Release and Persistence of the Brazilian Peppertree Biological Control Agent Pseudophilothrips ichini (Thysanoptera: Phlaeothripidae) in Florida

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Release and persistence of the Brazilian peppertree biological control agent *Pseudophilothrips ichini* (Thysanoptera: Phlaeothripidae) in Florida

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Abstract

Brazilian peppertree, *Schinus terebinthifolia* Raddi (Anacardiaceae), is an invasive weed of natural and agricultural areas of California, Florida, Hawaii, and Texas, USA. A thrips, *Pseudophilothrips ichini* (Hood) (Thysanoptera: Phlaeothripidae), was permitted and released in 2019 as the first biological control agent for this invasive weed in Florida, USA. The thrips feeds on flushing leaves that are produced during the vegetative season of the host. Together, the USDA–ARS, University of Florida, and Florida Department of Food and Consumer Services combined efforts to mass produce and release *P. ichini* throughout the Brazilian peppertree-invaded range in Florida. Between May 2019 and Dec 2021, more than 2 million *P. ichini* were released at 567 sites in Florida. Over this period, *P. ichini* persisted at up to 60% of the survey sites for at least 1 generation as indicated by recovery of thrips adults at least 60 d after release. These results indicate that this thrips, a classical biological control agent, has persisted in the invaded range of Brazilian peppertree in Florida with populations evident at many release sites. This biological control agent will provide land managers with a safe and cost-effective means of controlling Brazilian peppertree.

Key Words: weed biological control; *Schinus terebinthifolia*; release; persistence

Resumen

El pimentero brasileño, *Schinus terebinthifolia* Raddi (Anacardiaceae), es una maleza invasora de áreas naturales y agrícolas de California, Florida, Hawái y Texas, EE. UU. Un trips*, Pseudophilothrips ichini* (Hood) (Thysanoptera: Phlaeothripidae), fue autorizado y liberado en el 2019 como el primer agente de control biológico para esta maleza invasora en Florida, EE. UU. Los trips se alimentan de las hojas enrojecidas que se producen durante la temporada vegetativa del hospedero. Juntos, el USDA-ARS, la Universidad de Florida y el Departamento de Alimentos y Servicios al Consumidor de Florida combinaron esfuerzos para producir en masa y liberar *P. ichini* en toda la zona de distribución invadida por pimenteros brasileños en Florida. Entre mayo del 2019 y diciembre del 2021, se liberaron más de 2 millones de *P. ichini* en 567 sitios de Florida. Durante este período, *P. ichini* persistió en el 60% de los sitios de estudio durante al menos 1 generación, como lo indica la recuperación de adultos de trips al menos 60 días después de la liberación. Estos resultados indican que este trips, un agente de control biológico clásico, ha persistido en el área de distribución invadida por el pimentero brasileño en Florida con poblaciones evidentes en muchos de los sitios de liberación. Este agente de control biológico proporcionará a los administradores de tierras un medio seguro y rentable para controlar el pimentero brasileño.

Palabras Claves: control biológico de malezas; *Schinus terebinthifolia;* liberar; persistencia

Brazilian peppertree, *Schinus terebinthifolia* Raddi (Anacardiaceae), is one of the worst environmental and agricultural weeds worldwide (Boudjelas et al. 2000). In the US, this weed is one of the most aggressive and widespread invasive species in California, Florida, Hawaii, and Texas (Ewel 1986; Yoshioka & Markin 1991; Schmitz et al. 1997; Rodgers et al. 2014; EDDMapS 2021). Brazilian peppertree is a threat to diverse natural areas, agriculture, and cattle production (Morton 1978; Ewel 1986; Yoshioka & Markin 1991). This weed has colonized most of the Florida peninsula, covering more than 280,000 ha, often with dense monospecific stands that eliminate native plant growth (Ferriter 1997; Schmitz et al. 1997). Brazilian peppertree is a woody shrub that often grows in dense thickets in the invaded range (Ewel et al. 1982). The male flowers produce abundant pollen that is exploited by native and introduced pollinators, and is a source of nasal congestion, rhinitis, and other conditions in sensitive humans (Morton 1978). The fruit are toxic when consumed by birds and their volatiles can cause numerous respiratory and skin reactions in sensitive humans (Morton 1978).

