



Sustainability in Brazilian Citriculture: Three Decades of Successful Biological Control of Insect Pests

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Citrus insect pests has grown worldwide, concerning entomologists and farmers especially because of the high demand for food that makes it to our tables. The use of pesticides brought several issues, such as the negative impacts on the human health, pollution, and insect resistance. In this context, more environmentally-friendly strategies have been demanded by governments and consumers. In this review, we present three remarkable examples of pest management in Brazil that involved many researchers from different expertise areas to develop more sustainable strategies to reduce the damages to citrus production. The case studies consisted of high-quality research funded by the Brazilian government and private institutions. In the first case, we report on the monitoring of the citrus fruit borer with sex pheromones, which significantly improved the control of this insect pest. Based on behavioral studies, it was recommended that delta traps containing pheromone attractant be installed on the upper third part of the plant. A recent study indicates the promising use of the egg parasitoid *Trichogramma atopovirilia* for biological control. In the second case, the biocontrol of the leafminer using *Ageniaspis citricola* is discussed. The insect pest was introduced into Brazil in 1996, causing losses of up to 60 kg of fruit per tree. The solution for this problem was the introduction of the parasitoid *Ageniaspis citricola*, originally from Asia. The pest was successfully controlled by the parasitoid, which was able to adapt to different citrus-producing regions in Brazil. Finally, the most emblematic successful case in Brazil is the biological control of the Asian citrus psyllid (ACP), *Diaphorina citri*, using *Tamarixia radiata*. ACP is the most important citrus pest because it causes indirect damage by transmitting the gram-negative bacteria that cause HLB (huanglongbing) in citrus areas. HLB is a vicious disease that has no cure: after being infected, the plants present chlorosis of the leaves, production of yellow shoots, and die within 2–3 years. The combination of laboratory tests, insect monitoring and computational modeling showed the efficacy of *T. radiata* against *D. citri*, which reduces the number of infected trees.

Keywords: insect pest management, Asian citrus psyllid, parasitoid, citrus, citrus fruit borer

INTRODUCTION

Brazilian citrus, impacted by countless pests and diseases, is among the crops that suffer the most losses from attacks by insects, mites, and pathogens. More than 50 species of insects and mites cause direct and indirect damage (Parra et al., 2005). Some key pests are mites, fruit flies, mealybugs, CVC (Citrus Variegated Chlorosis) vectors (leafhoppers), the citrus leafminer, the citrus fruit borer, and the Asian citrus psyllid, which has increased the indiscriminate use of pesticides (Parra et al., 2004b; Raga et al., 2004; Jesus Junior et al., 2006; Bobot et al., 2011).

Since the first half of the 20th century, Brazilian citriculture has undergone pest attacks that have threatened its continuance. In 1937, the Tristeza virus, transmitted by the aphid *Aphis citricidus* (Hemiptera: Aphididae), was controlled by using resistant rootstocks (Parra et al., 2005). Some decades later, the mite *Brevipalpus phoenicis* (Acari: Tenuipalpidae), the vector of the leprosis virus, which caused qualitative and quantitative damage and was recently found to be disseminated by a complex of species of the genus *Brevipalpus* (Mineiro et al., 2018). The development of resistance management (especially rotation of chemical products) and determination of the most appropriate times and amounts of pesticides to use in controlling mites were welcomed by farmers (Muller et al., 2005).

The 1980s saw the appearance of CVC (Citrus Variegated Chlorosis), caused by the bacterium *Xylella fastidiosa* and transmitted by a group of leafhoppers of the family Cicadellidae. A research program to manage this disease was initiated, supported by FAPESP (São Paulo State Research Foundation), and resulted in sequencing of the bacterial genome, a significant advance in basic studies of CVC (Simpson et al., 2000).

By the end of that decade, citrus growers faced yet another challenge: an increase in attacks by the citrus fruit borer, *Gymnandrosoma aurantianum* (Lepidoptera: Tortricidae) (Bento et al., 2019). This insect pest caused losses of around 80–160 kg per tree, representing an impact of 50–80 million US dollars per year. The development of an attractant using a sex pheromone was important in rationalizing the management process, by using monitoring and enabling control strategies to be used at the appropriate time, helping to mitigate crop losses (Bento et al., 2016).

In 1996 a new pest was discovered, the citrus leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae), which directly damages sprouts and indirectly increases the occurrence of citrus canker; i.e., the damage caused by the larvae provides an access door to the bacterium responsible for this disease (Heppner, 1993; Gravena, 1994). Management of this pest was achieved through classical biological control, by introducing the larval parasitoid *Ageniaspis citricola* (Hymenoptera: Encyrtidae), imported from the USA and reared and released over 2 years in many different citrus-producing areas. After two more years, the parasitoid reached high parasitism rates of 70–80%, which reduced the incidence of citrus canker (Parra, 2021).

