ERDC/EL TR-24-10

Aquatic Plant Control Research Program (APCRP)

Initial Rearing, Release, and Establishment of Biological Control Agent *Pseudophilothrips ichini* to Control Brazilian Peppertree (*Schinus terebinthifolia*) in South Texas Ecosystem Restoration Projects

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June 2024

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Final Technical Report (TR)

Prepared for US Army Engineer Research and Development Center
3909 Halls Ferry Road, Vicksburg, MS 39180

Under 2020-E-1495, "Applying Biological Control for Invasive Weed Management in USACE Environmental Business Line/Program Projects: A Best Practices Framework and Case Study."

Abstract

Control of the invasive Brazilian peppertree (*Schinus terebinthifolia*) is a major cost component of US Army Corps of Engineers (USACE) ecosystem restoration (ER) projects in South Texas, specifically the USACE Galveston district (SWG) Resacas at Brownsville, Texas, ER Project. Biological control has been developed as a sustainable tool to lower long-term weed management costs. Although a biological control program for *S. terebinthifolia* has been in operation in Florida since 2019, no similar program existed in Texas until initiated by the Engineer Research and Development Center (ERDC) in 2020. Since 2021, the biological control agent *Pseudophilothrips ichini* has been reared at ERDC. This technical report details rearing, release, and establishment efforts from fall 2020 to spring 2023 to provide control of *S. terebinthifolia* in South Texas USACE ER project locations. Initial observations on impact and potential limitations to biological control in hot climates such as those of South Texas are also discussed.

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Preface

This study was conducted for the USACE Aquatic Plant Control Research Program (APCRP) under 2020-E-1495, “Applying Biological Control for Invasive Weed Management in USACE Environmental Business Line/Program Projects: A Best Practices Framework and Case Study.” The technical monitor was Mr. Michael Greer, APCRP program manager.

The work was performed by the Aquatic Ecology and Invasive Species Branch of the Ecosystem Evaluation and Engineering Division, US Army Engineer Research and Development Center–Environmental Laboratory (ERDC-EL). At the time of publication, Dr. Bradley Sartain was acting branch chief; and Mr. Mark Farr was division chief. The deputy director of ERDC-EL was Dr. Brandon J. Lafferty, and the director was Dr. Edmond J. Russo Jr.

Permission was granted by the Chief of Engineers to publish this information. The authors would also like to thank Dr. Ian Knight and Mrs. Julie Nachtrieb for their review of this manuscript and Ms. Caitlin Strickland, Ms. Lauren Paulson, and Ms. Sha Sallee for their laboratory assistance.

COL Christian Patterson was commander of ERDC, and Dr. David W. Pittman was the director.

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1 Introduction

1.1 Background

The United States Army Corps of Engineers (USACE) is authorized by the US Congress to conduct aquatic ecosystem restoration (ER) under several programs—including Continuing Authorities Program (CAP) projects (cost share with nonfederal sponsors), such as Section 1135 (Project Modifications for Improvement of the Environment), Section 206 (Aquatic Ecosystem Restoration), and Section 204 (Beneficial Use of Dredged Materials); Upper Mississippi River Restoration Program; and larger General Investigation studies (USACE 2018; Schad et al. 2023). Many of these projects include restoration or improvement of aquatic and near-aquatic habitats, including lakes, rivers, streams, riparian areas, wetlands, coastal environments, and bottomland hardwood forests. USACE ER project activities are framed in the following phases: Feasibility (planning), Preconstruction Engineering and Design (project development), Construction (project implementation), and Monitoring and Adaptive Management/Operations and Maintenance (O&M) (monitoring and maintenance). In one such USACE ER program—the USACE Galveston District (SWG) Resacas at Brownsville, Texas, ER Project—USACE is in collaboration with nonfederal sponsors, such as the City of Brownsville and Brownsville Public Utility Board, as well as other natural resource agencies (US Fish and Wildlife Service, Texas Parks and Wildlife Department, The Nature Conservancy, and the National Park Service) to implement restoration of former, naturally cutoff channels ("resacas") of the Lower Rio Grande River, Texas, for the benefit of fish and other wildlife (Schad et al. 2023). A primary goal of this ER project is to improve plant communities by increasing relative abundance of native species while reducing invasive species abundance, establishment, and spread in these unique ecosystems.

Resacas are paleochannels of the distributaries of the Rio Grande Delta that are naturally cut off from the main river. These paleochannels form oxbow-like linear water bodies across the landscape of Cameron County, including the city of Brownsville, Texas (Robinson 1995). Resacas provide habitat for a diverse community of fish and other wildlife, many of which are endemic to the South Texas region (Jahrsdoerfer and Leslie Jr. 1988). For example, Montezuma cypress (*Taxodium mucronatum*), a scarce conifer, and the Texas sabal palm (*Sabal mexicana*) only natively occur within the US in the Rio Grande Valley. The endangered, red-crowned parrot (*Amazona viridigenalis*) also uses the associated habitat—one of only a few locations within the US (IUCN 2021).

Over time, much of the riparian habitat along the resacas has been invaded by Brazilian peppertree (*Schinus terebinthifolia* Raddi [Sapindales: Anacardiaceae]). *Schinus terebinthifolia* is a subtropical tree species native to Brazil, Paraguay, and Argentina initially introduced to Florida on two separate occasions in the late 1800s (Ewel et al. 1982). The result was two distinct haplotypes with points of entry on either the west (haplotype A) or the east (haplotype B) coasts with widespread hybridization between and beyond Florida's boundaries (Williams et al. 2005, 2007). In its native range, *S. terebinthifolia* grows as a small, evergreen multitrunked tree or woody shrub (Barkley 1944; Ewel et al. 1982; Hight et al. 2002). As an invader to tropical and subtropical regions of the US, especially Florida, California, Hawaii, and coastal regions of South Texas, the invasive tree grows in dense monospecific stands of tangled branches that outcompete native vegetation (Figure 1) (UF-IFAS 2021; EDDMapS, n.d.). Native habitat and biodiversity are threatened by the ease and speed in which *S. terebinthifolia* spreads (i.e., vegetatively and by seed) (Ewel et al. 1982) and the allelopathic traits (i.e., chemical suppression of native plant populations) the tree is suspected to possess (Morgan and Overholt 2005).

Figure 1. *Schinus terebinthifolia* infestation along a resaca at Camp Rio, Brownsville, Texas.

In 2015, USACE began to evaluate the potential restoration of the aquatic and riparian habitats of resacas in Brownsville, Texas. By late 2020, a pilot restoration project (CAP Section 206 Resaca Blvd. Resaca ER Project) was completed as a proof of concept for the restoration of resaca habitats (Schad et al. 2023). Included in proposed restoration measures was the costly (approximately 50% of total construction costs) and labor-intensive removal of *S. terebinthifolia*. Treatment consisted of manual and mechanical removal followed by subsequent herbicide (triclopyr) applications (i.e., cut stump herbicide) (Schad et al. 2023). After treatments, some resprouting from trunks, exposed roots, and the existing seedbank was observed for the treated *S. terebinthifolia* stands and resulted in the need for continuous monitoring and follow-up treatments. This scenario is common in restored areas where this type of *S. terebinthifolia* treatment is implemented (Cuda et al. 2023). The cut stump herbicide strategy was initially successful for *S. terebinthifolia* reduction and allowed native vegetation to become established. However, given the observed *S. terebinthifolia* recruitment immediately following construction efforts, additional herbicide efforts were predicted to be necessary in the near-future and long-term, or an integrated pest management approach. Fortunately, biological control options had recently become available as a long-term, sustainable management tool to combat *S. terebinthifolia* regrowth and to suppress populations (USDA-APHIS 2019a, 2019b).

Biological control is the intentional introduction of a natural enemy (agent) from the invader's native range to control the target organism (Coombs et al. 2004). Prior to an approval for release, prospective biological control agents undergo extensive risk and safety evaluations, regulated by the US Department of Agriculture (USDA)–Animal and Plant Health Inspection Service (APHIS). Because of the rigor required, these programs can take upwards of 10–30 years to come to fruition (Coombs et al. 2004; Cuda 2016). During that time, host (target) specificity is vigorously tested and must be clearly demonstrated before release of the agent is permitted (Cuda et al. 2009). Ideally, biological control programs leverage these coevolved host-agent relationships to suppress invader populations below economically or ecologically harmful thresholds (Coombs et al. 2004). Biological control agents have the potential to reduce the economic and environmental costs associated with other management options (i.e., herbicides, physical labor, heavy machinery) and provide sustainable, long-term control (Gettys et al. 2014; Wainger et al. 2018). Land managers and restoration practitioners are hopeful that the addition of biological control as a management strategy will improve the efficacy of *S. terebinthifolia* management (Cuda et al. 2006).

