Brazilian Collections and Laboratory Biology of the Thrips Pseudophilothrips ichini (Thysanoptera: Phlaeothripidae): A Potential Biological Control Agent of the Invasive Weed Brazilian Peppertree (Sapindales: Anacardiaceae)

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Brazilian collections and laboratory biology of the thrips *Pseudophilothrips ichini* (Thysanoptera: Phlaeothripidae): a potential biological control agent of the invasive weed Brazilian peppertree (Sapindales: Anacardiaceae)

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Abstract

Brazilian peppertree, *Schinus terebinthifolia* Raddi (Sapindales: Anacardiaceae), is one of the most invasive weeds in Florida and Hawaii. In the invaded range, this fast-growing weed from South America poses a threat to agriculture and cattle production and decreases the biodiveristy of natural areas. The thrips *Pseudophilothrips ichini* (Hood) (Thysanoptera: Phlaeothripidae) is being studied as a potential agent for the biological control of this weed. The laboratory life history and native range of *P. ichini* in Brazil were examined over 10 yr. The thrips life history includes 2 feeding larval stages that occur on the plant and 3 non-feeding pupal stages that occur in the soil. Development time, body length, and distinct features of each life stage are described. The larva-to-adult development required 20 d, and adults lived for an average of 50 d. *Pseudophilothrips ichini* had a wide latitudinal range in Brazil along the eastern coast from Bahia (11.4°S) south to Santa Catarina State (27.1°S). It was collected from sea level to 1,329 m elevation. Observations in Brazil indicated that this thrips occurs year round and may occasionally reach high densities (>20 thrips/leaf). Despite searches in its native range of related plants, the thrips was found only on Brazilian peppertree. Considering the short generation time, broad environmental tolerance, host specificity, and damage caused to the host if this thrips is released for biological control, it will contribute significantly to the management of Brazilian peppertree.

Key Words: Anacardiaceae; biological control of weeds; invasive species; *Schinus terebinthifolia*

Resumen

El pimentero brasileño, *Schinus terebinthifolia* Raddi (Sapindales: Anacardiaceae), es una de las malezas más invasivas de la Florida y Hawai. En las áreas invadidas, esta mala hierba de América del Sur de crecimiento rápido representa una amenaza para la agricultura y la producción de ganado y disminuye la biodiversidad de áreas naturales. Se está estudiando el trips *Pseudophilothrips ichini* (Hood) (Thysanoptera: Phlaeothripidae) como un agente potencial para el control biológico de esta maleza. Se examinaron por más de 10 años la historia de vida en el laboratorio y la área de distribución natural de *P. ichini* en Brasil. La historia de vida de los trips incluye 2 estadios de larvas que se alimentan y ocurren sobre las plantas y 3 estadios de pupas que no se alimentan y que ocurren en el suelo. Se describen el tiempo de desarrollo, la longitud del cuerpo y las características distintivas de cada estadio de vida. El desarrollo de larva a adulto requiere 20 dias, y los adultos vivieron por un promedio de 50 dias. *Pseudophilothrips ichini* tiene un amplio rango latitudinal en Brasil a lo largo de la costa oriental de la Bahía (11.4°S) al sur hasta Santa Catarina (27.1°S). Este fue recolectado desde el nivel del mar hasta 1329 m de altitud. Las observaciones en Brasil indicaron que este trips se presenta por todo el año y en ocasiones puede alcanzar altas densidades (>20 trips/hoja). A pesar de las búsquedas en su área de distribución natural de las plantas relacionadas, los trips se encontraron sólo en el pimentero brasileño. Tomando en cuenta el corto tiempo generacional, amplia tolerancia ambiental, la especificidad del hospedero, y los daños causados al hospedero si este trips se libera para el control biológico, contribuirá significativamente al manejo de pimentero brasileño.