The most recent challenge in Brazilian citrus production was the arrival of HLB (huanglongbing) in 2004 (Teixeira et al., 2005a,b), a disease that led to the eradication of more than 50 million citrus trees in Brazil. The disease is disseminated by the

psyllid *Diaphorina citri* (Hemiptera: Psyllidae). Although this psyllid was detected in the country in the early 1940s (Costa Lima, 1942) and caused direct damage that was not initially worrisome, because it transmits the bacteria responsible for HLB it is now the key pest affecting this crop (Paiva and Yamamoto, 2014; Lee et al., 2015).

Despite these challenges, Brazilian citriculture leads the world in orange production (FAOSTAT, 2019), with well-structured programs to deal with the most varied problems, organized by research teams established in private and public institutions such as research centers and universities (Bassanezi et al., 2020).

In order to reduce the use of agrochemicals, in the last three decades more-sustainable strategies have been adopted in response to the concerns of consumers, such as biological control and pheromone applications (Turra et al., 2014). However, the continued preference of Brazilian citrus producers for agrochemicals (Gravena, 2011) has led to external pressure to change this scenario, given the lead role of Brazil in the exportation of oranges (Pinto et al., 2013). Examples are the increasing use of selective pesticides (for natural enemies and pollinators) and rotation of active compounds. These actions are based on the philosophy of Integrated Pest Management, which focuses on a combination of alternative and more environmentally friendly strategies with other control strategies that have been used in Brazil (Bassanezi et al., 2020).

The current review presents the results of three main pest-management programs in Brazilian citrus-producing areas in recent decades (for the citrus fruit borer, citrus leafminer, and Asian citrus psyllid), focusing on state of São Paulo. The following sections discuss the use of more-sustainable control strategies, such as biological-control programs in citrus production, advances in control strategies in general, and the continuing challenges to overcome.

THREE SUCCESSFUL CASES

Control of the Citrus Fruit Borer (*Gymnandrosoma aurantianum*) Based on Monitoring by Using Its Sex Pheromone

Reports of the citrus fruit borer in Brazil are from 1915 (Lima, 1927); however, this moth did not cause enough damage until the 1980s to justify the development of a management program to control it (Parra et al., 2004b). By the end of that decade this insect pest had become important, causing 50 million US dollars per year in damage (Bento et al., 2016) and losses around 80 kg of fruit per plant (Parra et al., 2004b). The citrus fruit borer is easily recognized because the larvae develop inside the fruit and release excreta through a hole in the skin (Gallo et al., 2002).

An initial taxonomic review concluded that the correct name of this insect is *Ecdytoplopha aurantiana* (Lepidoptera: Tortricidae) instead of *G. aurantianum*. This change was maintained during a few times, but currently taxonomists have returned to the original scientific name (Adamski and Brown, 2002).

Initially controlled with agrochemicals in a wrong manner, i.e., when the first attacked fruits were observed, the pest was

not controlled and the products eliminated natural enemies, increasing losses (Parra et al., 2004b). Given the increasing concern regarding this borer, a study involving several stages, from basic research to extension services and projects with growers has been conducted at the Luiz de Queiroz School of Agriculture at the University of São Paulo, aiming toward the development of a pest-management program (Parra et al., 2004b).

Different biological characteristics of this insect have been studied. An artificial diet was developed to allow continuous laboratory rearing (Garcia and Parra, 1998), since the pest naturally occurs mainly from October through April (Garcia et al., 1998). During its life span the citrus fruit borer lays 200 eggs on average, concentrating its oviposition activity between 16:00 and 19:00 h (76%) (Bento et al., 2001).

The length of the biological cycle ranges from 27.2 to 61.8 days at temperatures of 18 and 32°C, respectively. Besides temperature, other factors that affect the pest's biology have been studied, such as air relative humidity (RH) and soil moisture (Table 1). Oviposition does not occur and adult longevity is shorter at low levels (<50%) of RH. The pest is favored at intermediate soil moisture levels (Garcia, 1998).

Natural enemies of this insect pest include the hymenopterans *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) (egg parasitoid) and *Hymenochaonia* sp. (Hymenoptera: Braconidae) (larval parasitoid); however, their action is negatively affected by intensive use of non-selective chemical products (insecticides) (Molina and Parra, 2006).

The borer behaves differently according to the stage of the fruit; ripe fruits are preferred because of their basic pH (Parra et al., 2001). This insect is more frequent in areas close to forest fragments (Garcia et al., 1998). The annual number of generations varies according to the temperature, ranging between 7.1 and 8.3 in the state of São Paulo.