Research on the classical biological control of Brazilian peppertree was initiated in the 1950s in Hawaii (Yoshioka & Markin 1991) and continued more recently in Florida (Wheeler et al. 2016a). Native range surveys conducted in South America reported a diversity of potential agents (McKay et al. 2009; Wheeler et al. 2016a); however only a few

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species have been found to be suitable for release in Florida. These included the thrips *Pseudophilothrips ichini* (Hood) (Thysanoptera: Phlaeothripidae) and the leaf gall former, *Calophya latiforceps* Burckhardt (Hemiptera: Calophyidae) (Diaz et al. 2015; Wheeler et al. 2017). Feeding by the thrips *P. ichini* in the laboratory had a significant impact on plant performance (Wheeler et al. 2018). In 2019 a permit was issued from the USDA–Animal and Plant Health Inspection Service for field release of both agents. Since that time research has documented improved rearing methods of the thrips (Halbritter & Wheeler 2019; Halbritter et al. 2021) and life history trade-offs of the weed experiencing thrips herbivory (Halbritter & Wheeler 2021). Additional research is underway currently to examine the landscape level establishment of this agent, its impact on Brazilian peppertree populations, and biotic resistance that reduces its effectiveness. This biological control agent has potential to provide land managers with a safe and cost-effective means of controlling Brazilian peppertree by reducing reliance on herbicidal controls.

Seasonal fluctuations in plant quality and ambient field conditions doubtless will influence thrips adult longevity and development time. All feeding is done by the thrips adults and larvae. The immature *P. ichini* life history includes 2 larval stages and 3 non-feeding pupal stages. Under laboratory conditions (25 °C) development time from egg hatch to adult emergence was 20 d (± 1.4 d) and adults lived on average 50 d (± 3.8 d) (Wheeler et al. 2016b). Entire generation time ranged from 18.9 to 34.5 d when reared at constant temperatures of 30 °C and 20 °C in the laboratory, respectively (Manrique et al. 2014). Thrips reared in an outdoor screenhouse where temperatures ranged from 4.6 to 34.3 °C throughout the yr had an average generation time of 32.9 d (range 23 to 59 d) from the addition of founding adults to the harvest of F_1 adults for release (Halbritter et al. 2021). This life history information was used here to define persistence where we assumed that the discovery of thrips adults at sites 60 d after the most recent adult release suggests the completion of 1 or more generations and thus persistence. This metric is not the same as widespread, landscape-level establishment, which is the topic of other long-term monitoring research currently underway. The goals of this study were to document thrips mass production, release, and local persistence on Brazilian peppertree throughout the invaded range in Florida. While conducting these studies we also evaluated the effect of the number of individuals released and time interval since release on thrips persistence.

Materials and Methods

INSECTS

Thrips were reared continuously on live Brazilian peppertree plants since their collection in 2007 near Ouro Preto, Minas Gerais, Brazil (20.36911°S, 43.56029°W; 1,329 masl) (Wheeler et al. 2016b). For field release, adult *P. ichini* of unknown age and sex were collected from mass production colonies maintained at 3 laboratories within 3 locations: the USDA – ARS, Invasive Plant Research Laboratory, Fort Lauderdale, Florida; Florida Department of Agriculture and Consumer Services – Division of Plant Industry, Gainesville, Florida; and University of Florida – Institute of Food and Agricultural Sciences, Indian River Research and Education Center, Fort Pierce, Florida, USA.