In 2019, the biological control agent Brazilian peppertree thrips (*Pseudophilothrips ichini* (Hood) [Thysanoptera: Phlaeothripidae]) was approved for release in the US (Figure 2) (USDA-APHIS 2019b). *Pseudophilothrips ichini* feed on the flushing new leaves and stems of the plant, potentially slowing growth, and reducing seed production if thrips densities are high enough (Furmann et al. 2005; Wheeler, Mc Kay, et al. 2016). Laboratory studies have shown overwhelming negative impact to *S. terebinthifolia* (e.g., reduction in growth and the number of growing tips) caused by the thrips (Wheeler et al. 2018; Harlow et al. 2023), but field verification of these trends has just begun (Bowers et al. 2022; Wheeler et al. 2022). The first US releases of this agent occurred in Florida in 2019. Researchers from USDA–Agricultural Research Service (USDA-ARS), University of Florida (UF), and Florida Department of Agriculture and Consumer Services (FDACS) collaborated to rear and release over two million *P. ichini* at 567 sites between May 2019 and December 2021 (Wheeler et al. 2022). In Texas, the USACE Engineering Research and Development Center (ERDC) initiated a biological control program for *S. terebinthifolia* in late-2020 to aid in the ongoing USACE-SWG Resacas at Brownsville, Texas, Ecosystem Restoration Project.

Figure 2. *Pseudophilothrips ichini* adults (*black*) and first and second instar larvae (*orange*) feeding on *S. terebinthifolia* stems.

1.2 Objective

The goal of this technical report is to (1) provide background on the USACE biological control program for *S. terebinthifolia*; (2) report the initial results from mass-rearing, field releases, and establishment monitoring of *P. ichini* in South Texas; and (3) document initial observed benefits and other considerations of integrating invasive weed biological control into USACE ER phased-projects. This technical report represents year 1 and 2 efforts of a 5-year project.

1.3 Approach

To support efforts of *S. terebinthifolia* management in the USACE-SWG Resacas at Brownsville, Texas, Ecosystem Restoration Project the USACE ERDC initiated a biological control program for *S. terebinthifolia* in late-2020 and rearing of the *P. ichini* began in 2021. In March 2022, the first releases of *P. ichini* in Texas were made by ERDC in Brownsville, Texas. Release sites were each in different phases of ER project development (i.e., design, construction, and O&M), allowing a unique opportunity to integrate and assess the value of invasive weed biological control during multiple stages of the elaborate ER process (USACE 2016, 2018).

2 Materials and Methods

2.1 *Pseudophilothrips ichini*

The *P. ichini* life cycle consists of egg, two larval instars, three nonfeeding pupal instars, and the black, winged adult (2.5 mm in length)[*] (Wheeler, Silverson, et al. 2016). Eggs are laid on the surface of new leaflets near the apical meristem. Larvae feed and develop on the tip they were oviposited on by piercing plant tissues and consuming plant fluids (Figure 2) (Cuda et al. 2008). With high densities (>500 larvae), larvae progress down the stem as the fluids are depleted, resulting in dry, dead plant tips (Furmann et al. 2005). Late instar larvae are bright orange and will descend to the soil surface to pupate in the upper organic matter layer. Adults emerge and congregate on the underside of leaves (Wheeler, Silverson, et al. 2016). Development time is temperature dependent, ranging from 34.5 days at 20°C to 18.9 days at 30°C (Manrique et al. 2014).

2.2 Site Selection

Beginning in 2021, four *P. ichini* release sites were selected in Brownsville, Texas, based on past and future ER efforts and known infestations of *S. terebinthifolia* (Table 1; Figure 3). Sites were selected to assess the ability of the *P. ichini* to reduce growth after restoration (Resaca Blvd), during restoration efforts (Jagou plantation), and prior to restoration efforts (Brownsville Airport). Camp Rio was chosen as a control site where no restoration efforts were planned. Although control of mature trees may take decades, impact on young trees and seedlings was expected (Wheeler, Silverson, et al. 2016).

Table 1. Summary of US Army Corps of Engineers (USACE) ecosystem restoration sites and their current status.

Site	Restoration Phase	Restoration Status	Initial *P. ichini* Release
Resaca Blvd	Monitoring/maintenance	Restored 2019	March 2022
Jagou Plantation	Design	Estimated construction 2024	September 2022
Brownsville Airport	Planning	Estimated construction 2032	March 2022
Camp Rio	None	Unmanaged	September 2022

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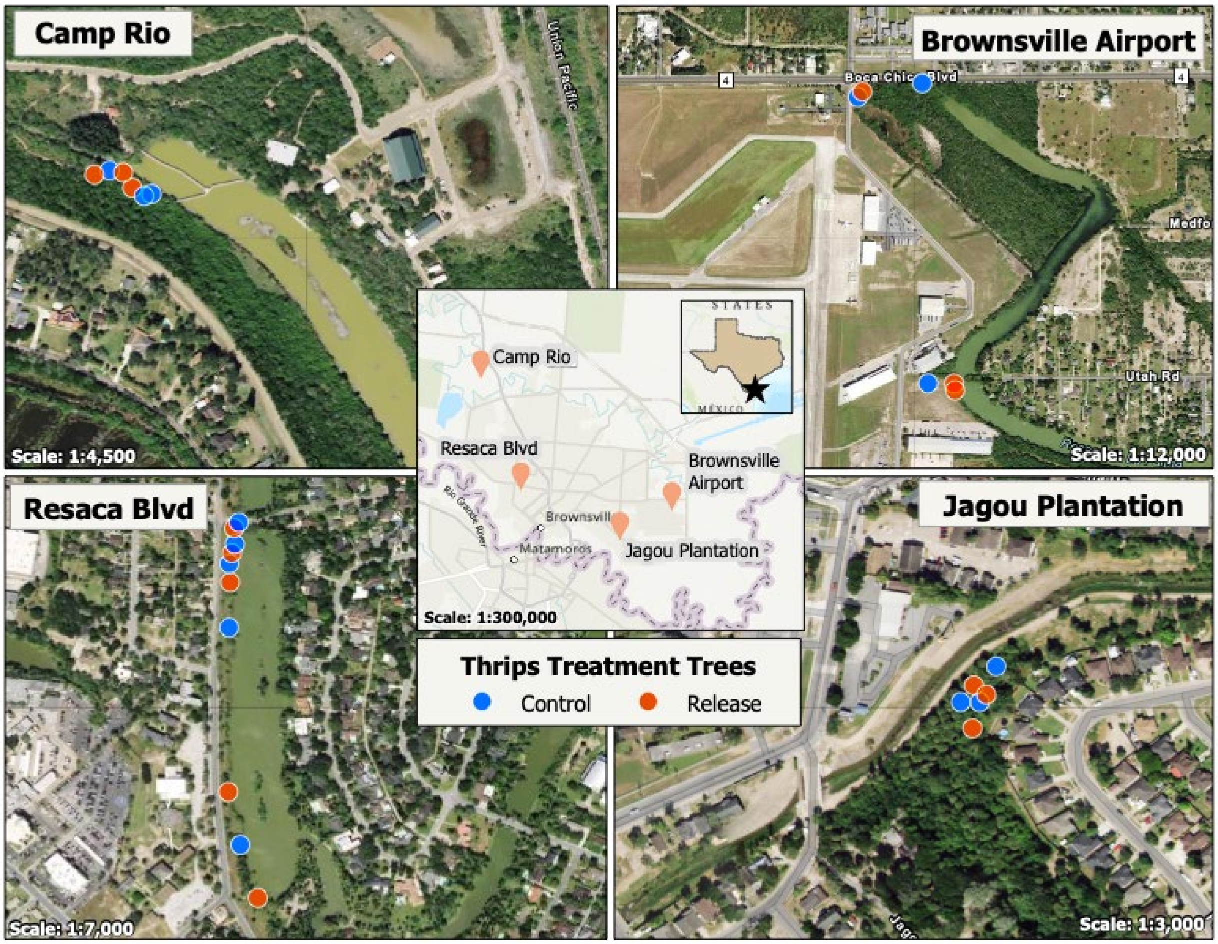
[*] For a full list of the spelled-out forms of the units of measure used in this document, please refer to *US Government Publishing Office Style Manual*, 31st ed. (Washington, DC: US Government Publishing Office, 2016), 248–52, https://www.govinfo.gov/content/pkg/GPO-STYLEMANUAL-2016/pdf/GPO-STYLEMANUAL-2016.pdf.

