Animal and Plant Health Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

Field Release of *Lophodiplosis indentata* (Diptera: Cecidomyiidae), for classical biological control of *Melaleuca quinquenervia* (Myrtaceae), in the contiguous United States

Final Environmental Assessment, January 2022

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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 release of the fly *Lophodiplosis indentata* (Diptera: Cecidomyiidae). *Lophodiplosis indentata* would be used for the classical biological control of *Melaleuca quinquenervia* (Myrtaceae) (hereafter referred to as melaleuca), in the contiguous United States.

Classical biological control of weeds is a 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.
5. Post-release monitoring.

This environmental assessment (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 *L. indentata* to control infestations of melalueca 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 December 16, 2021 for a 30-day public comment period. Five comments were received on the EA by the close of the comment period. Four comments were in favor of the proposed release of *L. indentata*, while the fifth was neither for nor against it and raised no concerns.

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 a permit application requesting environmental release of the fly *L. indentata* from Australia, and the PPBP is proposing to issue permits for this action. Before permits are issued, the PPBP must analyze the potential impacts of the release of this agent into the contiguous United States.

The applicant's purpose for releasing *L. indentata* is to reduce the severity of infestations of melaleuca in the contiguous United States. Australian punk tree or melaleuca is a large tree that grows predominantly in coastal wetlands and swamps along the east coast of tropical and sub-tropical Australia where it is native. It is also native to New Caledonia and Papua New Guinea. Melaleuca was imported into Florida as an ornamental street tree beginning in the last part of the 19th century (Dray et al., 2006) and was planted extensively in Palm Beach, Broward, Collier, and Miami-Dade Counties as they developed in the 1920's and 1930's (Pinardi, 1980). Additionally, the U.S. Army Corps of Engineers used melaleuca from 1938–1941 for levee stabilization to reduce flooding from Lake Okeechobee into agricultural fields (Dray et al., 2006). By the 1990's melaleuca covered over 200,000 hectares (ha) of wetlands in south Florida and dramatically disrupted normal water cycles, fire cycles, disturbance recovery cycles, nutrient cycling, light availability, and tree canopy structure, among other impacts, and was out competing native Florida Everglades vegetation (Bodle et al., 1994). South Florida provides nearly ideal conditions because melaleuca thrives in frequently flooded areas, its seeds begin to grow under water, and it prefers acidic, sandy soils (Myers, 1983).

Ecological impacts and economic costs of melaleuca removal are quite high, especially in sensitive wetlands. Removal entails felling mature trees, removing saplings by hand, herbicide application, and prescribed burning (Center et al., 2000). Several years of unsuccessful treatment campaigns during the 1970's and 1980's resulted in the listing of melaleuca as a state noxious weed. Due to the overwhelming costs (>$40 million cumulative) and advancing impacts of melaleuca, a biological control campaign began in 1986. This campaign produced three successful biological control agents that have been previously released into the United States: the beetle *Oxyops vitiosa*, the psyllid insect *Boreioglycaspis melaleucae*, and the fly *Lophodiplosis trifida*. Alone and in combination with other removal methods, the spread of melaleuca has been prevented and coverage in important habitats in south Florida has been reduced by nearly 50 percent (Rodgers, 2016).

Despite major gains in controlling melaleuca, many localized areas are still invaded by melaleuca. These regions are frequently treated with aerial herbicides, contributing to further non-target impacts. Hand spraying contracts cost between $1 million and $3 million annually. In regions where all three biological control insects are present, they effectively suppress both recruitment and size of existing trees. This suppression allows land managers to apply herbicides or other control efforts at much longer intervals than without biological control (Pratt et al., 2006; Tipping et al., 2016). However, in areas with low available resources for control or those where current biological control organisms are not adequately effective, biological control could be improved. Therefore, the applicant has a need to release *L. indentata,* a host-specific, biological control organism for the control of melaluca, into the environment to add to the impact of the three previously released biological control agents.

II. Alternatives

This section will explain the two alternatives available to the PPBP—no action and issuance of permits for environmental release of *L. indentata*. Although the PPBP's alternatives are limited to a decision on whether to issue permits for release of *L. indentata*, other methods available for control of melaleuca 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 *L. indentata*, depending on the efficacy of *L. indentata* to control melaleuca. These are methods presently being used to control melaleuca by public and private concerns.