Since only one egg per fruit is laid in the main citrus varieties (Valência, Pêra, Lima, Ponkan, Natal, and Hamlin), Bento et al. (2001) studied the behavior of the different life stages of the borer. They found that the adult is nocturnal and mates on the upper part of the plant; 53% of the fruits attacked are at heights between 1.0 and 2.0 m, mainly on the east side of the plant because of the trajectory of the sun (Bento et al., 2001).

Based on these studies, a pheromone was synthesized (Ferocitrus Furão) (Leal et al., 2001) to improve population monitoring. Prior to that time, monitoring was possible only after 10% of the fruits were attacked, which led to large losses of citrus.

Based on the behavioral studies by Bento et al. (2001), it was recommended that delta traps be installed on the upper third part of the plant. The traps contain a pheromone attractant (in the form of a tablet) with sticky trap to collect *G. aurantianum* males. The traps should be installed in the field at different periods, depending on the variety of citrus (Table 2). The tablet should be replaced every 30 days and one trap should be installed for every 10 ha (assuming a total of no more than 600 citrus plants).

A clear benefit of adopting sex pheromones in São Paulo was apparent during the 2002–2012 period, with estimated savings of US\$ 1.32 billion and a cost-benefit ratio equal to US\$

TABLE 1 | Effect of air relative humidity (RH) on the fecundity and longevity of *Gymnandrosoma aurantianum* (Tortricidae) (Garcia, 1998).

RH (%)	Eggs/female	Longevity (days)	
		Male	Female
30	–	5.0	6.5
50	3.9	9.3	12.9
70	34.1	16.4	19.4
90	53.6	14.9	16.8

TABLE 2 | Recommendation for citrus fruit borer monitoring according to the citrus variety (Bento et al., 2019).

Variety	Recommended period
Hamlin	February to July
Westin	February to July
Pêra	January to December
Valência	August to January
Natal	August to January

1,112 (investment/losses prevented) (Bento et al., 2016). The environmental benefit was also clear, since the use of pesticides was reduced by 50%, which protected the natural enemies.

If the control levels are reached, the use of *Bacillus thuringiensis* or another selective pesticide is recommended (Zorzetti et al., 2017). Recent studies have demonstrated the possible benefits of the use of the egg parasitoid *Trichogramma atopovirilia* (Hymenoptera: Trichogrammatidae) with 200,000–400,000 parasitoids released per ha before the first *G. aurantianum* larvae to enter into the fruit, which happens within 3–4 h after eclosion (Carvalho, 2003).

Biocontrol of the Leafminer (*Phyllocnistis citrella*) Using the Encyrtid *Ageniaspis citricola* (Hymenoptera: Encyrtidae)

This leafminer was introduced into Brazil in 1996 (Prates et al., 1996) and started to directly damage the leaves (drying) and indirectly facilitate the spread of citrus canker because of the increase in moisture next to the parts of the plant that are attacked (Chagas et al., 2001). The damage from the leafminer resulted in losses of up to 60 kg of fruit per tree.

In 1998, the parasitoid *Ageniaspis citricola*, originally from Asia, was introduced into Brazil, using specimens provided by Dr. Marjorie Hoy, who reared them in her laboratory at the University of Florida in Gainesville (Chagas et al., 2002). After a quarantine period, studies of the egg and first-instar larval parasitoids began. This parasitoid species is polyembryonic, producing 3–10 pupae per parasitized leafminer on citrus plants (Edwards and Hoy, 1998; Chagas et al., 2002). The thermal and moisture (RH) requirements of the pest were determined, and a rearing protocol was designed (Chagas and Parra, 2000) (Figure 1).

Parasitoid releases were initiated in 67 municipalities in São Paulo in 1998, using 50 pupae for every 50 ha, and within a short