Figure 3. *S. terebinthifolia* study populations in Brownsville, Texas.



2.2.1 Resaca Boulevard (Monitoring/Maintenance Phase) (N 25.921245, W 97.507625)

The Resaca Boulevard site consists of a 29.5-acre greenbelt situated between a residential neighborhood and a resaca. Prior to restoration in 2019, the riparian habitat of the resaca was dominated by *S. terebinthifolia.* The site served as a pilot project to assess the efficacy of restoring native riparian and aquatic plant species (Schad et al. 2023). As part of that work, *S. terebinthifolia,* and other invasive plant species, were physically removed along with the upper layers of topsoil to remove most of the existing invasive seedbank. However, by March 2022, several young sapling *S. terebinthifolia* had resprouted from old stumps and/or the remaining seedbank within the restoration footprint, and 10 of those saplings were selected for inclusion in this biological control study. The goal was to determine whether *P. ichini* could control recruitment/regrowth of

S. terebinthifolia on restored resacas. The first *P. ichini* releases at the Resaca Blvd site occurred in late March 2022.

2.2.2 Jagou Plantation (Design Phase) (N 25.891639, W 97.447863)

The Jagou plantation site is in a predominantly residential area near the historic site and architectural remains of a 120-year-old plantation (Clark 2018). The Jagou plantation resaca is included in the first phase of the USACE-SWG Resacas at Brownsville, Texas, Ecosystem Restoration Project, and the design phase of the restoration was concluded in 2023 with construction expected to occur in 2024. The release of *P. ichini* at Jagou will provide insight into the integration of *S. terebinthifolia* biological control in concert with active restoration measures, including the common cut-stump treatment as well as topsoil removal. If biological control is successfully integrated into an integrated pest management (IPM) strategy, it could reduce the cost and level of effort associated with the chemical and physical control of *S. terebinthifolia*. Trees and branches were selected and pruned in July 2022 and tree measurements (included in the final report) and *P. ichini* release began in September 2022. Pruning was done to encourage flushing new growth, which is preferred by adult *P. ichini* for feeding (Furmann et al. 2005; Wheeler, Mc Kay, et al. 2016).

2.2.3 Brownsville Airport (Planning Phase) (N 25.916442, W 97.418066)

The Brownsville Airport site is located along a resaca that is adjacent to an industrial area and major roadway. Brownsville Airport was selected to assess the establishment of *P. ichini* ahead of restoration efforts. The proposed restoration at the Brownsville Airport resaca is scheduled to begin in 2032. The release of the *P. ichini* at the Brownsville Airport site will be many years before restoration activities. Branch selection, initial measurements, and releases took place in March 2022.

2.2.4 Camp Rio (Unmanaged Control Site) (N 25.989056, W 97.531608)

The Camp Rio site is heavily infested with continuous stands of *S. terebinthifolia* (Figure 1). The survey sites are located along a hiking trail within a natural recreational area. Camp Rio is managed by the Brownsville charter school system as a youth outdoor education center. The Camp Rio resaca is not included in the USACE-SWG Resacas at Brownsville, Texas, Ecosystem Restoration Project and was chosen as a control site for the study. Camp Rio is the northern most site in the study area. Trees and branches were selected and pruned in July 2022 and measurements and release began in September 2022.

At Jagou, Brownsville Airport, and Camp Rio, six trees (three non-release control and three release trees) were chosen based on accessibility, similarity of size, and distance from other *S. terebinthifolia* (>5 m). Ten branches on each tree were tagged and labeled with metal forestry tags and orange flagging. Three temperature dataloggers (HOBO Pendant MX Temp/Light, Onset, Woburn, Massachusetts) were deployed at each release tree: one on the ground near the base of the tree, a second at breast height on the main trunk, and a third on an outer branch of the tree. At the Resaca Blvd site, smaller, younger (<2-year-old) trees were present post-restoration and therefore one branch on 10 individual trees (five control and five release trees) were tagged in the same manner. A single temperature datalogger was deployed approximately 2 ft from the ground on three of the five release trees.

2.3 Insect Rearing Colonies

After required transportation and rearing permits were acquired from appropriate resource agencies, such as the USDA in 2020 and the Texas Department of Agriculture in 2021, *Pseudophilothrips ichini*—originating from Ouro Preto, Mina Gerais, Brazil (USDA-APHIS 2019b)—were supplied to ERDC by the University of Florida, Gainesville, Florida, in October 2021. Rearing colonies were maintained in cages in greenhouses and environmental growth chambers at ERDC (Vicksburg, Mississippi) and the Lewisville Aquatic Ecosystem Research Facility (LAERF) (Lewisville, Texas). A continuous supply of *S. terebinthifolia* was produced from seeds collected from Brownsville, Texas (likely hybrid of the two Florida haplotypes A and B) (personal communication, D.A. Williams; Williams et al. 2005, 2007). Plant propagation and culturing methods were adapted from the standard operating procedures published by the FDACS in 2021 (FDACS-DPI 2021) and were similar to those used in Harlow et al. (2023). Plants were propagated by crushing the outer exocarp of the seeds before sowing into 36 cell germination trays (5 cm wide and 7 cm deep) filled with Pro-Mix BX Growing Medium (Premier Horticulture, Riviere-Du-Loup, Quebec, Canada). Trays were hand watered as needed. After 2 months of growth, seedlings were transferred into 0.8 L pots (Classic 100, Nursery Supplies, Kissimmee, Florida) with the same Pro-Mix BX potting soil. After 4 months of growth, plants were transplanted into 2.5 L pots (Classic 300S, Nursery Supplies, Kissimmee, Florida) and time release fertilizer (Florikan 14-14-14, Florikan ESA, Sarasota, Florida), minor nutrients (Southern Ag Minor Nutrients, Southern Agriculture Insecticides, Hendersonville, North Carolina), and pelletized lime (Soil Doctor, Oldcastle Lawn and Garden, Atlanta, Georgia) were added to the soil following label rates. From that point forward, plants were bottom watered 2–3 times a week. After 35–45 weeks of growth, plants were transplanted into 7.0 L pots (Classic 500S, Nursery Supplies, Kissimmee, Florida) for use in the larger cages. Peters Professional 20-20-20 (Everris NA, Dublin, Ohio) fertilizer was applied every 3–4 weeks at a rate of 2.1 g/L and was dispensed in 200, 300, or 400 mL of solution per 0.8, 2.5, and 7.0 L pot, respectively. Pest management consisted of alternating weekly applications of either 19.5 mL/L of Safer Soap (Woodstream Corporation, Lititz, Pennsylvania) or 7.8 mL/L Neem Oil (Bonide, Oriskany, New York) to control generalist herbivore pests. Pruning of the plants occurred occasionally to promote the growth of numerous new flushing tips. Plants were grown in a greenhouse for 6–11 months (20–100 cm tall) before use in rearing colonies.