A third alternative was considered but will not be analyzed further. Under this third alternative, the PPBP would have issued permits for the field release of *L. indentata*; 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, PPBP would not issue permits for the field release of *L. indentata* for the biological control of melaleuca. The release of this biological control agent would not take place. The following methods are presently being used to control melaleuca; these methods will continue under the "No Action" alternative and will likely continue even if permits are issued for release of *L. indentata*, depending on the efficacy of the organism to control melaleuca.

1. Chemical Control

The primary method used to remove large melaleuca trees involves cutting into the trunks then squirting herbicide into the wounds. The cuts can either girdle the bark on large trees or completely sever the trunk of small trees. Herbicides, such as imazapyr or imazapyr combined with glyphosate, are applied by hand directly onto the exposed cambial layer. Fairly low concentrations of triclopyr products also work on cut stumps and greatly reduce non-target damage (Center, 2007).

2. Mechanical Control

Trees are removed with heavy equipment in accessible areas, such as along canals, utility rights-of-way, and in new developments. Seedlings and small saplings may be hand pulled, especially after the older trees are killed or removed. Mechanical removal is often followed by herbicide applications (Langeland and Craddock Burks, 1998). Prescribed fire to manage melaleuca by burning of mature stands should be timed either at the beginning of the wet season when soil is moist enough to induce germination and seasonal flooding will soon submerge and kill seedlings, or at the end of the wet season when soil moisture is still high enough for germination but drought will soon kill seedlings (University of Florida, IFAS, 2007).

3. Biological Control

Three biological control organisms have been released in Florida against melaleuca: *Oxyops vitiosa*, a leaf-feeding weevil, the psyllid, *Boreioglycaspis melaleucae* Moore (Hemiptera: Psyllidae), and the fly *Lophodiplosis trifida* Gagné (Diptera: Cecidomyiidae). Plant feeding by these three insects cause premature leaf drop (Morath et al., 2006), and reductions in above-ground biomass (Rayamajhi et al., 2016), tree and seedling height (Pratt et al., 2014), seed production (Pratt et al., 2006), and seedling density (Franks et al., 2006). *Oxyops vitiosa* provides the greatest amount of control through extensive feeding on meristematic tissue (areas of plant tissue where active growth occurs), but all three insects have contributed significantly to the reduction of melaleuca throughout south Florida (Rayamajhi et al., 2007; 2008).

B. Issue Permits for Environmental Release of *Lophodiplosis indentata.*

Under this alternative, the PPBP would issue permits for the field release of the fly *L. indentata* for the biological control of melaleuca. These permits would contain no special provisions or requirements concerning release procedures or mitigating measures.

Biological Control Organism Information

1. Taxonomy and Description

The proposed biological control organism, *Lophodiplosis indentata* Gagné, was described, along with four other species in the same genus, from surveys in Australia (Gagné et al., 1997). All of the species in this genus are gall-forming[2] melaleuca specialists within the insect family Cecidomyiidae, suborder Nematocera, order Diptera (Gagné et al., 1997). The Nematocera include black flies, mosquitoes, crane flies, gnats, and a group of "midge" families incuding gall-forming Cecidomyiidae. The members of the Nematocera are characterized by slender, finely jointed antennae with six or more segments, very often plumose in the males (Borror et al., 2005).

All type specimens of *L. indentata* are housed at the USDA-Agricultural Research Service (ARS) Systematic Entomology Laboratory collections at the Smithsonian National History Museum in Washington, DC. Additional specimens are housed at the Florida Department of Plant Industry in Gainesville, Florida and at the USDA-ARS Invasive Plant Research Laboratory in Fort Lauderdale, Florida.

[2] Galls are abnormal growths that occur on leaves, twigs, roots, or flowers of many plants. Most galls are caused by irritation and/or stimulation of plant cells due to feeding or egg-laying by insects such as aphids, midges, wasps, or mites (Morton Arboretum, 2020).

