	0.086	59	54	64
30	18.125	18.071	0.076	0.059	0.089	52	46	59
31	19.861	19.923	0.088	0.065	0.109	49	43	55
32	24.028	24.506	0.133	0.087	0.171	45	37	56
33	28.083	28.305	0.096	0.072	0.123	41	36	47
Overall	8.01	8.07	0.097	0.076	0.117	106	91	123

[1] Data sets resampled with replacement because of low mean *P. japonica* densities for some field sites (data sets 1-5); resampling conducted using Resampling for Validation of Sample Plans (RVSP) simulation software (25).

TABLE 3 Resampling simulations used to validate a fixed precision, sequential sampling plan (23), for *P. japonica* adult density (1-m-row), by using a pre-set precision level of 0.22 (desired 0.25), via Taylor's Power Law (*a* = 1.83, and *b* = 1.38).

Validation Data Set	Observed Mean Density	Avg. statistics for 500 sequential sampling simulations[1]						
		Mean Density	Precision			Avg. Sample no.		
			Mean	Min.	Max	Mean	Min.	Max
1	0.750	0.813	0.421	0.211	0.657	37	19	74
2	0.833	0.857	0.204	0.141	0.274	33	24	46
3	1.333	1.405	0.336	0.222	0.480	27	17	48
4	1.500	1.565	0.239	0.101	0.343	25	17	35
5	1.833	1.867	0.187	0.107	0.253	23	17	29
6	2.000	2.105	0.291	0.159	0.403	22	14	33
7	2.187	2.251	0.270	0.158	0.392	21	14	35
8	2.437	2.514	0.252	0.155	0.357	20	13	30
9	2.750	2.847	0.276	0.156	0.391	19	13	27
10	2.917	3.010	0.272	0.141	0.398	18	13	26
11	3.500	3.556	0.197	0.086	0.280	17	12	22
12	4.000	4.154	0.272	0.122	0.405	16	10	25
13	4.667	5.485	0.438	0.075	0.600	15	7	25
14	5.083	5.119	0.146	0.065	0.237	14	11	18
15	5.333	5.437	0.229	0.100	0.345	14	9	19
16	6.000	6.058	0.129	0.042	0.205	13	11	16
17	6.667	6.976	0.260	0.049	0.407	12	8	17
18	6.960	7.079	0.208	0.097	0.348	12	8	16
19	7.420	8.078	0.296	0.140	0.519	11	7	19
20	8.170	8.248	0.239	0.105	0.410	11	8	16
21	8.290	8.330	0.196	0.091	0.312	11	8	17
22	8.300	8.803	0.281	0.156	0.532	11	7	17
23	9.515	9.864	0.264	0.125	0.460	10	7	15
24	10.212	10.543	0.201	0.062	0.424	10	7	13
25	10.828	11.407	0.279	0.083	0.570	10	6	14
26	11.529	11.885	0.258	0.109	0.407	9	7	14
27	12.430	12.522	0.158	0.058	0.281	9	6	12
28	13.333	13.833	0.240	0.051	0.365	9	6	13
29	13.667	13.929	0.182	0.060	0.295	9	7	12
30	18.125	18.527	0.197	0.057	0.320	8	6	10
31	19.861	20.687	0.231	0.066	0.413	7	5	10
32	24.028	25.483	0.316	0.105	0.628	7	5	10
33	28.083	28.936	0.248	0.049	0.399	6	5	9
Overall	8.010	8.310	0.249	0.106	0.398	15	11	23

[1] Data sets resampled with replacement because of low mean *P. japonica* densities for selected field sites; resampling conducted using Resampling for Validation of Sample Plans (RVSP) simulation software (25).