Palabras Clave: Anacardiaceae; control biológico de malezas; especies invasivas; *Schinus terebinthifolia*

Brazilian peppertree, *Schinus terebinthifolia* Raddi (Sapindales: Anacardiaceae), native to South America, is one of the most aggressive and widespread invasive species in Florida and Hawaii impacting agriculture, cattle production, and natural areas (Ewel 1986; Rodgers et al. 2014). Brazilian peppertree is an opportunistic invader forming dense stands in disturbed and natural ecosystems of hardwood hammocks, pine flatwoods, pine rocklands, sawgrass marshes, and coastal mangrove forests (Ewel et al. 1982; Gordon & Thomas 1997; Spector & Putz 2006; Donnelly et al. 2008). Many of these habitats (e.g., coastal mangrove forests) are critically important ecosystems in Florida because of their high productivity, their value as habitat to vertebrate and invertebrate species, and their function in shoreline protection and stabilization. These vital ecosystems are constantly being threatened by urbanization and invasive species such as Brazilian peppertree (Armentano et al. 1995; Doren & Jones 1997). Brazilian peppertree infests more natural areas in Florida than any other invasive species (Gann et al. 2008). In Hawaii, Brazilian peppertree is widespread in mesic to dry areas (Motooka et al. 2003). There are few management options avail-

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able against Brazilian peppertree in these natural habitats. Previous attempts to control Brazilian peppertree have included fire, mowing, disking, rolling, chopping, and bulldozing (Doren & Whiteaker 1990; LaRosa et al. 1992). None of these techniques proved effective at slowing the spread of this invasive species (T. Pernas, Everglades National Park, Florida, personal communication). Currently, the main approach for controlling Brazilian peppertree in the U.S. is herbicidal (glyphosate or triclopyr), which is only a temporary measure (Bossard et al. 2000).

Biological control research for Brazilian peppertree began in Hawaii in 1954, continued between 1960 and 1961 (Yoshioka & Markin 1991), and has been conducted in Florida more recently (Hight et al. 2002; Wheeler et al. 2016). Three biological control agents were released in Hawaii, the seed feeder *Lithraeus atronotatus* (Pic) (Coleoptera: Chrysomelidae: Bruchinae), the leaf folder *Episimus unguiculus* Clarke (Lepidoptera: Tortricidae), and the stem borer *Crasimorpha infuscata* Hodges (Lepidoptera: Gelechiidae) (Davis & Krauss 1962; Krauss 1962, 1963; Yoshioka & Markin 1991). Despite the establishment of the first 2 species in Hawaii, their feeding has not sufficiently reduced the weed problem prompting the need for additional species (Yoshioka & Markin 1991; Julien & Griffiths 1998; Hight et al. 2002). No biological control releases have occurred on the U.S. mainland (Wheeler et al. 2016).

The thrips *Pseudophilothrips ichini* (Hood) (Thysanoptera: Phlaeothripidae) is being proposed as a biological control agent of the invasive weed Brazilian peppertree for Florida and Hawaii (Wheeler et al. 2016). This thrips species was first recorded on leaves of *S. terebinthifolia* near Rio de Janeiro, Brazil, and assigned to the genus *Liothrips* (Hood 1949). Several members of this genus have since been assigned to *Pseudophilothrips* (Johansen 1979). Previously published literature incorrectly applied the name *P. ichini* to a different species, *Pseudophilothrips gandolfoi* Mound, Wheeler, & Williams (Garcia 1977; Hight et al. 2002; Cuda et al. 2008, 2009). The identity of these 2 species was recently clarified through molecular and morphological techniques (Mound et al. 2010), and the name *P. ichini* is applied herein. Little is known about the biology or distribution of *P. ichini* in its native range. The goal of this study was to describe the life history of this thrips species and its native geographic range in Brazil in order to assess its suitability as a biological control agent of *S. terebinthifolia* in the U.S. mainland. Additional details about these field collections are provided here. Documentation about the quarantine host range of this thrips species and its value for biological control of Brazilian peppertree will be covered elsewhere.