2. Geographical Range of *L. indentata*

Lophodiplosis indentata occurs throughout the range of melaleuca in coastal eastern Australia from northern Queensland to New South Wales (Gagné et al., 1997). *Lophodiplosis indentata* was originally collected and cultivated from populations near and around Brisbane, Queensland. All of the populations used for host specificity testing (discussed later in this document) are a mixture of populations collected throughout southeast Queensland and coastal New South Wales.

3. Life History of *L. indentata*

Lophodiplosis indentata lays eggs (oviposits) on new, tender leaves at the stem tips of melaleuca (Fig. 1A). Eggs hatch and the newly-hatched (neonate) larvae bore into leaf tissue, causing a pea gall or blister gall to form around the developing larva. A single larva goes through three developmental stages (instars) within a two-chambered gall (Fig. 1B). As the larva nears pupation, it prepares an exit window by chewing through the hardened gall tissue (Fig. 1C). Adults emerge at the prepared exit holes in the leaf tissue and leave behind the pupal "skin" (exuviae) that sticks out from the exit hole (Fig. 1B). Adults live between 3–5 days during which time females oviposit between 100 and 300 eggs (~226 ± 36 eggs average). Eggs hatch in 2 ± 0.5 days. Total time from egg to adult is approximately 47 ± 1.2 days at 25°Celcius (C). The time from *L. indentata* egg laying (oviposition) to the first observance of galls on those plants is 15–17 days under optimal conditions, but gall development may take as long as 30 days. Due to their overlapping ranges in the native range, it is anticipated that *L. indentata* will closely mirror the range and expansion observed for *L. trifida*.

Adult *L. indentata* flies oviposit on newly flushed plant material that is soft enough for neonate mouth parts to penetrate. The potential range and feeding preferences of *L. indentata* overlap with those of *Oxyops vitiosa*, the melaleuca snout beetle, (e.g., newly emerged leaves), but resource limitations are not anticipated for either insect. This is primarily due to 1) an abundance of plant material, especially in areas with managed melaleuca populations (e.g., burning and chemical treatment regrowth) and 2) gaps in *O. vitiosa* range due to unfavorable pupation conditions. Additionally, Raghu et al. (2012) found that previous plant use by other galling or leaf-feeding insects did not influence the plant material available for *L. indentata* to use.

Figure 1. A) Lophodiplosis indentata (female with newly oviposited eggs on melaleuca foliage). B) Mature pea gall with emergent adult. C) Dissected galls showing larva within the gall and developing exit windows. D) Uninfested (Left) and infested (Right) melaleuca saplings.

III. Affected Environment

A. Taxonomy and Description of Melaleuca

Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Rosidae
Order: Myrtales
Family: Myrtaceae
Subfamily: Myrtoideae
Tribe: Melaleuceae
Genus: *Melaleuca*
Species: *quinquenervia* (Cav.) Blake

Melaleuca quinquenervia (Cav.) Blake has several common names, primarily originating from Australia. These include melaleuca, cajeput, punk tree, bottle brush tree, niaouli, paper bark tree, and broad-leaved paperbark. Many of these names are not specific to *M. quinquenervia* and are regularly used for other species in the genus (Bodle et al., 1994). Synonyms include *M. leucadendra* L., *M. viridiflora* var. *angustifolia* (L.f.) Byrnes, *M. viridiflora* var. *rubiflora* Brong. and Gris., and *Metrosideros quinquenervia* Cav.

Melaleuca is part of the diverse and widespread plant family Myrtaceae in the order Myrtales. Myrtaceae contains the subtribes, Myrtoideae, Heteropyxidae, and Psiloxyleae. The Myrtaceae is sister to the plant family Vochysiaceae, which has a South American origin (Sytsma et al., 2004). The family Myrtaceae is diverse and has wet tropical, dry tropical, and temperate representatives (Wilson et al., 2005; Thornhill et al., 2015).

Melaleuca is a small to medium sized tree, though heights frequently reach 20 meters (m) or higher in the introduced range, depending on growing conditions. The bark is thick and papery, made up of many layers and prone to large splits in more mature trees. Mature leaves are dull green, leathery, a narrow oval shape tapering to a point at each end, and 6–24 millimeters (mm)) long. Seedlings and new growth are covered in fine hairs, giving them a silvery appearance. In south Florida, populations flower twice per year, once in fall and again in early summer, although flowering can occur year-round. Flowers have thick, puffy spikes made of clustered stamens, 10–20 mm in length. Once pollinated, melaleuca seeds are formed in small, woody capsules that drop seeds relatively close to the maternal tree (frequently less than 5 m away) (Blake, 1968; Laroche, 1999).