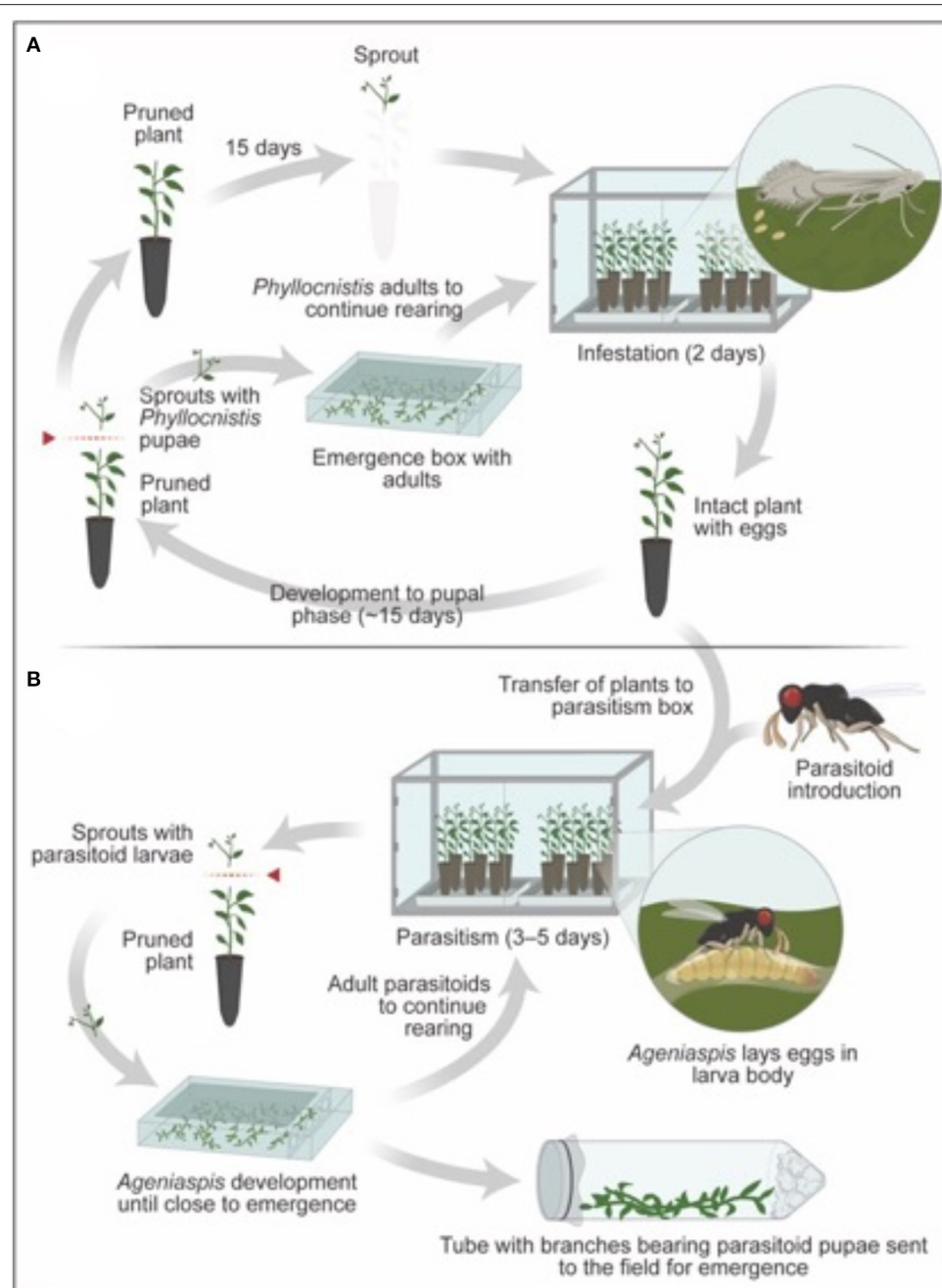


FIGURE 1 | Scheme of the production process of *Phyllocnistis citrella* (A) for the rearing of its parasitoid *Ageniaspis citricola* (B) for inoculative releases in a biological-control program (Parra, 2021).

time the parasitoid had spread throughout the state (Parra, 2021) (Figure 2).

The introduction of the parasitoid succeeded in containing the growth of the pest (Parra, 2021). Even after HLB appeared in Brazil, resulting in systematic applications of pesticides to control the Asian citrus psyllid, studies suggest that this parasitoid was able to survive in the field, since the percentage of citrus plants with *A. citricola* oscillated only between 56.23 and 60.55% (Paiva and Yamamoto, 2015). Figure 3 shows that the parasitoid has adapted to different citrus-producing regions in Brazil,

suggesting that it is resistant to the agrochemicals applied in the field.

Control of the Psyllid Vector of HLB (Huanglongbing) Using External Management With the Parasitoid *Tamarixia radiata*

Diaphorina citri was first reported in Brazil in 1942 (Costa Lima, 1942), although only minor damage to citrus plants was observed.

