United States
Department of
Agriculture

Marketing and
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Animal and
Plant Health
Inspection
Service

Field Release of the Insects *Calophya latiforceps* (Hemiptera: Calophyidae) and *Pseudophilothrips ichini* (Thysanoptera: Phlaeothripidae) for Classical Biological Control of Brazilian Peppertree in the Contiguous United States

Environmental Assessment, May 2019

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Contents

I. Purpose and Need for the Proposed Action

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Pests, Pathogens, and Biocontrol Permits (PPBP) is proposing to issue permits for environmental release of two insects, *Calophya latiforceps* (Hemiptera: Calophyidae) and *Pseudophilothrips ichini* (Thysanoptera: Phlaeothripidae). These agents would be used for the biological control of Brazilian peppertree, *Schinus terebinthifolia* (Anacardiaceae), in the contiguous United States.

This environmental assessment[1] (EA) has been prepared, consistent with USDA, APHIS' National Environmental Policy Act of 1969 (NEPA) implementing procedures (Title 7 of the Code of Federal Regulations (CFR), part 372). It examines the potential effects on the quality of the human environment that may be associated with the release of *C. latiforceps* and *P. ichini* to control infestations of Brazilian pepperweed within the contiguous United States. This EA considers the potential effects of the proposed action and its alternatives, including no action. Notice of this EA was made available in the Federal Register on February 27, 2019 for a 30-day public comment period. APHIS received a total of 129 comments on the EA by the close of the comment period. Most comments (120) were in favor of the release of the biological control agents. Nine comments were either not in favor of or raised concerns regarding the release of the two agents. These comments are addressed in appendix 7 of this document.

Classical biological control of weeds is a weed control method where natural enemies from a foreign country are used to reduce exotic weeds that have become established in the United States. Several different kinds of organisms have been used as biological control agents of weeds: insects, mites, nematodes, and plant pathogens. Efforts to study and release an organism for classical biological control of weeds consist of the following steps (TAG, 2016):

1. Foreign exploration in the weed's area of origin.
2. Host specificity studies.
3. Approval of the exotic agent by PPBP.
4. Release and establishment in areas of the United States invaded by the target weed.

[1] Regulations implementing the National Environmental Policy Act of 1969 (42 United States Code 4321 et seq.) provide that an environmental assessment "shall include brief discussions of the need for the proposal, of alternatives as required by section 102(2)(E), of the environmental impacts of the proposed action and alternatives, and a listing of agencies and persons consulted." 40 CFR § 1508.9.

5. Post-release monitoring.

APHIS has the authority to regulate biological control organisms under the Plant Protection Act of 2000 (Title IV of Pub. L. 106–224). Applicants who wish to study and release biological control organisms into the United States must receive PPQ Form 526 permits for such activities. The PPBP received permit applications requesting environmental release of two insects for the biological control of Brazilian peppertree, and the PPBP is proposing to issue permits for these actions. Before permits are issued, the PPBP must analyze the potential impacts of the release of these insects into the contiguous United States.

The applicants' purpose for releasing *C. latiforceps* and *P. ichini* is to reduce the severity of infestations of Brazilian peppertree in the contiguous United States. Brazilian peppertree is one of the worst invasive species in Florida (Schmitz et al., 1997). Rodgers et al. (2012) estimate that approximately 283,000 hectares of south and central Florida are invaded by Brazilian peppertree, and expenditures to control the tree by the South Florida Water Management District alone were approximately $1.7 million in 2011. In the same year, the Florida Fish and Wildlife Conservation Commission reported that nearly $7 million were spent by governmental agencies in Florida to control terrestrial invasive plants, including Brazilian peppertree (FWC, 2011).

Brazilian peppertree has been nominated as one of the 100 worst invasive species worldwide by the Global Invasive Species Database (2014). In Florida, Brazilian peppertree is listed as a noxious weed (FLDACS, 1999), a prohibited plant (FLDEP, 1993), and is classified as a Category I invasive plant species by the Florida Exotic Plant Pest Council (FLEPPC, 2009). This weed invades disturbed sites such as canal banks, fallow farmlands, and also natural communities including pinelands, hardwood hammocks, and mangrove forests (Cuda et al., 2006). Several attributes may contribute to its invasiveness, including a large number of drupes (fruits with thin skin and a central stone containing the seed) produced per female plant, an effective mechanism of dispersal by birds (Panetta and McKee, 1997), tolerance to shade (Ewel, 1978), fire (Doren et al., 1991), and drought (Nilsen and Muller, 1980a), allelopathic effects on neighboring plants (Gogue et al., 1974; Nilsen and Muller, 1980b; Morgan and Overholt, 2005; Overholt et al., 2012), and tolerance to saline conditions (Ewe, 2001; Ewe and Sternberg, 2002). The invasion and displacement of native species by Brazilian peppertree poses a serious threat to biodiversity in many ecosystems in Florida (Morton, 1978; Cuda et al., 2006).

Existing options for management of Brazilian peppertree, including chemical, mechanical, and physical control measures, have been used with some success against this weed, but permanent maintenance programs are required to prevent regrowth, which are costly, labor intensive, and may be detrimental to native vegetation (Koepp, 1978; Doren and Jones, 1997). For these reasons, the applicants have a need to release *C. latiforceps* and *P. ichini,* host-specific, biological control organisms for the control of Brazilian peppertree, into the environment.

II. Alternatives

This section will explain the four alternatives available to the PPBP—no action, issuance of permits for environmental release of *C. latiforceps*, issuance of permits for environmental release of *P. ichini,* and issuance of permits for environmental release of both *C. latiforceps* and *P. ichini* (preferred alternative). Although the PPBP's alternatives are limited to a decision on whether to issue permits for release of *C. latiforceps* and *P. ichini*, other methods available for control of Brazilian peppertree are also described. These control methods are not decisions to be made by the PPBP, and their use is likely to continue whether or not permits are issued for environmental release of *C. latiforceps* and *P. ichini*, depending on the efficacy of these insects to control Brazilian peppertree. These are methods presently being used to control Brazilian peppertree by public and private concerns.

A fifth alternative was considered, but will not be analyzed further. Under this fifth alternative, the PPBP would have issued permits for the field release of *C. latiforceps* and *P. ichini*; however, the permits would contain special provisions or requirements concerning release procedures or mitigating measures. No issues have been raised that would indicate special provisions or requirements are necessary.

A. No Action

Under the no action alternative, the PPBP would not issue permits for the field release of either *C. latiforceps* and *P. ichini* for the control of Brazilian peppertree. The release of these biological control agents would not take place. The following methods are presently being used to control Brazilian peppertree; these methods will continue under the "No Action" alternative and will likely continue even if permits are issued for release of *C. latiforceps* and *P. ichini*, depending on the efficacy of the organisms to control Brazilian peppertree. Chemical, mechanical, and physical control methods are employed to control Brazilian peppertree in Florida. Biological control of Brazilian peppertree in Hawaii is also discussed.

1. Chemical Control

Chemical control is the most common and cost-effective method employed for controlling Brazilian peppertree in Florida (Gioeli and Langeland, 1997; Randall, 2000; Langeland and Stocker, 2001;

Langeland, 2002). Foliar applications of triclopyr, glyphosate or imazapyr are usually employed to control seedlings (Gioeli and Langeland, 1997), and only approved products such as glyphosate or imazapyr can be used in aquatic systems (Langeland and Stocker, 2001). However, higher amounts of herbicides are needed during foliar applications. Basal soil applications of both hexazinone and tebuthiuron resulted in 80–90 percent mortality of Brazilian peppertree (Laroche and Baker, 1994). Basal bark applications of triclopyr ester were also effective, in particular during the fall when Brazilian peppertree is flowering due to the high level of translocation (Gioeli and Langeland, 1997). Cut-stumps treatments are also employed by cutting the trunk with a saw or machete and treating the stumps with herbicide (Gioeli and Langeland, 1997).

2. Mechanical Control

Mechanical control such as manual removal is often used to control Brazilian peppertree in low densities, and is particularly effective for small saplings. However, heavy equipment such as bulldozers, front end loaders, root rakes and other specialized equipment are needed in order to remove larger plants (DiTomaso et al., 2013). When using heavy equipment, the entire root system has to be removed in order to prevent resprouting (Doren et al., 1990; Dalrymple et al., 2003). Soil disturbance during mechanical control may favor Brazilian peppertree recolonization and therefore, other methods of control and restoration should follow.

3. Physical Control

Physical control has been also used to control Brazilian peppertree including soil removal, prescribed burning, and flooding (Randall, 2000). There have been mixed results from fire treatments; seed failed to germinate after burning but plants resprouted from the crowns and roots. Fire treatments were evaluated to control Brazilian peppertree infestations at the Everglades National Park, and repeated burning did not significantly decrease the rate of invasion which suggests that fire is not an appropriate management tool in this area (Doren et al., 1991). Brazilian peppertree seedlings are vulnerable to prolonged flooding (Ewel et al., 1982), while mature plants can survive long periods submerged and exhibit some tolerance to salinity (Ewe, 2001). The Sanibel Restoration Project illustrates the effects of flooding in managing Brazilian peppertree stands (Clark, 1999). A system of weirs was installed in 1995 at a cost of $4.5 million in order to maintain surface water at 3.2 feet above National Geodetic Vertical Datum (NGVD). The return of the hydrology to historical levels has stressed and killed some Brazilian peppertree plants at transitional areas and tropical hardwood hammock ridges.

4. Biological Control

Several biological control agents of Brazilian peppertree were released in Hawaii in the 1950s and 1960s including *Episimus unguiculus* Clarke (= *E. utilis* Zimmerman) (Lepidoptera: Tortricidae), *Lithraeus atronotatus* Pic (Coleoptera: Bruchidae), and *Crasimorpha infuscata* Hodges (Lepidoptera: Gelechiidae) (Julien and Griffiths, 1998). Despite the successful establishment of two of these agents, little impact has been reported on Brazilian peppertree populations in Hawaii (Yoshioka and Markin, 1991; Julien and Griffiths, 1998). In Florida, biological control efforts have been ongoing since the 1980s (Bennett and Habeck, 1991; Habeck et al., 1994; Cuda et al., 2006), but no agents have yet been released. Research on a defoliating sawfly that was shown to be highly host specific to Brazilian peppertree (Medal et al., 1999), was halted after the larvae were found to contain compounds toxic to mammals (Dittrich et al., 2004), and possibly birds. Other candidate agents performed poorly on Florida Brazilian peppertree (Manrique et al., 2008) or were shown to feed on related non-target plants during quarantine studies (Wheeler et al., 2011; McKay et al., 2012, Manrique et al., 2014).

B. Issue Permits for Environmental Release of *C. latiforceps*

Under this alternative, the PPBP would issue permits for the field release of the leaf galling psyllid, *C. latiforceps*, for the control of Brazilian peppertree. These permits would contain no special provisions or requirements concerning release procedures or mitigating measures.

Information about *Calophya latiforceps*

1. Taxonomy

The taxonomy of *Calophya latiforceps* Burckhardt follows the classification by Burckhardt and Ouvrard (2012):

Class Insecta
Division Neoptera
Order Hemiptera
Suborder Sternorrhyncha
Superfamily Psylloidea
Family Calophyidae Vondráček 1957
Genus *Calophya* Löw 1879
Species *Calophya latiforceps* Burckhardt 2011

Because *Calophya latiforceps* is a new species (Burckhardt et al., 2011), there are no synonyms or common names. However, the common name for species in the superfamily Psylloidea is "jumping plant-lice" (Burckhardt and Ouvrard, 2012).

2. Description of *C. latiforceps*

Trees containing galls of *C. latiforceps* can be recognized by yellowish foliage. Leaves with galls are pale yellow and open pit-galls are visible to the naked eye (Fig. 1a). Adults are bright yellow in coloration and are found on new flushes (Fig. 1b, c, d). The sex of the adults can be recognized by the shape of the tip of the abdomen (Fig. 1c, d, e). Eggs are laid along the margins and veins of new leaf flushes, and are white in color (less than 3 days) and turn black before hatching (Fig. 1f). Three days after crawlers settle, the cells around the nymphs turn yellow (Fig. 1g). Late instars are bright yellow (Fig. 2h) and secrete waxy droplets (Fig. 1i). When removed from the leaf, the fifth instar resembles a half-sphere (Fig. 1j). Upon adult emergence (Fig. 1k), empty pits can be observed on the leaf (Fig. 1l).

Voucher specimens of *C. latiforceps* originating from a colony maintained at the quarantine facility of University of Florida were deposited at the London Natural History Museum [accession: BMNH(E) 2013-24)] in April 2013, the United States National Museum Entomology Collection (accession: 2064054) in August 2013, and the Florida State Collection of Arthropods (accession: E2013-3192) in May 2013. Voucher specimens consisted of adults preserved in 70 percent ethyl alcohol and pinned adults.

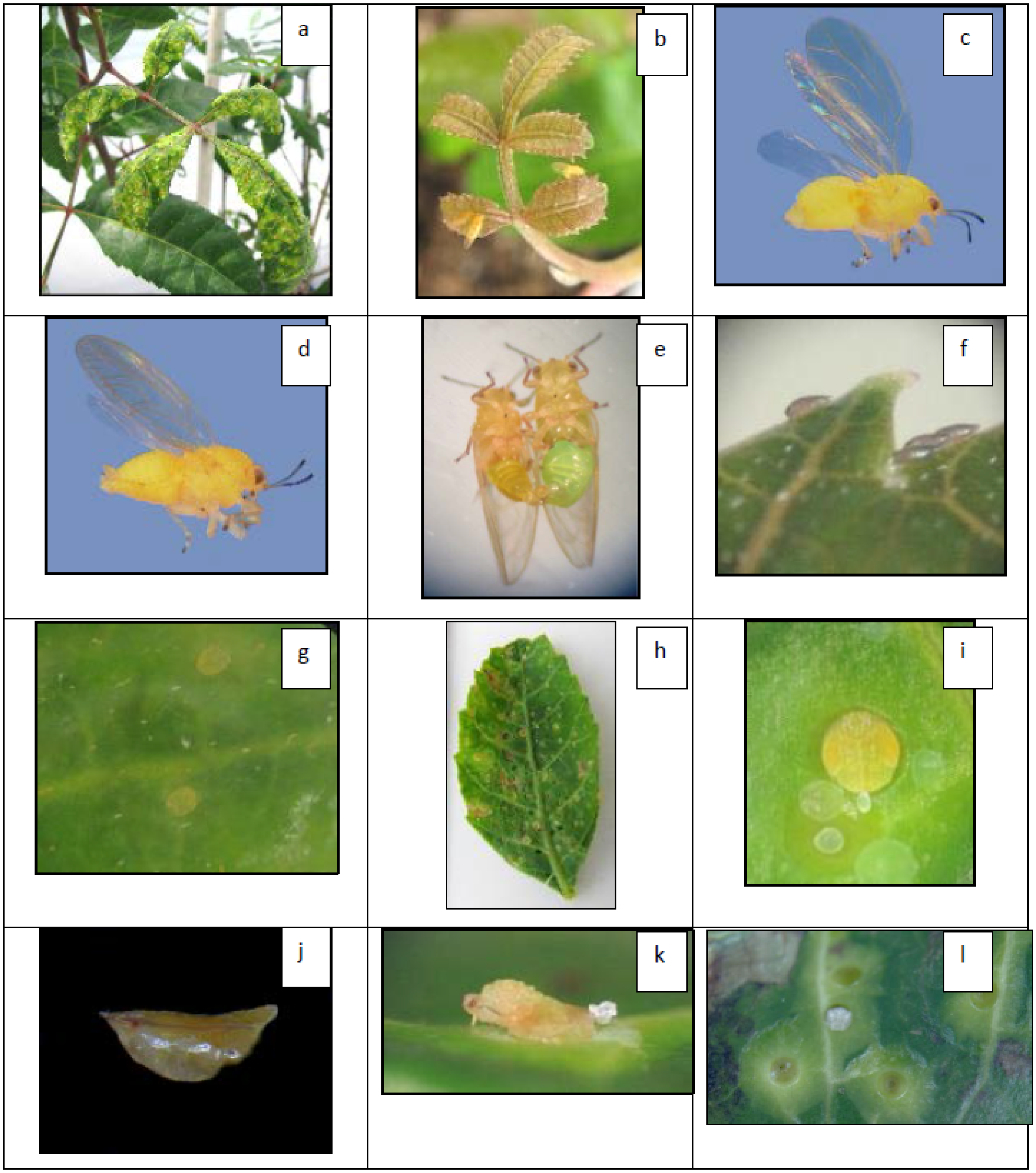

Figure 1. *Calophya latiforceps*: a) Leaf damage, b) adults on a new leaf flush, c) female, note the pointed abdomen, d) male, note the claspers, e) mating pair, male is smaller than female, f) eggs, g) crawlers, h) leaflet with nymphs, i) fifth instar on leaflet, j) fifth instar removed from gall, k) empty galls, l) adult emerging (Overholt et al., 2015).

3. Geographical Range of *C. latiforceps*

a. Native Range

Specimens in the genus *Calophya* have been collected in Africa, Europe, Oceania, and America. Based on the number of specimens of locality records, the Americas have the largest diversity of *Calophya* spp. (Burckhardt and Basset, 2000). *Calophya latiforceps* was found in the states of Bahia and Espirito Santo in Brazil during collecting trips conducted from 2010 to 2013 (Diaz et al., 2014). The distribution of *C. latiforceps* covers at least 954 kilometers (km) between the latitudes -12.32705°S and -20.91264°S.

b. Expected Attainable Range of *C. latiforceps* in North America

Based on cold tolerance studies, regions predicted to be suitable for *C. latiforceps* survival include Hawaii, Puerto Rico, peninsular Florida, southern Texas, and western Arizona, and California. Using this information and the current distribution of Brazilian peppertree, it is predicted that *C. latiforceps* can colonize all regions infested with Brazilian peppertree in North America.

3. Life History of *C. latiforceps*

The life history of *C. latiforceps* was described by Diaz et al. (2014). Newly emerged adults were pale green in coloration and remained inactive for approximately 30 minutes on the leaflet from which they emerged. On several occasions, adults were observed feeding directly on galls located on the abaxial side of the leaflet (the surface of a leaf that faces away from the stem) and several of these galls had live nymphs. Adults were poor fliers and mostly moved less than 30 centimeters (cm) in apparently random jumps. Groups of adults were observed feeding and searching for mates on new flushes, and mating occurred a few hours after emergence. Once a male found a female, both started walking side by side for a short distance, and mating lasted 3 to 5 minutes. Oviposition (egg laying) occurred mostly on new leaflets (less than 2 cm long); eggs were laid individually along the leaflet margins and veins as well as along leaf petiole). Adults lived in average for 9.3 ± 0.6 days; and females laid 85.8 ± 16.4 eggs (Diaz et al., 2014).

After eggs hatched, first instars (crawlers) walked slowly on the adaxial side of leaflets (the surface of a leaf that faces the stem) and settled after a couple of hours. Most of the crawlers settled on the same leaflet where the eggs were laid. Crawlers exhibited a strong preference for settling on the adaxial leaf surface (greater than 90 percent). No behaviors or morphological adaptations associated with long distance dispersal of crawlers, such as standing on hind legs, or presence of long legs and antennae, were observed (Gullan and Kosztarab, 1997). Twenty-four hours after settling, a yellow halo was noticeable around nymphs. Susceptible plants responded to nymphal feeding by forming a slight depression whereas resistant plants responded by killing the cells at the point of feeding. This latter response was noticeable after 2 or 3 days after nymphs settled, and resulted in 100 percent mortality of the psyllids. Immature survival and development time ranged from 11 to 75 percent (average 40 percent), and 35 to 53 days (average 38.6 days), respectively (Diaz et al., 2014).

C. Issue Permits for Environmental Release of *P. ichini*

Under this alternative, the PPBP would issue permits for the field release of *Pseudophilothrips ichini* for the control of Brazilian peppertree. These permits would contain no special provisions or requirements concerning release procedures or mitigating measures.