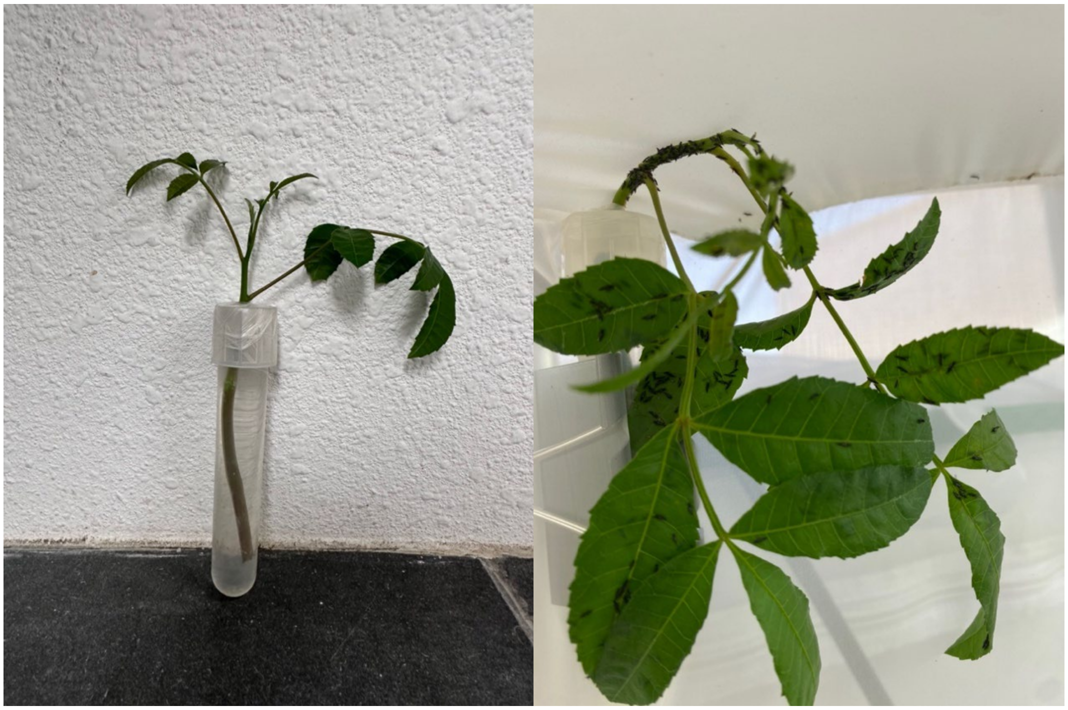
Prior to setting up each rearing cage, plants were cleaned by submerging the aboveground portions of the plants into soapy water (0.3 mL/L of Dawn dishwashing liquid) for 90 s before rinsing with clean water and allowing to air dry. The soil in each pot was topped with a layer of peatmoss to provide a humid substrate for thrips pupation. Eight or 16 plants, respectively, were placed into cages of two different dimensions (Figure 4): smaller tabletop cages that were white, constructed with 160 µm mesh, 1 × 1 × 1 m (Bugdorm 6E1010 Insect Rearing Cage, Taichung, Taiwan); and larger walk-in cages that were brown, constructed with 280 µm mesh, 1.8 × 1.8 × 1.8 m (BioQuip, Rancho Dominguez, California) on top of nursery trays (52 × 25 × 6.3 cm) with additional peatmoss packed around the pots. Recently emerged (within 2 weeks) adult thrips were dispersed within each cage at a rate of approximately 20–25 thrips per plant. After cages were set, plants were bottom watered 2–3 times a week. During periods of field releases, 2–3 cages were set up for each generation of thrips, hereafter referred to as a cohort. During the winter months, when development times were longer and monthly *P. ichini* releases were scheduled, the timing of cohorts was staggered so that a generation was produced each month. Air temperature within rearing cages was monitored using wireless dataloggers (HOBO Pendant MX Temp/Light, Onset, Woburn, Massachusetts).

Figure 4. *P. ichini* rearing colonies in Lewisville, Texas. Tabletop cages are to the *left*, walk-in cages (brown mesh) are in the *center*, and *S. terebinthifolia* growing in 7.0 L pots (9–11 months of growth) is to the *right*.

One to two weeks before the new cohort was expected to emerge (6–11 weeks depending on season and ambient temperatures), floral tubes containing water and a fresh *S. terebinthifolia* plant cutting were clipped to the upper corners of each cage and pressed into the soil of plant pots, to promote aggregation of adult thrips and facilitate easy collection. Floral tubes were constructed using freshly cut and washed *S. terebinthifolia* tips (15–20 cm long) inserted into plastic floral tubes (17 × 100 mm culture tubes with closures, VWR International, Radnor, Pennsylvania) filled with water, and sealed with parafilm around the lid (Figure 5). The stem of each plant was then cut at the base to allow the tree to dry out and encourage thrips to disperse to the newly added plant cuttings.

At the end of the development period, as new adults were observed in the cages, thrips were collected in increments of approximately 300 by gathering plant cuttings, and/or other plant material where thrips were congregated and placing them directly into 1 L plastic cups (13 cm tall × 11 cm diameter) that were lined with paper towels. Paper towels were held in place with rubber bands and helped to reduce thrips mortality in transit due to condensation. A square of Dacron chiffon netting (approximately 240 microns; BioQuip, Rancho Dominguez, California) was then placed over each cup and secured with a rubber band. Thrips were stored in a plant growth chamber (Percival Model E-36L, Percival Scientific, Perry, Iowa) for less than 2 weeks while awaiting transport to a release site. The plant growth chambers were set to a diurnal cycle (14 h light, 10 h dark) and held at 15°C. To prepare for overnight shipment, plastic cups were packed tightly into coolers to prevent movement and with ice packs to keep them cool during transit.

Figure 5. Floral tube used to promote thrips aggregation and facilitate easy thrips removal from cages.



2.4 *Pseudophilothrips ichini* Releases

The goal was to release 4,000–5,000 *P. ichini* per site per release. Therefore, each of five branches received approximately 300–400 thrips, or 1,500–2,000 for each tree. The first phase of releases occurred every 8–9 weeks at the Resaca Blvd and Brownsville Airport locations in spring 2022. During fall and winter 2022, releases were made every 5–7 weeks across all four sites (Table 2). Releases were halted in March 2023 to assess thrips persistence.

Table 2. Dates of thrips monitoring and releases.

Date	Weeks Since Previous Release	Persistence Monitoring	Releases
28 March 2022	–	–	X
30 May 2022	9	X	X
29 July 2022	8	X	–
30 September 2022	9	X	X
6 November 2022	5	X	X
5 December 2022	5	X	X
23 January 2023	7	X	X
1 March 2023	5	X	–
3 April 2023	5	X	–

On each release tree at Jagou, Brownsville Airport, and Camp Rio, five branches with newly flushing, growing tips received 300–400 thrips each. During the first release at a site, thrips were released onto five of the 10 tagged branches. During the second release, thrips were released on the remaining five tagged branches that did not receive thrips initially. All subsequent releases occurred on untagged branches of the release tree that had new growing tips. At the Resaca Blvd site, 300–500 thrips were released onto the tagged branch on each of the five release trees. If the tagged branch tip was damaged or dead, then an alternate healthy branch was chosen for release on the same tree. At each release tree, plastic cups were carefully opened, and the mesh cover checked for wandering thrips. Most thrips were found on the *S. terebinthifolia* cutting(s) inside the container and that cutting was gently draped over the branch (after removing it from the floral tube). If thrips remained on the paper towel inside the cup, the paper towel was relocated to the branch and secured with a clothes pin to allow thrips to disperse.

2.5 *Pseudophilothrips ichini* Persistence Monitoring

On each return trip to a site (every 5–9 weeks), *P. ichini* persistence was assessed before making additional releases (Table 2). On each tagged branch, *P. ichini* individuals from each life stage were counted during a 1 min search. Next, a 10 min search was conducted on untagged branches, giving particular focus to branches with flushing new tips and those that were close to the ground. On control trees that did not receive thrips, tagged branches were searched for *P. ichini* individuals for 1 min each. If no thrips were found, then no additional searching near control trees was conducted. If thrips were found on nearby or control trees, data were recorded in the same manner as mentioned previously.

2.6 Statistical Analysis

Pseudophilothrips ichini rearing efforts at LAERF from March 2022 to March 2023 are reported and analyzed in Section 3. For each cohort duration, individual daily mean cage temperatures were averaged to characterize temperature conditions experienced by *P. ichini* between introduction of thrips onto plants and the date of peak thrips harvest (generation time). Regression analysis was used to detect relationships between thrips production, generation time, and mean cage temperature. Table A-2 reports temperature data averaged among cages before calculating average daily maximum, minimum, and daily average temperature. Those data were used to calculate monthly averages. Temperature ranges reported are the absolute maximum and minimum temperature detected each month.