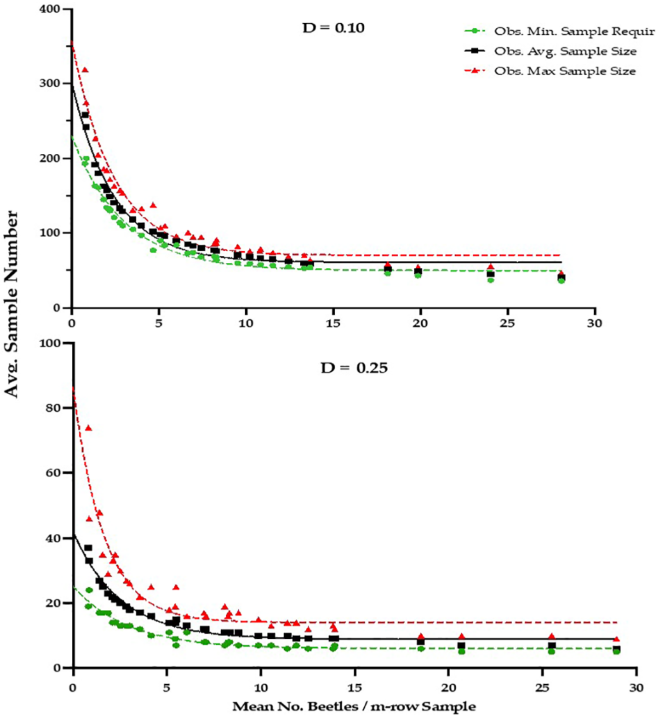


FIGURE 2
Observed Average, Minimum and Maximum Sample size results from RSVP Validation analysis for desired precision levels of D=0.10 and D=0.25, based on Green's Sequential Sampling Plan for *P. japonica* adults, using a 1-m-row sampling unit in raspberry, Rosemount MN, 2020-2021 (see Supplementary Table S1 for fitted equations for each precision level).

species is attributed to the release of volatile organic compounds (VOCs) in response to beetle feeding. For example, Loughrin et al. (40, 41) quantified the attraction of *P. japonica* adult aggregations to several VOCs emanating from crab apple and wine grapes, including floral kairomones (e.g., pbenethanol, linalool) or fruit-2like volatiles [e.g., (Z)-3-hexenyl butyrate, (Z)-3-hexenyl benzoate)]. The VOCs are released soon after *P. japonica* adults initiate feeding. Although VOCs have not been documented for raspberry, a similar phenomenon could be responsible for the aggregation phenomenon observed on raspberry (see also 11). Finally, once high numbers of adult *P. japonica* adults have colonized host plants, the release of the female sex pheromone also attracts additional males to the same feeding sites (1).

An extensive entomological literature has shown that the use of Taylor's Power Law regression provides a reliable approach to characterizing spatial pattern across a diversity of arthropod taxa (21, 24, 25, 31). Moreover, with the development of Green's (23) sequential sampling plan for estimating population density, the slope of the TPL regression was found to be useful for developing practical sampling plans, with designated average precision levels (21, 26). However, despite the reliability of the TPL regression, and other measures of spatial aggregation, additional research affirmed that such measures serve only as initial *estimates of spatial pattern*, reflecting a continuum from random to aggregated, rather than a fixed index (24). Like other ecological parameters spatial pattern estimates are dependent on sample size, host crops, or external variables such as insecticide use. Importantly, an additional key factor affecting the final performance of a sampling plan is the stochastic nature of the sequential sampling process itself; i.e., each time a plan is implemented (sampling bout), a different set of plants are sampled and different arthropod densities are encountered, all of which yields a slightly different estimate of pest density and the final precision level obtained (21, 24, 25).

These findings prompted the development of a bootstrap, or resampling approach to develop and validate sequential sampling plans that would incorporate both sources of variation (24). As illustrated by Naranjo and Hutchison (25), the model RVSP was developed to provide a validation process for sequential sampling plans using actual insect sampling data sets, versus a theoretical distribution such as the negative binomial. The resampling approach is a form of bootstrap sampling, where independent data sets for a given species are used to assess the actual precision of a sampling plan, allowing for more flexibility in building sampling plans based on realistic spatial patterns (22, 25, 26). In addition, as an iterative process it is used to modify the pre-set precision levels, to eventually achieve the desired precision and reasonable ASN. This is particularly useful for IPM applications. By contrast, traditional plans that are not validated can lead to unnecessarily high sample sizes, that are too time-consuming (25). The simulations can also be processed in a matter of seconds. Thus, the initial pre-set precision can and should be adjusted as needed to reach the desired observed precision levels for the sampling plan to be effective. For *P. japonica* we therefore adjusted

TABLE 4 Efficiency of two fixed-precision sequential sampling plans for *P. japonica* adults in raspberry, as measured by relative net precision (RNP).