PLANTS

All thrips were reared on live plants and our production methods generally followed the protocol described previously (Halbritter et al. 2021). Plants were grown at the same 3 locations noted above mostly in outdoor gardens but occasionally in environmental chambers. Plant production varied slightly at each location but generally plants were fertilized with both liquid (Southern Agricultural Insecticides, Inc., Palmetto, Florida, USA; 20N-20P-20K; 2.5 g per 3.8 L) and slow-release (Osmocote Blend, Summerville, South Carolina, USA; 21N-4P-8K) fertilizers applied (30–60 g per pot) every 6 to 8 wk. Plants were pruned periodically to stimulate the flush growth known to accelerate *P. ichini* feeding and development (Fig. 1). Plants were moved into mass production areas to feed thrips colonies. During 2019 to 2020 the production of *P. ichini* was routinely conducted in indoor rearing facilities under ambient conditions (27 °C; 50% RH; 14:10 h [L:D] photoperiod). During this period, thrips were fed live Brazilian peppertree plants inside vented acrylic cylindrical cages (locally fabricated) (45 cm long × 15 cm diam) (Wheeler et al. 2017). The thrips completed their entire life cycle inside these cylinders. We also reared thrips in larger, vented acrylic boxes (Suncoast Plastic Fabrication, Inc., Valrico, Florida, USA) (81.5 × 39.5 × 39.5 cm) with 2 live Brazilian peppertree plants inside. During 2021, thrips were primarily produced and maintained in thrips-proof Lumite (BioQuip® Products, Compton, California, USA) screen cages (1.8 × 1.8 × 1.8 m) that each held 6 plants in 11.4 L pots (Greenhouse Megastore, Danville, Illinois, USA) (Halbritter et al. 2021). All screen cages were placed in a screenhouse exposed to ambient conditions. All plants inside screen cages were watered daily by hand.

THRIPS RELEASE AND PERSISTENCE

Thrips adults were transferred under ambient conditions from the rearing facility to the field in plastic food containers (946.4 mL) (WebstaurantStore, Tampa, Florida, USA) fitted with vented lids and provisioned with a double-ply folded paper towel to absorb condensation. Containers were provisioned with 2 fresh Brazilian peppertree flushing leaf tips (10–15 cm in length) inserted into 2 water picks (7.6 cm; Aquatube, Wholesalefloral, Buffalo, New York, USA). No more than 2,000 thrips adults were included per container. Once at release sites, thrips always were kept shaded while in containers to prevent overheating. During release, we removed the infested plant material from the water picks and containers, and draped the tips onto Brazilian peppertree branches with flushing leaf tips. This minimized damage from handling and likely improved survival of thrips. As long as the plant material was fresh, 90% of the thrips aggregated on leaf tips. One large (> 3 m tall) tree was selected and marked at each site. Thrips were released onto foliage within about 5 m of either side of the marker. This range often fell within the same tree, but there were some cases where it extended onto adjacent trees. Release points at sites typically were surrounded by other Brazilian peppertree plants for 10 m or more in any direction, but often along linear access paths.

Thrips release protocols were developed as improvements were made in mass rearing. Previous results indicated larger thrips release numbers increased the probability of local persistence (Minteer et al., unpublished data). Therefore, as thrips production allowed, our protocol included the release of 2,000 or more thrips adults at each site for at least 3 consecutive visits. If 2,000 thrips per release were not available, releases of reduced numbers were conducted for at least 6 consecutive mo. As a range of release numbers were employed, we examined the effect of thrips numbers released on their persistence.

To determine thrips local persistence, we examined a subset of 223 of the total 567 sites. Although multiple releases and surveys were conducted for the long-term monitoring objective, only the first follow-up surveys are analyzed here. During these surveys the original trees and neighboring trees were visually inspected for the presence of thrips. The follow-up surveys for thrips ranged from 1 to 24 mo after release. Additionally, we gently beat the plant branches 20 times and recovered insects that fell onto a beat sheet (1 m^2 Ripstop beating sheet 2840R; Bio-