The number of F_0 thrips (F_0 refers to the initial generation of *P. ichini* thrips introduced to cages whereas F_1 refers to the resulting generation produced) and plants used to initiate each cage varied based on the number of adult thrips and high-quality plants available. Therefore, in addition to the number of thrips produced per plant, we report F_1 thrips production, adjusted for the number of F_0 thrips per plant used to initiate each cage, as well as fold productivity (defined as the number of F_1 thrips produced divided by the number of F_0 thrips introduced). Differences in the metrics listed above between rearing cage types were compared via Student's t-test ($p = 0.05$) in JASP (JASP Team 2022). One cage in cohort 6 was setup 2 weeks earlier than the others to transition back to producing only one cohort every 2 months (Table A-1). That cage is excluded from averaged metrics and analysis.

3 Results

3.1 Rearing

In total, 156,695 adult *P. ichini* thrips were produced from March 2022 to March 2023 and released in Brownsville, Texas and/or used in experiments. An average of 19,586 ± 2,222.1 (mean ± standard error [SE]) adult thrips were produced per cohort. A cohort corresponds to a generation of thrips produced in two to three cages with the same setup date. An overview of rearing colony production by cohort and cage type can be found in Table A-1. The overall average number of F_1 thrips produced per plant was 680.9 ± 64.8 thrips. When adjusted for the number of F_0 thrips per plant used to initiate each cage, the average was 268.2 ± 21.9 thrips. Average fold productivity across all cages and cohorts was 26.4 ± 2.3 F_1 thrips per F_0 thrips introduced.

When split between the different cage sizes, the smaller tabletop cages produced 88% more F_1 thrips per plant (852.2 ± 71.4) than the larger walk-in cages (452.7 ± 61.4) ($t[19] = -4.06$, $p < .001$) (Table 3). Fold productivity was 65% greater in the tabletop cages (31.8 ± 2.5 F_1 thrips per F_0 thrips introduced) than walk-in cages (19.2 ± 2.6) ($t[19] = -3.38$, $p = .002$) (Table 3). However, when thrips production was adjusted for the number of F_0 thrips per plant used to initiate each cage, no difference was detected (Table 3).

Table 3. Differences in thrips production between cage types reported at mean ± SE (standard error).

Cage Type	Number of Plants	Number of F_0 Adults	Number of F_1 Adults	F_1 Thrips per Plant[a]	F_1 Thrips per (F_0 Thrips/Plant)	Fold Productivity[a]
Small Tabletop	7–8	209.8 ± 8.3	6,637.3 ± 528.3	852.2 ± 71.4	248.1 ± 19.3	31.8 ± 2.5
Large Walk-In	14–16	360.6 ± 28.6	6,937.6 ± 997.8	452.7 ± 61.5	295.1 ± 44.1	19.2 ± 2.6

[a] $p < .01$.

Thrips production varied (9,831–28,506 F_1 thrips) with seasonal temperature (Table 4 and Table A-1). Average cage temperature during development was weakly correlated with the number of thrips produced within each cage ($R^2 = 0.181$, $p = .054$), but strongly correlated with generation time ($R^2 = 0.782$, $p < .001$) (Figure 6). When the number of thrips produced was adjusted for the initial number of F_0 thrips introduced per plant, winter cohorts (cohorts 1, 6, 7, and 8) were less productive than summer cohorts (cohorts 2, 3, 4, and 5) (Figure 7).

Table 4. *P. ichini* production per cohort from April 2022 to January 2023. See Table A-1 for details of each cage. Production metrics presented as mean ± SE.

Cohort–Start Month	Plants–Cages	Sum F_0 Adults	Average Generation Time (weeks)	Sum F_1 Adults	F_1 Thrips per Plant	F_1 Thrips per (F_0 Thrips/Plant)	Fold Productivity
1–April 2022	36–3	863	6.90	14,895	453.2 ± 118.6	204.3 ± 13.7	18.2 ± 3.7
2–June 2022	39–3	1,300	7.33	28,506	786.6 ± 145.0	282.4 ± 29.2	22.8 ± 3.0
3–July 2022	24–2	500	6.50	16,953	767.7 ± 183.8	401.3 ± 96.9	34.6 ± 3.5
4–August 2022	31–3	800	6.76	18,030	592.4 ± 70.8	233.6 ± 42.2	23.2 ± 3.0
5–September 2022	32–3	700	9.29	27,362	943.4 ± 181.5	406.8 ± 50.2	40.4 ± 4.7
6–October 2022	32–3	2,000[a]	9.38	22,568[a]	409.4 ± 233.5	177.9 ± 27.8	17.6 ± 8.2
7–November 2022	29–3	700	11.48	18,550	797.0 ± 288.4	233.4 ± 33.4	29.0 ± 9.0
8–January 2023	16–2	400	10.29	9,831	614.4 ± 99.7	196.6 ± 31.9	24.6 ± 4.0

[a] One cage in cohort 6 was setup two weeks early to transition back to producing only one cohort every two months. That cage is excluded from averaged metrics and analysis.

Figure 6. Relationship between mean cage temperature during a cohort and (*A*) the total number of F_1 *P. ichini* thrips produced per cage; and (*B*) the generation time of each cohort in weeks.

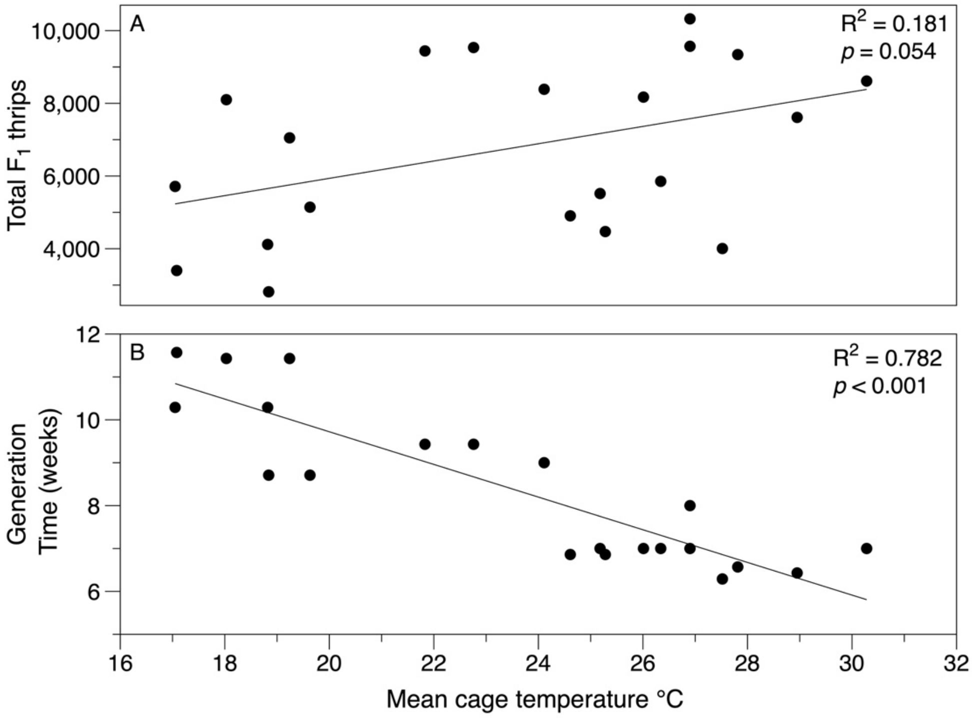
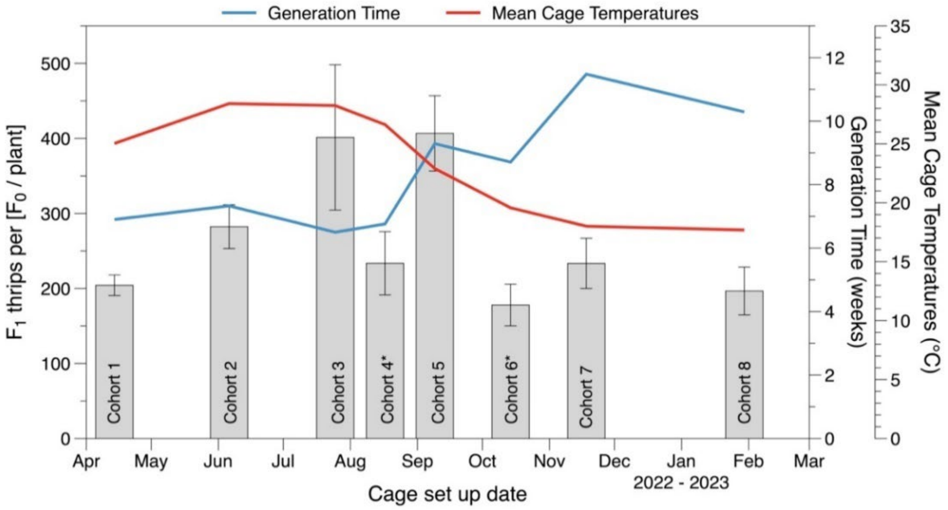


Figure 7. Number of F_1 thrips produced per cohort from April 2022 through February 2023 based on harvest date. Number of F_1 thrips produced is adjusted for the initial number of thrips per plant. Mean cage temperatures were averaged daily for each cohort duration. Generation time is the time between introduction of thrips onto plants and the date of peak thrips harvest. Cohorts 4 and 6 were harvested and reset before peak emergence.