Biological Control Agent Information

1. Taxonomy

Phylum: Arthropoda
Class: Insecta
Order: Thysanopters
Suborder: Tubulifera
Family: Phlaeothripidae
Genus: *Pseudophilothrips*
Species: *Pseudophilothrips ichini* (Hood)

2. Description of *P. ichini*

Following the intial 1949 description of the *Liothrips ichini* thrips by Hood, it was later reassigned to a new genus *Pseudophilothrips* (Johansen, 1979). This new genus *Pseudophilothrips* represents a discrete New World, mainly Central and South American, lineage that was derived from the genus *Liothrips*. Prepared paratypes of *Pseudophilothrips ichini* are deposited in Departamento Entomologia, University of São Paulo, Luiz de Queiroz College of Agriculture; U.S. National Museum, Washington DC; Entomology Department, University of California, Riverside; Natural History Museum, London; Senckenberg Museum, Frankfurt; Australian National Insect Collection, Canberra. Additionally, voucher collections of these thrips are deposited in the Florida State Collection of Arthropods, Gainesville, FL. Quarantine collections were identified morphologically by Dr. L.A. Mound, Commonwealth Scientific and Industrial Research Organisation, Entomology, Canberra, Australia.

Individuals from *P. ichini* were characterized by molecular methods and these sequences are posted in National Center for Biotechnology Information, GenBank under accessions GU942812- GU942818. Briefly, to characterize these collections molecularly, a total of 589 thrips were sequenced at 410 base pairs of the mitochondrial cytochrome oxidase I gene using primers LCO1490 and HC02190, C1-J-1718 and C1-N-2191. Thrips were collected from 207 individual Brazilian peppertrees with a median number of two thrips collected from a single tree. This analysis indicated that there were six *P. ichini* thrips haplotypes from Brazilian field samples. More detailed methods are provided in Mound et al. (2010). Collections in quarantine were identified by molecular methods by Dr. D.A. Williams, Department of Biology, Texas Christian University, Fort Worth, TX.

3. Geographical Range of *P. ichini*

a. Native Range

The geographic range of the host and thrips was determined by frequent survey trips conducted by the researchers to Argentina, Brazil, Paraguay, and Uruguay during 2005 to 2014. These surveys were mostly concentrated in Brazil and ranged from Natal, Rio Grande do Norte, to Pelotas, Rio Grande do Sul states at the northern and the southern extremes of the known host plant range, respectively. Surveys also extended into Argentina, Paraguay, and Uruguay. These surveys covered the entire South American range of Brazilian peppertree.

In Bahia state the western range of the host extended to near Feira de Santana, and in Minas Gerais state, Brazilian peppertree populations extended west to Belo Horizonte then south to western Rio Grande do Sul and northeastern Argentina and eastern Paraguay. However, the thrips distribution did not entirely overlap that of the host. It was never found north of Sergipe or south of Santa Catarina states of Brazil. Furthermore, the thrips was never found west of Parana, Santa Catatina, or Rio Grande Do Sul states of Brazil in adjacent Argentina or Paraguay. As mentioned above, six thrips haplotypes were discovered in Brazil. Results presented here are only on the thrips haplotype number one collected near Ouro Preto, Minas Gerais, Brazil.

b. Expected Attainable Range of *P. ichini* in North America

Temperature-based physiological models indicated that *P. ichini* could establish throughout the Brazilian peppertree-invaded range in the United States (Manrique et al., 2014).

3. Life History of *P. ichini*

The life history stages of *P. ichini* include an egg, two larval stages, three pupal stages, and the adult (Fig. 2). The larval and adult stages only feed on the tips and leaves of Brazilian peppertree. The pupal stages are non-feeding, resting stages that occur in the soil, whereas all other stages occur on the plant. A freshly laid egg required on average (± SE) 5.5 (± 0.1) days to hatch. The first and second larval instars required 5.0 (± 0.3) days and 8.1 (±1.0) days, respectively followed by the pupal stages which required

6.3 (± 0.2) days. Total development time at 27°C from egg hatch to adult emergence was 20.0 (± 1.4) days. Thrips can complete development at temperatures ranging from 20 to 30°C (Manrique et al., 2014), which coincides with temperatures found in Florida and Hawaii. Physiological models based on cold tolerance suggest that *P. ichini* may establish throughout the geographical range of Brazilian peppertree in the United States (Manrique et al., 2014).

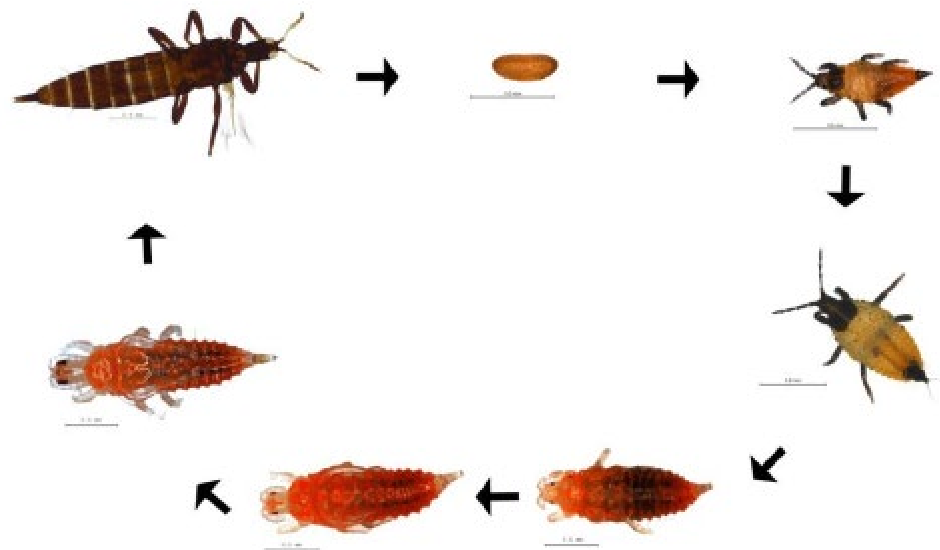

Figure 2. Life history stages of the thrips, *Pseudophilothrips ichini* reared on host leaves of *Schinus terebinthifolia* in quarantine at USDA-Agricultural Research Service, Invasive Plant Research Laboratory (IPRL) (horizontal bar = 0.5 mm) (Wheeler et al., 2014).

Thrips population dynamics observed from Brazilian field surveys indicate that this species is present year round and its densities are influenced more by the availability of host flushing tips than by seasons. Thrips were present during every Brazilian survey (n = 19) conducted between 2005 and 2014, and these surveys occurred during every month of the year, except May. These field observations indicated that this thrips will typically be found feeding on the Brazilian peppertree expanded flush leaves produced periodically at branch tips. Adults colonize first by feeding on the flush leaves, followed by egg deposition on the leaves, and two larval stages which feed preferentially on the stems of the growing tip. Typically these attacked stems and tips produce leaves that are distorted and wrinkled eventually leading to death of the plant tip. Branches that had these dead tips were never seen flowering which precludes reproduction of the attacked tissues. If released in its invaded range, the thrips should have abundant food supply. Surveys conducted in south

Florida indicated Brazilian peppertrees flush new leaves year round. The thrips are expected to reproduce year round especially in the weed's southern regions of the invaded range. With the short generation time (20 days), at least 12 generations per year are expected.

D. Issue Permits for Environmental Release of Both *C. latiforceps* and *P. ichini* (Preferred Alternative)

Under this alternative, the PPBP would issue permits for the field release of both *C. latiforceps* and *P. ichini* for the control of Brazilian peppertree, as described in alternatives B and C. These permits would contain no special provisions or requirements concerning release procedures or mitigating measures.

III. Affected Environment

A. Taxonomy of Brazilian Peppertree

Kingdom Plantae
Division Magnoliophyta
Class Dicotyledonae (Magnoliopsida)
Subclass Rosidae
Order Sapindales
Family Anacardiaceae
Subfamily Anacardioideae
Tribe Rhoeae
Genus *Schinus* L.
Subgenus *Euschinus*
Species *Schinus terebinthifolia* Raddi 1820

Schinus terebinthifolia has several common names including Brazilian peppertree, Brazilian pepper, Christmas berry (Hawaii and Guam), false pepper or faux poivrier (France), aroeira, aroeira negra, aroeira vermelha, aroeira da Minas, aroeira da praia, corneiba (Brazil), chichita (Argentina), copal (Cuba); pimienta de Brazil (Puerto Rico), and Florida holly (Morton, 1978; USDA-NRCS, 2002; Wunderlin and Hansen, 2008). Many synonyms of this species are from Brazilian collections and are over 100 years old. These include *Lithraea chichita*, *Sarcotheca bahiensis*, *Schinus aroeira*, *Schinus chichita*, and *Schinus mucronuleata* (Wunderlin and Hansen, 2008). The *Schinus* genus is endemic to South America where about 30 species are known from Ecuador to Patagonia (Pell et al., 2011).

Brazilian peppertree is a woody perennial shrub or small tree that can exceed 13 m in height and can live more than 30 years (Ewel et al., 1982). Leaves are alternate, 2.5–5 cm (1–2 inch) in length, elliptical, pinnately compound with 5–15 finely toothed leaflets. The plants are generally dioecious with either male or female white flower clusters that occur on separate trees. Flower pollination occurs primarily by insects. The glossy fruits (drupes) are borne in clusters that are initially green, becoming bright red when ripe. Seeds are individual within each fruit and brown in color. Flowering occurs predominantly in late fall to early winter in Florida with a second smaller pulse in late spring (Ewel et al., 1982).

B. Areas Affected by Brazilian Peppertree

1. Native Range of Brazilian Peppertree

The genus *Schinus* is native to Argentina, southern Brazil, Uruguay, Paraguay, Chile, Bolivia, and Peru (Barkley, 1944). The center of origin for the genus *Schinus* is northern Argentina (Barkley, 1944). Brazilian peppertree is native to Brazil, Argentina, Paraguay and Uruguay (Barkley 1944, 1957; Ewel 1986).

2. Introduced Range of Brazilian Peppertree

Since the late 1800s, Brazilian peppertree has been introduced as an ornamental plant into many tropical and subtropical regions including parts of Australia, New Zealand, the Bahamas, Bermuda, Fiji, Island of Mauritius, Kenya, Micronesia, New Caledonia, Reunion Island, South Africa, Asia, and Tahiti (Cuda et al., 2006; Scheffer and Grissell, 2003). In the United States, Brazilian peppertree occurs in Florida, Texas, California, Hawaii, the Commonwealth of Puerto Rico, the U.S. Virgin Islands (EDDMapS, 2014) and Georgia (Gray et al., 2009). In Florida, Brazilian peppertree is widely distributed from Monroe County in the south to St. Johns and Levy Counties in the north. In addition, it has been reported in Franklin County in the Panhandle (Wunderlin and Hansen, 2014) and Nassau County on the northeast coast (Salco, 2007). Brazilian peppertree is more abundant in south and central Florida due to its sensitivity to cold temperatures (Langeland and Burks, 1998). Ecological niche modeling suggests that cold tolerance may have increased since the introduction of Brazilian peppertree, which may explain the recent northward spread of this species (EDDmapS, 2014; Mukherjee et al., 2012).

3. Life History of Brazilian Peppertree

Brazilian peppertree is an evergreen perennial shrub or small tree that typically grows to a height of 3–7 meters (m), but can reach up to 13 m (Langeland and Burks, 1998; Cuda et al., 2006). Some individuals can live as long as 35 years (Hall et al., 2006). Brazilian peppertree is dioecious (having the male and female reproductive organs in separate individuals), and the main flowering period in Florida is from September to October with a much-reduced bloom occurring from March to May (Ewel et al., 1982). Small white flowers are borne in branching clusters of flowers near the end of branches. Flowers produce abundant amounts of pollen and nectar, and are primarily insect-pollinated. A large number of bright red drupes are typically produced on the plants from November to February. The drupes are eaten and dispersed primarily by birds and mammals, although some dispersal occurs by gravity or water (Ewel et al., 1982). Migrating robins (*Turdus migratorius*) consume numerous drupes of Brazilian peppertree and can disperse the seeds long distances from the parent tree. Seeds remain viable in the soil in Florida for 6 months (Ewel et. al., 1982) and in Australia for 9 months (Panetta and McKee, 1997). Seed germination occurs from November to April and seed viability ranges from 30–60 percent (Ewel et al., 1982). Seedlings are able to tolerate a broad range of extreme soil moisture conditions (Ewel, 1978), and survival of established seedlings ranges from 66–100 percent (Ewel et al., 1982). It has been reported that three-year old plants were capable of producing seeds (Ewel et al., 1982).

Brazilian peppertree often forms large monocultural stands displacing native vegetation in south and central Florida (Ewel 1986, Cuda et al. 2006). This species has invaded nearly every upland habitat type in the state including disturbed sites (e.g., canal banks, fallow farmlands, and along roadsides), and natural communities (e.g., pinelands, hardwood hammocks and mangrove forests) (Cuda et al., 2006). Several attributes may contribute to its invasiveness, including prolific seed production, seed dispersal by birds (Panetta and McKee, 1997), and tolerance to shade (Ewel, 1978), fire (Doren et al., 1991), drought (Nilsen and Muller, 1980a), and saline conditions (Ewe, 2001; Ewe and Sternberg, 2002). In addition to the massive production of drupes, Brazilian peppertree is capable of resprouting from above-ground stems and crowns after damage from cutting, fire, or herbicide treatment (Cuda et al., 2006). The ability of this species to tolerate a wide range of conditions makes it a superior competitor and enables it to invade a variety of habitats (Cuda et al., 2006). For example, abandoned farmlands enriched with phosphorous facilitated invasion of this weed in Everglades National Park (Li and Nordland, 2001). Brazilian peppertree can tolerate saline conditions and is also present in mangrove forests along shorelines (Ewe, 2001). Many invasive species, including Brazilian peppertree, produce allelopathic compounds which negatively affect the growth of neighboring plants (Gogue et al., 1974; Nilsen and Muller, 1980b; Morgan and Overholt, 2005; Overholt et al., 2012). For example, drupes of Brazilian peppertree reduced growth and biomass of two Florida native mangrove species (Donnelly et al., 2008).

C. Plants Related to Brazilian Peppertree and Their Distribution

There are no native species in the genus *Schinus* in the United States, although four *Schinus* species have been introduced historically into the continental United States: 1) *Schinus longifolius* (Lindl.) Spreg. in Texas, 2) *Schinus molle* L. in California, Florida, and Texas, 3) *Schinus* *polygamus* (Cav.) Cabrera in California, and 4) *Schinus terebinthifolia* in California, Florida, and Texas (Bargeron and Swearingen, 2010). Even though Barkley (1944) reported specimens of *S. longifolius* in Texas, later surveys were unable to detect its presence. Similarly, although *S. molle* was recorded in a single collection in Central Florida from 1931 (UNC Herbarium), it should be considered an historic introduction which did not establish. *Schinus polygamus* is considered a weedy species in southern California but little is known about the extent of its invasive status (Cal-IPC, 2007). Of the four *Schinus* species that have been introduced, only *S. molle* in California has ornamental value. However, the California Exotic Pest Plant Council has listed this species as a 'limited' category invasive species (Cal-IPC, 2006).

Members of the Anacardiaceae include the agricultural mango (*Mangifera indica* L.), pistachio (*Pistacia* spp.), and cashew (*Anacardium occidentale* L.) (Mitchell and Mori, 1987). Additionally, several native North American species include the sumacs (e.g., *Rhus* spp*)*, poison oaks (*Toxicodendron* spp.*)*, and species of the Neotropical mombin (*Spondias* spp.). Modern phylogenies have been compiled for several genera of the Anacardiaceae, including *Pistacia* (Al Saghir, 2010), *Rhus* (Yi et al., 2007), *Spondias* (Miller and Schaal, 2005), and *Toxicodendron* (Nie et al., 2009). However, the relationship among the genera of the family, e.g., which genera are most closely aligned with *Schinus*, remains uncertain (Pell, 2004).

The native geographic range of few of the Anacardiaceae species overlap that of Brazilian peppertree (USDA-NRCS, 2002; Wunderlin and Hansen, 2008). Those native, taxonomically related species that overlap with Brazilian peppertree include *Metopium toxiferum, Rhus copallina*, *R. sandwicensis* (Hawaii only), *Toxicodendron radicans*, *T. vernix*, *Spondias dulcis,* and *S. mombin* (both co-occur in Puerto Rico and Virgin Islands). Related native species with near or minimal overlap with the weed include *Cotinus obovatus*, *Pistacia mexicana*, *Rhus aromatica*, *R. glabra*, *R. michauxii*, *R. typhina*, and *Toxicodendron pubescens*. Related introduced species that overlap with Brazilian peppertree include *Anacardium occidentale* (Hawaii, Puerto Rico), *Mangifera indica* (Hawaii, Puerto Rico), *Schinus molle* (Puerto Rico), *Pistacia chinensis,* and *Spondias purpurea* (southwestern Florida, Puerto Rico, Virgin Islands). Related species that are introduced with near or minimal overlap with the weed include *Pistacia vera*.

The most important U.S. agricultural commodity in the Anacardiaceae is pistachio (*Pistacia vera*) which is cultivated primarily in California (98 percent of the U.S. acreage). This crop occupies 70,819 hectares (ha) (175,000 acres) and constitutes a $1 billion industry. California pistachios are the Kerman cultivar and with irrigation, grow in climates with long, dry, hot summers, low humidity and cool winters. Plants do not grow well in wet summer conditions. The primary area where pistachios are grown are deserts of the San Joaquin Valley of southern California. These include Kern, Madera, Kings, Fresno, Tulare, and to a lesser degree Tehama and San Bernardino counties. Other cultivated members of the Anacardiaceae include mango, *Mangifera indica* and cashew, *Anacardium occidentale*. Both mangoes and cashews are mostly imported into the United States (Evans, 2008; McLaughlin et al., 2013). However, they are cultivated in southern California, south Florida, Hawaii, Puerto Rico, and the Virgin Islands (USDA-NRCS, 2002).

IV. Environmental Consequences

A. No Action

1. Impact of Brazilian Peppertree

a. Wildlife and Domestic Animals

Dense stands of Brazilian peppertree shade out and may kill food plants used by the white-tailed deer (*Odocoileus virginianus* (Zimmerman)) in Florida Panther National Wildlife Reserve (Maffei, 1997). The decrease of the white-tailed deer may in turn affect the endangered Florida panther (*Felis concolor coryi* (Bangs)) because it serves as an important prey item. Brazilian peppertree is known to have toxic resins in the bark, leaves and fruits which may be poisonous to some mammals and birds (Lloyd et. al., 1977; Morton, 1978). Ingestion of the leaves and fruits can be fatal to grazing animals such as cattle and horses (Morton, 1978). The leaves and fruit of Brazilian peppertree are known to be toxic to wildlife. The resin in the bark, leaves, and fruit may be toxic to mammals and birds (Morton, 1978). Apparently some birds are unable to detect or avoid the toxins as mockingbirds, cedar-waxwings, and especially migrating robins in mid-winter, may feed heavily on the ripe fruits (Morton, 1978). Compounds in the fruit are known to have an intoxicating effect on migratory birds (Stahl et al., 1983).

b. Plants

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 and Thomas, 1997; Spector and Putz, 2006; Donnelly et al., 2008). Coastal mangrove forests are critically important ecosystems in Florida because of their high productivity, valued habitat to vertebrate and invertebrate species, and for shoreline protection and stabilization. This vital ecosystem is constantly being threatened by urbanization and invasive species such as Brazilian peppertree (Armentano et al., 1995; Doren and Jones, 1997). This species infests more natural areas in Florida than any other invasive species (Gann et al., 2008).

Invasive plant species that displace native vegetation such as Brazilian peppertree can also alter the habitat and modify the plant composition resulting in a new community structure (Gordon, 1998). As an example, Brazilian peppertree infestations result in increased soil development and elevation in shallow soil systems (Gordon, 1998). In addition, Brazilian peppertree grows rapidly and dominates the understory of unburned pine rocklands. Dense stands of this weed retain high moisture and reduce fire frequency which in turn affects pines and herbaceous species (Gordon, 1998; Stevens and Beckage, 2009). For example, in areas of the Everglades where fire had been suppressed, Brazilian peppertree comprised 40 percent of the trees of 2 m height and 66 percent of trees taller than 5 m (Loope and Dunevitz, 1981).