B. Areas Affected by Melaleuca

1. Native Range of Melaleuca

The center of origin of melaleuca is northeastern Australia. Its range includes much of the coastal region from Sydney northward as well as New Caledonia and Papua New Guinea (Craven and Lepschi, 1999).

2. Introduced Range of Melaleuca

It is present as an ornamental in Brazil, China, Hawaii, Mexico, Venezuela, Costa Rica. In Hong Kong, it was widely planted by local agencies and is now naturalized and spreading. This is also true in the Caribbean area (Pratt et al., 2005). In North America, melaleuca is naturalized in south Florida, Hawaii, and throughout the northern Caribbean islands (Cuba, Bahamas) with plantings in California, Texas, and Louisiana (Wheeler et al., 2007; University of Georgia, 2017). It has become invasive in Florida. Large trees in central Florida die back to the trunk after hard freezes then refoliate. Freezing temperatures kill smaller trees so melaleuca probably could not invade areas far outside the current naturalized and cultivated distribution. However, within the current distribution, it could expand into coastal marshes of California and wetlands of Louisiana and Texas if seed sources were present.

3. Habitats Where Melaleuca is Found in North America

Throughout south Florida, melaleuca invades wet and moderatley moist areas in both disturbed and undisturbed areas (Dray et al., 2006). Melaleuca causes significant damage to wetland habitats, particularly the Everglades
ecosystem. Large tracts of trees are common from the Florida Keys north to Daytona (University of Georgia, 2017). Melaleuca has had several introductions into Florida, but it was first introduced in the late 1800's through the horticulture trade (Dray et al., 2006). By the 1920's, melaleuca had spread from plantings near Naples, FL into the adjacent cypress swamps (Dray et al., 2006). The infestation reached peak density in the 1980's and 1990's before an integrative management plan was proposed to address the worsening problem.

When the first biological control agent, *Oxyops vitiosa*, was released in 1996, melaleuca covered 200,000 hectares of land in south Florida, much of which was in the historical Everglades fooprint. In contrast, during more recent reconnaissance flights, Rodgers et al. (2014) determined that 90 percent of the infestation area had coverage values of less than 10 percent, and the current infested area is reported at approximately 36,000 hectares (University of Georgia, 2017). The areas where melaleuca still persists frequently lie within privately managed lands where management resources are limited.

C. Plants Related to Melaleuca and Their Distribution

1. Native and Non-Native Relatives

No native species of *Melaleuca* exist in North America. *Melaleuca alternifolia* Cheel is grown in plantations for essential oil, though not in large quantities within the United States. Species formerly in the genus *Callistemon* and now in the genus *Melaleuca*, *Melaleuca citrina* (synonym (syn.) *Callistemon citrinus*) (crimson bottlebrush) and *Melaleuca viminalis* (weeping bottlebrush), are frequently used as ornamental plants. These species are small trees with attractive bright red flowers, but like, *M. quinquenervia*, also show potential for invasiveness with infestations in California, Arizona, and Florida.

2. Distribution of Plants Related to Melaleuca

Native species in the genera *Eugenia*, *Calyptranthes*, *Myrcianthes*, and *Mosiera* make up a large part of the plants that occur in hardwood hammock understories in the Florida landscape. These are common in areas of higher ground in far south Florida and into the Florida Keys. Several of these, including *Calyptranthes pallens* (syn. *Myrcia neopallens*), are state-listed as threatened or endangered due to habitat loss. For many of these species, Florida is the northernmost extent of their range. Many of these species range throughout the West Indies, and even into Mexico and Central America. *Eugenia uniflora* and *Rhodomyrtus tomentosa* are also invasive in Florida, though in upland and scrub habitats rather than wetlands where melaleuca is prevalent. Guava (*Psidium* species) and rose apples (*Syzygium* species) are commonly cultivated in Florida, but only in small orchards or individual trees on private residences. *Syzygium jambos* is native to Southeast Asia, but is cultivated, along with *Syzygium malaccense* in the Caribbean as a food crop.