Sampling plan Precision level	ASN[1]	Avg. Sample Time (hr)[2]	Total Sample Time (hr)[3]	RNP[4]
D = 0.10	106	0.0125	1.325	7.55
D = 0.25	15	0.0125	0.187	21.40

[1] Average sample number (ASN) was estimated based on 500 iterative sampling runs (bouts), as part of the resampling analysis using RVSP (25), as per Tables 2, 3. The ASN shown here is the average observed for 500 validation sampling runs.

[2] Average time to record a single 1-m-row sample (45 sec) per person, which includes the time to walk between samples (<5 sec); total sampling time = 45 sec, or 0.0125 person hr.

[3] Time in person-hours to sample the ASN, including the time to walk between samples, for a given precision level.

[4] Relative net precision = (1/(RV*c))*100, where RV is relative variation (D*100), and c is the total cost (time) related to collecting total samples for ASN, usually measured in person-hours.

the precision levels to 0.08 and 0.21 in the final RVSP simulations to achieve the desired actual precision of D=0.10 and 0.25, respectively. The results of Green's sequential sampling plan also indicate that as the density of *P. japonica* increases, fewer samples are required to determine adult density (Tables 2, 3, Figure 2).

The strata study indicated that the majority of *P. japonica* adults are found in the top third of the canopy (Table 5). Feeding by *P. japonica* in the upper strata of crop canopies has also been documented in wine grapes (5) and soybean (42). Feeding in the upper strata of various crops has been attributed to their attraction to sunlight (UV) or the nutritional value of feeding on younger leaves (1, 11). This information will be helpful for growers and crop consultants as they can focus their crop inspections more efficiently within the upper canopy strata. Finally, although a formal time-of-day study was not conducted, we found that beetle activity was most noticeable between 11am to 5pm, and that beetle counts too early in the morning could lead to underestimates of the actual infestation levels.

To our knowledge, this is the first study to document the spatial pattern of *P. japonica* adults in raspberry. However, previous work with *P. japonica* has shown that the adults also exhibit aggregated distributions, when beetles were sampled in wine grapes where a strong "edge effect" was noted (13), or when trapping beetles in semiochemical-baited traps (5). It is also notable that the strong linear TPL relationship for *P. japonica* adults is similar to the TPL results found for other beetle species (e.g., 26). Regardless of the biological basis for aggregation pattern in the field, it is well known that the subsequent sampling plans for such species, often necessitates higher sample sizes and costs, compared to sampling plans for species characterized by random spatial patterns (25).

Our sequential sampling plan indicates that, on average, only 15 samples are necessary to estimate adult *P. japonica* population density in raspberry, when using the IPM based precision level (D) of 0.25 (Table 3). With a sample time of 30-45 sec, and the time to walk between samples averaging 5 sec, we assumed a final conservative estimate of 45 sec for total time to take a 1-m-row sample. Thus a sample size of 15 would equate to ~11 minutes per field site, which is a reasonable time frame for growers and crop consultants to make control decisions (Table 4). When beetle densities are relatively moderate to high, the sampling time will be much less (Figure 2). The combination of a brief sampling time and a validated precision level (0.25 for IPM), suggests a high level of efficiency in sampling adults. *Popillia japonica* often overwinter in loam-clay soils and prefer moist turf (1, 43), which is commonly grown between raspberry rows. This should inform growers that once a population is established, there will continue to be moderate to high beetle pressure in the foreseeable future making an effective sampling plan imperative. Recent studies in Minnesota suggest that *P. japonica* adult emergence begins during late June (6, 7); therefore growers and consultants in the upper Midwest region should begin sampling by mid-July to catch peak beetle activity, and begin sampling efforts.