Allelopathy may exacerbate plant invasion by facilitating exotic species to outcompete natives and disrupt invaded habitat structure (Hierro and Callaway, 2003; Callaway and Ridenour, 2004). This mechanism occurs where plants produce compounds that kill or affect the growth or germination of associated plants. Leaves and fruit of Brazilian peppertree produce an unidentified compound(s) that reduces germination, growth, and leaf production of Florida native species (Morgan and Overholt, 2005; Donnelly et al., 2008).

c. Human Health

The leaves and fruit of Brazilian peppertree are known to be toxic to humans. The sap can cause dermatitis and edema in sensitive people and the resin in the bark, leaves, and fruit may be toxic to humans (Morton, 1978).

d. Beneficial Uses

Historically, Brazilian peppertree was introduced in Florida and Hawaii as an attractive ornamental and source for honeybee nectar (Morton, 1978). Beekeepers consider Brazilian peppertree a primary nectar producer in the fall, but other plant species bloom during that period and provide alternate nectar sources in Florida and Hawaii (Roddy and Arita-Tsutsumi, 1997; Ellis and Nalen, 2013). The dried fruits of Brazilian peppertree have been used as a spice for cooking and are sold in the United States and elsewhere (Wheeler et al., 2001). However, the ingestion of these fruits can be dangerous due to its toxic properties (Stahl et al., 1983). In South America, all parts of the tree have been used in traditional herbal medicines (Morton, 1978). For example, Brazilian peppertree can be used as an antioxidant, for wound healing, antitumor, and an antimicrobial among other uses (e.g., de Lima et al., 2006; Barbosa et al., 2007). Finally, Brazilian peppertree wood has little commercial value because of its low quality, small trunk size, and difficulty of harvesting due to the clumped plant structure (Morton, 1978). However, the wood has been used in toothpicks, posts, railway ties, and construction (Morton, 1978).

2. Impact from Use of Other Control Methods

The continued use of chemical, mechanical, physical, and biological controls at current levels would be a result if the "no action" alternative is chosen. These environmental consequences may occur even with the implementation of the biological control alternative, depending on the efficacy of either *Calophya latiforceps* or *Pseudophilothrips ichini* to reduce Brazilian peppertree populations in the contiguous United States.

a. Chemical Control

Chemical control is the most common method employed against Brazilian peppertree in Florida (Gioeli and Langeland, 1997; Randall, 2000). Even though successful control can be achieved, foliar and soil applications of herbicides have been reported to damage neighboring non-target plants (Gioeli and Langeland, 1997). For example, Arsenal® (imazapyr) is known to move through the soil causing leaf deformation in some native species.

Brazilian peppertree invades salt-tolerant communities such as mangrove forest along the shorelines in Florida (Doren and Jones, 1997; Rodgers et al., 2014). There are few management options available against Brazilian peppertree in these critically important habitats. Mangrove species are particularly vulnerable to foliar herbicide applications (Doren and Jones, 1997).

b. Mechanical Control

Heavy equipment is not suitable for sensitive natural areas such as mangrove communities where alternative control measures are required. Disturbance resulting from mechanical removal favors reestablishment of Brazilian peppertree (Doren and Jones, 1997).

c. Physical Control

There have been mixed results from fire treatments; seed failed to germinate after burning but plants resprouted from the crowns and roots of plants. Fire treatments were evaluated to control Brazilian peppertree infestations at the Everglades National Park, and repeated burning did not significantly decrease the rate of invasion, suggesting that fire is not an appropriate management tool in this area (Doren et al., 1991). Other physical methods of Brazilian peppertree control have been ineffective.

d. Biological Control

Three biological control agents were released in Hawaii, a seed feeder *Lithraeus atronotatus* Pic (Coleoptera: Bruchidae), leaf folder *Episimus unguiculus* Clarke; (Lepidoptera: Tortricidae), and a defoliator *Crasimorpha infuscata* Hodges (Lepidoptera: Gelechiidae) (Davis and Krauss, 1962; Krauss, 1962; Krauss, 1963; Yoshioka and Markin, 1991). Despite the establishment of the first two species in Hawaii, their feeding has not sufficiently reduced the weed problem (Yoshioka and Markin, 1991; Julien and Griffiths, 1998; Hight et al., 2002).

B. Issue Permits for Environmental Release of *C. latiforceps*

1. Impact of *C. latiforceps* on Nontarget Plants

Host specificity of *C. latiforceps* to Brazilian peppertree has been demonstrated through scientific literature and host specificity testing. If the candidate biological control agent only attacks one or a few closely related plant species, it is considered to be very host specific. Host specificity is an essential trait for a biological control organism proposed for environmental release.

a. Scientific Literature

Most insects that induce galls are specialists and are associated with a single plant species or only a few closely related hosts (Hardy and Cook, 2010). This narrow host range is explained by the intimate physiological interactions required for successful gall formation, which involves the control and redirection of the host plant resources to the advantage of the gall inducer (Shorthouse et al., 2005). Galls are energy sinks for the plant (Weis et al., 1988), causing a reduction in photosynthesis, stunted growth, and defoliation, which in turn reduces plant biomass and reproduction (Harris and Shorthouse, 1996; Florentine et al., 2005; Marini and Fernandes, 2012).

The host associations of species in the family Calophyidae show a high level of monophagy (feeding on one plant species) (Ouvrard, 2014). Species of Calophyidae are associated with hosts in the families Anacardiaceae, Boraginaceae, Burseraceae, Cunoniaceae, Fouquieriaceae, Meliaceae, Proteaceae, Rubiaceae, Rutaceae, Sapotaceae, Simaroubaceae and Theaceae. Among these, the highest diversity of calophyids is associated with the family Anacardiaceae (Ouvrard, 2014).

b. Host Specificity Testing

Host specificity tests are tests to determine how many plant species *C. latiforceps* attacks, and whether nontarget species may be at risk.

(1) Site of Quarantine Studies

Host specificity testing of *C. latiforceps* was conducted in Salvador City, State of Bahia, Brazil, and the Biological Control Research and Containment Laboratory, University of Florida, Fort Pierce, Florida.

(2) Population of the Insect Studied

The population proposed for initial release will be from the same colony used for the experiments in quarantine, which originated from several locations in the municipality of Salvador, Bahia.

(3) Test Plant List

Test plant lists are developed by researchers for determining the host specificity of biocontrol agents of weeds in North America. Test plant lists are usually developed on the basis of phylogenetic relationships between the target weed and other plant species (Wapshere, 1974). It is generally assumed that plant species more closely related to the target weed species are at greater risk of attack than more distantly related species.

The centrifugal phylogenic method (Wapshere, 1989) was used to select the plants to be tested. Dr. Dan Parfitt, a pistachio expert from the University of California, recommended the *Pistacia* spp. and cultivars which were tested. Most of the species tested belong to the family Anacardiaceae and included several known hosts of *Calophya* spp. In addition, special consideration was given to economically (crops) and ecologically important (threatened and endangered) species (Appendix 1). Plants were obtained from native or crop plant nurseries, Drs. Jim Cuda (University of Florida- Gainesville) and Greg Wheeler (USDA-Fort Lauderdale), and in a few cases, collected from wild populations. Three new *Pistacia vera* cultivars (Lost Hills, Golden Hills, and Randy) developed by the University of California/Davis were obtained from the Acemi Nursery in Kerman, California under permission from Foundation Plant Services at University of California - Davis. A rootstock, UCB1 was obtained directly from Foundation Plant Services, along with budwood of the parental species of UCB1 (*P. atlantica* and *P. integerrima*). The budwood was grafted on to UCB1 rootstock at the quarantine facility in Fort Pierce. *Schinus terebinthifolia* plants were grown from seeds collected in different areas in Florida, and were genetically characterized as haplotypes A, B, or hybrids (for more information, see Williams et al. 2005; 2007). Seeds or cuttings were sown in a germination soil mixture and maintained in a greenhouse. After the appearance of leaves, young plants were transplanted to 3.8 or 7.6 liter pots containing regular potting media and maintained in a screenhouse. All plants were fertilized once with 14 grams (g) of slow-release fertilizer (OsmocotePlus®, 15N-9P-12K), and every two months with 400 millileters (ml) of liquid fertilizer

(1g per liter of water) (Miracle Grow® 24N-8P-16K). Because *C. latiforceps* adults feed and oviposit on young leaf flushes, plants used for experiments had at least one flush of new growth. All experiments were conducted from October 2012 to November 2013 (see appendix 2 for details about host specificity testing).

(4) Discussion of Host Specificity Testing

Gall-forming insects, such as *C. latiforceps*, are known to be highly host specific due to their intimate relationships with their host-plants (Hardy and Cook, 2010). The no-choice and paired-choice experiments demonstrated that females laid significantly more eggs on Brazilian peppertree compared to non-target species. Nevertheless, females also laid eggs on non-target species in the families Anacardiaceae, Meliaceae, Zygophyllaceae, Sapindaceae, Hamamelidaceae, Rosaceae, and Fabaceae. Despite the close relationship with their host plants, non-target oviposition seems to be a common behavior among psyllids (Hodkinson, 2009). For example, psyllids evaluated as biological control agents of other plants, including *Prosopidopsylla flava* Burckhardt released for control of *Prosopis* spp. in Australia, *Boreioglycaspis melaleucae* Moore released in Florida against *Melaleuca quinqueneria* (Cav.) S. F. Blake, and *Aphalara itadori* Shinji released in the United Kingdom against *Fallopia* spp. completed development in only a few plant species, however, oviposition occurred in 57 of 58 species (van Klinken, 2000), 27 of 43 species (Wineriter et al., 2003), and 38 of 70 species (Shaw et al., 2011; Grevstad et al., 2013), respectively. Under field conditions, *C. latiforceps* is expected to oviposit preferentially on Brazilian peppertree. The oviposition on non-targets observed in the laboratory may be infrequent in nature because adults emerging on Brazilian peppertree trees most likely will remain and oviposit on their natal tree.

Upon egg hatching, crawlers of *C. latiforceps* settled, stimulated gall formation and completed development to adult only on Brazilian peppertree. There was no gall formation even on the congeners *Schinus polygamus* and *Schinus molle*, which are hosts of other *Calophya* species (Burckhardt and De Queiroz, 2012). This monophagy, especially at the nymphal stage, has been extensively documented in the genus *Calophya* from host rearing records from several continents (Burckhardt and Basset, 2000; Burckhardt and De Queiroz, 2012; Ouvrard, 2014) and for many species in the Psylloidea (Burckhardt et al., 2014). On non-target plants, all crawlers of *C. latiforceps* died in less than three days after hatching. Researchers observations suggest that the crawlers died due to starvation caused either by the lack of a feeding stimulus in some plants or by a hypersensitive host plant reaction in other plants (Overholt et al., 2015). These results suggest that if spillover oviposition occurs in the field, *C. latif*orceps crawlers do not pose a threat to non-target plants (Overholt et al., 2015).

Long distance dispersal of psyllid adults is usually by wind (Hodkinson, 2009), which would allow adults to potentially come in contact with non-target plants. In the adult survival experiment, most of the adults of *C. latiforceps* died by the third day of exposure on non-target plants, whereas adults were still alive after eleven days on Brazilian peppertree. Poor adult survival was observed even on the congeners *S. molle* and *S. polygamus*, strongly suggesting that the host range for adult feeding is restricted to Brazilian peppertree.

Due to the importance of pistachio production in the southwestern United States, several varieties/cultivars of *Pistacia* were tested, including *P. chinensis*, *P. texana*, *P. atlantica*, *P. intergerrima*, *P. vera* var. Kerman, *P. vera* var. Peters, *P. vera* var. Lost Hills, *P. vera* var. Golden Hills, *P. vera* var. Randy and UCB-1, a hybrid of *P. atlantica* and *P. integerrima.* Very few eggs were laid on the *Pistacia* species/cultivars, but most importantly, there was no gall formation and all first instars died.

2. Impact of *C. latiforceps* on Brazilian Peppertree

Damage by *C. latiforceps* reduced leaf performance and the growth of Brazilian peppertree. Under field conditions, high densities of galls are predicted to have an impact on biomass accumulation and eventually reproductive output. Under greenhouse conditions, plants with galls were 30 percent shorter, had more than 3 times greater leaf abscission, and 11 percent less biomass accumulation compared with plants without galls. Similarly, in a study in the native range, *C. terebinthifolii* (a leaf galling psyllid similar to *C. latiforceps*) was shown to reduce biomass accumulation of Brazilian peppertrees by 40 percent over a three month period (Vitorino et al., 2011). Prade et al. (2016) found that plants by *C. latiforceps* had lower photosynthesis compared to uninfested plants 30 and 45 days after gall initiation, and chlorophyll content was lower over a 70-day period. Plant height was reduced 31 percent and biomass 11 percent after three months of infestation (Prade et al., 2016). In the adventive range, the absence of coevolved natural enemies and higher host plant abundance are likely to favor rapid population growth and subsequent negative impacts to plant performance. Although it is not possible to predict with certainty the effect *C. latiforceps* will have on Brazilian peppertree in Florida, some indication may be provided by the accidental introduction of a congener of *C. latiforceps* into California. *Calophya schini* invaded California in the 1980s and caused major damage to its host plant *Schinus molle,* an exotic ornamental. The psyllid reached extremely high gall densities and resulted in severe defoliation of trees (Downer et al., 1988). Because of the severity of the problem, a classical biological control program was initiated with the release of a parasitoid from Chile (*Tamarixia schina* Zuparko) (Zuparko et al., 2011), which reportedly provided satifactory control of the psyllid (Kabashima et al., 2014).

3. Potential for Pathogen Transmission

The psyllid (Psylloidea) families Psyllidae and Triozidae include several members that transmit plant pathogenic bacteria (Hodkinson, 2009), including Phytoplasmas and Liberibacters (Weintraub and Beanland, 2006). Although no records of any plant pathogen transmission by species in the family Calophyidae were found, the researchers examined *C. calophya* and other *Calophya* spp. to determine whether plant pathogenic bacteria could be detected. First, specific primers were used to determine the presence of four liberibacters; L. solanacearum (Lso), L. asiaticus (Las), L. americanus (Lam) and L. africanus (Laf). All the tests were negative (Diaz et al., 2015a). The researchers went further and sequenced the 16S ribosomal RNA gene to more broadly examine bacteria associated with four populations of *Calophya* spp., including two populations of *C. latiforceps*. While this assay revealed many bacteria in samples of *Calophya* spp., no liberibacters or phytoplasmas were detected. The insects examined were from laboratory colonies maintained on Brazilian peppertree grown outside in Fort Pierce, Florida. Brazilian peppertree was not assayed for 16S ribosomal sequenes, but if the trees were infected with phytoplasmas or liberibacters, the bacteria were not taken up by the psyllids during feeding. Because the insect is monophagous (Diaz et al., 2014), the researchers were not able to rear them on plants known to be infected with liberibacters or phytoplasmas to determine whether transission is possible.

4. Uncertainties Regarding the Environmental Release of *C. latiforceps*

Once a biological control agent such as *C. latiforceps* is released into the environment and becomes established, there is a slight possibility that it could move from the target plant (Brazilian peppertree) to attack nontarget plants, such as *Schinus molle*. Host shifts by introduced weed biological control agents to unrelated plants are rare (Pemberton, 2000). Native species that are closely related to the target species are the most likely to be attacked (Louda et al., 2003). If other plant species were to be attacked by *C. latiforceps*, the resulting effects could be environmental impacts that may not be easily reversed. Biological control agents such as *C. latiforceps* generally spread without intervention by man. In principle, therefore, release of this biological control agent at even one site must be considered equivalent to release over the entire area in which potential hosts occur, and in which the climate is suitable for reproduction and survival. However, significant non-target impacts on plant populations from previous releases of weed biological control agents are unusual (Suckling and Sforza, 2014).

In addition, this agent may not be successful in reducing Brazilian peppertree populations in the contiguous United States. Worldwide, biological weed control programs have had an overall success rate of 33 percent; success rates have been considerably higher for programs in individual countries (Culliney, 2005). Actual impacts on Brazilian peppertree by *C. latiforceps* will not be known until after release occurs and post-release monitoring has been conducted (see appendix 3 for release protocol and post-release monitoring plan). However, it is expected that *C. latiforceps* will reduce the biomass and reproductive output of Brazilian peppertree.

5. Wildlife and Domestic Animals

The gradual reduction of Brazilian peppertree is expected to be beneficial to wildlife and domestic animals including white-tailed deer, the Florida panther, migratory birds, horses, and cattle. *Calophya latiforceps* is a plant-feeding insect and poses no risk to wildlife species.

6. Human Health

Reduction of Brazilian peppertree would be beneficial for human health by reducing the leaves, fruit, and sap of Brazilian peppertree that are known to be toxic to or cause dermatitis and edema in humans.

7. Beneficial Uses

Calophya latiforceps would reduce (but not eliminate) the presence of Brazilian peppertree in the environment; thus, it would still be available for beneficial uses, including honey production, herbal medicine, as a spice, and as an ornamental planting. However, it may cause damage to ornamental plantings of Brazilian peppertree.

8. Cumulative Impacts

"Cumulative impacts are defined as the impact on the environment which results from the incremental impact of the action when added to other past, present and reasonably foreseeable future actions regardless of what agencies or person undertakes such other actions" (40 CFR 1508.7).

Other private and public concerns work to control Brazilian peppertree in invaded areas using available chemical, mechanical, physical, and biological control methods. Release of *C. latiforceps* is not expected to have any negative cumulative impacts in the contiguous United States because of its host specificity to Brazilian peppertree. Effective biological control of Brazilian peppertree will have beneficial effects for Federal, State, local, and private weed management programs, and may result in a long-term, non-damaging method to assist in the control of Brazilian peppertree in the contiguous United States.

9. Endangered Species Act

Section 7 of the Endangered Species Act (ESA) and ESA's implementing regulations require Federal agencies to ensure that their actions are not likely to jeopardize the continued existence of federally listed threatened and endangered species or result in the destruction or adverse modification of critical habitat.

In the contiguous United States, there is one plant that is federally-listed in the family Anacardiaceae (Michaux's sumac (*Rhus michauxii*)), the same family as the target weed. Based on the host specificity of *C. latiforceps* reported in testing, field observations, and in the scientific literature, APHIS has determined that environmental release of *C. latiforceps* may affect, but is not likely to adversely affect Michaux's sumac or the

Everglade snail kite and its critical habitat. APHIS has also determined that *C. latiforceps* may affect beneficially, the Florida panther, Key deer, Florida scrub-jay, gopher tortoise, Bartram's hairstreak butterfly and its critical habitat, Florida leafwing butterfly and its critical habitat, Miami blue butterfly, Schaus swallowtail butterfly, and beach jacquemontia.

APHIS prepared a biological assessment and requested concurrence from the U.S. Fish and Wildlife Service on these determinations, and received a concurrence letter dated January 10, 2018.

C. Issue Permits for Environmental Release of *P. ichini*

1. Impact of *P. ichini* on Nontarget Plants

Host specificity of *P. ichini* to Brazilian peppertree has been demonstrated through scientific literature and host specificity testing.

b. Scientific Literature

The only known published report of the host range of *P. ichini* was from the original collection (originally described as *Liothrips ichini*) on leaves of Brazilian peppertree near Rio de Janeiro, Brazil (Hood, 1949; d'Araujo et al., 1968). Prior to the studies reported by the researchers, no formal quarantine host testing had been conducted on this thrips species. However, field host range was examined by the authors in its native range of Brazil during periodic surveys for prospective biological control agents. This effort focused, not only on the target weed, but on neighboring species, especially members of the Anacardiaceae that co-occur with Brazilian peppertree in its native range. These species included several South American endemic *Schinus* and *Lithrea* species, *Anacarium occidentale* (cashew), and the introduced *Mangifera indica* (mango). The *Schinus* species that overlap with Brazilian peppertree include *S. molle*, *S. lentiscifolius*, *S. longifolius*, *S. polygamus*, and *S. weinmannifolius* (JBRJ, 2011). The other species of the sympatric Anacardiaceae include *Lithrea molleoides*, and *L. brasiliensis*. A few other species, such as *Anacardium humile*, *Astronium glaziovii*, *Astronium gracile*, *Astronium graveolens*, *Myracrodruon urundeuva*, *Schinopsis brasiliensis*, *Tapirira guianenses*, and *Thrysodium spruceanum*, occur in the coastal region of Brazil (JBRJ, 2011), but were never found sympatric with Brazilian peppertree.

The results of this field host range assessment indicated a high degree of specificity where this thrips was never found on any species other than the target weed. However, it needs to be mentioned that the areas where the thrips occurs have few other natural populations of Anacardiaceae members. Although *S. molle* does not naturally occur in the area where thrips occur, ornamental *S. molle* plants were found and searched at four locations that also had thrips. These included four sites, one each in

Parana, Minas Gerais, Rio de Janeiro, and Sao Paulo states. The plants were visually inspected and shaken to collect insects as described above but thrips were never found on *S. molle*.

b. Host Specificity Testing

Host specificity tests are tests to determine how many plant species *P. ichini* attacks, and whether nontarget species may be at risk.