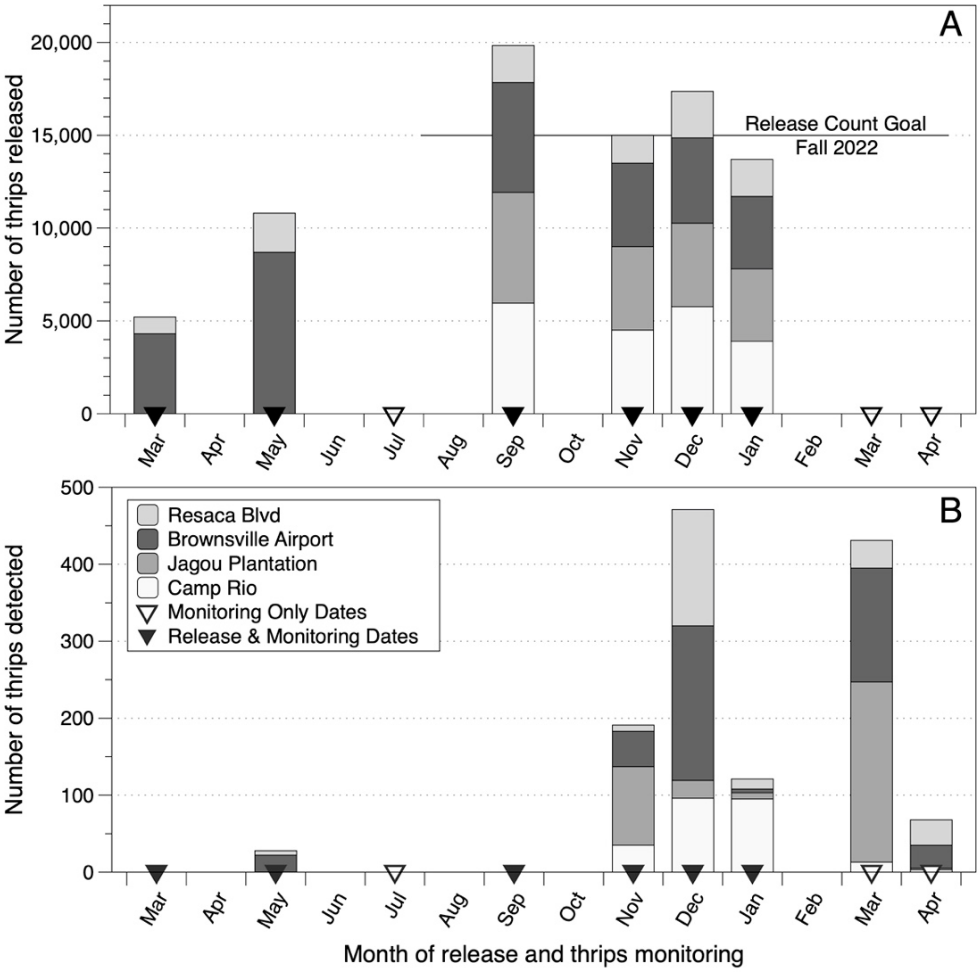
3.2 Release

Between March 2022 and January 2023, 86,409 adult thrips were released among four sites in Brownsville, Texas (Table 5). The number of thrips released during each visit varied based on the availability of thrips from rearing operations. Mean number of thrips released during each visit was 1,833 ± 228.8 at Resaca Blvd and 5,064 ± 440.6 thrips at each of the other three sites (Jagou, Brownsville Airport, and Camp Rio). The first summer of rearing *P. ichini* at LAERF indicated that high temperatures (>35°C) were limiting for thrips production in North Texas and may be a problem for thrips persistence in South Texas. Therefore, no thrips were released during July 2022 (Table 5 and Figure 8*A*).

Table 5. Number of thrips released at each site from March 2022 to January 2023.

Release Month	Resaca Blvd	Brownsville Airport	Camp Rio	Jagou Plantation	Released per Month
March 2022	898	4,310	—	—	5,208
May 2022	2,100	8,700	—	—	10,800
July 2022	0	0	—	—	0
September 2022	1,993	5,920	5,953	5,969	19,835
November 2022	1,500	4,500	4,500	4,498	14,998
December 2022	2,512	4,591	5,765	4,500	17,368
January 2023	2,000	3,900	3,900	3,900	13,700
Totals per site	**11,003**	**31,921**	**20,118**	**18,867**	**86,409**

Figure 8. Number of *P. ichini* thrips (*A*) released and (*B*) detected at each site visit from March 2022 to April 2023.



3.3 Persistence

Adult *P. ichini* were detected during every site visit except the July and September 2022 visits (Figure 8*B*). Larval *P. ichini* were detected during 54% (14 of 26) of surveys. There were no clear differences between sites in detection of thrips. Jagou and Brownsville Airport had the greatest number of thrips detected (452 and 370 thrips, respectively) whereas Resaca Blvd and Camp Rio had the least (245 and 242, respectively) (Table 6). Throughout the year, 68.9% of thrips were detected during 2 months, December (471 thrips) and March (431 thrips). The greatest number of larvae were detected (363 larvae across all sites) during March (Table 6 and Figure 8*B*).

Table 6. Summary of *P. ichini* detected during each survey in Brownsville, Texas, from March 2022 to April 2023.

Survey Month	Weeks Since Last Release	Resaca Blvd		Brownsville Airport		Camp Rio		Jagou Plantation		Monthly Total
		Adults	Larvae	Adults	Larvae	Adults	Larvae	Adults	Larvae	
May 2022	9	6	0	13	9	—	—	—	—	28
July 2022	8	0	0	0	0	—	—	—	—	0
September 2022	17	0	0	0	0	—	—	—	—	0
November 2022	5	8	0	44	2	35	0	102	1	192
December 2022	5	151	0	200	1	54	42	23	0	471
January 2023	7	13	0	5	0	37	58	8	0	121
March 2023	5	12	24	30	118	6	7	20	214	431
April 2023	10	9	22	22	8	2	1	1	1	66
Totals per site		199	46	314	138	134	108	154	216	1,309
		245		452		242		370		

4 Discussion

4.1 Rearing

ERDC's *P. ichini* mass rearing program builds on the work of USDA and UF researchers in Florida who began investigating *P. ichini* as biological control agents over 30 years ago (Bennett et al. 1988; Cuda et al. 2008; Cuda 2016; Wheeler, Mc Kay, et al. 2016; Halbritter et al. 2021). Based on the work of Halbritter et al. (2021) using the same cages, it was expected that the large walk-in cages would be the most productive way to rear *P. ichini*. Our production per plant (452.7 ± 61.4 F_1 thrips per plant) was substantially less than reported by Halbritter et al. (2021) using identical large walk-in cages (2,310 ± 174.1 F_1 thrips per plant). However, when adjusted for the number of F_0 thrips introduced, our productivity (19.2 ± 2.6 F_1 thrips per F_0 thrips introduced) was 108% greater than the Halbritter et al. (2021) cages (9.2 ± 0.7 F_1 thrips per F_0 thrips introduced), because substantially less F_0 thrips were used to initiate each cage in our cultures (360 F_0 thrips in our study versus 1,500 F_0 thrips reported by Halbritter et al. 2021; Table 3). The difference in productivity between low (the current study) and high thrips starting densities (Halbritter et al. 2021) may be due to density-dependent limitations on *P. ichini* productivity under rearing conditions. Intraspecific competition between F_0 individuals of an insect population can influence the quantity and fitness of the F_1 generation (Zehnder and Hunter 2009; Rezaei et al. 2020; Than et al. 2020). Overcrowding or competition for ideal feeding and oviposit locations may limit the efficiency of *P. ichini* mass rearing programs (Peters and Barbosa 1977; Hayamizu 1984; Applebaum and Heifetz 1999; Awmack and Leather 2002), a phenomenon that we previously demonstrated (Harlow et al. 2023).