IV. Environmental Consequences

A. No Action

1. Impact of Melaleuca

a. Animals

Melaleuca invades freshwater marshes, including sawgrass prairies, and transforms them into forested areas (Laroche, 1994). Melaleuca forests provide limited food and habitat value for native wildlife (Dray et al 2006; Bodle et al., 1994, O'Hare and Dalrymple, 1997; Dray et al., 2009).

b. Native Plants

Melaleuca invades wetlands throughout south Florida, including vast areas within Everglades National Park (Gordon, 1998). Volatile oils contained in melaleuca leaves and branches increase fire severity, which alters native plant communities (Austin, 1978; Martin et al., 2011). While melaleuca invades human disturbed and altered habitats (e.g. canal banks, roadsides, pastures, water catchment areas), its ability to invade and impact intact natural areas is perhaps its most problematic trait (Turner et al., 1997). Melaleuca invades freshwater marshes, including sawgrass prairies, and transforms them into forested tracts. While huge, dense stands are now rare for melaleuca invasions, populations persist in wet areas. These areas with melaleuca are extremely costly to access and treat. Due to changing land use, surface flow water dynamics, and shifting climate limits, the potential habitat for melaleuca has expanded from its original invasive range (Watt et al., 2009).

c. Human Health

Despite previous reports of allergic sensitives, Stablein et al. (2002) found that melaleuca is not a significant allergen nor is the odor from leaves, flowers and wood a respiratory irritant. However, oils stored within branches, leaves and wood tissue are extremely volatile and can act as a fire accelerant (Flowers, 1991). Melaleuca invasion increases fire frequency, intensity and spread, which has direct health impacts on nearby human populations (Flowers, 1991).

d. Social and Recreational Uses

Melaleuca was widely planted throughout Florida, especially in coastal areas, where it grew quickly and withstood flooding. Some of these trees planted in the 1920's–1940's can still be seen in south Florida neighborhoods (e.g., Coral Gables and Naples) (Dray et al., 2006). Melaleuca began escaping cultivation by the 1930's and mechanical and chemical control efforts were begun in the 1970's. In 1990 the Florida Exotic Pest Plant Council listed it as a major invader, Florida banned its sale or transport, and a concerted effort to control the spread and severity of the invasion began. In the United States, melaleuca provides no known commercial, social, or recreational service.

2. Impact from Use of Other Control Methods

The continued use of chemical, mechanical, 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 ability of *L. indentata* to reduce melaleuca populations in the contiguous United States.

a. Chemical Control

Herbicide applications are effective at killing larger melaleuca trees, but do not suppress the regrowth from the large seed bank in the soil, making multiple reapplications necessary (Thayer and Laroche, 1994). In addition, non-target species, especially native species, suffer damage from non-selective products.

b. Mechanical Control

Mechanical control methods will have no adverse effects outside of the treatment areas. However, mechanical control is not appropriate for sensitive natural areas due to habitat destruction caused by heavy equipment. Also, mechanical removal often requires a followup chemical treatment to be effective.

c. Biological Control

In regions in Florida where all three previously released biological control insects (*Oxyops vitiosa, Boreioglycaspis melaleucae,* and *Lophodiplosis trifida*) are present and environmental conditions are favorable for them, they effectively suppress both recruitment and size of existing trees. This suppression allows land managers to apply herbicides or other control measures at much longer intervals than without biological control (Pratt et al., 2006; Tipping et al., 2016). However, in areas with low available resources for control or areas where the three biological control organisms are not adequately effective (areas where habitat flooding interferes with their survival and development) biological control could be improved.

B. Issue Permits for Environmental Release of *Lophodiplosis indentata*

1. Impact of *L. indentata* on Nontarget Plants

Host specificity of *L. indentata* to melaleuca has been demonstrated through scientific literature and host range testing. If the candidate biological control agent only attacks one or a few plant species closely related to the target weed, 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

Lophodiplosis species are *Melaleuca*-species specialists restricted to Australia. *Lophodiplosis trifida,* an approved and established biological control organism for melaleuca has an extremely narrow host range with development only occurring on *M. quinquenervia* and two other *Melaleuca* species (*M. dealbata* and *M. viridiflora*) (Purcell et al., 2007; Pratt et al., 2013) (table 1).