TABLE 5 Mean (+/-SEM)[1] number of *P. japonica* adults per m-row for three strata (15-cm intervals) within a raspberry ('Heritage') canopy (no insecticide sprays), Rosemount MN, 2021.

Strata	Mean No. Adults	Mean Proportion of Total
Top	9.45 (± 1.08) a	0.788 (± 2.52) a
Middle	2.10 (± 0.43) b	0.175 (± 2.18) b
Bottom	0.45 (± 0.14) c	0.037 (± 0.98) b

[1] Means followed by the same letter are not significantly different, Tukey's HSD (P=0.05). Analysis completed based on proportion of *P. japonica* adults per stratum, using the arcsine transformation; back-transformed means presented in Table.

Although the pattern for aggregation of *P. japonica* adults was consistent among all insecticides tested (b >1.0), the differences observed in population density and corresponding ASNs (Tables 2, 3) was not surprising given the known differences in efficacy of insecticides for this species (36); there may also be sub-lethal or behavioral effects on the beetles in treated plots, that may also differentially affect spatial pattern. For example, Burkness et al. (36) found that zeta-cypermethrin (Mustang Maxx) consistently reduces beetle populations to <5 beetles/m-row, for up to 2 weeks following an application, resulting in several sample dates with low densities. As shown for other insect species, low densities may lead to a more random spatial pattern. Moreover, the change from aggregated to random is more of a continuum versus an abrupt change of the TPL slope from b=1.0 to b>1.0 (22).

The primary production practice for raspberries in Minnesota is the use of fall bearing varieties (14) because they yield well, and are less labor-intensive than summer raspberries that require intense labor for pruning each season. In recent years, because of the establishment of another invasive, spotted-wing Drosophila (*Drosophila suzukii*), more growers are beginning to transition toward summer bearing raspberries (14). Although our research was conducted primarily with the fall bearing 'Heritage' variety, we believe the proposed sampling method should be applicable to summer bearing raspberries as well. Fall and summer bearing raspberries share similar canopy growth patterns, with ample foliage produced during summer-fall growth periods, when *P.*

japonica adults are most active in the Midwest region (7). However, because multiple varieties are grown in the region, further research is needed to determine the degree to which *P. japonica* adults show similar aggregation behavior, and thus changes in spatial aggregation that could affect sampling plan recommendations. In addition, more work should be done with other varieties to evaluate the degree of fruit feeding by *P. japonica* beetles, as this may require more targeted sampling on fruit and flowers versus foliage late in the season. As *P. japonica* continues to colonize crops in Europe (44–48), and global climate change continues to facilitate invasive pest expansion (49, 50), it will be critical for researchers to develop innovative monitoring tools for both surveillance and IPM applications to minimize excess insecticide use and respond to grower challenges.

In summary, our study shows that *P. japonica* adults exhibit an aggregated distribution on fall bearing 'Heritage' raspberries in Minnesota. Despite a high level of aggregation, the validation analysis, when using Green's sequential sampling plan, requires an average of only 15 1-m-row samples to estimate the population density, at an average precision level (D) of 0.25, recommended for IPM decision-making (25). As with other sequential sampling plans, more samples are necessary at low densities (e.g., < 5 beetles/sample). For the high density range of 10 to 25 beetles/sample unit, the ASN continued to decline, especially for D=0.25, but ranged from 8-15 samples, respectively (Table 3, Figure 2). The sequential sampling plan for *P. japonica* on raspberry should be useful to growers and crop consultants, by providing statistically sound estimates of population density, with a reasonable sample size and cost. Finally, research is underway in Minnesota to determine economic injury levels, and a practical economic threshold for *P. japonica* in raspberry. The sampling plan can then be used in tandem with an effective economic threshold for *P. japonica* adults, to further build an IPM program for fall bearing raspberry in the Midwest U.S.