(1) Site of Quarantine Studies

Host specificity testing of *P. ichini* was conducted in Salvador City, State of Bahia, Brazil, and the Biological Control Research and Containment Laboratory, University of Florida, Fort Pierce, Florida.

(2) Population of the Insect Studied

The *P. ichini* population introduced under quarantine for testing was originally field collected from Brazilian peppertree leaves in November 2007. The original collection occurred at a site (-20.36911 latitude; -43.56029 longitude; 1,329 meter elevation) near Ouro Preto, Minas Gerais state in Brazil. Of the six thrips DNA variants found, this collection was identified by molecular techniques as haplotype number one. Upon arrival in the United States, *P. ichini* collection was divided between the two quarantine test facilities. All laboratory studies were conducted at these two quarantines from this single introduction. The identity of these colonies was confirmed with genetic analysis (Williams, D. Texas Christian University, unpublished data). The thrips were collected from a haplotype A Brazilian peppertree plant, one of the two parental lines that are invasive in Florida and Hawaii (Williams et al., 2007). Extensive molecular analysis described above indicated this thrips was one of the two main haplotypes of this species found in Brazil and was collected throughout much of the native range of the host. This same thrips haplotype was also found in Sergipe, Bahia, Espirto Santo, Rio de Janeiro, Sao Paulo and Minas Gerais states. Sites ranged from sea level to 1,329 meters elevation, from 11.43° to 24.30° south latitude. Field observations reported here were conducted throughout this geographic range.

(3) Test Plant List

The strategy for developing this test plant list generally followed that recommended by Wapshere (1974) with modifications described in Briese and Walker (2008). A test plant list for Brazilian peppertree was compiled using North American, Caribbean, and Mexican flora. In total, 116 species from 45 plant families from 33 plant orders were tested. These included mostly members of the order Sapindales to which the family Anacardiaceae is assigned. The researchers tested 23 species of the

Anacardiaceae and 60 species of the families of the Sapindales. Due to their economic importance and occurrence in the invaded range, four known and one unknown mango variety and the primary cultivated pistachio variety were tested (appendix 4).

(4) Discussion of Host Specificity Testing

The results of no-choice tests indicated that adults were produced on the congener of the target weed, *Schinus molle*. Seven of nine (78 percent) of the replicate plants of this species were accepted by the thrips. The number of adults produced on *S. molle* averaged 20.3, whereas 124.9 adults on were obtained on average from the Brazilian peppertree control. Adults were also produced on eight other species of the Anacardiaceae (*Cotinus coggygria, Malosoma laurina, Metopium toxiferum, Pistacea chinensis, Pistacea vera, Rhus glabra, Rhus sandwicensis,* and *Rhus typhina*) and one species outside the family (*Dodonaea viscosa*). These averages ranged from 0.4–5.5 thrips per plant. The percent of the replicate test plants accepted by the thrips ranged from 7–50 percent of the plants. The average number of adults produced when fed these test plants was generally less than 2, except for the 5.5 thrips produced on *Rhus sandwicensis*. Only one species outside the Anacardiaceae produced adults. This was *Dodonaea viscosa*, a member of the Sapindaceae, where 30 percent of the plants were accepted and an average of 2.8 adults was produced on the plants tested. Overall, feeding damage on these non-target species was negligible compared to the target weed that had dried stems and damaged leaves by the end of the experiment.

Those test plant species that produced adults in the no-choice tests above were then tested in choice tests. When given a choice, *P. ichini* preferred to feed and lay eggs on the target weed, resulting in high numbers of offspring produced. As is typically the case, choice tests indicated that the thrips host range was narrower when compared to no-choice tests. For example, nine test plant species produced progeny in no-choice tests while only four species produced progeny under choice testing (*Schinus molle, Metopium toxiferum, Rhus glabra,* and *Rhus sandwicensis*). The number of thrips adults produced on these non-target species was significantly lower than on the target weed. For example, an average of two adult thrips (range 0–12) were produced on only one of the six *S. molle* plants tested. Only one adult thrips was produced on only one plant of *M. toxiferum*. Similarly, three adult thrips were produced on a single plant of *R. glabra* and 1–4 thrips were produced on 4 plants of *R. sandwicensis*. These results suggest that the test plant species lack the ovipositional cues used by ovipositing females and that these non-target species are generally inadequate nutritionally for completion of thrips development.

Multiple generation tests indicated that *P. ichini* sustained a population for more than one generation only on the two weedy exotic species, the

Brazilian peppertree control and *S. molle*. For the remaining non-target species, none supported development of thrips past the first generation. The number of multiple generation replications ranged from 1 to 8 for different species. However, adults were successfully produced on at most three replicate plants of *M. laurina*, *R. glabra*, *R. typhina*, and *D. viscosa*. A single F_1 replicate was obtained for the remainder of the species, except none were obtained for *C. coggygria*. The results indicate however, that no larvae or adults were produced from the F_1 generation. For example, as many as 63 larvae were produced on *D. viscosa* and these matured into 16 adults but the adults failed to produce another generation of thrips. Though fewer F_1 larvae were obtained, the same final result was found for the other species tested.

A total of 116 plant species (and 5 varieties of mango) were tested as potential hosts of *P. ichini.* In no-choice and choice tests they demonstrated a high degree of specificity toward the target weed, Brazilian peppertree. There was minor use and a relatively small amount of reproduction in no-choice tests on several North American and Hawaiian plant species. However, significant F_1 thrips production was only found on the Brazilian congener *S. molle.* This species is an ornamental, and a close relative of the weed that has also become invasive in California and Hawaii (Nilsen and Muller, 1980a; Howard and Minnich, 1989). Most (seven of nine) of the replicate plants of this species were suitable hosts. However, relative to the controls, the average number of F_1 offspring produced on *S. molle* (20.3 F_1 adults) was 16 percent of that on the Brazilian peppertree control plants (124.9 F_1 adults). Choice data indicated that one of the six *S. molle* plants tested produced F_1 adults, but as before, few (average of 2 adults) F_1 adults were produced.

In Brazil the distribution of *Schinus molle* and the thrips do not overlap. *Schinus molle* occurs in the southern state of Rio Grande do Sul, south of the thrips natural range. Climatic differences in these regions probably influence these distributions as *S. molle* is adapted to more temperate, arid conditions while the thrips occupies more subtropical and tropical regions. In its invaded range, Brazilian peppertree occurs in the southwestern corner of California near San Diego, and *S. molle* occurs from the San Francisco area south to San Diego. If permission is granted to release the thrips and it disperses naturally to California it could establish on the Brazilian peppertree plants near San Diego. In the San Diego area, spill over may occur from infested Brazilian peppertrees onto neighboring *S. molle* trees. Considering ornamental *S. molle* was not observed to be damaged by thrips in Brazil it seems unlikely that this will happen. However, the host specificity test results suggest some thrips feeding and development could occur on *S. molle*. Together, the Brazilian field observations and quarantine choice data suggest that *S. molle* is rarely selected by the thrips under more natural conditions and the no-choice starvation results indicate that when this species is used, it is a poor host.

The remaining plants that produced F_1 adults in no-choice tests included *Cotinus coggygria*, *Malosma laurina, Metopium toxiferum*, *Pistacia chinensis*, *Pistacia vera, Rhus glabra*, *Rhus sandwicensis*, *Rhus typhina*, and *Dodonea viscosa*. The average number of F_1 adult thrips produced was never higher than 5.5 on any non-target plant (*R. sandwicensis*), compared with the average 124.9 thrips produced per plant on the control. Multiple choice testing further narrowed the number of non-target species that were used by the thrips. In these cases, generally only a single plant replicate of *M. laurina, M. toxiferum* and *R. glabra* produced less than 4 F_1 adults compared with an average of 56.5 F_1 adults produced on Brazilian peppertree. The choice test results showed that under these conditions the most-used test plant, besides *S. molle*, was the Hawaiian plant *R. sandwicensis* where 4 out of 9 of the plants were used. However, these plants produced few thrips, less than 5 adults per plant. In choice tests, no F_1 adult thrips were produced on *C. coggygria, M. laurina, P. chinensis*, *P. vera, R. typhina,* or *D. viscosa*.

These results suggest that, although the host range of the thrips is restricted to Brazilian peppertree, a small amount of non-target feeding may occur. However, these non-target species cannot sustain more than one generation and thus slight damage might occur to these species in the form of spill over from adjacent infestations on Brazilian peppertree. Of these plant species, such spill over will be restricted as the geographic distribution of only *D. viscosa*, *M. laurina, M. toxiferum*, and *R. sandwicensis* overlaps that of Brazilian peppertree. It must be noted that *R. sandwicensis* occurs on Hawaii, and *P. ichini* will not be released there. See appendix 5 for host specificity test design.

2. Impact of *P. ichini* on Brazilian Peppertree

Potential impacts of *P. ichini* on Brazilian peppertree are difficult to predict. Greenhouse studies showed that plant height and number of stems were reduced following thrips feeding, and plants were not able to recover after 2.5 months (Manrique et al., 2014). In its native Brazil and in the laboratory environment, feeding leads to plant tips wilting followed by tip death. These damaged tips will not flower or produce fruit until the plant can direct resources again to produce fresh foliage. As Brazilian peppertree is not known to reproduce vegetatively, decreasing the sexual reproduction will reduce the number of seeds, and thus, seedlings produced. This same type of damage occurred with the successful release of the *Melaleuca quinquenervia* biological control agent *Oxyops vitiosa* where tip damage by the agent prevented the weed's ability to regenerate and reinvade (Center et al., 2012). The thrips may also increase the plant's susceptibility to disease and abiotic stresses.

3. Uncertainties Regarding the Environmental Release of *P. ichini*

The uncertainties regarding release of *P. ichini* are the same as those for release of *C. latiforceps.* Actual impacts on Brazilian peppertree by *P. ichini* will not be known until after release occurs and post-release monitoring has been conducted (see appendix 6 for release protocol and post-release monitoring plan for *P. ichini*). However, it is expected that *P. ichini* will reduce plant height and number of stems of Brazilian peppertree. It may also reduce the number of seeds, and thus, seedlings produced.

5. Wildlife and Domestic Animals

The gradual reduction of Brazilian peppertree by *P. ichini* may be beneficial to wildlife and domestic animals including white-tailed deer, the Florida panther, migratory birds, horses, and cattle. *Pseudophilothrips ichini* is a plant-feeding insect and poses no risk to wildlife species.

6. Human Health

Reduction of Brazilian peppertree by *P. ichini* would be beneficial for human health by reducing the leaves, fruit, and sap of Brazilian peppertree that are known to be toxic to or cause dermatitis and edema in humans.

7. Beneficial Uses

Pseudophilothrips ichini would reduce (but not eliminate) the presence of Brazilian peppertree in the environment; thus, it would still be available for beneficial uses, including honey production, herbal medicine, as a spice, and as an ornamental planting. However, it may cause damage to ornamental plantings of Brazilian peppertree and to a lesser extent, *Schinus molle*.

8. Cumulative Impacts

"Cumulative impacts are defined as the impact on the environment which results from the incremental impact of the action when added to other past, present and reasonably foreseeable future actions regardless of what agencies or person undertakes such other actions" (40 CFR 1508.7).

Other private and public concerns work to control Brazilian peppertree in invaded areas using available chemical, mechanical, physical, and biological control methods. Release of *P. ichini* is not expected to have any negative cumulative impacts in the contiguous United States because of its host specificity to Brazilian peppertree. Effective biological control of Brazilian peppertree will have beneficial effects for Federal, State, local, and private weed management programs, and may result in a long-term, non-damaging method to assist in the control of Brazilian peppertree in the contiguous United States.

9. Endangered Species Act

Section 7 of the Endangered Species Act (ESA) and ESA's implementing regulations require Federal agencies to ensure that their actions are not likely to jeopardize the continued existence of federally listed threatened and endangered species or result in the destruction or adverse modification of critical habitat.

In the contiguous United States, there is one plant that is federally listed in the family Anacardiaceae (Michaux's sumac (*Rhus michauxii*)), the same family as the target weed. Based on the host specificity of *P. ichini* reported in testing, field observations, and in the scientific literature, APHIS has determined that environmental release of *P. ichini* may affect, but is not likely to adversely affect Michaux's sumac or the Everglade snail kite and its critical habitat. APHIS has also determined that *P. ichini* may affect beneficially, the Florida panther, Key deer, Florida scrub-jay, gopher tortoise, Bartram's hairstreak butterfly and its critical habitat, Florida leafwing butterfly and its critical habitat, Miami blue butterfly, Schaus swallowtail butterfly, beach jacquemontia, Everglades bully, Florida pineland crabgrass, pineland sandmat, and Florida prairie-clover.

APHIS prepared a biological assessment and requested concurrence from the U.S. Fish and Wildlife Service on these determinations, and received a concurrence letter dated January 10, 2018.

D. Issue Permits for Environmental Release of Both *C. latiforceps* and *P. ichini*

The environmental consequences would be the same as described under alternatives B and C. The main difference would be that the release of both agents that target different areas of the Brazilian peppertree plant may be more effective at controlling the target weed than if only one of the agents was released. The two species use different leaves to avoid competing directly with each other. *Pseudophilothrips ichini* attacks the youngest leaf tips while a major impact of *C. latiforceps* feeding is the premature abscission of older leaves (G. Wheeler, pers. comm., August 9, 2017).

V. Other Issues

Consistent with Executive Order (EO) 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-income Populations," APHIS considered the potential for disproportionately high and adverse human health or environmental effects on any minority populations and low-income populations. There are no adverse environmental or human health effects from the field release of *C. latiforceps* and *P. ichini* and will not have disproportionate adverse effects to any minority or low-income populations.

Consistent with EO 13045, "Protection of Children from Environmental Health Risks and Safety Risks," APHIS considered the potential for disproportionately high and adverse environmental health and safety risks to children. No circumstances that would trigger the need for special environmental reviews are involved in implementing the preferred alternative. Therefore, it is expected that no disproportionate effects on children are anticipated as a consequence of the field release of *C.*

latiforceps and *P. ichini*.

EO 13175, "Consultation and Coordination with Indian Tribal Governments," was issued to ensure that there would be "meaningful consultation and collaboration with tribal officials in the development of Federal policies that have tribal implications…."

APHIS is consulting and collaborating with Indian tribal officials to ensure that they are well-informed and represented in policy and program decisions that may impact their agricultural interests in accordance with EO 13175.

VI. Agencies, Organizations, and Individuals Consulted

The Technical Advisory Group for the Biological Control Agents of Weeds (TAG) recommended the release of *C. latiforceps* on April 8, 2016 TAG members that reviewed the release petition (15-02) (Overholt et al., 2015) included USDA representatives from the National Institute of Food and Agriculture, Animal and Plant Health Inspection Service, and U.S. Forest Service; U.S. Department of Interior's Bureau of Land Management and U.S. Fish and Wildlife Service; U.S. Army Corps of Engineers; and representatives from California Department of Food and Agriculture, Mexico Secretariat of Agriculture, Livestock, Rural Development, and Fisheries, and Food and Agriculture and Agri-Food Canada.

The TAG recommended the release of *P. ichini* on May 26, 2016. TAG members that reviewed the release petition (14-02) (Wheeler et al., 2014) included USDA representatives from the National Institute of Food and Agriculture, Animal and Plant Health Inspection Service, and U.S. Forest Service; U.S. Department of Interior's Bureau of Land Management and U.S. Fish and Wildlife Service; U.S. Army Corps of Engineers; and representatives from California Department of Food and Agriculture, Mexico Secretariat of Agriculture, Livestock, Rural Development, and Fisheries, and Food and Agriculture and Agri-Food Canada.

This EA was prepared by personnel at APHIS, University of Florida, and the USDA, Agricultural Research Service, Invasive Plant Research Laboratory. The addresses of participating APHIS units, cooperators, and consultants follow.

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Appendix 1. **Oviposition, plant response to crawler probing, and survival to adult of *Calophya latiforceps* on various plant species (Overholt et al., 2015).**

Scientific name	Common name	Importance	n	No. eggs/female	Plant response[1]	% survival[2]
Category 1 - Genetic types of the target weed species found in North America						
SAPINDALES						
Anacardiaceae						
Schinus terebinthifolia – Haplotype A	Brazilian peppertree	Invasive weed	7	6.2 ± 1.2a	Gall initiation	26.7 ± 0.1a
Haplotype B		Invasive weed	7	7.8 ± 1.5a	Gall initiation	34.2 ± 0.1a
Hybrids		Invasive weed	11	6.8 ± 1.0a	Gall initiation	29.2 ± 0.1a
Haplotype not determined		Invasive weed	10	8.3 ± 3.2	Gall initiation	not measured
Category 2 - North American species in the same genus as the target weed						
SAPINDALES						
Anacardiaceae						
Schinus molle L.	Peruvian peppertree	Invasive weed	12	0.5 ± 0.3b	Hypersensitive	0
Schinus polygamus (Cav.) Cabrera	Hardee peppertree	Introduced	10	0.6 ± 0.2b	Hypersensitive	0
Category 3 - North American species in other genera in the same family as the target weed						
SAPINDALES						
Anacardiaceae						
Anacardium occidentale L.	Cashew	Cultivated	10	0		
Cotinus coggyria Scop.	European smoke tree	Introduced	10	0		
Cotinus obovatus Raf.	American smoke tree	Endangered	6	0		

Appendix 1 (cont.)

Mangifera indica L.	Mango	Cultivated	10	0.2 ± 0.2b	No response	0
Malosma laurina (Nutt.) Nutt. Ex Abrams	Laurel sumac	Native	6	0		
Metopium toxiferum (L.) Krug & Urb.	Florida poisontree	Native	10	0		
Pistacia texana Kunth	American pistachio	Native	10	0		
Pistacia chinensis Bunge	Chinese pistache	Introduced	10	0		
Pistacia atlantica Desf.[/3]	Mt. Atlas mastic tree	Introduced	10	0		
Pistacia integerrima J. L. Stewart[/3]	Zebrawood	Introduced	10	0.003 ± 0.003b	No response	0
Pistacia atlantica × *Pistacia integerrima* (UCB1)[/3]	Pistacio	Cultivated	4	0.16 ± 0.05b	No response	0
Pistacia vera L. var Kerman[/3]	Pistachio	Cultivated	12	0.31 ± 0.22b	Hypersensitive	0
Pistacia vera L. var Peters[/3]	Pistachio	Cultivated	4	0.16 ± 0.03b	No response	0
Pistacia vera L. var. Lost Hills[/3]	Pistachio	Cultivated	10	0		
Pistacia vera L. var. Golden Hills[/3]	Pistachio	Cultivated	5	0.63 ± 0.46b	No response	0
Pistacia vera L. var. Randy[/3]	Pistachio	Cultivated	10	0.008 ± 0.008b	No response	0
Rhus aromatica Aiton	Fragrant sumac	Native	11	1.4 ± 0.8b	No response	0
Rhus copallinum L.	Winged sumac	Native	10	0.3 ± 0.3b	Hypersensitive	0
Rhus sandwicensis A. Gray	Neneleau	Native (HI, USA)	10	0.1 ± 0.1b	No response	0
Rhus typhina L.	Stoghorn sumac	Native	10	0.2 ± 0.2b	No response	0
Spondias dulcis Parkinson	Jewish plum	Introduced	10	0		
Spondias purpurea L.	Purple mombin	Introduced	10	0		
Toxicodendron radicans (L.) Kuntze	Eastern poison ivy	Native	10	2.7 ± 1.0b	Hypersensitive	0

Toxicodendron pubescens Mill.	Poison oak	Native	10	0		
Category 4 - Threatened and endangered species in the same or closely-related families to the target weed						
SAPINDALES						
Anacardiaceae						
Comocladia dodonaea (L.) Urb.	Poison ash	Native	10	0		
Rhus glabra L.	Smooth sumac	Native	10	0.5 ± 0.3b	No response	0
Rhus michauxi Sarg.	False poison sumac	Native	10	0		
Toxicodendron vernix (L.) Kuntze	Poison sumac	Native	10	0.1 ± 0.1b	No response	0
Hippocastanaceae						
Aesculus pavia	Red buckeye	Threatened	4	0		
Staphyleaceae						
Staphylea trifolia L.	American bladdernut	Native	6	0		
Meliaceae						
Sweetenia mahogani (L.) Jacq.	Mahogany	Native	6	0.1 ±0.1b	No response	0
Sapindaceae						
Hypelate trifoliata Sw.	White ironwood	Native	4	0		
Simarougaceae						
Leitneria floridana Chapman	Corkwood	Native	4	0		
Zygophyllaceae						
Guaiacum sanctum L.	Hollywood lignum vitae	Native	4	0.1 ± 0.1b	No response	0
MAGNOLIALES						