Materials and Methods

FIELD COLLECTIONS

Field surveys in Brazil for potential biological control agents of Brazilian peppertree began in 2005 and continued through 2015. Brazilian peppertree was also intensively surveyed in the northeastern provinces of Argentina, and details regarding the results there were provided by Mc Kay et al. (2009). In Brazil, 20 surveys were conducted during this period documenting the distribution of the thrips as part of a broader project to describe the native range of Brazilian peppertree and associated herbivores. Surveys established over 900 sites (Fig. 1) typically lasted 15 d and included 2 to 3 collectors. Searches for and collections of thrips were conducted by visual inspection and by shaking tree branches and capturing fallen individuals below with a beating sheet. A subsample of individuals was quickly transferred to 95% ethanol for later morphological examination. Thrips field collections were most commonly characterized as either present or absent, and, occasionally, large populations were noted.

To examine the ecological host range of the thrips in their native range, adjacent plants, especially members of the Anacardiaceae, were also searched at each site. Members of the Anacardiaceae sympatric with Brazilian peppertree included several South American endemic *Schinus* and *Lithraea* species, *Anacardium occidentale* L. (cashew), and the introduced *Mangifera indica* L. (mango). The *Schinus* species that overlap with Brazilian peppertree include *S. molle* L.*, S. lentiscifolia* Marchand*, S. longifolia* (Lindl.) Speg.*, S. polygama* (Cav.) Cabrera*,* and *S. weinmannifolia* Engl*.* (Muñoz 2000; JBRJ 2015). Other species of sympatric Anacardiaceae include *Lithraea molleoides* (Vell.) Engl. and *L. brasiliensis* Marchand*.* Other species, such as *Anacardium humile* St.-Hil., *Astronium glaziovii* Mattick*, Astronium gracile* Engl., *Astronium graveolens* Jacq., *Myracrodruon urundeuva* Allemo, *Schinopsis brasiliensis* Engl., and *Tapirira guianenses* Aubl., occur in the native range of Brazilian peppertree in Brazil (JBRJ 2015) but were never found sympatric with the host.

THRIPS REARING CONDITIONS

Colony thrips were routinely reared on live Brazilian peppertree plants inside vented acrylic cylindrical cages (45 cm length × 15 cm diameter). The vent at the top of the cylinder was sealed with expanding foam to prevent thrips escape (Jones et al. 2010). Experimental thrips were reared in Petri dishes (9 cm diameter) under ambient conditions of temperature (27 ± 2 °C) and photoperiod (12:12 h L:D). To observe the thrips life stages, each Petri dish was lined with black filter paper (Rundfilter 551; Schleicher & Schuell, Dassel, Germany). The filter paper was moistened and the thrips were recorded daily to determine numbers and duration of each life stage. The thrips were fed a freshly collected leaf tip of Brazilian peppertree. Leaves were replaced as needed, generally every 2 to 3 d. As with other thrips (Lewis 1973), members of this species have 2 larval and 3 pupal stages, the latter defined as the pre-pupal, 1st pupal, and 2nd pupal stages.

THRIPS SLIDE MOUNTS

For detailed observations and measurements of thrips life stages, laboratory specimens were mounted on microscope slides and viewed at 7 to 100× magnification under a Leica MZ-16 stereomicroscope (ThripsWiki 2014). Briefly, specimens were soaked in a NaOH (2%) solution for 12 to 15 h at room temperature. Specimens were then transferred to clean water, where they were soaked for 1 h, and then to ethanol (60%) for 12 to 24 h. The thrips were dehydrated in a series of increased ethanol concentrations, 70% (1 h), 80% (20 min), 95% (10 min), 100% (5 min) and then transferred to clove oil for 30 min. To make microscope mounts, specimens were transferred to a drop of Canada balsam placed on a cover slip with the thrips' ventral side up. A microscope slide was firmly lowered onto each specimen in the Canada balsam on the cover slip. The slide was inverted and placed onto a hotplate (45 °C) for 3 wk.