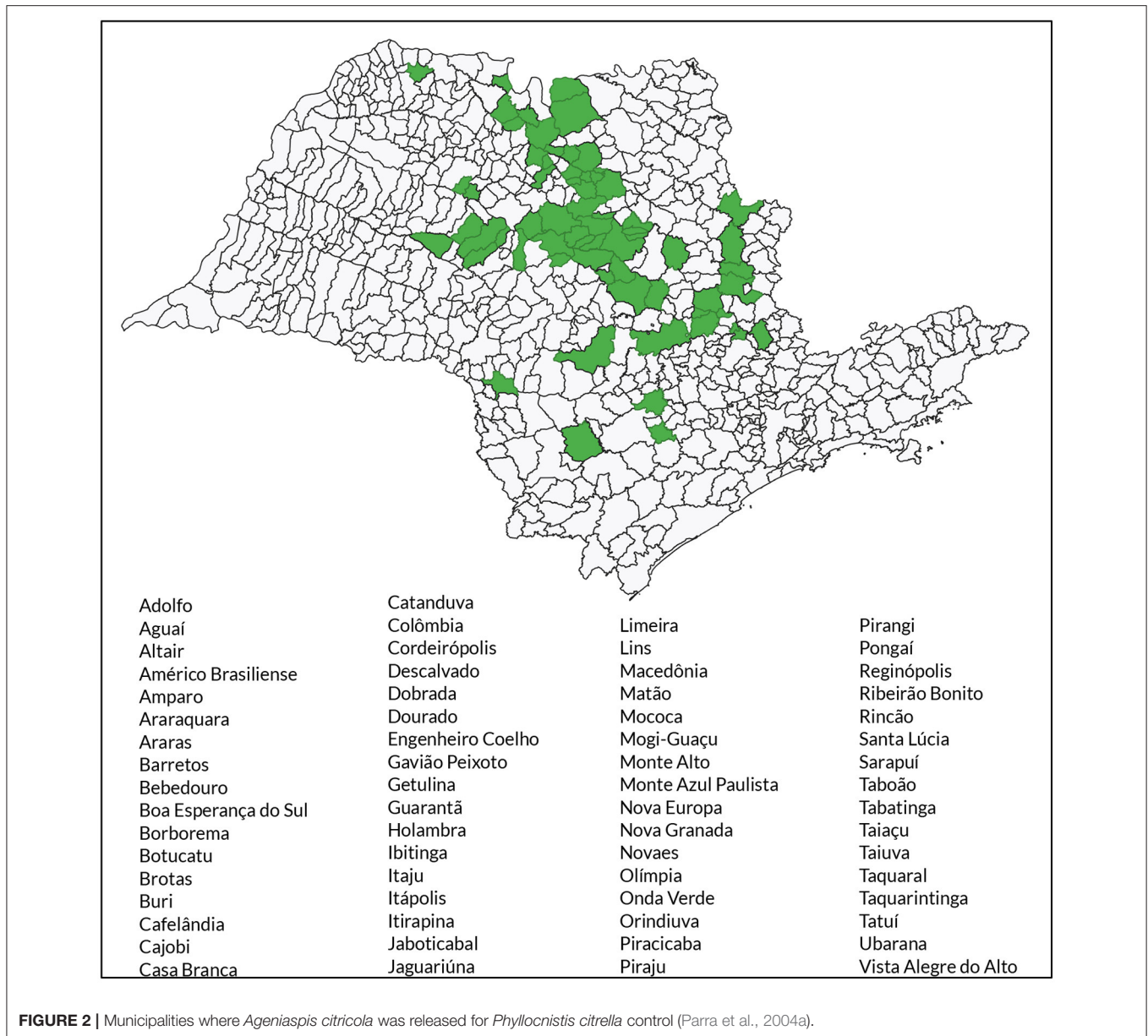


FIGURE 2 | Municipalities where *Ageniaspis citricola* was released for *Phyllocnistis citrella* control (Parra et al., 2004a).

The psyllid damages plants directly by sucking the phloem, which causes leaf curling and leads to the development of fumagine mold (Lavigne, 1957; Nakano et al., 1999; Gallo et al., 2002).

This situation changed in 2004, when the first cases of huanglongbing (HLB), a disease transmitted by this psyllid (Bové, 2006), were reported in Brazil (Coletta-Filho et al., 2004; Teixeira et al., 2005a). In 2005, HLB reached the USA (Halbert, 2005), although it has been reported in other regions around the world since the beginning of the 20th century (Grafton-Cardwell et al., 2013).

Since 2004, *D. citri* has become the most important citrus pest because it causes indirect damage by transmitting the gram-negative bacteria *Candidatus Liberibacter asiaticus* (Coletta-Filho et al., 2004) and *Ca. Liberibacter americanus* (Teixeira et al.,

2005b; Wulff et al., 2014), as well as a phytoplasma (Teixeira et al., 2008). HLB, caused by these bacteria, has necessitated the eradication of more than 55 million plants in the state of São Paulo alone (CDA, 2019).