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.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

AT: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. EB: Conceptualization, Data curation, Methodology, Supervision, Validation, Writing – review & editing. WH: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The study was funded by support from the AES (Agric. Expt. Station) Rapid Agric. Response Fund (award, 081823) and USDA-Minn. Dept. of Agric. Specialty Crops Block grants (awards: 0070811, 0092341), and the University of Minnesota, AES, St. Paul, MN.

Acknowledgments

We thank Oliver McEllistrem, Sally GA Nelson, and Dr. Dominique Ebbenga (UMN Fruit & Vegetable IPM Lab) for assistance with the field research, and acknowledge Drs. Robert Koch and Mary Rogers (UMN) for providing helpful reviews for a previous version of the manuscript.

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.

The author WH declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/finsc.2024.1465829/full#supplementary-material

References

1. Potter DA, Held DW. Biology and management of the Japanese beetle. *Annu Rev Entomol.* (2002) 47:175–205. doi: 10.1146/annurev.ento.47.091201.145153

2. Althoff EM, Rice KB. Japanese beetle (Coleoptera: Scarabaeidae) invasion of North America: history, ecology, and management. *J Integ Pest Manage.* (2022) 13:1–11. doi: 10.1093/jipm/pmab043

3. Shanovich HN, Dean AN, Koch RL, Hodgson E. Biology and management of Japanese Beetle (Coleoptera: Scarabaeidae) in corn and soybean. *J Integr Pest Manage.* (2019) 10:9. doi: 10.1093/jipm/pmz009

4. Wawrzynski RP, Ascerno ME. Mass trapping for Japanese beetle (Coleoptera: Scarabaeidae) suppression in isolated areas. *J Arboricult.* (1998) . 24:303–7. doi: 10.48044/jauf.1998.038

5. Ebbenga DN, Burkness EC, Clark MD, Hutchison WD. Impact of adult Popillia japonica (Coleoptera: Scarabaeidae) foliar feeding injury on fruit yield and quality of a temperate, cold-hardy wine grape, 'Frontenac'. *Front Insect Sci.* (2022) 2:887659. doi: 10.3389/finsc.2022.887659

6. Ebbenga DN, Wold-Burkness SJ, Burkness EC, Hutchison WD. Japanese beetle as an Emerging Fruit Pest, in: *Fruit Edge* (2023). St. Paul, MN: University of Minnesota, Extension. Available online at: https://fruitedge.umn.edu/Japanese-beetle/Japanese-beetle-emerging-pest-fruit-crops (Accessed June 15, 2024).

7. Ebbenga DN, Burkness EC, Hutchison WD. Optimizing the use of semiochemical-based traps for efficient monitoring of Popillia japonica (Coleoptera: Scarabaeidae): Validation of a volumetric approach. *J Econ Entomol.* (2022) 115:869–76. doi: 10.1093/jee/toac049

8. Hammons DL, Kurtural SK, Newman MC, Potter DA. Invasive Japanese beetles facilitate aggregation and injury by a native scarab pest of ripening fruits. *Proc Nat Acad Sci.* (2009) 106:3686–91. doi: 10.1073/pnas.0811097106

9. Leskey TC, Nielsen AL. Impact of the invasive Brown Marmorated Stink Bug in North America and Europe: History, biology, ecology, and management. *Annu Rev Entomol.* (2018) 63:599–618. doi: 10.1146/annurev-ento-020117-043226

10. Venette RC, Hutchison WD. Invasive insect species: Grand challenges, strategies and opportunities. *Front Insect Sci.* (2021) 1:650520. doi: 10.3389/finsc.2021.650520

11. Burkness EC, Ebbenga DN, Toninato AG, Hutchison WD. Exclusion and repulsion of *Popillia japonica* (Coleoptera: Scarabaeidae) using various coverings on high tunnel structures for primocane raspberry. *Insects.* (2022) . 13:771. doi: 10.3390/insects13090771