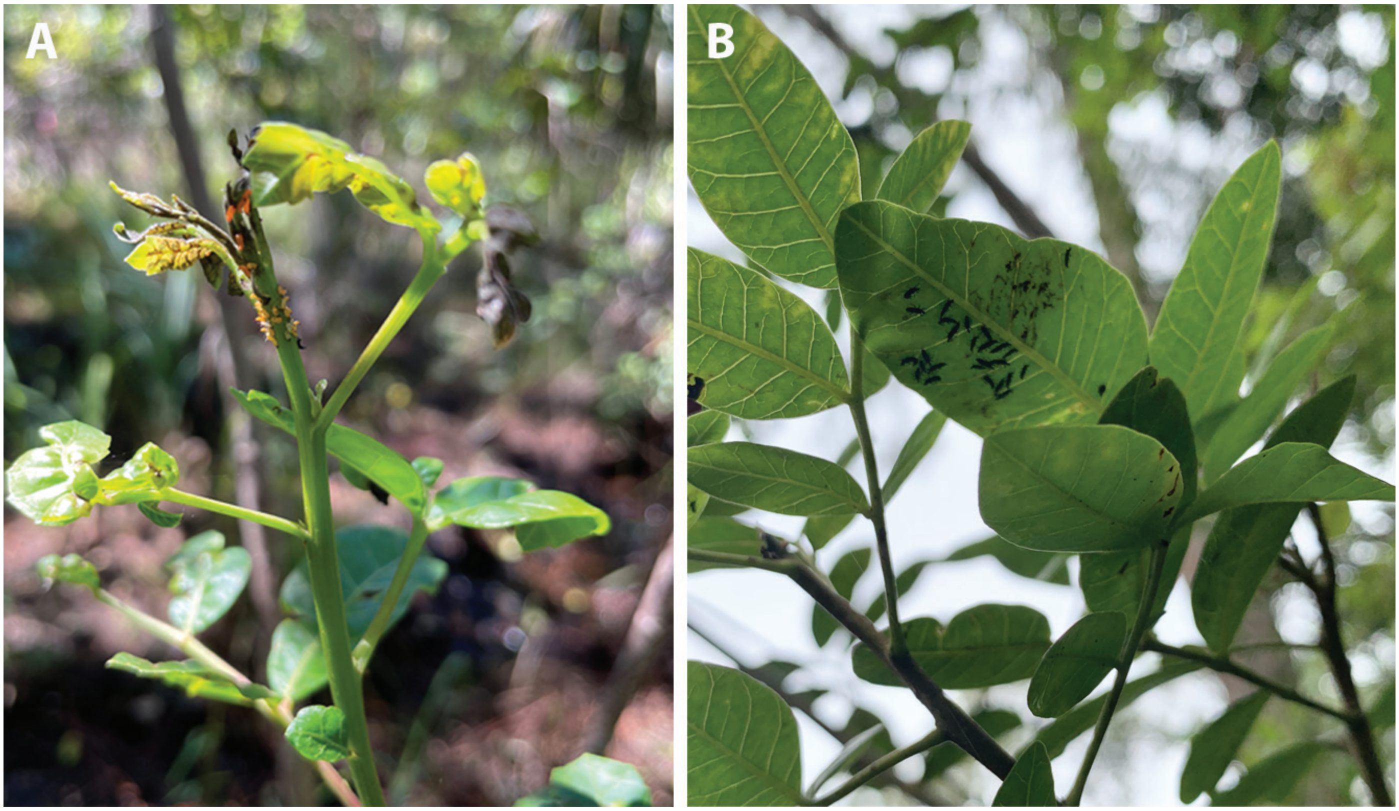

Fig. 1. *Pseudophilothrips ichini*, a biological control agent of Brazilian peppertree released during May 2019 to Dec 2021. Thrips orange larvae (A) and black adults (B) aggregated on Brazilian peppertree leaves at a release site.

Quip, Rancho Dominguez, California, USA). Following counting, all thrips were returned to the plant. Thrips presence or absence was noted, and thrips were considered to have persisted if they were recovered more than 2 mo (60 d) after a release, indicating emergence of 1 or more generations. To determine if persistence was a short-term event, we also examined the effect of time since release on thrips persistence.

STATISTICAL ANALYSIS

Regression was used to model the numbers of thrips released from May 2019 to Dec 2021. To determine if the number thrips released influenced the proportion of sites where thrips were persisting, regression was used to examine the proportion of sites where thrips were recovered across a range of release numbers ($P = 0.05$). Similarly, to determine if time interval after release influenced persistence, regression was used to examine the proportion of sites where thrips persisted across a range of survey times. All statistical analyses were conducted with SAS ver. 9.4 (SAS Institute 2016).