When considering the efficiency of *P. ichini* rearing between cage type, tabletop cages were ideal. They contained fewer (7–8 trees) and shorter (60–90 cm) plants, which translated to less time and space required to maintain them. In addition, when harvesting F_1 *P. ichini*, less time was required to search for thrips within the smaller trees because there were fewer branches and leaves. The larger walk-in cages were initiated with 14–16 plants, most of which were older (7–11 months of growth) and larger (80–120 cm tall), and therefore required substantially more effort from plant maintenance to cage breakdown. The smaller tabletop cages were overall easier to work with and produced a comparable number of thrips as the walk-in cages (6,637.3 ± 528.3 versus 6,937.6 ± 997.8, respectively) (Table 4).

Seasonal changes in temperature can influence rearing program productivity and release availability, timing, and frequency, because insect development is dependent on temperature (Chapman 1982; Manrique et al. 2014; Halbritter et al. 2021). If rearing is subject to ambient temperatures, then controlling for temperature extremes becomes paramount. Cages occasionally reached extreme temperatures (from 40°C to 50°C) for short periods in March, May, June, July, and August (Table A-2). If temperatures stayed above 40°C for an extended time, it was common to have major thrips mortalities in rearing cultures. Low temperatures experienced by the thrips in short durations ranged from 5°C to 7°C and did not result in any apparent thrips mortality (Table A-2).

Mean temperature within cages had a strong relationship with generation time, in that higher temperatures generally resulted in shorter generation time (Figure 6*B*). Thrips production was also logistically affected by the amount of time allowed between cage set up and planned timing of thrips harvest. As daylength shortened and temperatures decreased in the fall (Table A-2), the timing of adult emergence did not coincide with planned release dates. As a result, August and October cohorts (cohorts 4 and 6) were harvested prior to peak emergence, and production during those months was less than other months (Figure 7). In response, adjustments were made by extending the collection dates by 2 weeks, thus improving the number of F_1 thrips harvested in subsequent cohorts (Table 4 and Figure 7). Winter months required nearly double the length of time between generations than spring and summer months (from 6 to 11 weeks) (Table 4, Figures 6*B* and 7, and Table A-2). An important lesson learned was that rearing operations subject to ambient temperatures should consider the seasonal differences in agent development. In North Texas, *P. ichini* development is reduced during winter months, which required increasing the number of rearing cages if large numbers of thrips were needed for release.

4.2 Release

Overall, *P. ichini* production was sufficient to meet our release goal of 4,000–5,000 thrips per site and visit at infested ecosystem restoration sites in Brownsville, Texas (Table 3 and Figure 8*A*). The resulting number of thrips released onto each tree ranged from 1,500 to 2,000 thrips. This number per tree is similar to what has been reported for widespread releases of *P. ichini* throughout Florida (Wheeler et al. 2022). Future releases in Brownsville and associated monitoring are scheduled to continue through 2025. Determination of the impact of biological control agents on *S. terebinthifolia* is ongoing.

Releases at Camp Rio and Jagou began after Resaca Blvd and Brownsville Airport (Table 1). Two months before the planned September release (during the July 2022 site visit), all tagged branches at Camp Rio and Jagou were pruned to encourage new growth and increase the number of fresh tips. During subsequent site visits, it was observed that multiple tagged branches failed to regrow new apical tips for thrips feeding and oviposition. Branches that grew new tips in the preceding months were those that had the best light exposure, such as those along the border of a trail or within the gap of a fallen tree. In the future, any pruning of branches prior to release should occur in the spring and/or in areas where overstory shading is absent.

4.3 Persistence

Monthly releases in the fall of 2022 were expected to boost *P. ichini* population levels during a mild South Texas winter and spring, resulting in increased numbers before summer. Unfortunately, all sites experienced temperatures at or below freezing for approximately 24 h between December and January surveys. This resulted in a dieback of new *S. terebinthifolia* growing tips. The reduced food supply may explain the reduction in thrips abundance detected during the January 2023 survey (Table 6 and Figure 8*B*). Conversely, the presence of a few thrips after the freeze event suggests overwintering may not be a problem for thrips persistence in this area (lengthy hard freezes are rare in the Rio Grande Valley). Biological control programs of tropical or subtropical weeds in temperate latitudes often face obstacles from an agent’s inability to overwinter (Hughes et al. 2009; Knight and Harms 2022). Whereas upper thermal limits of the biological control agent are less often a concern (Grodowitz et al. 2017). However, a changing climate may result in summer and winter weather extremes that become increasingly problematic for biological control program success (Harms et al. 2020, 2021).

The greatest number of thrips were detected in December 2022 and March 2023 (Table 6 and Figure 8*B*). These may have been adult thrips from the previous release five weeks prior (Table 6). The typical longevity of *P. ichini* is 4–7 weeks, depending on factors such as temperature and host plant quality (Manrique et al. 2014; Wheeler, Silverson, et al. 2016; Halbritter and Wheeler 2021). Thrips larval abundance peaked in March 2023 at most sites (Table 6), indicating successful reproduction that coincided with abundant spring growth of new tips (M. Harlow, unpublished data). However, the survey 10 weeks later (April 2023) detected an 85% decrease in the number of thrips across all sites (Table 6). Although the reason for such a rapid decline is unclear, a limitation may occur at the pupation stage. Researchers have highlighted the importance of moderate soil moisture and humid pupation substrate for successful *P. ichini* pupation (Halbritter and Wheeler 2019; Telmadarrehei et al. 2023). The Brownsville ER study/release sites vary in the amount of organic matter (plant litter) on the soil surface and suitable substrate available for thrips pupation. Further investigation is needed to understand life stage-specific stressors limiting thrips establishment.

In addition to reduced pupation success, factors such as predation (Reimer 1988; Halbritter et al. 2023) and high temperatures (Li et al. 2011; Ramanand et al. 2017) may contribute to limitations on *P. ichini* establishment. An increased presence of natural enemies, such as predacious arthropods (ants, spiders, and hemipterans) and anoles (*Anolis sp.*) were noted during field observations on release trees but not control trees (M. Harlow, personal observation). The minute pirate bug, reported to predate on *P. ichini* in Florida (Halbritter et al. 2023) has been detected in South Texas as well (M. Harlow and D. Flores, personal observation). Additionally, high temperatures in South Texas can be quite extreme compared to other areas where *P. ichini* releases are ongoing. Temperatures measured in the current study indicated spikes as high as 42.8°C during summer months on exposed vegetation, and as high as 40.7°C below trees near the soil. Further work is needed to understand the role of high temperatures on survival and population growth of the *P. ichini*. Investigations into the factors that have the potential to limit *P. ichini* establishment are ongoing.

4.4 Future Work

Release and monitoring of *P. ichini* populations at the USACE-SWG Resacas at Brownsville ER sites will continue through 2025. Assessment of the thrips impact on *S. terebinthifolia* at the branch, tree, and community level is ongoing. The culmination of this work is to inform best management practices (BMP) for incorporating biological control into USACE ecosystem restoration projects, and guidance for use of the *P. ichini* against the invasive *S. terebinthifolia* in the South Texas region.

5 Conclusion

From March 2022 to April 2023, more than 156,600 adult *P. ichini* were produced at ERDC, between facilities in Mississippi and Texas, to initiate the first *S. terebinthifolia* biological control program outside Florida. Over that period, more than 86,000 thrips were released in Brownsville, Texas, across three USACE ecosystem restoration project sites and one control site to help combat the invasive *S. terebinthifolia*. Establishment of the thrips in South Texas remains tenuous and will require further evaluation. However, preliminary results indicate successful overwintering of *P. ichini* over the first year of releases. The best way to use the agent in managing *S. terebinthifolia* in habitat restoration projects and other land management activities is still under investigation.