Magnoliaceae						
Magnolia virginiana L.	Sweetbay	Native	4	0		
Category 5 - North American species in other families in the same order that have some phylogenetic, morphological, or biochemical similarities to the target weed						
SAPINDALES						
Aceraceae						
Acer rubrum L.	Red maple	Native	4	0		
Acer sacharidum L.	Silver maple	Native	4	0		
Sapindaceae						
Litchi chinensis Sonn.	Lychee	Introduced	4	0.1 ± 0.1b	No response	0
Sapindus saponaria L.	Soapberry	Native	4	0		
Burseraceae						
Bursera simaruba (L.) Sarg.	Gumbo limbo	Native	4	0		
Simaroubaceae						
Simarouba glauca DC	Paradise tree	Native	4	0		
CELASTRALES						
Aquifoliaceae						
Ilex cassine L.	Dahoon holly	Native	4	0		
Category 6 - North American species in other orders that have some morphological or biochemical similarities to the target weed						
ASTERALES						
Asteraceae						
Ambrosia trifida L.	Giant ragweed	Native	4	0		

Solidago rugosa Mill.	Goldenrod	Native	4	0
ARIALES				
Apiaceae				
Daucus carota L.	Carrot	Introduced, cultivated	4	0
ERICALES				
Ericaceae				
Arctostaphylos densiflora M.S. Baker	Vine hill manzanita	Endangered	4	0
FAGALES				
Betulaceae				
Alnus serrulata (Aiton) Willd.	Hazel alder	Native	4	0
CORNALES				
Nyssaceae				
Nyssa sylvatica Marshall	Blackgum	native	4	0
Fagaceae				
Quercus virginiana Mill.	Live oak	Native	6	0
DIPSACALES				
Adoxaceae				
Sambucus nigra L.	Black elderberry	Native	4	0
EUPHORBIALES				
Euphorbiaceae				
Chamaesyce hyssopifolia (L.) Small	Hyssopleaf sand mat	Native	4	0

Euphorbia pulcherrima Willd. ex Klotzsch	Poinsettia	Introduced, cultivated	4	0		
HAMAMELIDALES						
Hamamelidaceae						
Hamamelis virginiana L.	Witch hazel	Native	6	0.1 ± 0.1b	No response	0
JUNGLANDALES						
Juglandaceae						
Carya glabra (Mill.) Sweet	Pignut hickory	Native	4	0		
MYRTALES						
Combretaceae						
Langucularia racemosa (L.) C.F. Gaertn.	White mangrove	Native	4	0		
Myrtaceae						
Eucalyptus camaldulensis Dehnh.	Rose gum	Introduced	4	0		
Eugenia uniflora L.	Surinam cherry	Introduced	4	0		
Eugenia axillaris (L.) C.F. Gaertn.	White stopper	Native	4	0		
THEALES						
Theaceae						
Gordonia lasianthus (L.) J. Ellis	Loblolly bay	Native	4	0		
URTICALES						
Ulmaceae						
Ulmus alata Michx.	Winged elm	Native	4	0		

LAMIALES						
Verbenaceae						
Vitex agnus-cactus L.	Lilac chastetree	Native	4	0		
MYRICALES						
Myricaceae						
Myrica cerifera (L.) Small	Wax myrtle	Native	5	0		
PRIMULALES						
Myrsinaceae						
Ardisia escallonioides Schltdl. & Cham.	Marlberry	Native	4	0		
ROSALES						
Rosaceae						
Prunus caroliniana (Mill.) Aiton	Cherry laurel	Native	4	0.1 ± 0.1b	No response	0
Crataegus spathulata Michx.	Hawthorn	Native	5	0		
Category 7 - Plants not closely related to weed, which have agricultural significance and are grown in same range as the weed in North America						
ARALES						
Araceae						
Alocasia macrorrhizos (L.) G. Don	Giant taro	Cultivated	4	0		
ASTERALES						
Asteraceae						
Lactuca sativa L.	Lettuce	Cultivated	4	0		
BROMELIALES						
Bromeliaceae						

Ananas comosus (L.) Merr.	Pineapple	Cultivated	4	0		
EUPHORBIALES						
Euphorbiaceae						
Manihot esculenta Crantz	Cassava	Cultivated	4	0		
SAPINDALES						
Rutaceae						
Citrus aurantifolia (Christm.) Swingle	Lime	Cultivated	4	0		
Citrus × *sinensis* (L.) Osbeck (pro sp.)	Sweet orange	Cultivated	4	0		
[*maxima* × *reticulata*]						
LAMIALES						
Verbenaceae						
Tectona grandis L. f.	Teak	Cultivated	4	0		
FABALES						
Fabaceae						
Arachis hypogaea L.	Peanut	Cultivated	4	0		
Phaseolus vulgaris L.	Pinto bean	Cultivated	4	0		
Vigna unguiculata (L.) Walp	Cowpea	Cultivated	4	0.1 ± 0.1b	No response	0
MALVALES						
Malvaceae						
Abelmoschus esculentus (L.) Moench	Okra	Cultivated	4	0		
Gossypium hirsutum L.	Upland cotton	Cultivated	4	0		
Hibiscus rosa-sinensis L.	Shoeblackplant	Cultivated	4	0		

Verbenaceae				
Clerodendrum L.	Glorybower	Cultivated	4	0
Vitex agnus-castus L.	Chaste tree	Cultivated	4	0
RUBIALES				
Rubiaceae				
Coffea arabica L.	Arabian coffee	Cultivated	4	0
SOLANALES				
Convolvulaceae				
Ipomoea batatas (L.) Lam.	Sweet potato	Cultivated	4	0
Solanaceae				
Capsicum annuum L.	Bell pepper	Cultivated	4	0
Solanum melongena L.	Eggplant	Cultivated	4	0
Lycopersicon esculentum Miller	Tomato	Cultivated	4	0
BRASSICALES				
Brassicaceae				
Brassica oleracea L.	Broccoli/cauliflower	Cultivated	4	0
MAGNOLIAES				
Lauraceae				
Persea americana Mill.	Avocado	Cultivated	4	0
POALES				
Poaceae				
Zea mays L.	Corn	Cultivated	4	0

Appendix 1 (cont.)

Oryza sativa L.	Rice	Cultivated	4	0
Saccharum officinarum L.	Sugar cane	Cultivated	4	0
VIOLALES				
Caricaeae				
Carica papaya L.	Papaya	Cultivated	4	0
GINKOALES				
Ginkoaceae				
Ginkgo biloba L.	Maidenhair tree	Introduced	4	0
PROEALES				
Proateaceae				
Macadamia integrifolia Maiden & Betche	Macadamia	Cultivated	4	0
ZINGIBERALES				
Musaceae				
Musa sp.	Banana	Cultivated	4	0

[1]Response to host probing: 'no response' means no visible response, 'hypersensitivity' means necrosis was visible at point of crawler feeding.

[2]Based on the number of adults emerged from two tagged leaves per plant.

/[3]Forty insects were released on these plant species/varieties.

Appendix 2. **Host-specificity testing methods (Overholt et al., 2015).**

The process of host-plant colonization by gall-forming psyllids involves two critical life history adaptations; oviposition on rapidly growing plant tissues, and the ability of first instars (crawlers) to stimulate gall production in the host plant (Hodkinson 2009, Rohfritsch 1992). Using no-choice and paired-choice experiments, the researchers determined: 1) which plant species were suitable for adult oviposition, gall initiation and complete immature development, 2) whether females exhibited ovipositional preference, and 3) the survival of adults on non-target plants.

No-choice oviposition and gall formation.

The objective of this experiment was to determine the plant species acceptable for adult oviposition, and subsequently nymphal establishment and gall formation. All plant species identified in the plant list were tested, along with *S. terebinthifolia* haplotypes A, B, and intraspecific hybrids. Due to the economic importance of pistachio production in the western United States, several cultivars of *P. vera,* a hybrid of two *Pistacia* species used as a rootstock, and the parents of the rootstock were tested. Twenty adults from the colonies were released in mesh cages (61×61×91 cm) containing one potted plant, except for some of the *Pistachio* species/cultivars on which 40 adults were released in order to increase the rigor of the test. Because *C. latiforceps* has an average sex ratio of 1:1 (females: males) (Diaz et al. 2014), it was assumed that each release included 10 females and 10 males, or 20 males and 20 females in cases where 40 insects were released. After 3 days, adults were removed with an aspirator and the total number of eggs per plant was counted using a 30× magnifying hand lens. Ten days after oviposition, the plant reaction to crawler feeding was classified as: 1) gall initiation with the crawler embedded on the leaf surface, 2) host rejection with the presence of a brown necrotic spot at the point of feeding (indicative of a hypersensitive response), and 3) no signs of hypersensitity or crawler settling. Hypersensitivity is a defense mechanism where plant cells necrose at and around the point of damage by a foreign organism (Fernandes, 1990). Upon gall initiation, survival to adult was followed on two tagged leaves per plant. These leaves had at least five nymphs (galls) and were selected haphazardly.

Adult emergence was confirmed 45 days after oviposition by the presence of exuvia on the galls. Plant species were tested in groups based on the availability of young shoots and insects, and in all cases at least one *S. terebinthifolia* plant was included as control. The number of replicates per plant species was ten for the Anacardiaceae and four for other plant families. The total number of eggs per plant species per female was compared using one-way analysis of variance for those species on which oviposition occurred; species without eggs were not included in the analysis. Post-hoc mean comparisons were conducted using Tukey-Kramer HSD method (JMP v. 10, SAS software). Survival to adult on each *S. terebinthifolia* haplotype was compared using one-way ANOVA (arcsin transformation) and means are presented as untransformed values.

Appendix 3. Release Protocol and Post-Release Monitoring Plan for *C. latiforceps* (Overholt et al., 2015).

Release Protocol

The researchers propose to release *Calophya latiforceps* Buckhardt (Hemiptera: Calophyidae) adults from a laboratory colony maintained at the UF/IFAS Biological Control Research and Containment Laboratory in Fort Pierce, Florida. This population originated from several field locations in the municipality of Salvador, Bahia. To maintain clean and healthy colonies, a staged rearing method was used where a new plant was inoculated with adults every week. Cages were labeled with name of the species and the inoculation date. After adult emergence or 40–60 days after inoculation, all the leaves of the plant were removed and the plant was allowed to recover for 60 days. The identification of the species maintained in the colonies has been confirmed on several occasions by experts in Switzerland and Florida, and more recently by sequencing the cytochrome oxidase, subunit 1 gene (Diaz et al., 2015b). Voucher specimens of *C. latiforceps* originating from the University of Florida colony were deposited at the London Natural History Museum [accession: BMNH(E) 2013-24)] on April 2013, the United States National Museum Entomology Collection (accession: 2064054) on August 2013, and the Florida State Collection of Arthropods (accession: E2013-3192) on May 2013.

Samples collected in Brazil contained two nymphal parasitoids (Diaz et al., 2014). To start a parasitoid-free colony, all parasitoids that emerged from the samples were removed. Additionally, all shipments of nymphs originating in Brazil were kept in a separate room. Because of these containment measures, there has never been any contamination by parasitoids in the *Calophya* colonies in more than three years.

The mass rearing and release process will start by establishing outdoors field insectaries at the Biological Control Research and Containment Laboratory. The specific location for the insectaries will be: 7930 Pruitt Research Road, Fort Pierce, FL 34945. *Calophya latiforceps* adults will be released in walk-in screen cages containing potted saplings of Brazilian peppertree. This mass rearing procedure will ensure the production of large numbers of adults. One hundred adults will be placed in plastic vials containing a shoot of Brazilian peppertree. Vials will be placed in a cooler and transported to field release sites. To secure initial colonization, adults will be released inside cages covering branches of Brazilian peppertree. After 10 days, the cage will be removed.

Releases of *C. latiforceps* will be conducted initially in Florida. Adults will be first released at six baseline monitoring sites established by Dr. Paul Pratt at the USDA-ARS Invasive Plant Research Laboratory in Fort Lauderdale, Florida, and at two sites established at the University of Florida Institute of Food and Agriculture Sciences (UF/IFAS) research stations in Fort Pierce and Immokalee. Demographic data on plant performance has been continuously collected from the USDA sites from 2006 to 2014, and data collection at the UF/IFAS sites began in November, 2014 (Fig. A). The initial number of adults released per site will be approximately 1,000. The releases will be conducted from May to September when new leaf flushes are typically plentiful.

Once enough data is gathered on establishment rates and plant impact in Florida, the researchers will work with authorities in Texas to determine their interest in releases.

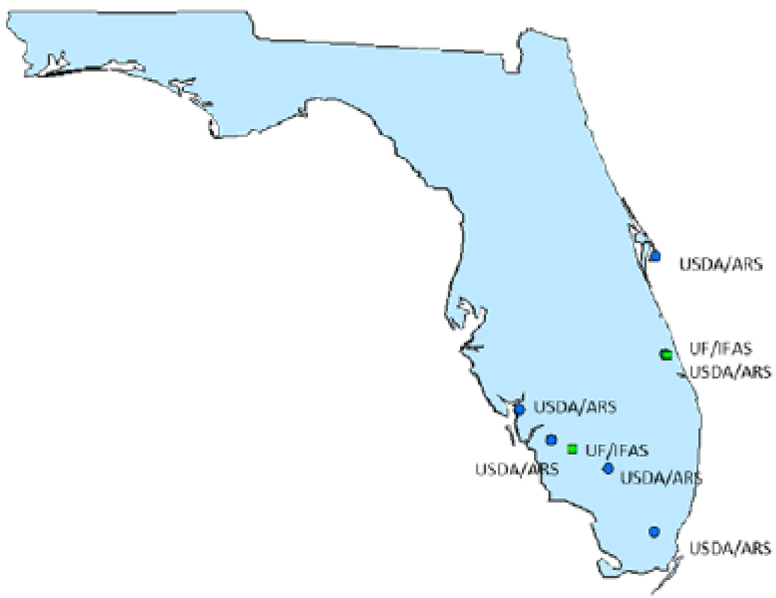

Figure A. Sites selected for initial releases of *C. latiforceps* in Florida (Overholt et al., 2015).

Post-Release Monitoring

To determine if *C. latiforceps* is established in Florida, gall formation will be monitored on sentinel plants located at each field site. Branches with eggs will be tagged and the fate of the eggs will be monitored over time. The presence of large galls and the signs of exuvia from the last instars will be considered successful plant colonization. Establishment will be defined as the recovery of adults after two winters from first release. After four years, establishment will be evaluated at random locations in Florida with the assistance of county agents. Sampling will occur along linear transects through patches of Brazilian peppertree to monitor the presence or absence of *C. latiforceps* galls. By selecting random locations, the rates of establishment and spread of the biological control agent across Florida will be documented (see Overholt et al., 2009 for methods).

The impact of *C. latiforceps* on Brazilian peppertree will be measured at long-term monitoring plots at the campuses of the University of Florida in Fort Pierce (St. Lucie Co.), and Immokalee (Collier Co.). These plots were planted in November, 2014, and include insecticide protected and unprotected plants. The purpose of these plots is twofold. First, before the release of the agent, these plots will allow the impact of extant herbivores and diseases on plant performance to be measured. Second, by following the performance of these trees after the releases, the direct impact of the biological control agents will be quantified. Plant and insect data will be collected every trimester to monitor plant growth and insect feeding.

Even though host range tests strongly suggest that *C. latiforceps* can establish permanent populations only on Brazilian peppertree, non-target plants in the family Anacardiaceae will be monitored. Populations of *Rhus copallinum* (sumac) and *Mangifera indica* (mango) will be identified in Florida. The presence or absence of gall formation will be evaluated from June to August.

Appendix 4. North American and Caribbean species of Sapindales and other test plants that are native or introduced and were tested against the thrips *Pseudophilothrips ichini*, prospective biological control agents of Brazilian peppertree. Listed Anacardiaceae species compiled from Pell et al. (2011). The remaining species names and their distributions from USDA-NRCS (2002) and Wunderlin and Hansen (2008).

Spp	Order	Family	Sub-Family	Species [1]	Authority	Common Name	Distribution [2]	Status [3]	Listed species US T/E [4]	Listed species State T/E [5,6,7]
Category 1: Genetic types of the target weed <u>species</u>										
1	Sapindales	Anacardiaceae	Anacardioideae	*Schinus terebinthifolia*	Raddi	Brazilian Peppertree	CA, FL, HI, TX, PR, VI	I		
	Three invasive genotypes of *S. terebinthifolia*, A, B, and hybrid were found to be suitable hosts of *P. ichini* (Manrique et al. 2008).									
Category 2: Species in the same (or closely related) <u>genus</u> as the target weed, including environmentally and economically important										
2	Sapindales	Anacardiaceae	Anacardioideae	*Schinus molle*	L.	Peruvian Peppertree	AZ, CA, FL, HI, TX, PR	I		
Category 3: Species in other genera in the same <u>family</u> as the target weed, divided by Subfamily and tribes, including environmentally and economically important										
3	Sapindales	Anacardiaceae	Anacardioideae	*Anacardium occidentale*	L.	Cashew	PR, VI	I		
4	Sapindales	Anacardiaceae	Anacardioideae	*Comocladia dodonaea*	(L.) Urb.	Poison ash	PR, VI	N		
5	Sapindales	Anacardiaceae	Anacardioideae	*Cotinus coggygria*	Scop.	Eurasian Smoketree	NE-US, UT	I		
6	Sapindales	Anacardiaceae	Anacardioideae	*Malosma larina*	(Nutt.) Nutt. Ex Abrahms	Laurel sumac	CA	N		
7	Sapindales	Anacardiaceae	Anacardioideae	*Mangifera indica*	L.	Mango	FL, HI, PR, VI	I		
8	Sapindales	Anacardiaceae	Anacardioideae	*Mangifera indica*-Carrie	L.	Mango	FL, HI, PR, VI	I		
9	Sapindales	Anacardiaceae	Anacardioideae	*Mangifera indica*-Common	L.	Mango	FL, HI, PR, VI	I		
10	Sapindales	Anacardiaceae	Anacardioideae	*Mangifera indica*-Haden	L.	Mango	FL, HI, PR, VI	I		
11	Sapindales	Anacardiaceae	Anacardioideae	*Mangifera indica*-Ice Cream	L.	Mango	FL, HI, PR, VI	I		
12	Sapindales	Anacardiaceae	Anacardioideae	*Metopium toxiferum*	(L.) Krug & Urb.	Poisonwood	FL, PR	N		
13	Sapindales	Anacardiaceae	Anacardioideae	*Pistacia chinensis*	Bunge	Chinese Pistacio	SE-US, TX, CA	I		
14	Sapindales	Anacardiaceae	Anacardioideae	*Pistacia mexicana*	Kunth	American Pistacio	TX	N		
15	Sapindales	Anacardiaceae	Anacardioideae	*Pistacia vera*-Kerman	L.	Pistacio nut	CA	I		
16	Sapindales	Anacardiaceae	Anacardioideae	*Rhus aromatica*	Aiton	Fragrant Sumac	NW-FL, E-US	N		