Results

BRAZILIAN PEPPERTREE AND THRIPS GEOGRAPHIC RANGE

The geographic range of the host Brazilian peppertree and the thrips *P. ichini* were determined by frequent surveys in Brazil, Paraguay, and Argentina. The host plant naturally occurred along the eastern coast of Brazil from Natal in Rio Grande do Norte State, south to Entre Rios Province in northeastern Argentina and adjacent Paraguay (Fig. 1). In Bahia State, Brazilian peppertree extended west to near Feira de Santana, and in Minas Gerais State, populations extended west to

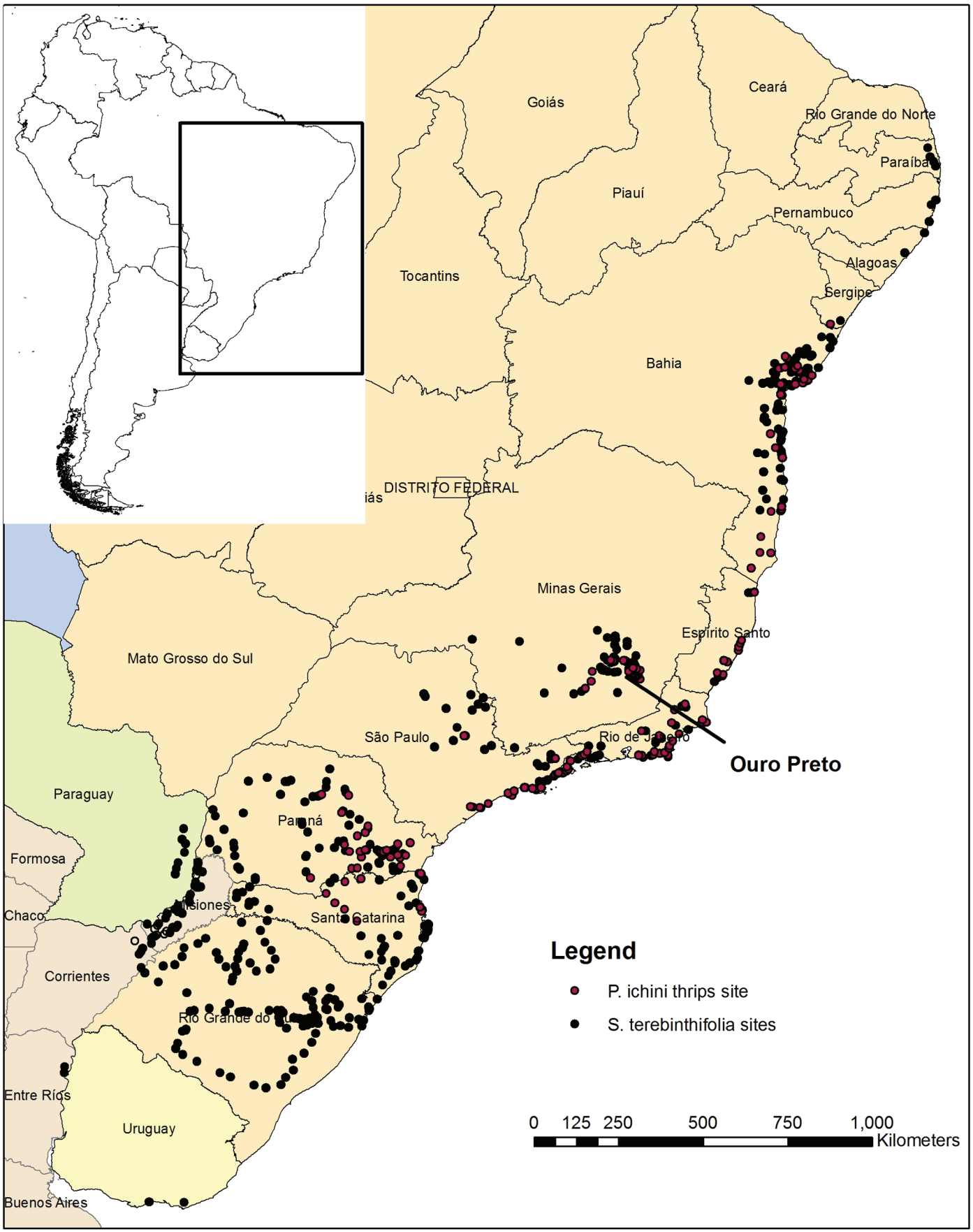

Fig. 1. Map showing the distribution of the host Brazilian peppertree, *Schinus terebinthifolia*, in its native range (black dots) and the thrips *Pseudophilothrips ichini* (red dots). Thrips introduced to quarantine for life history studies were collected from a population near Ouro Preto, Minas Gerais, Brazil.