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Appendix: Supplemental Information

Table A-1. Rearing cage totals to compare differences among cages (large walk-in and small tabletop) and cohorts.

Cohort–Month	Cage Type	Number of Plants	F_0 Adults	Generation Time (weeks)	F_1 Adults	Cohort F_1 Total	F_1 Thrips per Plant	F_1 Thrips per (F_0 Thrips /Plant)	Fold Productivity
1–April 2022	Small	8	218	7.00	5,518	–	689.75	202.50	25.31
	Large	14	300	6.86	4,905	–	350.35	228.90	16.35
	Large	14	345	6.86	4,472	14,895	319.43	181.47	12.96
2–June 2022	Small	8	300	7.00	8,613	–	1076.63	229.68	28.71
	Large	15	500	8.00	9,569	–	637.93	287.07	19.14
	Large	16	500	7.00	10,324	28,506	645.25	330.37	20.65
3–July 2022	Small	8	200	6.43	7,612	–	951.50	304.48	38.06
	Large	16	300	6.57	9,341	16,953	583.81	498.19	31.14
4–August 2022	Small	8	200	6.29	4,005	–	500.63	160.20	20.03
	Small	8	200	7.00	5,854	–	731.75	234.16	29.27
	Large	15	400	7.00	8,171	18,030	544.73	306.41	20.43
5–September 2022	Small	8	200	9.00	8,386	–	1048.25	335.44	41.93
	Small	8	200	9.43	9,535	–	1191.88	381.40	47.68
	Large	16	300	9.43	9,441	27,362	590.06	503.52	31.47
6–October 2022	Small	8	1,500[a]	10.71	14,610	–	1826.25	77.92	9.74
	Small	8	200	8.71	5,143	–	642.88	205.72	25.72
	Large	16	300	8.71	2,815	22,568	175.94	150.13	9.38
7–November 2022	Small	7	200	11.43	7,050	–	1007.14	246.75	35.25
	Small	7	200	11.43	8,100	–	1157.14	283.50	40.50
	Large	15	300	11.57	3,400	18,550	226.67	170.00	11.33
8–January 2023	Small	8	200	10.29	4,118	–	514.75	164.72	20.59
	Small	8	200	10.29	5,713	9,831	714.13	228.52	28.57

[a] Held adults from August cohort until ready for the October cohort. This cage is not included in any analysis.

Table A-2. *P. ichini* rearing cage temperatures (°C) (Lewisville Aquatic Ecosystem Research Facility [LAERF], Lewisville, Texas) averaged across each month.

Month	Max	Average	Min	Range
March 2022	28.89 ± 1.39	19.01 ± 0.67	11.28 ± 0.73	5.75–50.19
April 2022	30.75 ± 0.53	22.47 ± 0.41	15.79 ± 0.66	7.42–39.85
May 2022	33.75 ± 0.65	26.22 ± 0.48	20.33 ± 0.49	13.55–41.18
June 2022	35.41 ± 0.57	27.96 ± 0.27	22.83 ± 0.23	19.82–46.80
July 2022	35.15 ± 0.23	28.96 ± 0.12	24.24 ± 0.16	22.01–41.83
August 2022	35.79 ± 0.45	28.34 ± 0.25	23.95 ± 0.11	21.62–40.97
September 2022	31.74 ± 0.55	25.45 ± 0.37	21.23 ± 0.35	16.34–39.98
October 2022	28.09 ± 0.66	21.63 ± 0.52	16.90 ± 0.55	9.82–39.55
November 2022	23.99 ± 0.64	18.53 ± 0.63	15.33 ± 0.64	10.59–33.33
December 2022	23.46 ± 0.50	17.94 ± 0.54	15.36 ± 0.64	7.25–31.10
January 2023	23.86 ± 0.74	18.34 ± 0.45	15.43 ± 0.40	10.59–31.83
February 2023	23.67 ± 0.92	16.96 ± 0.52	12.74 ± 0.34	9.86–37.88
March 2023	25.34 ± 0.44	18.68 ± 0.37	14.29 ± 0.42	10.55–38.44

Note: Calculated (mean ± SEM) mean daily maximum, average, and minimum. Range is the absolute maximum and minimum temperature.

Abbreviations

APCRP	Aquatic Plant Control Research Program
APHIS	Animal and Plant Health Inspection Service
ARS	Agricultural Research Service
BMP	Best management practices
CAP	Continuing Authorities Program
DPI	Department of Plant Industry
EDDMapS	Early Detection and Distribution Mapping System
ER	Ecosystem restoration
ERDC	Engineer Research and Development Center
FDACS	Florida Department of Agriculture and Consumer Services
IFAS	Institute of Food and Agricultural Sciences
IPM	Integrated pest management
IUCN	International Union for Conservation of Nature and Natural Resources
LAERF	Lewisville Aquatic Ecosystem Research Facility
O&M	Operation and maintenance
SE	Standard error
SWG	Galveston District
UF	University of Florida
USACE	US Army Corps of Engineers
USDA	US Department of Agriculture

REPORT DOCUMENTATION PAGE

1. REPORT DATE June 2024	**2. REPORT TYPE** Final Technical Report (TR)	**3. DATES COVERED** **START DATE** FY20	**END DATE** FY23

4. TITLE AND SUBTITLE

Initial Rearing, Release, and Establishment of Biological Control Agent *Pseudophilothrips ichini* to Control Brazilian Peppertree (*Schinus terebinthifolia*) in South Texas Ecosystem Restoration Projects

5a. CONTRACT NUMBER	**5b. GRANT NUMBER**	**5c. PROGRAM ELEMENT**
5d. PROJECT NUMBER	**5e. TASK NUMBER**	**5f. WORK UNIT NUMBER**

6. AUTHOR(S)

Megann M. Harlow, Nathan E. Harms, Aaron N. Schad, Danny L. Allen, William A. LeVan, and Ashton B. DeRossette

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) See reverse.	**8. PERFORMING ORGANIZATION REPORT NUMBER** ERDC/EL TR-24-10

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) US Army Engineer Research and Development Center 3909 Halls Ferry Road, Vicksburg, MS 39180	**10. SPONSOR/MONITOR'S ACRONYM(S)**	**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

12. DISTRIBUTION/AVAILABILITY STATEMENT

Distribution Statement A. Approved for public release: distribution is unlimited.

13. SUPPLEMENTARY NOTES

2020-E-1495, "Applying Biological Control for Invasive Weed Management in USACE Environmental Business Line/Program Projects: A Best Practices Framework and Case Study."

14. ABSTRACT

Control of the invasive Brazilian peppertree (*Schinus terebinthifolia*) is a major cost component of US Army Corps of Engineers (USACE) ecosystem restoration (ER) projects in South Texas, specifically the USACE Galveston district (SWG) Resacas at Brownsville, Texas, ER Project. Biological control has been developed as a sustainable tool to lower long-term weed management costs. Although a biological control program for *S. terebinthifolia* has been in operation in Florida since 2019, no similar program existed in Texas until initiated by the Engineer Research and Development Center (ERDC) in 2020. Since 2021, the biological control agent *Pseudophilothrips ichini* has been reared at ERDC. This technical report details rearing, release, and establishment efforts from fall 2020 to spring 2023 to provide control of *S. terebinthifolia* in South Texas USACE ER project locations. Initial observations on impact and potential limitations to biological control in hot climates such as those of South Texas are also discussed.

15. SUBJECT TERMS

Aquatic plants; Brazilian pepper tree--Biological control; Insects as biological pest control agents; Introduced organisms; Invasive plants

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**
a. REPORT Unclassified	**b. ABSTRACT** Unclassified	**C. THIS PAGE** Unclassified	SAR	44

19a. NAME OF RESPONSIBLE PERSON Megann M. Harlow	**19b. TELEPHONE NUMBER (include area code)** (214) 878-3377

STANDARD FORM 298 (REV. 5/2020)

PREVIOUS EDITION IS OBSOLETE.

Prescribed by ANSI Std. Z39.18

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES (concluded)

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