12. Henden J, Guédot C. Effect of surrounding landscape on *Popillia japonica* abundance and their spatial pattern within Wisconsin vineyards. *Front Insect Sci.* (2022) 2:961437. doi: 10.3389/finsc.2022.961437

13. Lannan MC, Guédot C. Attract-and-kill for managing *Popillia japonica* (Coleoptera: Scarabaeidae) abundance and leaf injury in commercial vineyards. *J Econ Entomol.* (2024) 117:508–15. doi: 10.1093/jee/toae031

14. DiGiacomo G, Hadrich J, Hutchison WD, Peterson H, Rogers M. Economic impact of spotted wing drosophila (Diptera: Drosophilidae) yield loss on Minnesota raspberry farms: a grower survey. *J Integr Pest Manage.* (2019) 10:11. doi: 10.1093/jipm/pmz006

15. Moyer RA, Hummer KE, Finn CE, Frei B, Wrolstad RE. Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: *Vaccinium, Rubus*, and *Ribes*. *J Agric Food Chem.* (2002) 50:519–25. doi: 10.1021/jf011062r

16. Ladd TL Jr. Japanese beetle (Coleoptera: Scarabaeidae): Influence of favored food plants on feeding response. *J Econ Entomol.* (1987) 80:1014–7. doi: 10.1093/jee/80.5.1014

17. Pehluvan M, Güleryüz M. Research on the adaptation of some raspberry (*Rubus ideaus* L.) cultivars in Oltu (Erzurum) ecological conditions. *Alinteri Zirai Bilimler Dergisi.* (2010) 18:7–13.

18. Ebbenga DN, Hanson AA, Burkness EC, Hutchison WD. A degree-day model for forecasting adult phenology of *Popillia japonica* (Coleoptera: Scarabaeidae) in a temperate climate. *Front Insect Sci.* (2022) 2:1075807. doi: 10.3389/finsc.2022.1075807

19. Fleming WE. *Biology of the Japanese Beetle. Technical Bulletin No. 1449.* Washington, DC: USDA (1972).

20. Radcliffe EB, Hutchison WD, Cancelado R eds. *Integrated Pest Management: Concepts, Tactics, Strategies and Case Studies*. Cambridge UK: Cambridge Univ. Press. UK (2009). 450 pp.

21. Binns MR, Nyrop JP. Sampling insect populations for the purpose of IPM decision making. *Annu Rev Entomol.* (1992) 37:427–53. doi: 10.1146/annurev.en.37.010192.002235

22. Hutchison WD. Sequential sampling to determine population density. In: Pedigo L, Buntin D, editors. *Handbook of sampling methods for arthropods in agriculture*. CRC, Boca Raton, FL (1994). p. 207–44.

23. Green RH. On fixed precision sequential sampling. *Res Popul. Ecol.* (1970) 12:249–51. doi: 10.1007/BF02511568

24. Hutchison WD, Hogg DB, Poswall MA, Berberet RC, Cuperus GW. Implications of the stochastic nature of Kuno's and Green's fixed-precision stop-lines: Sampling plans for the pea aphid (Homoptera: Aphididae) in alfalfa as an example. *J Econ Entomol.* (1988) 81:749–58. doi: 10.1093/jee/81.3.749

25. Naranjo SE, Hutchison WD. Validation of arthropod sampling plans using a resampling approach: software and analysis. *Am Entomol.* (1997) 43:48–57. https://www.ars.usda.gov/research/software/download/?softwareid=129&modecode=20-20-05-05. (Accessed September 15, 2024).