In 2005, the eulophid *Tamarixia radiata* (Hymenoptera: Eulophidae) was introduced into Brazil (Gomez-Torres et al., 2006) and soon afterward into Argentina (Lizondo et al., 2007) as an important component of HLB management. This parasitoid had been successfully released on Réunion Island (1978) and Guadalupe (1999) for *D. citri* control, leading to elimination of the insect pest from those isolated islands (Étienne and Aubert, 1980; Étienne et al., 2001).

Tamarixia radiata parasitizes more-advanced instars of *D. citri*, consuming the host to develop and grow. It is easily

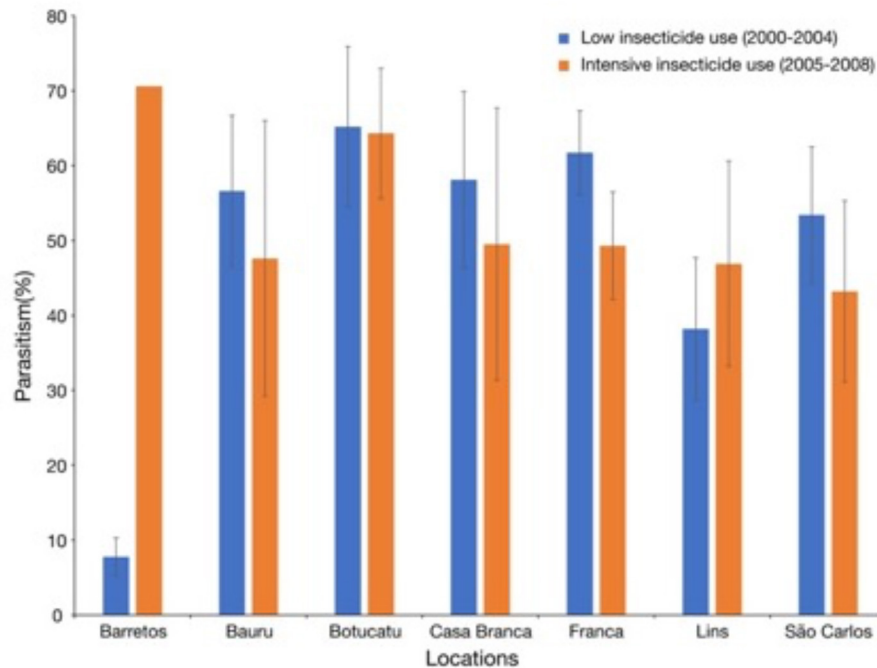


FIGURE 3 | Parasitism rate of *Ageniaspis citricola* on *Phyllocnistis citrella* at different locations with different pesticide application levels (Paiva and Yamamoto, 2015).

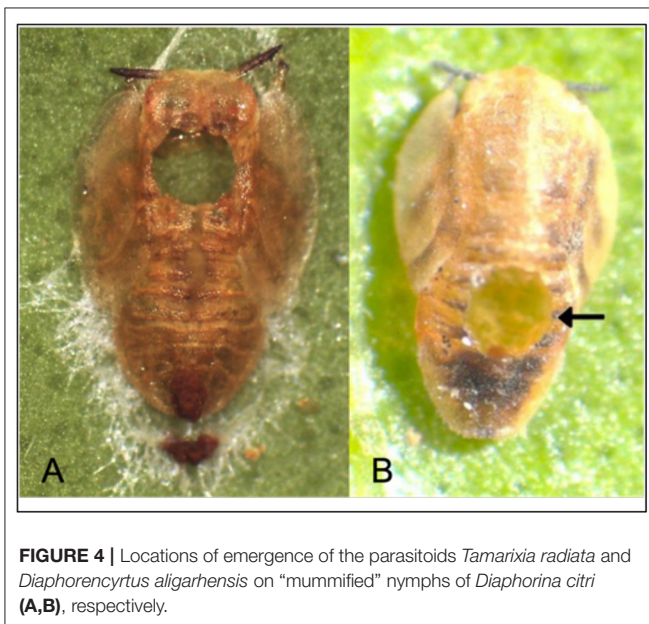


FIGURE 4 | Locations of emergence of the parasitoids *Tamarixia radiata* and *Diaphorencyrtus aligarhensis* on "mummified" nymphs of *Diaphorina citri* (A,B), respectively.

detected, since the emerging adult parasitoid leaves a hole on the upper part of the nymph. Another parasitoid, *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae), leaves a hole on the underside of the nymph (Diniz et al., 2021) (Figure 4).