Results

Thrips releases

Thrips releases occurred from the northern range of Brazilian peppertree in peninsular Florida near Cedar Key, on the Atlantic coast near Palm Coast, and south to the most southern extent of the range near Key West (Fig. 2). A total of 2,136,583 *P. ichini* were released between May 2019 and Dec 2021 at 567 sites in Florida on the invasive weed Brazilian peppertree. During this period the number of release sites increased from 50 in 2019, to 567 in 2021 (Fig. 3). Initial monthly releases of thrips during 2019 were relatively low, however, as rearing methods were developed, and the scale of production increased, average monthly releases during 2020 increased 7-fold. During 2021 the average monthly number of *P. ichini* released increased an additional 2.5-fold. The number of thrips released per site also increased initially from 1,167 during 2019, to 1,465 and 2,482 thrips released per site during 2020 and 2021, respectively. This constituted a significant increase in the number of thrips released each mo ($Y = -16073.4 + 4846.3 * X$, where X = numeric mo in sequence after initial release and Y = predicted number of thrips released. $r^2 = 0.64$; $P < 0.0001$). The greatest number released in a single mo was 208,500 thrips in May 2021. However, during 2020 and 2021, lower release numbers were noted during the cooler months of Dec and Jan (Fig. 4).

THRIPS PERSISTENCE

When examining release sites surveyed after thrips releases, up to 60% of sites surveyed after 60 d since the initial thrips release had thrips detected. Thrips were detected at nearly all-time intervals surveyed, up to 703 d (24 mo) since the initial release (Table 1). Overall, thrips were detected at 77 of the 223 sites, or 35%, where releases were made 60 or more d earlier. However, there were 2 time intervals where thrips were not detected at any site (e.g., survey mo 8 and 10; Table 1). The proportion of sites where thrips persisted was not influenced by release number ($F_{1,12} = 0.69$; $P = 0.4234$) and the proportion of sites where thrips persisted did not change significantly over the 24-mo survey ($F_{1,12} = 0.91$; $P = 0.3597$).

Discussion

More than 2 million *P. ichini* were released in Florida between the May 2019 and Dec 2021 for the biological control of the invasive