									Listed species	
									US	State
Spp	Order	Family	Sub-Family	Species [1]	Authority	Common Name	Distribution [2]	Status [3]	T/E [4]	T/E [5,6,7]
17	Sapindales	Anacardiaceae	Anacardioideae	*Rhus copallina*	L.	Winged Sumac	E-US	N		
Category 3 (continued)										
18	Sapindales	Anacardiaceae	Anacardioideae	*Rhus glabra*	L.	Smooth Sumac	US, NW-FL	N		
20	Sapindales	Anacardiaceae	Anacardioideae	*Rhus sandwicensis*	A. Gray	Neneleau	HI	N		
21	Sapindales	Anacardiaceae	Anacardioideae	*Rhus typhina*	L.	Staghorn sumac	SE-US, not FL	N		
22	Sapindales	Anacardiaceae	Anacardioideae	*Toxicodendron pubescens*	Mill.	Atlantic Poison Oak	NW-FL, E-US	N		
23	Sapindales	Anacardiaceae	Anacardioideae	*Toxicodendron radicans*	(L.) Kuntze	Eastern Poison Ivy	E-US	N		
25	Sapindales	Anacardiaceae	Spondioideae	*Spondias dulcis*	Parkinson	Jewish plum	PR	N		
26	Sapindales	Anacardiaceae	Spondioideae	*Spondias mombin*	L.	Yellow Mombin	PR,VI	N		
27	Sapindales	Anacardiaceae	Spondioideae	*Spondias purpurea*	L.	Purple Mombin	FL, PR, VI	I		
Category 4: Threatened and endangered species in the same family as the target weed										
6	Sapindales	Anacardiaceae	Anacardioideae	*Cotinus obovatus*	Raf	American Smoketree	SE-US, not FL	N		Protected in TN
19	Sapindales	Anacardiaceae	Anacardioideae	*Rhus michauxii*	Sarg.	Michauxii's Sumac	SE-US [7]	N	Endang-ered	Protected in FL, GA, NC
24	Sapindales	Anacardiaceae	Anacardioideae	*Toxicodendron vernix*	(L.) Kuntze	Poison Sumac	E-US, TX	N		Endangered in KY
Category 5– North American or introduced species in other families that have some phylogenetic, morphological, or biochemical relationship to the target weed, economically and environmentally important plants										
28	Sapindales	Aceraceae	N/A	*Acer rubrum*	L	Red maple	E-US	N		
29	Sapindales	Aceraceae	N/A	*Acer saccharinum*	L	Silver Maple	US & Canada	N		
30	Sapindales	Burseraceae	N/A	*Bursera simaruba*	(L.) Sarg.	Gumbo limbo	FL, PR,VI	N		
31	Sapindales	Hippocastanaceae	N/A	*Aesculus pavia*	L.	Red Buckeye	E-US	N		
32	Sapindales	Meliaceae	N/A	*Aglaia odorata*	Lour.	Chinese Perfume Plant	HI	I		
33	Sapindales	Meliaceae	N/A	*Azadirachta indica*	A. Juss.	Neem	HI, PR	I		
34	Sapindales	Meliaceae	N/A	*Entandrophragma caudatum*	Sprague	Mountain Mahogany	HI	I		
35	Sapindales	Meliaceae	N/A	*Khaya senegalensis*	(Desr.) A. Juss.	African mahogany	FL, HI, PR	I		
36	Sapindales	Meliaceae	N/A	*Lansium domesticum*	Correa	Langsat	HI	I		
37	Sapindales	Meliaceae	N/A	*Sandoricum koetjape*	(Burm. f.) Merr.	Santol	HI	I		

Spp	Order	Family	Sub-Family	Species [1]	Authority	Common Name	Distribution [2]	Status [3]	Listed species US T/E [4]	Listed species State T/E [5,6,7]
38	Sapindales	Meliaceae	N/A	*Swietenia macrophylla*	King	Big-leaf Mahogany	HI	I		
Category 5 (continued)										
39	Sapindales	Meliaceae	N/A	*Swietenia mahagani*	(L.) Jacq.	West Indian Mahogany	FL, HI, PR,VI	N/I		
40	Sapindales	Meliaceae	N/A	*Toona ciliata*	Roem.	Australian Red Cedar	HI,PR	I		
41	Sapindales	Rutaceae	N/A	*Casimiroa edulis* (Redlands)	Llave & Lex.	White Sapote	HI	I		
42	Sapindales	Rutaceae	N/A	*Citrofortunella microcarpa*	(Bunge) Wijnands	Calamondin	Hi	I		
43	Sapindales	Rutaceae	N/A	*Citrus x aurantifolia*	Swingle	Key Lime	FL,PR,VI	I		
44	Sapindales	Rutaceae	N/A	*Citrus x sinensis*	(L.) Osbeck	Sweet orange	FL,LA,PR,VI	I		
45	Sapindales	Rutaceae	N/A	*Flindersia brayleyana*	F. Muell.	Queensland Maple	HI	I		
46	Sapindales	Rutaceae	N/A	*Fortunella japonica*	(Thunb.) Swingle	Kumquat	CA,HI	I		
47	Sapindales	Rutaceae	N/A	*Murraya exotica (=paniculata)*	L.	Orange Jessamine	FL, HI, PR,VI	I		
48	Sapindales	Rutaceae	N/A	*Zanthoxylum fagara* [9]	(L.) Sarg.	Lime Pricklyash	FL,HI, TX	I		
49	Sapindales	Sapindaceae	N/A	*Dimocarpus longan*/Biew Kieuw	Lour.	Longan	FL,HI	I		
50	Sapindales	Sapindaceae	N/A	*Dodonaea viscosa*	(L.) Jacq.	Hopbrush	AZ, FL, HI,TX, PR,VI	N/I		
51	Sapindales	Sapindaceae	N/A	*Exothea paniculata*	(Juss.) Radlk.	Inkwood	FL, HI,PR,VI	N/I		
52	Sapindales	Sapindaceae	N/A	*Filicium decipiens*	(Wight & Arn.) Thwaites	Japanese Fern Tree	HI	I		
53	Sapindales	Sapindaceae	N/A	*Harpullia pendula*	Planch. Ex F.Muell.	Tulipwood	HI	I		
54	Sapindales	Sapindaceae	N/A	*Hypelate trifoliata*	Sw.	White Ironwood	FL,PR,VI	N		
55	Sapindales	Sapindaceae	N/A	*Koelreuteria paniculata*	Laxm.	Goldenrain Tree	E-US, HI	I		
56	Sapindales	Sapindaceae	N/A	*Litchi chinensis* var. mauritius	Sonn.	Lychee	FL, HI	I		
57	Sapindales	Sapindaceae	N/A	*Majidea zanguebarica*	J.Kirk	Mgambo Tree	HI	I		
58	Sapindales	Sapindaceae	N/A	*Sapindus oahuensis*	Hillebr. Ex Radlk.	Lonomea	HI	N		
59	Sapindales	Sapindaceae	N/A	*Sapindus saponaria*	L.	Soapberry	SE-US, HI	N		
60	Sapindales	Simaroubaceae	N/A	*Leitneria floridana*	Chapm.	Corkwood	SE-US, HI	N		
61	Sapindales	Simaroubaceae	N/A	*Simarouba glauca*	DC.	Paradise Tree	FL	N		
62	Sapindales	Staphyleaceae	N/A	*Staphylea trifolia*	L.	American bladdernut	E-US	N		
63	Sapindales	Zygophyllaceae	N/A	*Guaiacum sanctum*	L.	Lignum-vitae	FL,PR	N		
64	Sapindales	Zygophyllaceae	N/A	*Tribulus cistoides*	L.	Jamaican Feverplant	S-US,HI,PR,VI	I		

Spp	Order	Family	Sub-Family	Species [1]	Authority	Common Name	Distribution [2]	Status [3]	Listed species US T/E [4]	Listed species State T/E [5,6,7]
Category 6. North American or introduced species in other orders that have some phylogenetic, morphological, or biochemical relationship to the target weed, incl economically and environmentally important plants										
65	Alismatales	Araceae	N/A	*Alocasia macrorrhizos*	(L.) G.Don	Giant taro	FL, HI, TX,PR	I		
66	Apiales	Apiaceae	N/A	*Daucus carota*	L	Carrot	US	I		
67	Aquifoliales	Aquifoliaceae	N/A	*Ilex cassine*	L	Dahoon Holly	SE-US	N		
68	Asterales	Asteraceae	N/A	*Ambrosia trifida*	L	Giant Ragweed	US & Canada	N		
69	Asterales	Asteraceae	N/A	*Lactuca sativa*	L	Head Lettuce	US	I		
70	Asterales	Asteraceae	N/A	*Solidago arguta*	Aiton	Goldenrod	E-US	N		
71	Brasicales	Brassicaceae	N/A	*Brassica oleracea*	L.	Califlower	US	I		
72	Bromeliales	Bromeliaceae	N/A	*Ananas comosus*	(L.) Merr.	Pineapple	FL, PR	I		
73	Cornales	Cornaceae	N/A	*Nyssa sylvatica*	Marshall	Blackgum	E-US	N		
74	Cyperales	Poaceae	N/A	*Oryza sativa*	L.	Rice	FL,PR,VI	I		
75	Cyperales	Poaceae	N/A	*Saccharum officinarum*	L.	Sugarcane	SE-US	I		
76	Cyperales	Poaceae	N/A	*Zea mays*	L.	Corn	US,PR,VI	I		
77	Dipsacales	Adoxaceae	N/A	*Sambucus nigra*	L	Elderberry	US & Canada	N/I		
78	Ebenales	Sapotaceae	N/A	*Planchonella sandwicensis*	(A. Gray) Baehni & O. Deg.	'ala'a	HI	N		
79	Ericales	Ericaceae	N/A	*Arctostaphylos densiflora*	M.S. Baker	Manzanita	CA	N		
80	Ericales	Primulaceae	N/A	*Ardisia escallonioides*	Schitdl. & Cham.	Marlberry	FL	N		
81	Fabales	Fabaceae	N/A	*Acacia koa*	A. Gray	Koa	HI	N		
82	Fabales	Fabaceae	N/A	*Arachis hypogaea*	L.	Peanut	SE-US	I		
83	Fabales	Fabaceae	N/A	*Phaseolus vulgaris*	L.	Pinto bean	US	I		
84	Fabales	Fabaceae	N/A	*Sophora chrysophylla*	(Salisb.) Seem.	Mamane	HI	N		
85	Fagales	Betulaceae	N/A	*Alnus serrulata*	(Aiton) Willd.	Hazel Alder	E-US	N		
86	Fagales	Fagaceae	N/A	*Quercus virginiana*	Mill.	Live oak	SE-US	N		
87	Ginkgoales	Ginkgoaceae	N/A	*Ginkgo biloba*	L.	Maidenhair tree	E-US	I		
88	Hamamelidales	Hamamelidaceae	N/A	*Hamamelis virginiana*	L.	Witch hazel	E-US	N		
89	Juglandales	Juglandaceae	N/A	*Carya glabra*	(Mill.) Sweet	Pignut hickory	E-US	N		
90	Lamiales	Verbenaceae	N/A	*Clerodendrum sp.*	N/A	Glorybower	SE-US	I		
91	Lamiales	Verbenaceae	N/A	*Tectona grandis*	L. f.	Teak	HI, PR,VI,	I		
92	Lamiales	Verbenaceae	N/A	*Vitex sp.*	N/A	Chastetree	S-US	I		

94	Magnoliales	Magnoliaceae	N/A	*Magnolia virginiana*	L.	Sweetbay	SE-US	N		
95	Malpighiales	Euphorbiaceae [10]	N/A	*Chamaesyce hypericifolia*	(L.) Millsp.	Graceful Sandmat	HI, S-US,PR, VI	N/I		

			N/A						Listed species	
			N/A						US	State
Spp	Order	Family	Sub-Family	Species [1]	Authority	Common Name	Distribution [2]	Status [3]	T/E [4]	T/E [5,6,7]
Category 6 (continued)										
96	Malpighiales	Euphorbiaceae	N/A	*Euphorbia pulcherrima*	Willd. ex Klotzsch	Poinsettia	HI, PR	I		
97	Malpighiales	Euphorbiaceae	N/A	*Hippomane mancinella*	L.	Manchineel	FL, PR, VI	N		Protected in FL
98	Malpighiales	Euphorbiaceae	N/A	*Manihot esculenta*	Crantz	Cassava	SE-US	I		
99	Malvales	Malvaceae	N/A	*Abelmoschus esculentus*	(L.) Moench	Okra	E-US, PR, VI	I		
100	Malvales	Malvaceae	N/A	*Gossypium hirsutum*	L.	Cotton	S-US, HI, PR,VI	N/I		
101	Malvales	Malvaceae	N/A	*Hibiscus sp.*		Hibiscus				
102	Myricales	Malvaceae	N/A	*Morella (=Myrica) cerifera*	(L.) Small	Wax myrtle	SE-US	N		
103	Myrtales	Combretaceae	N/A	*Laguncularia racemosa*	(L.) C.F. Gaertn.	White Mangrove	FL, PR, VI	N		
104	Myrtales	Myrtaceae	N/A	*Eucalyptus camaldulensis*	Dehnh.	Red Gum	CA, FL, HI, PR, VI,	I		
105	Myrtales	Myrtaceae	N/A	*Eugenia axillaris*	(Sw.) Willd.	White Stopper	FL,PR,VI	N		
106	Myrtales	Myrtaceae	N/A	*Eugenia uniflora*	L.	Surinam cherry	FL, HI, PR,VI	I		
107	Myrtales	Myrtaceae	N/A	*Metrosideros polymorpha*	Gaudich.	Ohi'a lehua	HI	N		
108	Proteales	Proteaceae	N/A	*Macadamia integrifolia*	Maiden & Betche	Macadamia nut	PR	I		
109	Rosales	Rosaceae	N/A	*Crataegus spathulata*	Michx.	Hawthorn	SE-US	N		
110	Rosales	Rosaceae	N/A	*Prunus caroliniana*	Aiton	Cherry laurel	SE-US	N		
111	Rubiales	Rubiaceae	N/A	*Coffea arabica*	L.	Arabian coffee	HI, PR,VI	I		
112	Scrophulariales	Myoporaceae	N/A	*Myoporum sandwicense*	(A. DC.) A. Gray	Naio	HI	N		
113	Solanales	Convolvulaceae	N/A	*Ipomoea batatas*	(L.) Lam.	Sweet potato	US,HI,PR,VI	I		
114	Solanales	Solanaceae	N/A	*Capsicum annuum*	L.	Bell Pepper	S-US, HI, PR,VI	I		
115	Solanales	Solanaceae	N/A	*Solanum lycopersicum*	L.	Tomato	US, HI, PR,VI,	I		
116	Solanales	Solanaceae	N/A	*Solanum tuberosum*	L.	Potato	US, HI, PR	I		
117	Theales	Theaceae	N/A	*Gordonia lasianthus*	(L.) Ellis	Loblolly bay	SE-US	N		
118	Urticales	Ulmaceae	N/A	*Ulmus alata*	Michx.	Florida elm	SE-US	N		
119	Violales	Caricaceae	N/A	*Carica papaya*	L.	Papaya	FL, HI, PR, VI	I		
120	Zingiberales	Musaceae	N/A	*Musa acuminata*	Colla	Edible Banana	FL	I		

Category 7: Any plant on which the biological control agent or its close relatives (within the same genus) have been previously recorded to feed and/or reproduce

93	Laurales	Lauraceae	N/A	*Persea americana*	Mill.	Avocado	FL, HI, PR,VI	I	

[1] Species names follow Pell et al (2011) USDA/NRCS (2002) and Wunderlin and Hansen (2008)

[2] Distributions from USDA/NRCS (2002) and from personal observations.

[3] I: Introduced; N: Native

[4] Federal endangered plant list from USFWS Endangered Species Program (2006). (Accessed 15 Apr 2014) (http://ecos.fws.gov/)

[5] Florida state endangered plant list from Coile and Garland (2004); Nongame and Rare Species Program: Federal/State Threatened and Endangered Species (Accessed 15 Apr 2013). Texas Parks and Wildlife Department, Texas. www.tpwd.state.tx.us/huntwild/wild/wildlife_diversity/texas_rare_species/listed_species/

[6] Tennessee Natural Heritage Program (2002). Rare plant list (20 October 2002). Department of Environment and Conservation, Tennessee.

[7] Kentucky State Nature Preserves Commission (2000). Endangered, threatened, and special concern species (20 October 2002). Kentucky State Nature Preserves Commission, Kentucky.

[8] *Rhus michauxii* collected only once, from Alachua Co, FL in 1961 from a male clone plant.

[9] Several Hawaiian species of *Zanthoxylum* are listed as federally endangered. We test here, *Z. fagara*, a surrogate of these species.

[10] We place the Euphorbiaceae in the Malpigiales after APG III (2009) instead of Euphorbiales as in USDA/NRCS (2002)

Appendix 5. **Design of host specificity tests for *P. ichini* (Wheeler et al., 2014).**

1. Plant parts and growth stages tested – All tests were conducted on whole plants that were pruned and fertilized to stimulate the flush growth needed for thrips feeding and development. Plants were kept free of pests with periodic insecticide applications, however, no plants were used within 3 months of treatment.

2. Source populations of the test plants - Most test plants were obtained from plant nurseries. These included Pine Island Nursery (Miami, FL), Silent Native Nursery (Palmetto Bay, FL), Pine Ridge Gardens (London, AR) and seed catalog Sheffelds Seed Co (New York, NY). For species not available commercially, seeds were collected from local wild populations. The listed species, *Rhus michauxii*, was generously provided by Dr. J.L. Randall (University of North Carolina, Chapel Hill, NC). *Pistacia mexicana* seeds were generously provided by Mr. Jack Skiles (Langtry, TX) and Ms. Ann Vacek (USDA/Agricultural Research Service, Weslaco, TX). *Pistacia vera* seeds were donated by Mr. Craig Kallsen (Univ. California, Davis) and seedlings were provided by Mr. Lane Miller (S&J Ranch, Pinedale, CA). *Toxicodendron pubescens* were collected and provided by Dr. Richard Weaver (retired botanist, Department of Plant Industry, Gainesville, FL). *Comocladia dodonaea* plants were collected and provided by Mr. Tony Pernas (National Park Service, Homestead, FL). Plants from Hawaii were provided by Mr. Pat Conant (retired, HI Department of Agriculture, Hilo, HI) and Dr. Tracy Johnson (USDA/Forest Service, Volcano, HI). The Brazilian peppertree control plants were grown from locally collected seeds.

3. Numbers of replicates – In general, the number of replicates for no-choice tests was 10 for species of the closest relatives, such as species from the Anacardiaceae family; otherwise 4 replicates were included. In several examples, plants were difficult to obtain and grow and sufficient numbers for the desired replication were not achieved. Examples include the endangered plant species, *Rhus michauxii* (n=7), *Toxicodendron vernix* (n=6), and *Spondias mombin* (n=4). Only healthy plants with flushing growth were tested, which was a challenge for several species under subtropical south Florida growing conditions. Despite fewer than the target number of replicates, there was no variation in the thrips testing results with these species (see results). Moreover, other representative of these genera were tested with the target number of replicates. Although *Dodonaea viscosa* is assigned to the Sapindaceae and thus not a close relative, 10 replicates were included because of possible *P. ichini* feeding and development on this species. To confirm results obtained by each quarantine group, half the replicates of the Anacardiaceae were tested in each quarantine (Ft. Lauderdale and Ft. Pierce).

4. Number, stage, and age of individuals – In no choice and two choice tests, 20 adult thrips from the quarantine colony were introduced into vented cages with plants. These cages were constructed of clear Plexiglas®, measured 45 centimeters (cm) in height x 15 cm in diameter (Fig. B), and were fitted with a mesh vent at the top and 2–3 vents (4 cm diameter) on the cylinder side. The open end of these cages fit tightly inside the upper rim of a 3.8 liter (1 gallon) pot. All experiments were conducted in quarantine greenhouses at 27 ± 2°C at 60–70

percent relative humidity (RH) under ambient photoperiod. All plants were watered as needed and inspected visually at least three times per week. To prevent the production of a second generation, adults were removed as soon as larvae were found on the plants. The total number of F_1 adults produced was counted. Thus, data on *P. ichini* oviposition and completion of development on different test plant species was obtained.

5. Details of experimental setup – The protocol for host range tests can be divided into no-choice, choice and multiple generation tests. To predict the host range of *P. ichini*, the field host range observed in Brazil was supplemented with this series of tests. Here simultaneous no-choice starvation tests were conducted that extended for a complete generation of the thrips. These starvation no-choice tests are the most rigorous and conservative test design used to define a candidate's fundamental or physiological host range (Van Klinken and Heard, 2000; Schaffner, 2001). The primary criticism of these tests is that they potentially overlook candidates that would be safe to release (Cullen, 1990; Schaffner, 2001). Two choice tests were also conducted as they may complement the no-choice tests and are often considered to better simulate more natural conditions than no-choice tests (Harley, 1969). Choice tests may be accepted as better predictors of risk than other testing methods (Cullen, 1990). The two choice tests conducted were the 'normal choice tests' as they simultaneously exposed the target weed and a single test species (Schaffner, 2001). These tests were conducted during the candidate's most mobile life stage, the adult, where 'decisions' are made for oviposition. When F_1 offspring resulted from a no-choice test, the individuals were followed where possible, with multiple generation tests. These tests determined the number of generations a population of the agent could sustain solely on the non-target species.

Figure B. Plexiglas cylinders (45 cm x 15 cm diameter) used to conduct no-choice tests for the thrips *Pseudophilothrips ichini*.