The psyllid has an egg-adult period ranging from 43.5 to 12.1 days in temperatures of 18–32°C, respectively, and its viability decreases sharply in temperatures exceeding 30°C (Nava et al., 2007) (Table 3).

TABLE 3 | Duration and viability of *Diaphorina citri* life cycle (egg-adult) reared in "Cravo" lemon at different temperatures.

Temperature (°C)	Duration (days)	Viability (%)
18	43.5a	69.9a
20	30.9b	66.6a
22	29.6b	64.1a
25	17.1c	69.4a
28	15.4cd	69.5a
30	12.4d	66.8a
32	12.1d	12.2b

RH: 70% and photophase of 14 h (Nava et al., 2007). Mean values followed by the same letter in the column are not different by Tukey test ($P > 0.05$). RH: 70 ± 10%, and 14 : 10 h (light : dark) photoperiod.

The thermal constant of this insect is 210.91 degree-days and the lower threshold temperature is 13.5°C (Table 4). The eggs are also affected by the relative humidity (RH), and especially the nymphal stage can die in lower RH levels (Nava et al., 2010).

The biological parameters of the psyllid vary according to the host, which affects its management in the field, as well as laboratory production of the psyllid for parasitoid rearing (Oliveira et al., 2022).

The development of *T. radiata* is affected by temperature, ranging from 17.31 to 7.59 days at 18 to 32°C (Gomez-Torres et al., 2012; Gómez-Torres et al., 2014).

The parasitism rate is also affected by temperature and is highest at 25°C, although adult emergence is similar over the range of 25–35°C (Gómez-Torres, 2009).

In order to store the parasitoid for later release, since the storage conditions required may affect transport and distribution over long distances, the lowest temperature tolerated by *T. radiata* was also investigated. The study revealed that this insect can be stored at 11°C for more than 6 days without affecting survivorship (Bertanha et al., 2021).

Knowledge of the thermal and hygrometric requirements of *T. radiata* and *D. citri* from previous studies made it possible to develop a fuzzy-based index that indicated the most appropriate

areas of the state of São Paulo for biological control, considering the plant phenology and biological parameters of both host and parasitoid (Figure 5). This study allowed researchers to concentrate their efforts and resources on certain privately owned areas, resulting in more efficient programs (Garcia et al., 2019). An app based on this index is under development for distribution to farms.

Production of the parasitoid in the laboratory (Parra et al., 2016; Diniz et al., 2021) allowed it to be included in recommendations for HLB management by the Fund for Citrus Protection (Fundecitrus), using a new approach called “external management,” which consists of periodic releases of the parasitoid in non-commercial areas, i.e., abandoned groves, areas with myrtle (*Murraya paniculata*), backyards, and sites where no control measures are being used (Diniz et al., 2020).

By now, more than 10 “biofactories” for parasitoid rearing have been established in the state of São Paulo, by private companies, research institutes, and universities, in order to meet their own requirements or to support small farmers (Diniz et al., 2021).

TABLE 4 | Lower threshold temperature (Tb, °C), thermal constant (K, degree-days), development rate (1/D), and determination coefficient (R²) of *Diaphorina citri* reared on “Cravo” lemon (Nava et al., 2007).

Stage	Tb (°C)	K (DD)	Equation (1/D)	R ² (%)
Egg	12.07	52.61	y = 0.019007x - 0.229488	97.73
Nymph	13.94	156.88	y = 0.006374x - 0.088836	94.65
Egg-adult	13.53	210.91	y = 0.004741x - 0.064134	96.70