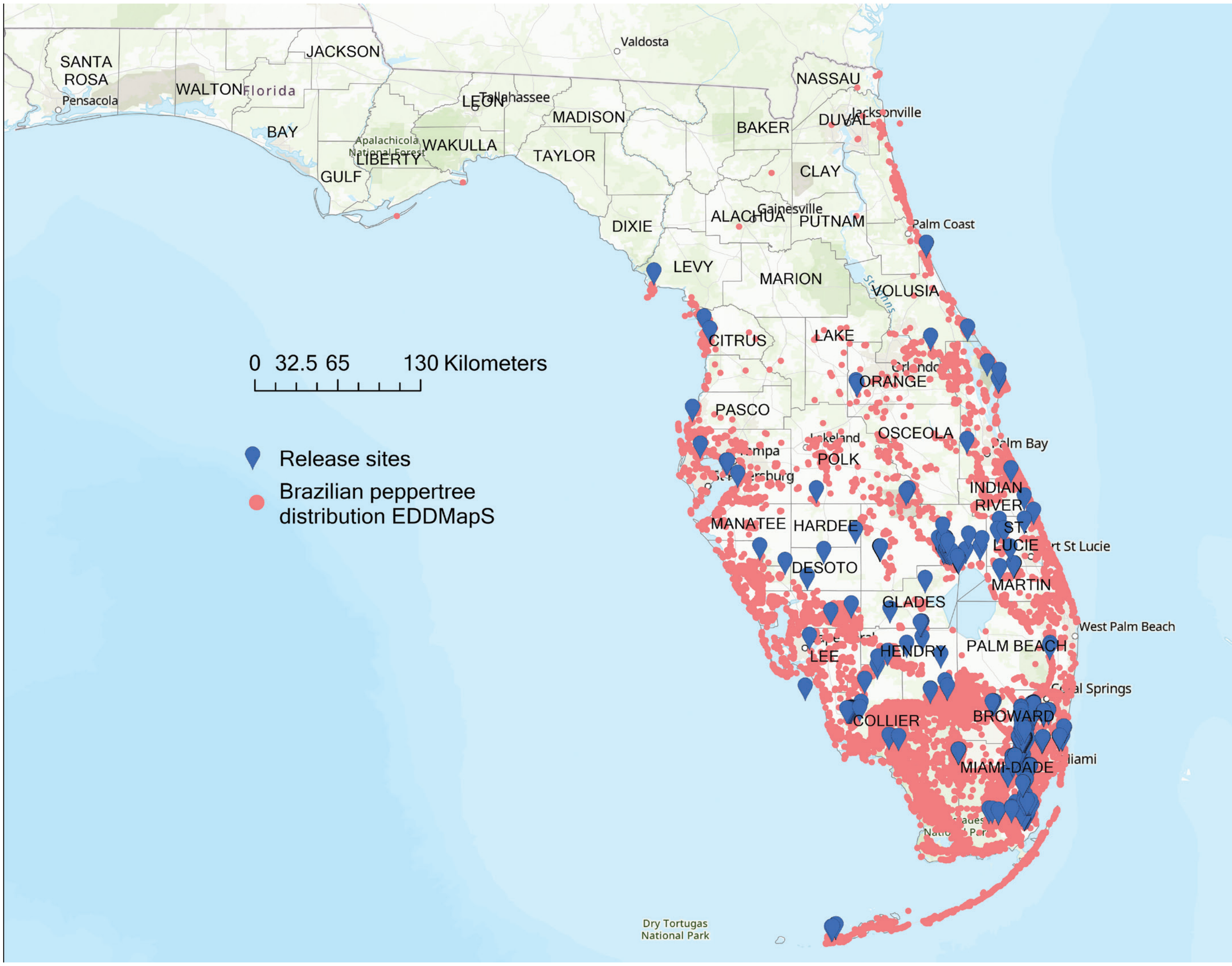

Fig. 2. Release sites of *Pseudophilothrips ichini* during May 2019 to Dec 2021. A total of 2,136,583 thrips were released at 567 sites. Blue points represent thrips release sites, pink dots represent the distribution of Brazilian peppertree (EDDMapS 2021).

weed Brazilian peppertree. These releases were made throughout the invaded range in Florida. Both the number of thrips released per mo and the number of sites where releases were made increased from 2019 to 2021 as production of the colonies improved. Thrips were detected overall at 35% of the sites when releases were made 60 or more d earlier. The proportion of sites where thrips persisted did not change significantly over time suggesting that many of these initial thrips populations may be self-sustaining and stable. Future research will continue to release and monitor thrips populations in diverse habitats to identify factors that limit their abundance throughout the invaded range.

The numbers of thrips released was influenced by many factors, among them the number of thrips produced from colonies. Though labor inputs may be relatively high (Halbritter et al. 2021), production of thrips increased from 2019 to 2021 by rearing in large screen cages (1.8 × 1.8 × 1.8 m). Low production is a result of several factors that affect insect health and fecundity, including low ambient temperatures, poor plant quality, and outbreaks of other plant pests (DeGraff & Wood 2009; Moran et al. 2014; Schwarzländer et al. 2018; Wahl & Diaz 2020; Harms et al. 2021). These factors were mitigated as they were discovered to minimize their impact on thrips production. For example, when an outbreak of the predacious pirate bug, *Montandoniola confusa* Streito & Matocq (Hemiptera: Anthocoridae), occurred in rearing facilities, sanitation and isolation efforts quickly ameliorated the problem (Halbritter et al., submitted manuscript). Seasonal temperatures affected thrips rearing; however, indoor facilities mitigated the lower production influenced by lower ambient temperatures. Moreover, considerable sharing of resources among cooperating agencies proved critical and facilitated a continuous supply of healthy plants and thrips.

Numerous factors may have influenced the persistence of thrips populations at field sites (Newman et al. 1998; Landis et al. 2000). Field observations suggested that sites providing a more humid microclimate, such as those with more leaf litter, herbaceous understory, denser canopies, and protection from wind or extreme weather, had better persistence. Other factors like higher soil organic matter may have improved pupal survival and emergence. Natural enemies such as predacious ants and bugs may affect thrips persistence and population density (Reimer 1988; Funderburk et al. 2016). Numerous ant species are found commonly on Florida Brazilian peppertree and likely are important predators of small sap-feeding insects (Halbritter et al. 2022). The pirate bug, *M.*