<u>No-choice tests</u>

No-choice tests assessed oviposition, completion of development, and reproduction on test plant species. Twenty thrips adults were introduced into the standard vented cage (Fig. B) that contained either a single control or test plant. These P_1 adults were allowed to feed, mate and oviposit until second instar larvae were observed (~23 days), after which time the cage was removed and the P_1 adults were collected. The cage was replaced and the exposed plants were observed over the next 27 days (one generation plus a 7-day buffer) for the maturation of F_1 offspring. The number of F_1 adults were counted and the experiment was terminated.

<u>Choice tests</u>

If F_1 offspring were produced on a non-target species during no-choice tests, a choice test was conducted that included that non-target species and Brazilian peppertree. The choice tests were conducted by releasing 20 adult thrips into a medium size Plexiglas® cage (50 x 50 x 50 cm; Fig. C) or a large fabric cage (100 x 100 x 100 cm). The thrips were allowed to feed and oviposit on either the target weed or the same species of non-target plant. The thrips adults were released on the base of the cage, between the weed and test plants. Cages were checked three times per week. When second instars were visible, the adults were removed and each plant was placed separately into the standard vented cage (Fig. B). The exposed plants were left undisturbed for at least 27 days then the number of F_1 adults produced in each cage was counted and the experiment was terminated. This way preference of *P. ichini* could be determined and again (as in the no-choice tests) whether the test plant was a developmental host.

Figure C. Plexiglas® cages (50 cm x 50 cm x 50 cm) used to conduct choice tests for *P. ichini*. This replicate contains one plant of the target, Brazilian peppertree and one plant of *Rhus sandwicensis*. When second instars were observed, the adults were removed and the plants were separated into individual cylindrical cages for emergence of F_1 adults.

Multiple generation tests

The protocol for multiple generation tests was to maintain F_1 adults produced during no-choice or choice tests to subsequent generations on the same test plant species. This was attempted by transferring whatever F_1 adults were produced to a fresh plant of the same species. It was planned to transfer up to 20 F_1 adults to new cages that contained the same non-target species. If fewer than 20 F_1 adults were produced, transfers were made with what was available. Simultaneous controls were treated identically with 20 F_1 adults. To determine the feasibility of continuing a thrips population on each non-target, this process was to be repeated for three generations or until no futher F_1 adults were found. The plan was to compare the number of larvae and adults produced at each generation between the test plants and the Brazilian peppertree controls. Although this was the planned protocol for muliple generation tests, obtaining sufficient numbers of F_1 thrips from a non-target and additional plants to test them was often difficult. Most often too few F_1 adults were produced from these species. Additionally, for some species (e.g., *S. molle*) insufficient test plants were available to continue to the next generation.

Positive controls

Positive controls were used in all studies simulataneously with each test plant where thrips were fed Brazilian peppertree plants. These positive controls were included to ensure that lack of feeding and development on the test plant was not due to problems with insect quality or unfavorable conditions. Similar data were recorded on the control thrips for F_1 production on the target weed. If thrips failed to reproduce and fewer than 10 F_1 adults were observed on the control, experimental results were discarded and the test plants were re-evaluated.

Rationale for study design and execution

Thrips feed by puncturing the cell walls of healthy flushing plant tips with a mandible and subsequently suck the plant juices that leak from the wound (Kirk, 1995). Plants are known to respond to thrips damage by inducing anti-herbivore defenses that change host selection by thrips (Delphia et al., 2007). For these reasons, tests were conducted on whole, live plants, not bouquets or leaf disks (Palmer, 1999). The thrips are small (~3 mm in length) delicate arthropods that are easily lost or damaged by removing cages and handling. Once thrips adults were introduced into each cage, they were left undisturbed as much as possible to avoid false negative results where thrips mortality was caused by handling and not a lack of host plant suitability. A test was considered successful if at least 10 F_1 adults were produced on the Brazilian peppertree positive control plant.

All tests of the members of the Anacardiaceae were conducted independently at quarantine facilities at Ft. Lauderdale and Ft. Pierce. To detect laboratory-induced factors that could influence the results, half the replicated tests were conducted at each lab and their results were shared by the team members.

Appendix 6. Release Protocol and Post-Release Monitoring Plan for *P. ichini* (Wheeler et al., 2014).

Protocol for Releasing the Agent

Mass rearing of the agent will be conducted by Wheeler and colleagues (USDA-Agricultural Research Service (ARS)) and Overholt and colleagues (University of Florida). Field releases will be conducted throughout Florida in collaboration with extension agents and land managers. Several field sites will be established in the invaded area and post-monitoring of establishment and impact will be conducted (see below). Release strategies for recently introduced biological control agents consider whether it is best to make a few number of large agent releases or a large number of small agent releases. These decisions will be influenced by the number of agent individuals available and the number of prepared release sites (Grevstad, 1999). Previous research concluded that for a similar species, the gorse thrips, numerous relatively small releases of 250 adults per release was successful (Memmott et al., 1998). Initially, the researchers will follow this recommendation but leave open the possibility of adjusting this number as deemed appropriate. Other research has indicated that it may be important to consider a mixture of strategies during the initial release phase allowing time to learn and improve chances of finding the optimum approach (Shea and Possingham, 2000).

Post-release Monitoring

Determination of the impact of a released biological control agent is time consuming and labor intensive. Several pre-release studies were conducted by the researchers in preparation for release of *P. ichini*. Long term, pre-release demographic studies were established in 2008 at six locations in Florida. At each site annual demographic data are being collected that include plant growth, survival, and reproduction of 80 plants. Following the tagging and initial description, plants have been monitored yearly. Additional data collection includes litterfall that captures the normal foliar and reproductive output and senescence of the population. If approved, thrips will be released at all sites and their populations and impact will be monitored. These data represent pre-release characterizations needed to determine the impact of an agent on the weed population after release (Paynter, 2006; Raghu et al., 2006; Dauer et al., 2012; Evans et al., 2012).

A Brazilian peppertree garden was established at the location in Ft. Lauderdale, FL. Previous research investigated the residual control of systemic insecticides to protect Brazilian peppertrees from *P. ichini* thrips damage. To determine this, treated and untreated leaves at different time intervals post-application were fed to *P. ichini* thrips in quarantine to determine how long the insecticide remained effective. If granted a release permit, similar garden trees planted in 2008 at will be experimentally treated with the same methods and infested with thrips. By protecting Brazilian peppertrees against *P. ichini*, tree survival, reproduction, and growth with and without thrips will be compared.

Additionally, a common garden, field host range experiment was established at the USDA-ARS, Ft. Lauderdale location in 2011 consisting of related plant species of the Anacardiaceae that grow in South Florida. These include the species of Anacardiaceae *Anacardium occidentale*, *Comocladia dodonaea*, *Malosma laurina*, *Mangifera indica*, *Metopium toxiferum*, *Pistacia chinensis*, *Rhus copallinum*, *Spondias dulcis*, *S. purpurea*, *S. mombin*, and *Toxicodendron radicans*. Additionally, members of related plant families in the Sapindales order include *Citrus* spp. (Rutaceae), *Bursera simaruba* (Burseraceae), *Swietenia mahagoni* (Meliaceae), and *Dodonaea viscosa* (Sapindaceae). Plant species can be purchased and added to the garden if the need arises and a release date approaches. This garden is designed to confirm the results of quarantine specificity tests by defining the thrips ecological host range under multi-choice field conditions (Pratt et al., 2009).

Appendix 7. **Response to comments on this Environmental Assessment.**

Notice of this Environmental Assessment (EA) was made available in the Federal Register on February 27, 2019 for a 30-day public comment period. APHIS received a total of 129 comments on the EA by the close of the comment period. Most comments (120) were in favor of the release of the biological control agents. Nine comments were either not in favor of or raised concerns regarding the release of the two agents. The issues raised in the 9 comments are addressed below.

1. One commenter questioned whether the psyllid *Calophya latiforceps* would be able to provide effective control given its extremely sedentary life style. It would seem necessary to implement a large program to raise and release the psyllid for it to impact hundreds of thousands of invaded hectares. The description of the release protocol in Appendix 3 of the EA does not indicate that the program will be sufficiently large to achieve desired results.

Response: *Calophya latiforceps* reduces the height and biomass of Brazilian peppertree by 31 and 11 percent, respectively, over just one psyllid generation (Prade et al., 2016). Sufficient data have been collected from laboratory studies (Prade et al., 2016) that indicate *C. latiforceps* and *Pseudophilothrips ichini* (Wheeler et al., 2017) will be damaging agents.

The researchers have recently initiated a mass production and redistribution effort for both Brazilian pepper biological control agents for Florida at the USDA/ARS laboratory in Ft. Lauderdale. Although no agents are yet available outside quarantine, preparations are being made for large-scale release. Recent meetings have identified partners from University of Florida and Florida Department Agriculture and Consumer Services. These details were not included in previously in this EA as they are currently in the development stages.

References:

Prade, P., R. Diaz, M.D. Vitorino, J.P. Cuda, P. Kumar, B. Gruber, and W.A. Overholt. 2016. Galls induced by *Calophya latiforceps* (Hemiptera: Calophyidae) reduce leaf performance and growth of Brazilian peppertree. Biocontrol Sci. Technol. 26: 23–34.

Wheeler, G.S., V. Manrique, W.A. Overholt, F. Mc Kay, and K. Dyer. 2017. Quarantine host range testing of *Pseudophilothrips ichini*, A potential biological control agent of Brazilian peppertree, *Schinus terebinthifolia,* in North America and Hawaii Entomol. Exp. Appl. 162: 204–217.

2. A commenter indicated that the EA says that, once officials have sufficient data on the efficacy of *Calophya latiforceps* in controlling *S. terebinthifolius* in Florida, they will consult with authorities in Texas about whether to release the psyllid there, too. It is unclear who will undertake this consultation on behalf of either the biocontrol proponents or – especially – Texas. Nor is it clear whether such possible expansion will occur after two years – assuming the *C. latiforceps*' establishment is determined at that time; or after four years, when establishment is further assessed with the assistance of county agricultural agents. Regarding possible releases of

Calophya latiforceps in Texas, the commenter urges APHIS to amend the release and monitoring protocol to clarify what form consultation will take. Topics to be addressed should include, for example, whether a separate environmental assessment will be prepared. Also, how will APHIS reach out to the full range of conservationists and other stakeholders?

Response: The release protocol described in the EA is the initial release protocol. These sites will be used to release and monitor the establishment and impact of psyllids. These sites were chosen based on the long-term (pre-release) data that has been collected at each of these sites. Mass-rearing and release programs (which will be instituted after data collection on initial establishment and spread) for both the psyllids and thrips will be modeled after the air potato leaf beetle rearing and release program. The air potato leaf beetle rearing and release program was a collaborative effort among USDA-ARS, University of Florida, the Florida Department of Agriculture and Consumer Services, and University of Florida Extension. This program was successful in establishing the beetle throughout the state (Overholt et al., 2016).

Once data on establishment, spread, and early impact are collected in Florida and field colonies of the insects are at levels that will tolerate harvesting, officials involved with the project in Florida will determine if authorities in Texas are interested in releasing the agents in Texas. This EA covers the APHIS permitting of these two biological control agents anywhere in the contiguous United States, and has been made available to interested parties throughout the United States for their comment and input. In addition, for either of these biological control agents to be moved from Florida to Texas or to any other State, a PPQ 526 permit issued by APHIS is required. As part of the permitting process, the appropriate State Plant Regulatory Official from the State Department of Agriculture as well as the State Plant Health Director for APHIS in the destination State is notified of the permit application and has an opportunity to comment on it. If there are any additional regulatory concerns, they will be addressed at that time by those individuals in combination with the APHIS permitting scientist.

Reference:

Overholt, W.A., M. Rayamajhi, M., E. Rohrig, S. Hight, S., F.A. Dray, E. Lake, M. Smith, K. Hibbard, G.P. Bhattarai, K. Bowers, R. Poffenberger, M. Clark, B. Curry, B. Stange, E. Calise, T. Wasylik, C. Martinez, and J. Leidi. 2016. Release and distribution of *Lilioceris cheni* (Coleoptera: Chrysomelidae), a biological control agent of air potato (*Dioscorea bulbifera*: Dioscoreaceae), in Florida. Biocontrol Science and Technology. 26: 1087–1099, DOI: 10.1080/09583157.2016.1185090

3. A commenter raised a concern regarding the host specificity of *Pseudophilothrips ichini*. The EA notes that this thrips can reproduce in low numbers on several non-target plant species. Of greatest concern to the commenter is that two of those species are Hawaiian native plants. The Hawaiian sumac *Rhus sandwicensis* is found on all the main Hawaiian Islands. The thrips also attacks a second Hawaiian native plant, *Dodonea viscosa*. While the EA says the thrips causes minimal damage to *D. viscosa*, the plant species' ecological importance raises concern. True, the proposal is to release the biocontrol agents on the continental United States and not in Hawai`i. But insects have often been transported inadvertently to Hawai`i – and the Islands' plant species

have often proved highly vulnerable to attack by non-native species. One example is the *Erythrina* gall wasp (*Quadrasticus erythrinae*), which has caused enormous losses to one native tree species (the wiliwili tree, *Erythrina sandwicensis*) as well as to introduced species in the same genus on the Islands. Another example is the Myoporum thrips (*Klambothrips myopori*), which has caused widespread mortality of the native naio trees (*Myoporum sandwichense*). Naio are an important component of native mesic and dryland forests in Hawai`i, which cover about 140,000 acres on Hawai`i Island alone. They are also economically important in landscaping. Outbreaks of *Klambothrips* were first reported on Hawai`i Island in 2009; by 2019 they have spread across Hawai`i Island and have devastated local naio populations, causing between 20% and 80% mortality. In 2019 they were first reported on O`ahu. The thrips had previously been detected in California on landscaping plants and are thought to have hitchhiked to Hawai`i via visitors from California.

Pseudophilothrips ichini could reach the Hawaiian Islands by hitchhiking in air transportation of people and goods from Florida. It is also possible that the thrips could move West across the continent, leapfrogging on other populations of both wildland and cultivated *Schinus,* to California, where hosts are common. Unintentional transport of *P. ichini* from California to Hawai`i would be even more likely to occur than from Florida. Should *P. ichini* reach Hawai`i – even years after its release in Florida – it could threaten the ecological role if perhaps not the biological survival of *R. sandwichensis* and *Dodonea viscosa*. The thrips is likely to thrive on the Islands because several good hosts, including both *Schinus terebinthifolius* and *S. molle,* are widespread there. In this way, the thrips could put constant pressure on *R. sandwichensis* populations, despite the fact that *R. sandwichensis* and *Dodonea viscosa* themselves can support only one or a few generations of the thrips.

The commenter recommends that APHIS undertake additional host testing of *Pseudophilothrips ichini* on biologically or ecologically related plants growing in Hawai`i before allowing release of this possible biocontrol agent, particularly for *R. sandwichensis.* The commenter also suggests that APHIS should amend the release and monitoring protocols to enhance protections with the goal of preventing *P. ichini* from reaching Hawai`i. For example, APHIS should quickly assemble information on the origins, pathways, and destinations by which *Schinus* species and other non-target host species are moved in interstate trade – with the aim of shutting down pathways that are most likely to transmit biocontrol agents to Hawai`i.

Response: These examples of insects transported to Hawaii were not proposed weed biological control agents, the subjects of intensive host range evaluation under replicated experimental conditions. Unlike the biological control agents that are proposed for release here, these inadvertent introductions were not tested for host range prior to release. Without these studies, these inadvertent introductions would not be considered for field release as biological control agents.

Regarding *Dodonea viscosa*, no-choice tests (where the minimal feeding was reported on the non-target species noted here) are unnatural and extremely conservative. This type of test does not offer an insect a choice. It forces the insect to feed on the plant or die. During these no-choice tests, 20 adult thrips were placed on each of the non-target plants and allowed to feed, mate, and lay eggs for 23 days. After 23 days, the adult thrips were removed and the plants were monitored for an additional 27 days to monitor any development of immature thrips. During the no-choice tests with *Dodonea viscosa* (over 10 repetitions) very few F1 thrips were produced (average 2.8). During multi-generation tests, the thrips were not able to survive past the first generation. This demonstrates that the thrips will not be able to survive in the absence of their host, Brazilian peppertree. During choice tests (which are designed to be a more realistic scenario where both host and non-host plants are present) the thrips clearly choose Brazilian peppertree over *D. viscosa* as no thrips were produced on the non-target. (Wheeler et al., 2017)

The same tests described above for *D. viscosa* were also performed on *Rhus sandwichensis*. With *R. sandwichensis* an average of 3.3 F1 thrips were produced (from 20 adult thrips placed on each plant) and no thrips survived beyond the first generation on the non-target. Like *D. viscosa*, *R. sandwichensis* will not be able to support thrips populations in the absence of their host, Brazilian peppertree. During choice tests (designed to mimic the presence of both host and non-host co-occurring in nature) the thrips clearly chose Brazilian peppertree (average of 66.1 F1 thrips on Brazilian peppertree and 1.1 on *R. sandwichensis*). These insects have a clear host preference for Brazilian peppertree and populations of the thrips are unable to survive on either *D. viscosa* or *R. sandwichensis* if Brazilian peppertree is absent (Wheeler et al., 2017).

The only other plant that *P. ichini* can damage is the other weed species *Schinus molle*. But in Brazil, despite many visits to this species in the area where the thrips is common, we never found thrips feeding on *S. molle*.

Though the laboratory results accurately predict a high degree of safety, the researchers plan to conduct a field host range study. Once APHIS issues release permits for Florida, and releases have been made, the researchers/permittees will evaluate the fidelity of the agents under field conditions in areas with high agent densities. These field gardens will include *R. sandwicensis* and *D. viscosa.*

APHIS would require testing of additional Hawaiian plants for a proposal to release these biological control agents into Hawaii.

Importation of plants into Hawaii is already very tightly regulated. It is unlikely that evaluation of interstate movement of *Schinus* would reduce the already extremely remote possibility that these species would reach Hawaii.

Reference:

Wheeler, G.S., V. Manrique, W.A. Overholt, F. McKay, and K. Dyer. 2017. Quarantine host range testing of *Pseudophilothrips ichini*, A potential biological control agent of Brazilian peppertree, *Schinus terebinthifolia,* in North America and Hawaii Entomol. Exp. App. 162: 204–217. doi:10.1111/eea.12506

4. Two commenters were concerned about the impact of Brazilian pepper biological control on beekeepers. Some beekeepers rely on Brazilian pepper for their honey crop in central to southern

Florida. The commenters are concerned that other potential plants that could provide forage for bees (i.e., saw palmetto, citrus, gallberry, Chinese tallow, etc.) are either unavailable, unsuitable, or reduced due to development or insecticide sprays. A commenter suggested that native plants, food crops, or other plants, that provide important nutrition for pollinators be introduced to offset the loss when Brazilian pepper is reduced.

Response: Biological control will not eliminate Brazilian pepper (BP) in the United States. Biological control will just reduce the density of BP and restore a more natural balance. The plant will still be present on the landscape and available for use by beekeepers. Brazilian peppertree is still an important (major) nectar resource for beekeepers in Brazil, where dozens of species of natural enemies (including *P. ichini* and *C. latiforceps*) feed on the plant (da Silva Santos et al., 2015). *Calophya latiforceps* and *P. ichini* will have their greatest impact on small, pre-reproductive seedlings and saplings. There are already aggressive chemical control activities that have been ongoing for many years. These biocontrol agents will work together in integrated management programs and with other approaches, will help in reducing, but not eliminating, this invader.

In addition, numerous other plant species are flowering during the same time of year as Brazilian pepper in Florida many of which are listed as major or minor nectar producers important to beekeepers. These include: *Euthamia minor, Helianthus agrestis, Bidens alba, Solidago leavenworthii, Solidago sempervirens, Solidago stricta, Bigelowia nudata, Bigelowia nuttallii, Richardia scabra, Pontederia cordata, Polygonum punctatum, Bidens pilosa* var. *radiata, Liatris spicata, Baccharis halimifolia, Jussiaea peruviana, Oxypolis filiformis,* and *Funastrum clausum* (From: Morton, 1964 and others).

Brazilian peppertree forms dense monocultures in the invaded area. As it is reduced in density other plant species will rebound and biodiversity in the invaded region will likely increase. Higher biodiversity is important to maintain healthy bee colonies (Nicholls and Altieri, 2013). The introduction of native plants, food crops, or other plants, that provide important nutrition for pollinators be introduced to offset the reduction of Brazilian pepper is beyond the scope of this proposed action and not necessary to carry out.