Table 1. *Lophodiplosis* species and their host plants (From: Smith et al., 2019).

L. trifida (Gagné) - Described from Townsville, Queensland from stem galls on *M. quinquenervia*
L. denticulata (Gagné) - Described from Townsville, Queensland from blister galls on leaves of *Melaleuca* species.
L. bidentata (Gagné) - Described from Townsville, Queensland from rosette galls on *Melaleuca* species.
L. cornuata (Gagné) - Described from Townsville, Queensland from trumpet galls on *Melaleuca viridiflora*
L. indentata (Gagné) - Described from Townsville, Queensland from blister galls on leaves of *Melaleuca* species.

b. Host Range Testing

Quarantine host range testing was conducted to determine the specificity of *L. indentata* to melaleuca and to determine if plants in the contiguous United States could be at risk of attack by *L. indentata.*

(1) Site of Quarantine Studies in the United States

Quarantine host specificity studies were conducted at the USDA-ARS Invasive Plant Research Lab, Gainesville, Florida, and the USDA-ARS Invasive Plant Research Lab, Fort Lauderdale, Florida.

(2) Test Plant List

Test plant lists are developed by researchers for determining the host specificity of biological control organisms of weeds in North America. Test plant lists are usually developed on the basis of the relatedness 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 host specificity test strategy as described by Wapshere (1974) is "a centrifugal phylogenetic testing method which involves exposing to the organism a sequence of plants from those most closely related to the weed species, progressing to successively more and more distantly related plants until the host range has been adequately circumscribed." Researchers do not pursue release of biological control organisms that do not demonstrate high host specificity to the target weed. In the case of *L. indentata, r*esearchers tested 46 plant species as potential hosts for *L.*

indentata. See appendix 1 for details of the plant selection process and plants tested.

(3) Discussion of Host Specificity Testing

See appendix 1 for a complete description of host specificity test design and results.

Researchers conducted no-choice host specificity tests to determine the host range of *L. indentata.* In no-choice tests, the biological control organism is not offered any other choice for egg-laying except the test plant and not its preferred host plant. In these tests, *L. indentata* displayed a high level of developmental specialization on the target weed, *Melaleuca quinquenervia*. No gall development or adult emergence occurred on any plant species except *M. quinquenervia*, including three other *Melaleuca* species (see table 1-3 in appendix 1). However, egg laying occurred on several non-target species including *Melaleuca citrina, Calyptranthes pallens, Eugenia axillaris, Pittosporum tobira, Persea americana, Mosiera longipes, Myrcianthes fragrans, Tibouchina semidecandra, Illicium parviflorum, Eucalyptus camaldulensis,* and *Rhodomyrtus tomentosa.* This is likely due to the tendency of *L. indentata* and other similar insects to dump their eggs right before death, especially when they do not have the preferred host plant available to them (Wright and Center, 2008). Wright and Center (2008) found that the previously released biological control insect *L. trifida* behaved similarly in its unselective egg laying, but has proven to be very specific to melaleuca in laboratory host range tests, field host specificity tests, and in the field in Florida (Pratt et al., 2013).

2. Impact of *L. indentata* on Melaleuca

Field examples of the sole impact of *L. indentata* on melaleuca are not possible to determine in its native range because there are multiple gall formers on melaleuca there. Therefore, a study was conducted to determine the impact of *L. indentata* on melaleuca alone, and in combination with *L. trifida.* See appendix 1 for study details.

In the study, researchers found that in seedlings, *L. indentata* alone significantly reduced stem size of melaleuca. Galling from *L. indentata* along with *L. trifida*, significantly reduced the height growth rate in seedlings. Compared to controls and individual impacts, *L. indentata* and *L. trifida* impact sapling height growth rate more together than alone. No strong evidence was found to suggest that these insects would work against one another.