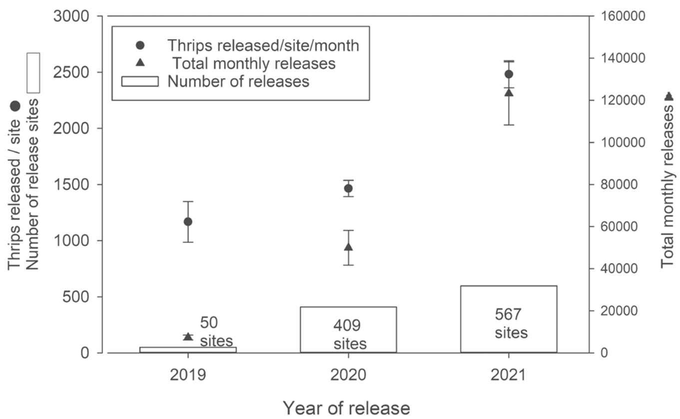

Fig. 3. Number of *Pseudophilothrips ichini* released per site per mo (solid circles), total monthly releases (solid triangles), and number of release sites from May 2019 to Dec 2021. Mean (± SE).

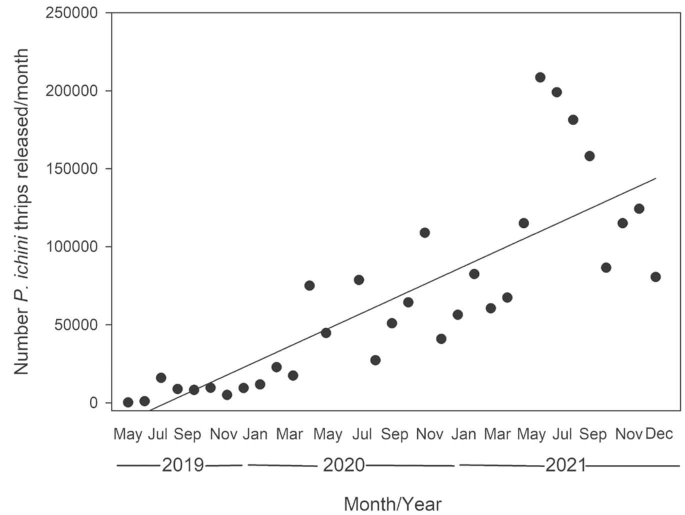

Fig. 4. Monthly *Pseudophilothrips ichini* released from May 2019 to Dec 2021. The number of thrips released increased significantly during this period (Y = −16073.4 + 4846.3 * X, where X = numeric mo in sequence after initial release and Y = predicted number of thrips released. $r^2 = 0.64$; $P < 0.0001$).

confusa, introduced in Florida for control of agronomic pest thrips (Bennett 1995), may impact field populations of *P. ichini* (Halbritter et al., submitted manuscript). Another thrips natural enemy released against agronomic pest thrips in Florida, the parasitoid *Thripastichus gentilei* (del Guercio) (Hymenoptera: Eulophidae) may attack *P. ichini* (Boyd & Held 2016). This parasitoid, or a congener, was reared from *P. ichini* larvae collected in Brazil (Wheeler et al. 2016b) and was reared in Florida from black vine thrips, *Retithrips syriacus* (Mayet) (Thysanoptera: Thripidae) feeding on Brazilian peppertree (Wheeler et al. 2022). The factors that influence *P. ichini* local persistence are currently under investigation. We hope to mitigate the factors that restrict thrips persistence to promote outbreak populations and greater impact on the target weed.

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Table 1. Percent of sites where the thrips *Pseudophilothrips ichini* (adults or larvae) persisted for 1 to 24 mo following release between May 2019 and Dec 2021. We examined 223 release sites at different time intervals since prior releases. The survey times were grouped into 30 d intervals. The average number of thrips released at each point within each time interval is presented in the far right column.

Survey time (mo)	Number sites examined	Number sites with thrips	Percent of sites with thrips[1]	Avg number thrips released[2]
1	19	10	52.6	4,374
2	30	20	66.7	2,837
3	20	12	60.0	2,312
4	24	14	58.3	1,523
5	33	11	33.3	1,739
6	25	11	44.0	1,710
7	6	2	33.3	1,083
8	8	0	0	1,488
9	8	3	37.5	469
10	2	0	0	2,031
11	8	3	37.5	1,000
12	25	14	56.0	1,150
18	12	6	50.0	665
24	3	1	33.3	1,337

[1]The proportion of sites where thrips persisted did not change significantly over the survey time ($P > 0.3$).
[2]The average number thrips released did not influence proportion of sites where thrips persisted ($P > 0.4$).

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