Belo Horizonte then south to western Rio Grande do Sul, northeastern Argentina, and eastern Paraguay (Fig. 1).

Despite systematic searches, the thrips distribution did not entirely overlap that of the host. The thrips species was found in the Brazilian states Sergipe, Bahia, Espírito Santo, Rio de Janeiro, São Paulo, and Minas Gerais (Fig. 1). Sites ranged from sea level to 1,329 m elevation and from 11.42864 to 27.04962 south latitude. However, *P. ichini* was never found north of Sergipe or south of Santa Catarina states of Brazil. Furthermore, it was never found west of Paraná, Santa Catarina, or Rio Grande do Sul states of Brazil or in adjacent Argentina or Paraguay. The thrips population introduced under quarantine for subsequent life history studies was field collected from Brazilian peppertree leaves in Nov 2007. This collection occurred at a site (20.36911°S, 43.56029°W; 1,329 m elevation) near Ouro Preto, Minas Gerais State, Brazil (Fig. 1).

Thrips seasonal observations from Brazilian field surveys indicated that *P. ichini* was present year round, and its presence was influenced more by the availability of host flushing tips than by seasons. Thrips were present during every Brazilian survey conducted between 2005 and 2015, and these surveys occurred during every month of the year, except May. Thrips were not found at every site during every visit, but within the thrips' distribution, populations were found at 40% of the sites surveyed. Although generally only the presence or absence of thrips was recorded, high densities of thrips (>20 thrips/leaf) were occasionally observed during the austral spring, summer, and fall.

THRIPS LIFE HISTORY

The life history stages of *P. ichini* include egg, 2 larval stages, 3 pupal stages, and the adult (Fig. 2). The pupal stages include the prepupal, 1st pupal, and 2nd pupal stages. Only the larval and adult stages feed on the tips and leaves of Brazilian peppertree. The pupal stages are non-feeding resting stages that normally occur in the soil, whereas all other stages occur on the plant. A freshly laid egg measured 0.4 mm in length and required on average (± SE) 4.75 (± 0.2) d until larval hatch (*n* = 24 eggs from 10 females). The body length of the 1st and 2nd larval stages measured 0.7 and 1.0 mm, respectively, and their development required 5.0 (± 0.2) and 7.6 (± 0.4) d, respectively. The prepupal stage measured 1.6 mm in body length and required 1.3 (± 0.1) d for development. The 1st and 2nd pupal stages measured 1.9 and 2.1 mm, respectively, in body length and required 1.6 (± 0.1) and 3.6 (± 0.1) d, respectively, for development. Total development time from larval hatch to adult emergence was 20.0 (± 1.4) d. To avoid damaging the live adults, their gender was not determined. Adults measured 2.5 mm in body length and, when fed leaves, lived 50.0 (± 3.8) d. The larval stages were distinguished by the 2nd larval stage having a line (lacking in the 1st) on the last segment extending almost completely around the abdomen. Each pupal stage was distinguished in the following ways. The pre-pupa lacked wing buds and had greatly reduced antennae; the 1st pupal stage had wing buds, the antennae barely reached the abdomen, and the head was square shaped; and the 2nd pupal stage had longer wings extending to the abdomen, the antennae reached beyond the thorax, and the head was trapezoidal, wider at the distal end than the base.