Observations from the native range indicate that in the field, *L. trifida* and *L. indentata* feed and form galls on different areas of the melaleuca plant. *Lophodiplosis trifida* is found in rapidly growing plant tissue close to the ground (i.e., seedlings and saplings), whereas *L. indentata* generally galls leaf tissue higher up in the plant. Melaleuca directs significant amounts of resources towards these galls which results in reduced sapling height when both insects are present. Leaf feeding by other biological control insects on melaleuca resulted in reductions in seed production, flowering, and seedling survival, among other things (Pratt et al., 2006; Rayamajhi et al., 2007; 2008; Center et al., 2012; Tipping et al., 2016; 2018). Thus, *Lophodiplosis indentata* could also have a significant impact on melaleuca by diverting plant resources away from reproductive structures.

3. Impact on Animals

Reduction of melaleuca by *L. indentata* could be beneficial to animals because it would reduce the potential of melaleuca to dominate animal habitats. Melaleuca forests provide limited food and habitat value for native wildlife (Dray et al 2006; Bodle et al., 1994, O'Hare and Dalrymple, 1997; Dray et al., 2009).

4. Impact on Human Health

No negative human impacts are anticipated from release of *L. indentata*. No reports of adverse interactions from Australia or the United States exist for any *Lophodiplosis* species. Adults are nectar feeders and cannot bite or pierce tissues (plant or animal). Larvae feed internally in their leaf galls and never come in contact with the environment. On the other hand, fewer melaleuca seedlings and trees would contribute significantly to alleviating human health problems associated with fires accelerated by melaleuca.

5. Social and Recreational Uses

Lophodiplosis indentata would not have any social, recreational, or commercial impacts other than potential improvement of habitat. Melaleuca has no social, recreational, or commercial uses; thus, release of *L. indentata* would not affect them.

6. Uncertainties Regarding the Environmental Release of *L. indentata*

Once a biological control agent such as *L. indentata* is released into the environment and becomes established, there is a slight possibility that it could move from the target plant (melaleuca) to attack nontarget plants. 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 *L. indentata*, the resulting effects could be environmental impacts that may not be easily reversed. Biological control agents such as *L. indentata* 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 melaluca populations in Florida. 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 melaleuca by *L. indentata* will not be known until after release occurs and post-release monitoring has been conducted (see Appendix 2 for release protocol and post-release monitoring plan). It is expected that *L. indentata* will work in concert with other previously released melaleuca biological control agents, likely restricting further spread of melaleuca in Florida.

7. 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 melaleuca in invaded areas in Florida using available chemical, mechanical, and biological control methods. Release of *L. indentata* is not expected to have any negative cumulative impacts in the contiguous United States because of its host specificity to melaleuca. Effective biological control of melaleuca 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 melaleuca.

8. 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 are no plants that are federally listed or proposed for listing in the family Myrtaceae, the same family as the target weed. In no-choice host specificity testing, egg laying by *L. indentata* occurred on a plant in the family Lauraceae (*Persea americana*). A federally listed plant (pondberry) occurs in the family Lauraceae. No gall formation, tissue swelling, development, or adult emergence occurred on any non-target species used in testing. Adults only emerged from melaleuca. *Lophodiplosis indentata* would not likely oviposit on pondberry if it were to encounter it under natural conditions, and no development, feeding, or gall formation would occur on it. In addition, pondberry does not occur within the range of melaleuca so would not likely be exposed to *L. indentata*. Therefore APHIS has determined that release of *L. indentata* may affect, but is not likely to adversely affect pondberry.

APHIS determined that release of *L. indentata* may affect, but is not likely to adversely affect the Florida bonneted bat (*Eumops floridanus*) and Everglade snail kite (*Rostrhamus sociabilis plumbeus*). These species are known to use melaleuca for roosting and nesting, but *L. indentata* would not affect large trees as used by these animals.

APHIS has also determined that the release of *L. indentata* may affect beneficially the Florida panther (*Puma* (=*Felis*) *concolor coryi*), Cape Sable seaside sparrow (*Ammodramus maritimus mirabilis*), wood stork (*Mycteria americana*), American crocodile (*Crocodylus acutus*), and Okeechobee gourd (*Cucurbita okeechobeensis* ssp. *okeechobeensis*) because melaleuca has been reported to negatively impact these species.

A biological assessment was prepared and submitted to the U.S. Fish and Wildlife Service (FWS) and is part of the administrative record for this EA (prepared by T.A. Willard, July 20, 2020). APHIS requested concurrence from the FWS on these determinations and received a concurrence letter dated January 14, 2021.