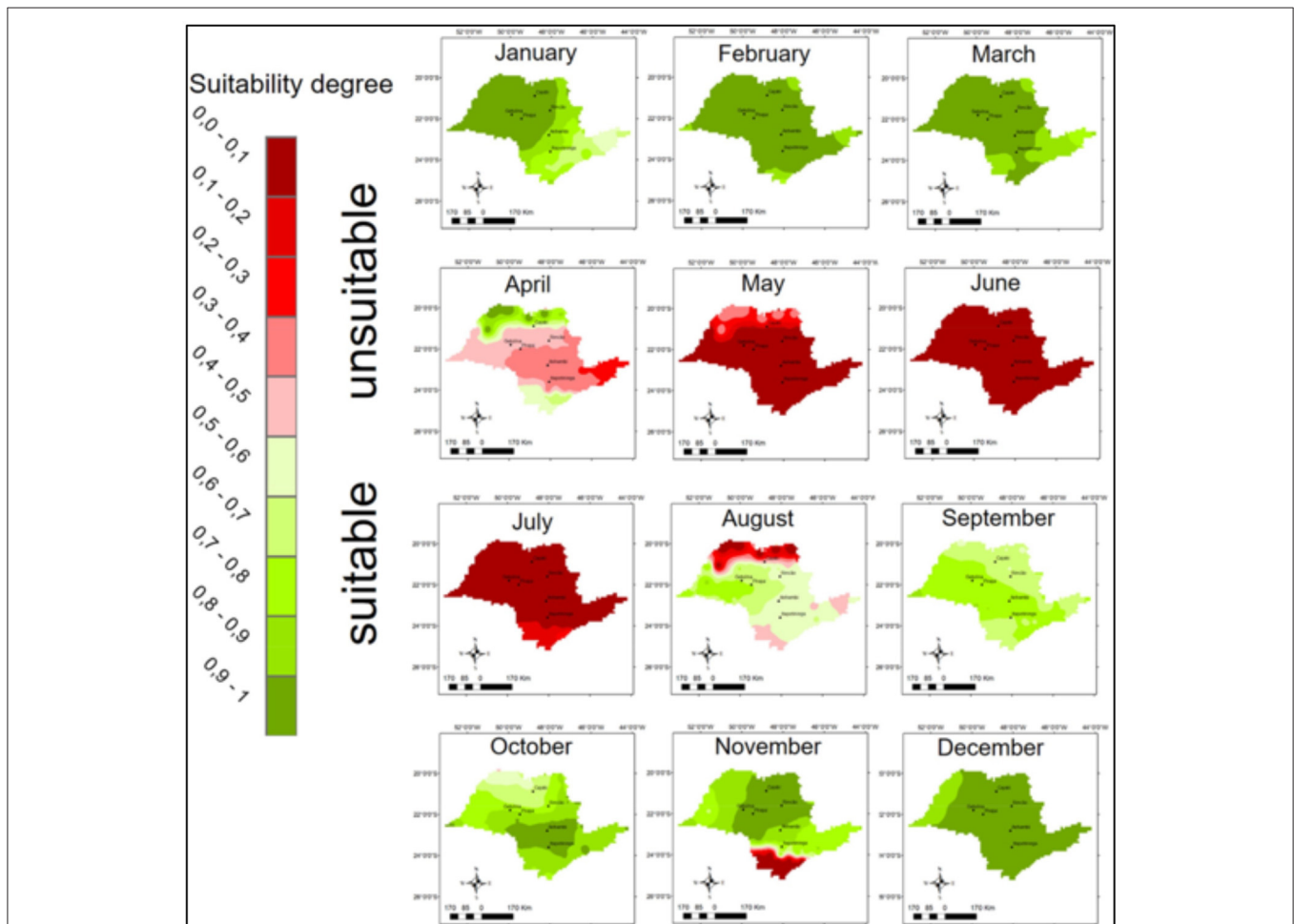


FIGURE 5 | Map of the state of São Paulo, Brazil (main citrus-producing region in the country), showing the fuzzy-based index values (suitability indexes) for different areas. The indexes indicate the more suitable areas for biological control (Garcia et al., 2019).

Based on the dispersal capacity and efficiency of the parasitoid, a release of 800–3,200 parasitoids/ha, which results in a reduction of up to 80% of the total number of nymphs, was found to be most efficient (Marin, 2019).

Finally, a predictive model was developed using the R programming language, combining all information produced by the previous studies to date. The model was used to simulate different scenarios, i.e., application of biological control, eradication of infected trees, and use of pesticides. The results of the model simulations showed that the combined use of these management strategies (chemical, biological, and cultural practices), the basic components of an Integrated Pest Management program, resulted in a 73.9% reduction in the infection rate, indicating that the combination of these management strategies could achieve effective control of HLB (Garcia et al., 2021).

FINAL CONSIDERATIONS

Although the application of chemical products continues to be standard procedure in Brazilian citriculture, we have presented three case studies as examples to follow in moving toward environmental sustainability in pest control. Environmental sustainability has recently come to the forefront as a requirement by consumers and because of the growing concern regarding current production methods, rejecting the intense use of agrochemicals.

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This concern is understandable given the increase in the use of agrochemicals, for instance to manage HLB in citrus-growing areas. However, biological control still has room to grow, with the entomopathogenic fungus *Cordyceps fumosorosea* for psyllid control combined with external management, i.e., releases of *T. radiata* in non-commercial areas, a case study described here.

Combining the actions recommended by Fundecitrus, such as eradication of infected plants, with external management can be an effective strategy to maintain Brazil as a leader in citriculture, controlling HLB and overcoming the most serious challenges in world citriculture.

AUTHOR CONTRIBUTIONS

JP was responsible for conceptualization of this review. All authors wrote, read, reviewed the literature, and agreed with the current format of this manuscript.

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