THRIPS HOST RANGE IN SOUTH AMERICA

The only known published report of the host range of this thrips was from the original collection on leaves of Brazilian peppertree near Rio de Janeiro, Brazil (Hood 1949; Silva et al. 1968). Similarly, the results of our field host range assessment indicated a high degree of specificity; this thrips was found only on Brazilian peppertree. However, in the thrips' native range, few other natural populations of Anacardiaceae

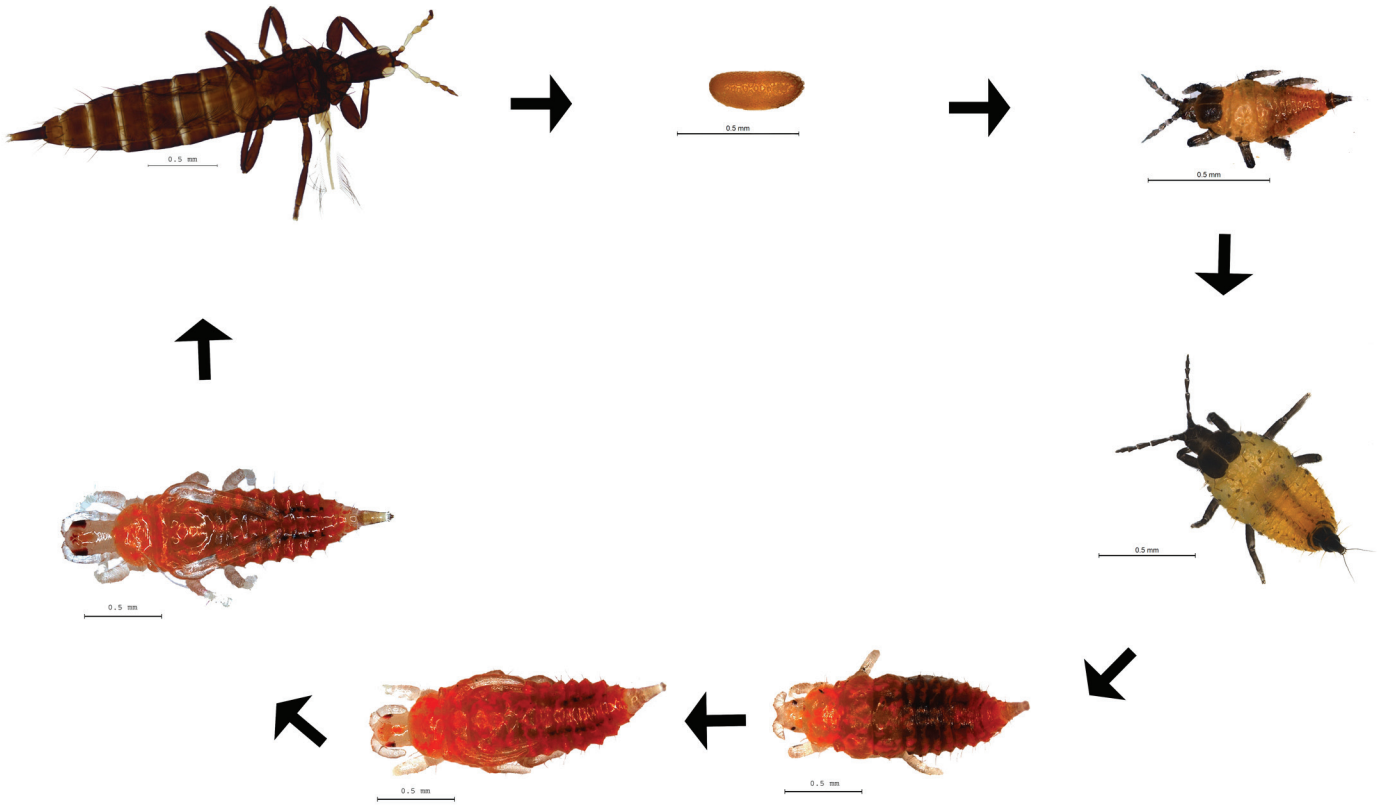

Fig. 2. Life history stages of the thrips *Pseudophilothrips ichini* reared on leaves of *Schinus terebinthifolia* in quarantine at the United States Department of Agriculture, Agricultural Research Service, Invasive Plant Research Laboratory (horizontal bars = 0.5 mm).