References:

da Silva Santos, K.C., A.B. Gonçalves, and M.P. Cereda. 2015. Polens importantes na flora apícola em uma região de Cerrado em Campo Grande–MS/Important pollens from Brazilian savannah bee flora in Campo Grande-MS. Revista de Biologia Neotropical. 12(2): 81–85.

Morton, J.F. 1964. Honeybee plants of South Florida. Florida State Horticultural Society. 415–436.

Nicholls, C.I., and M.A. Altieri. 2013. Plant biodiversity enhances bees and other insect pollinators in agroecosystems. A Review. Agronomy for Sustainable Development. 33: 257–274. http://dx.doi.org/10.1007/s13593-012-0092-y

5. A commenter expressed concern about biocontrol agents introduced from elsewhere often doing more harm than good when released into the wider environment. Examples included sparrows, starlings, *Cactoblastis cactorum* moths, brown tree snakes, cane toads, Asian ladybird beetles, mongooses, mosquitofish, etc. The fact that the thrips species lays eggs on a wide variety of plant species, and are able to reach adulthood, even at low rates, on our native and commercially important Anacardiaceae is of concern to the commenter. Insects, by nature of rapid life cycles, can evolve very quickly to take advantage of novel habitats and food chains. Although the species may prefer *Schinus* in their native Brazil, environmental conditions in Florida may have them targeting something entirely different once they are out of a controlled testing environment. These two may do more harm than good if they infect mangoes, cashews and our native *Rhus* and *Toxicodendron* species. Even more so if they expand beyond these to become yet another plant pest needing chemical poisons to control. Are there no native species that could be recruited? The commenter suggests that more testing is required before releasing these biocontrol agents into the environment in Florida.

Response: Of the examples cited above, only the *Cactoblastis cactorum* moth was a classical weed biological control agent; the others invaded by unknown means or by well-intentioned lay people without proper pre-release host range testing. This one example, *Cactoblastis* moth, was not an intentional release into North America. How this South American species arrived in Florida continues to be debated but it likely arrived with importations of infested ornamental cacti from the Caribbean.

Patterns of non-target use by biological control of weeds agents can be reliably judged before introduction (Pemberton, 2000). This has been demonstrated countless times and the process has become demonstrably safer and more robust since the start of new host testing methodologies in the 1970s. These tests determining the physiological host range of an agent are placed under intense scrutiny by other scientists, regulators, and land managers. Further, multiple studies have demonstrated that the physiological host range (conservative tests done in the lab pre-release) of these biological control agents is broader than the actual ecological host range (post-release). Host testing clearly showed that these agents will not sustain a population on any species outside the genus *Schinus*.

The native insects that feed on Brazilian pepper in Florida have been surveyed and are not controlling the weed. Therefore, native species cannot be used to control Brazilian pepper.

Reference:

Pemberton, R.W. 2000. Predictable risk to native plants in weed biological control. Oecologia. 125: 489–494.

6. A commenter stated “I'm not sure exactly where they decided to release the Asian beetles but they were supposed to get rid of aphids on soybeans and so far they're just a menace it seems like every time they release something to combat something it becomes a problem and you brought the Brazilian pepper tree in to help the honey bees and now it’s a problem so bringing in some

kind of lice and other insect doesn't seem to be the answer because somehow it will become a problem they always do!”

Response: Brazilian pepper was brought into Florida in the mid-1800s for use as an ornamental plant, not as a source for honey bees.

Classical biological control is a method to manage exotic invasive insects or plants (such as Brazilian pepper) by reuniting natural enemies (biocontrol agents) from the invasive plant or insect’s native range with the plant or insect in its introduced range. Classical biological control was first used more than 150 years ago and can be a cost-effective and sustainable control method for exotic invasive organisms, with some spectacular successes (Hinz et al., 2019). Although there are some instances where biological control agents released in the past have had some unintended impacts, the approval process for release of weed and insect biocontrol agents is much more stringent today. It is incorrect to state that all biological control agents become problems.

Since the beginning of classical biological control in the 19th century, the proportion of intentionally released weed biocontrol agents causing nontarget attack declined from 18.2 percent in the period until the 1960s to 9.9 percent in the period from 1991 to 2008, and the proportion of releases causing nontarget attacks decreased in the same periods from 14.8 to 5.3 percent (Hinz et al., 2019). In addition, the proportion of agents causing sustained attack of nontarget species declined from 12 to 1 percent (Hinz et al., 2019). The incidences of unpredicted nontarget attack have decreased over time and this trend is expected continue with advancements of molecular tools that clarify evolutionary relationships within target plant families (Hinz et al., 2019).

Reference:

Hinz, H.L., R.L. Winston, and M. Schwarzländer. 2019. How safe is weed biological control? A global review of direct nontarget attack. The Quarterly Review of Biology. 94: 1–27.

7. A commenter indicated that he feels more research may be needed to see if there would be any negative impacts by the release of these insects to other desirable plant species. The release may not have a direct impact on humans but may have an indirect impact if the insects were to cause an economic impact on plant foods produced in the US for human consumption.

Response: This work was undertaken by scientists, each with more than 20 years of experience conducting this type biological control research. The protocols followed no-choice, choice, and multigeneration tests that are accepted by the international scientific community. The research was conducted in a modern state of the art facility using the most conservative methods known. Plants that were tested included native and introduced plants related to the target weed, plants that occur in similar habitats as the target weed, threatened and endangered plants related to the target weed, and many plants of ornamental, agricultural or economic importance. The Technical Advisory Group for Biocontrol Agents of Weeds reviewed the test results and recommended the release of both agents. The results of both studies were published in peer-reviewed scientific journals. APHIS is satisfied with the extensive testing and review that has been conducted by the researchers.

8. One commenter asked how long the study of biocontrol has been for. How do we know the effects of releasing the biocontrol beyond this time frame? 10 years down the road or 20 years?

Response: Biocontrol has been practiced for more than 115 years. More specifically, biocontrol of Brazilian peppertree has been ongoing since the 1950s as Hawaii Department of Agriculture studied the safety and introduced the moth *Episimus unguiculus* from Brazil. Work on the proposed agents, *Pseudophilothrips ichini* started at the USDA in 2005. Work on the second proposed agent, *Calophya latiforceps*, was initiated in 2010. Research on both species continues.

9. A commenter stated that the species has ranges across varying ecotypes in the United States. How do we know that the insect will react similarly in Florida and California? Also in the EA it stated that "worldwide, biocontrol releases have had a 33% success rate, but it is a much higher success rate within individual countries." The US has vast differences in climate, topography, etc. across individual states and the country. What individual countries are referenced in the above quote?

Response: Based on cold tolerance studies, regions predicted to be suitable for *C. latiforceps* survival in the contiguous United States include peninsular Florida, southern Texas, western Arizona, and California. Also, temperature-based physiological models indicated that *P. ichini* could establish throughout the Brazilian peppertree-invaded range in the United States (Manrique et al., 2014). However, whether the proposed biological control agents will survive and establish throughout the range of Brazilian pepper in the contiguous United States will not be known with certainty until they are released into the environment.

Success rates vary by the country of introduction for many reasons, including differing ecotypes. The individual countries referenced in the above quote include Australia, Canada, New Zealand, South Africa, and the United States.

Reference:

Manrique, V., J.P. Cuda, W.A. Overholt, D.A. Williams, and G.S. Wheeler. 2008. Effect of host-plant genotypes on the performance of three candidate biological control agents of *Schinus terebinthifolius* in Florida. Biol. Control. 47: 167–171.

10. A commenter asked "During the host specificity tests they used nursery plants, how would they plan for genetic differences that may be present?"

Response: Some ornamental plants were purchased from nurseries. However, when possible, native plants were collected from local wild sources. Multiple plants (repetitions) of each species were tested to ensure that a range of genetic differences were tested.

11. A commenter asked how the researchers will quantify the amount of data collected as described in appendix 3 of the EA?

Response: The collected data, in support of these biocontrol releases, were published in peer-reviewed journal articles:

Diaz, R., D. Moscoso, V. Manrique, and W.A. Overholt. 2014. Field density, host utilization and life history of *Calophya latiforceps* (Hemiptera: Calophyidae): An herbivore of Brazilian Peppertree (*Schinus terebinthifolia*). Biocontrol Science and Technology. 24: 536–553.

Diaz, R., V. Manrique, J.E. Munyaneza, V.G. Sengoda, S. Adkins, K. Hendricks, P.D. Roberts, and W.A. Overholt. 2015. Host specificity testing and examination for plant diseases reveal that the gall-forming psyllid, *Calophya latiforceps* (Hemiptera: Calophyidae), is safe to release for biological control of *Schinus terebinthifolia* (Sapindales: Anacardiaceae). Entomologia Experimentalis et Applicata. 154: 1–14.

Wheeler, G.S., V. Manrique, W.A. Overholt, F. McKay, and K. Dyer. 2017. Quarantine host range testing of *Pseudophilothrips ichini*, A potential biological control agent of Brazilian peppertree, *Schinus terebinthifolia,* in North America and Hawaii Entomol. Exp. App. 162: 204–217. doi:10.1111/eea.12506

Post release data, as mentioned in Appendix 3, will also be published in peer-reviewed scientific journals for public review.

12. A commenter questioned that without the containment measures how would they manage any possible emergence of parasitoids in the *Calophya* colonies?

Response: All imported individuals were quarantined under strictly regulated conditions for at least one generation allowing for the emergence and exclusion of native parasitoids. Once the new introductions were cleared of natural enemies, the agents were typically reared in general quarantine for the duration of the host range studies. Host range testing was conducted on these parasitoid-free colonies. These same colonies will be used for field releases and will be parasitoid-free. However, once released into the environment, the biological control agents could possibly be attacked by parasitoids already occurring in the environment.

13. A commenter asked "It says they have reached a preliminary determination that there won't be a significant environmental impact, but how much research has been done to reach that preliminary determination, and what still needs to be done to reach a final determination?"

Response: Work on the proposed agents, *Pseudophilothrips ichini* began in 2005. Work on the second proposed agent, *Calophya latiforceps* was initiated in 2010. A total of 12 (six scientific papers for each agent *P. ichini* and *C. latiforceps*) were published by the primary researchers (Wheeler and Diaz). Additional research has been published on these species by other researchers. Research on both species continues. A final determination is considered following review of public comments.

APHIS has reviewed the comments made on this EA. Most comments were favorable and in support of the proposed release of the agents in the United States. Based on the comments that have been submitted, APHIS believes that a finding of no significant impact (fonsi) is appropriate for the proposed release of *C. latiforceps* and *P. ichini* into the environment of the continental United States. A separate EA would be prepared should APHIS receive a request for release of these agents into other areas of the United States (i.e., Hawaii, Puerto Rico) that were not evaluated in this EA and are not included in the fonsi prepared for this proposed action.

14. Two commenters asked that if the introduced insects establish and eliminate the target plant species, what happens after the target weed is gone?

Response: Biological control has never completely eliminated a target weed; thus, the target weed will never be gone. As the population of the target weed is reduced, the population of biological control agents would decrease in size as well.

15. Two commenters asked about what will happen if there is a loss of control over the introduced insects. What are the contingency plans put in place if there is a loss of control of the released species?

Response: Technically, once the agents are released into the environment, they are not “under control”. That is why extensive host specificity testing is conducted by the researchers to ensure that unexpected impacts to non-target species do not occur.

Both agents will be released into the Brazilian pepper infestation. The realized range of these agents in the United States after release will mostly depend on the distribution of the weed and secondarily on climatic constraints. Host range testing clearly showed these species will not be able to sustain populations without their host, Brazilian pepper.

16. A commenter asked about the details on methods used for data collection.

Response: The methods used for data collection were published in peer-reviewed journal articles:

Diaz, R., V. Manrique, J.E. Munyaneza, V.G. Sengoda, S. Adkins, K. Hendricks, P.D. Roberts, and W.A. Overholt. 2015. Host specificity testing and examination for plant diseases reveal that the gall-forming psyllid, *Calophya latiforceps* (Hemiptera: Calophyidae), is safe to release for biological control of *Schinus terebinthifolia* (Sapindales: Anacardiaceae). Entomologia Experimentalis et Applicata. 154: 1–14.

Wheeler G.S., V. Manrique, W.A. Overholt, F. Mc Kay, and K. Dyer. 2017. Quarantine host range testing of *Pseudophilothrips ichini*, A potential biological control agent of Brazilian peppertree, *Schinus terebinthifolia,* in North America and Hawaii. Entomologia Experimentalis et Applicata. 162: 204–217.

See these articles for the details of data collection used for both agents.

17. A commenter asks "Where are the alternatives that don't necessarily use a form of bio-control or the no action? There needs to be more alternatives or they need to be more clearly stated."

Response: The alternatives are defined as those that are in APHIS authority, in this case permitting or not permitting either one or both of the biocontrol agents. Any other actions are outside the scope of the decision to be made by APHIS and outside the scope of this EA. There are many individuals and agencies controlling Brazilian pepper and we have included various methods that are used by others to combat Brazilian peppertree infestations (primarily herbicides and mechanical controls). These are all part of the "no action" alternative and may continue even if the biological control agents are released into the environment. APHIS is not making a decision on any other method for control of Brazilian pepper. These actions are simply described as part of the baseline.

18. A commenter asked "How well does the given data interpret the possible impacts of the bio-control?"

Until the 1960's host specificity testing of exotic weed biocontrol agents released in the United States did not occur. However, today, extensive nontarget testing of a wide range of plant species is required before APHIS approves the release of exotic biocontrol agents into the environment. Properly conducted biological control host range testing has an excellent track-record predicting the safety of the released agents. A recent review (Hinz et al., 2019) found that since the beginning of classical biological control in the 19th century, the proportion of intentionally released agents causing nontarget attacks declined from 18.2 percent in the period until the 1960s to 9.9 percent in the period from 1991 to 2008. This trend was also true for the proportion of releases causing nontarget attacks, despite an increase in the number of biological control agents released and releases made (Hinz et al., 2019). The authors conclude that this decline may be a result of improvement in methods over time that determine the environmental safety of weed biological control agents prior to their release, that regulations to import exotic biocontrol agents have become stricter, or a combination of both (Hinz et al., 2019). The authors of the review also anticipate that progress in research and technology will continue to improve environmental safety assessment methods in biological control (Hinz et al., 2019). The pre-release host range testing protocol presented here for *C. latiforceps* and *P. ichini* is an accurate predictor of the post release host range of these biological control agents.

Reference:

Hinz, H.L., R.L. Winston, and M. Schwarzländer. 2019. How safe is weed biological control? A global review of direct nontarget attack. The Quarterly Review of Biology. 94: 1–27.

19. A commenter asked what makes bio-control the best method to be used? What are the costs and how do they compare to other control methods?

Response: This question is beyond the scope of this EA. However, biological control is the safest, most cost effective means of weed control that works as a component of an integrated approach with other control methods. This method is self-sustaining, requiring a minimum of input from land managers post-release. Current control tactics have to be repeated frequently at great cost. Bio-control has been shown to have a 28 to 1 return on investment over historical timeframes.

20. A commenter asked what could be the possible direct/indirect impacts on the ecosystem or the wildlife with the reduction of the undesired Brazilian peppertree? The EA acknowledges that there could be an impact but the impact is insignificant even though there is no data given to back up this interpretation.

Response: The EA states that it is highly unlikely that there would be any impacts other than the reduction of the weed; this would likely have positive impacts on the ecosystem, but it is difficult to predict the scope or timing of these improvements. The direct effects of the release of these biological control agents will be the decrease in the community dominance presently exerted by the Brazilian pepper invasion on native habitats and agriculture. This reduction in Brazilian pepper competitive advantage will reduce the expense of, and human exposure to, herbicides. Moreover, this direct effect will include greater biodiversity and stability of invaded habitats.

There are few documented negative indirect effects of the release of these biological control agents (Hinz et al., 2019). Indirect effects are difficult to predict. One possible indirect effect may be the increase in native parasitoids that include the biological control agents in their diet. If this happens, the populations of these natural enemies could increase and spillback and increase the attack rate on their native host. However, pre-release studies in Brazil and in the invaded range indicate very few parasitoids attack these biological control agents or their surrogates.

For certain endangered and threatened species in Florida, indirect effects of Brazilian pepper biocontrol are expected to be beneficial. Gould and Hammer (1999) assert that butterflies native to pineland and hammock communities are threatened by the spread of Brazilian pepper due to replacement of host plants. Federally listed butterflies that could benefit from biocontrol of Brazilian pepper include Schaus swallowtail butterfly (*Heraclides aristodemus ponceanus*), Miami blue butterfly *Cyclargus* (=*Hemiargus*) *thomasi bethunebakeri*, Florida leafwing butterfly (*Anaea troglodyta floridalis*), and Bartram's hairstreak butterfly (*Strymon acis bartrami*). In the Everglades National Park, the nesting habitat of the threatened gopher tortoise (*Gopherus polyphemus*) is being encroached upon by Brazilian pepper (Doren and Jones, 1997), although Brazilian pepper leaves and berries are secondary or seasonal food for gopher tortoises (Ashton and Ashton, 2008). Brazilian pepper occurs on islands throughout the range of the endangered Key deer (*Odocoileus virginianus clavium*), and can out-compete native vegetation in large areas, reducing the availability of deer forage and degrading deer habitat (FWS, 2010). Brazilian pepper is likely to replace species used as food by white-tailed deer, which are important in the diet of Florida panthers (*Puma* (=*Felis*) *concolor coryi*) (Maffei, 1994). The invasion of some scrub habitat within Indian River, St. Lucie, and Martin counties by exotic plants and animals, including Brazilian peppertree, has degraded the threatened Florida scrub-jay (*Aphelocoma coerulescens*) habitat locally (FWS, 2007).

References:

Ashton, R.E., and P.S. Ashton. 2008. The natural history and management of the gopher tortoise *Gopherus polyphemus* (Daudin). Malibar, FL: Krieger Publishing Company. 275 p.

Doren, R.F., and D.T. Jones. 1997. Management in Everglades National Park. Pp. 275–286. *In:* D. Simberloff, D.C. Schmitz, and T.C. Brown (eds.), *Strangers in Paradise: Impact and Management of Nonindigenous Species in Florida*, Island Press, Washington, D.C.

Gould, W. and R. Hammer. 1999. Exotic plants and butterflies in southern Florida--the pros and cons. *In:* Jones, David T.; Gamble, Brandon W., eds. Florida's garden of good and evil: Proceedings of the 1998 joint symposium of the Florida Exotic Pest Plant Council and the Florida Native Plant Society; 1998 June 3-7; Palm Beach Gardens, FL. West Palm Beach, FL: South Florida Water Management District: 197–203.

Hinz, H.L., R.L. Winston, and M. Schwarzländer. 2019. How safe is weed biological control? A global review of direct nontarget attack. The Quarterly Review of Biology. 94: 1–27.

Maffei, M.D. 1997. Management in National Wildlife Refuges. Pp. 267–274. *In:* D. Simberloff, D.C. Schmitz, and T.C. Brown [eds.]. Strangers in Paradise: Impact and Management of Nonindigenous Species in Florida. Island Press, Washington, D.C.

U.S. Fish and Wildlife Service. 2007. Florida Scrub-Jay (*Aphelocoma coerulescens*) 5-Year Review: Summary and Evaluation. Southeast Region, Jacksonville Ecological Services Field Office, Jacksonville, Florida. 53 pp.

U.S. Fish and Wildlife Service. 2010. Key deer (*Odocoileus virginianus clavium*) 5-Year Review: Summary and Evaluation. Southeast Region, South Florida Ecological Services Office Vero Beach, Florida. 32 pp.

21. A commenter indicated that APHIS should be blamed for allowing Brazilian pepper into the United States. The commenter also states that APHIS should fine nurseries who sell Brazilian pepper and people who grow Brazilian pepper.

Response: APHIS is not responsible for the introduction of Brazilian pepper into the United States. Brazilian pepper was brought into Florida in the mid-1800s for use as an ornamental plant. APHIS was not formed as an agency until 1972. In addition, APHIS does not have the authority to fine nurseries that sell or people who grow Brazilian pepper. Only plants that are designated as Federal noxious weeds are in APHIS authority to regulate. In Florida, Brazilian peppertree is listed as a State noxious weed, a prohibited plant, and is classified as a Category I invasive plant species by the Florida Exotic Plant Pest Council. However, because Brazilian pepper is so widely distributed in the United States, it is not a candidate for the Federal noxious weed list that is regulated by APHIS under the Plant Protection Act of 2000.