26. Galvan TL, Burkness EC, Hutchison WD. Enumerative and binomial sequential sampling plans for the multicolored Asian lady beetle (Coleoptera: Coccinellidae) in wine grapes. *J Econ Entomol.* (2007) 100:1000–10. doi: 10.1093/jee/100.3.1000

27. Ribeiro AV, Aita RC, Pezzini DT, DiFonzo CD, Hunt TE, Knodel JJ, et al. Optimization of sample unit size for sampling stink bugs (Hemiptera: Pentatomidae) in soybean. *Crop Prot.* (2022) 157:105986. doi: 10.1016/j.cropro.2022.105986

28. Burkness EC, Hutchison WD. Development and validation of a binomial sequential sampling plan for striped cucumber beetle (Coleoptera: Chrysomelidae) in cucurbits. *J Econ Entomol.* (1997) 90:1590–4. doi: 10.1093/jee/90.6.1590

29. Hodgson EW, Burkness EC, Hutchison WD, Ragsdale DW. Enumerative and binomial sequential sampling plans for soybean aphid (Homoptera: Aphididae) in soybean. *J Econ Entomol.* (2004) 97:2127–36. doi: 10.1093/jee/97.6.2127

30. de Araújo TA, Pezzini DT, Ramos RS, Picanço MC, Bastos CS, Hunt TE, et al. Development and validation of sampling plans for *Frankliniella schultzei* on tomato. *Crop Prot.* (2020) 134:105163. doi: 10.1016/j.cropro.2020.105163

31. Taylor LR. Aggregation, variance and the mean. *Nature.* (1961) 189:732–5. doi: 10.1038/189732a0

32. Beckerman J, Bessin R, Welty C, Athey K, Wahle E, Lewis D, et al. *Midwest Fruit Management Guide*. West Lafayette, IN: Purdue Extension, Purdue University (2021). Available at: https://ag.purdue.edu/hla/hort/pages/sfg_sprayguide.aspx.

33. R Studio Team. *RStudio, Integrated Development for R. RStudio*. Boston, MA: PBC (2020). Available at: http://www.rstudio.com/.

34. Southwood TRE, Henderson PA. *Ecological methods. 3rd ed.* Oxford. UK: Blackwell Ltd. (2000).

35. Pedigo LP, Rice ME, Krell RK. *Entomology and Pest Management. 7th ed.* Long Grove IL: Waveland Press Inc. (2021). 584 pp.

36. Burkness EC, Ebbenga DN, Hutchison WD. Evaluation of foliar insecticide control of adult Japanese beetle in raspberry 2019. *Arthropod. Manage Tests.* (2020) 45 (1), 1–1. doi: 10.1093/amt/tsaa009

37. Ng YS, Trout RJ, Ahmad S. Spatial distribution of the larval populations of the Japanese Beetle (Coleoptera: Scarabaeidae) in turfgrass. *J Econ Entomol.* (1983) 76:26–32. doi: 10.1093/jee/76.1.26

38. Ng YS, Trout RJ, Ahmad S. Sequential sampling plans for larval populations of the Japanese beetle (Coleoptera: Scarabaeidae) in turfgrass. *J Econ Entomol.* (1983) 76:251–3. doi: 10.1093/jee/76.2.251

39. Sara SA, McCallen EB, Switzer PV. The spatial distribution of the Japanese beetle, *Popillia japonica*, in soybean fields. *J Insect Sci.* (2013) 13:36. doi: 10.1673/031.013.3601

40. Loughrin JH, Potter DA, Hamilton-Kemp TR. Volatile compounds induced by herbivory act as aggregation kairomones for the Japanese beetle (*Popillia japonica* Newman). *J Chem Ecol.* (1995) 21:1457–67. doi: 10.1007/BF02035145

41. Loughrin JH, Potter DA, Hamilton-Kemp TR, Byers ME. Role of feeding–induced plant volatiles in aggregative behavior of the Japanese Beetle (Coleoptera: Scarabaeidae). *Environ Entomol.* (1996) 25:1188–91. doi: 10.1093/ee/25.5.1188

42. Ribeiro AV, Cira TM, MacRae IV, Koch RL. Effects of feeding injury from Popillia japonica (Coleoptera: Scarabaeidae) on soybean spectral reflectance and yield. *Front Insect Sci.* (2022) 2:1006092. doi: 10.3389/finsc.2022.1006092