species co-occur. For example, the congener, *S. molle* was never found naturally at the same site with the thrips. However, ornamental plants of this non-target species were found and searched at 4 locations within the thrips range. These included 4 sites, 1 each in Paraná, Minas Gerais, Rio de Janeiro, and São Paulo states. The plants were visually inspected and shaken to collect insects as described in the methods, but thrips were never found on *S. molle*. Similar searches resulted in finding thrips on *Lithraea* species, but these were a different species of thrips as revealed by both morphology and DNA analysis (Wheeler et al., unpublished data).

THRIPS NATURAL ENEMIES IN SOUTH AMERICA

Although many insect predators, including ants and spiders, were seen abundantly on the host in Brazil, we never witnessed predation of any thrips life stage during these surveys. Collection of thrips larvae and rearing in quarantine indicated that very few parasitoids attack this stage. Collections of thrips larvae in Nov 2007 indicated that less than 5% of field-collected individuals (approximate *n* = 100 larvae) were attacked by the parasitoid *Thripastichus* sp. (Hymenoptera: Eulophidae). Additional collections made in Jun 2015 indicated that no thrips larvae (approximate *n* = 100 larvae) were parasitized. These parasitoids were excluded during quarantine colonization and have not been seen since this initial collection from Brazil.

Discussion

The species *P. ichini* was chosen as a potential biological control agent of Brazilian peppertree because 1) it appears to have a high level of specificity for the target weed (Wheeler et al., unpublished data); 2) it has wide environmental tolerance indicated by a broad climatic range as reported here and by Manrique et al. (2014); and 3) field observations and greenhouse studies indicate thrips feeding damage dramatically reduces survival, growth, and reproduction of the host (Manrique et al. 2014; Wheeler et al., unpublished data). Despite this apparent broad climatic tolerance, the thrips was not found in South America throughout the entire range occupied by the host Brazilian peppertree. Possibly the thrips' native range is limited by climate or host haplotype, and the tests of these hypotheses have yet to be published.

Thermal requirements and plant genotypes can be important factors in the establishment of newly introduced biological control agents (Ireson et al. 2008). Temperature-based physiological models indicated that *P. ichini* will establish throughout the invaded range in the U.S. (Manrique et al. 2014). Moreover, *P. ichini* is well adapted to the 2 Brazilian peppertree haplotypes and their hybrid that are invasive in Florida and Hawaii (Manrique et al. 2008).

Despite the thrips occasionally reaching high densities (>20 adults/leaf), they may be protected from predators by defensive allomones as is known from other thrips species (Blum et al. 1992; Suzuki et al. 2004). These defenses are characteristically produced in thrips when disturbed and can be recognized by the raised abdomen tip that secretes a droplet of repellent liquid (Lewis 1973). This same tip raising behavior was seen when we disturbed individuals of *P. ichini*. The only observed natural enemy of these thrips, namely, *Thripastichus* sp., was discovered during the initial field collection of larvae of *P. ichini*. The vulnerability of these thrips to natural enemies in the U.S. has yet to be determined.

Field observations indicated that this thrips was typically found feeding on the Brazilian peppertree's expanded flush leaves produced periodically at branch tips. Adults colonized first by feeding on the flush leaves, followed by egg deposition on the leaves, and 2 larval stages. Typically these attacked stems and tips produced leaves that are distorted and wrinkled eventually leading to death of the plant tip. Branches that had these dead tips were never seen flowering and thus did not produce fruit. If released in the Brazilian peppertree's invaded range, the thrips should have abundant food supply. Surveys conducted in south Florida indicated Brazilian peppertrees flush new leaves year round except late Oct and early Nov (Ewel et al. 1982). With abundant food supply and the short generation time (20 d), we expect the thrips biological control agent will contribute significantly to the control of this invasive weed.

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