43. Gould GE. Japanese beetle damage to soybeans and corn. *J Econ Entomol.* (1963) 56:776–81. doi: 10.1093/jee/56.6.776

44. Santoiemma G, Battisti A, Gusella G, Cortese G, Tosi L, Gilioli G, et al. Chemical control of Popillia japonica adults on high-value crops and landscape plants of northern Italy. *Crop Prot.* (2021) 150:105808. doi: 10.1016/j.cropro.2021.105808

45. Poggi S, Desneux N, Jactel H, Tayeh C and Verheggen F. A nationwide pest risk analysis in the context of the ongoing Japanese beetle invasion in Continental Europe: The case of metropolitan France. *Front Insect Sci.* (2022) 2:1079756. doi: 10.3389/finsc.2022.1079756

46. Gotta P, Ciampitti M, Cavagna B, Bosio G, Gilioli G, Alma A, et al. Roversi PF and Marianelli L. *Popillia japonica* – Italian outbreak management. *Front Insect Sci.* (2023) 3:1175138. doi: 10.3389/finsc.2023.1175138

47. Graf T, Scheibler F, Niklaus PA, Grabenweger G. From lab to field: biological control of the Japanese beetle with entomopathogenic fungi. *Front Insect Sci.* (2023) 3:1138427. doi: 10.3389/finsc.2023.1138427

48. Strangi A, Paoli F, Nardi F, Shimizu K, Kimoto T, Iovinella I, et al. Tracing the dispersal route of the invasive Japanese beetle. *Popillia japonica. J Pest Sci.* (2024) 97:613–29. doi: 10.1007/s10340-023-01653-1

49. Kistner-Thomas EJ. The potential global distribution and voltinism of the Japanese beetle (Coleoptera: Scarabaeidae) under current and future climates. *J Insect Sci.* (2019) 9:1–13. doi: 10.1093/jisesa/iez023

50. Liess S, Twine TE, Snyder PK, Hutchison WD, Konar-Steenberg G, Keeler BL, et al. High-resolution climate projections over Minnesota for the 21st century. *Earth Space Sci.* (2022) 9:e2021EA.001893. doi: 10.1029/2021EA001893

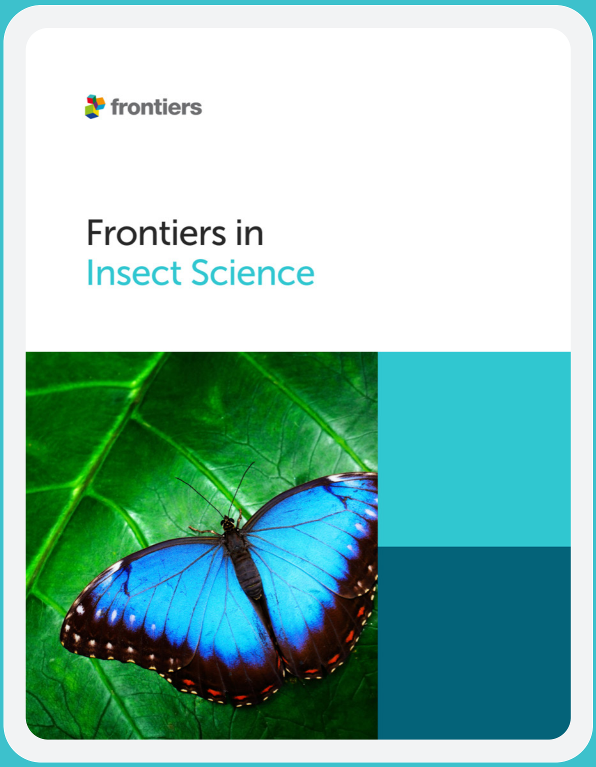
Frontiers in
Insect Science

Explores the biology of insects and their interaction with other organisms

Advances entomology, with research on the biology, physiology and neurobiology of insects. It explores commercial and industrial applications of insect research to global challenges such as climate change and nutrition.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact

frontiers | Research Topics