V. Other Issues

A. Equity and Underserved Communities

In Executive Order (EO) 13985, Advancing Racial Equity and Support for Underserved Communities Through the Federal Government, each agency must assess whether, and to what extent, its programs and policies perpetuate systemic barriers to opportunities and benefits for people of color and other underserved groups. In EO 12898, Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations, Federal agencies must identify and address disproportionately high and adverse human health or environmental impacts of proposed activities.

Consistent with these EOs, 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 *L. indentata* and will not have disproportionate adverse effects to any minority or low-income populations.

Federal agencies also comply with EO 13045, Protection of Children from Environmental Health Risks and Safety Risks. This EO requires each Federal agency, consistent with its mission, to identify and assess environmental health and safety risks that may disproportionately affect children and to ensure its policies, programs, activities, and standards address the potential for disproportionate risks to children. Consistent with this EO, 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 *L. indentata*.

B. Tribal Consultation and Coordination

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 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 *L. indentata* on May 22, 2020. The TAG members that reviewed the release petition (19-01) (Smith et al., 2019) included USDA representatives from the Animal and Plant Health Inspection Service, National Institute of Food and Agriculture, U.S. Forest Service, and Agricultural Research Service; U.S. Department of Interior's U.S. Geological Survey, Bureau of Land Management, and U.S. Fish and Wildlife Service; U.S. Army Corps of Engineers; and representatives from the National Plant Board, Weed Science Society of America, Mexico Secretariat of Agriculture, Livestock, Rural Development, and Fisheries, and Agriculture and Agri-Food Canada.

This EA was prepared by personnel at APHIS and ARS. The addresses of participating APHIS units, cooperators, and consultants follow.

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Policy and Program Development
Environmental and Risk Analysis Services
4700 River Road, Unit 149
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U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine
Pests, Pathogens, and Biocontrol Permits
4700 River Road, Unit 133
Riverdale, MD 20737

U.S. Department of Agriculture
Agricultural Research Service
Invasive Plant Research Laboratory,
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Ft. Lauderdale, FL 33314

U.S. Fish and Wildlife Service
Branch of Environmental Review
5275 Leesburg Pike, MS:ES

Falls Church, VA 22041

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Appendix 1. U.S. host-specificity testing methods and results (Smith et al., 2019).

All references cited in this appendix are included in section VII. References.

1. Known Host Specificity

Lophodiplosis species are multivoltine *Melaleuca* specialists restricted to Australia (Table 1-1). *Lophodiplosis trifida*, an approved and established biological control for *M. quinquenervia*, has an extremely narrow host range with development only occurring on *M. quinquenervia* and two other species within the *M. leucadendron* complex (*M. dealbata* and *M. viridiflora*) (Purcell et al., 2007; Pratt et al., 2013).

Table 1-1. *Lophodiplosis* species and their host plants.

L. trifida (Gagné) Described from Townsville, Queensland from stem galls on *M. quinquenervia*
L. denticulata (Gagné) Described from Townsville, Queensland from blister galls on leaves of *Melaleuca* spp.
L. bidentata (Gagné) Described from Townsville, Queensland from rosette galls on *Melaleuca* spp.
L. cornuata (Gagné) Described from Townsville, Queensland from trumpet galls on *M. viridiflora*
L. indentata (Gagné) Described from Townsville, Queensland from blister galls on leaves of *Melaleuca* spp.

2. Populations of the Agent Studied

The population of *L. indentata* used for testing was sourced from several shipments of insects collected on test trees in Indooroopilly, Australia from 2011–2013 (mixed populations from Queensland). All shipments were sent to the Invasive Plant Research Laboratory quarantine facility in Gainesville, Florida. The identity of the insect was confirmed by R.J. Gagné through morphological characters. Several individuals were also used for DNA extractions and those sequences are now stored in GenBank.

3. Test Plant List

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. Standard protocols for selecting the test plant list were used as laid out in the TAG manual based on the Wapshere (1974) approach with modifications described in Briese and Walker (2008). The host specificity test strategy as described by Wapshere (1974) is “a centrifugal phylogenetic testing method which involves exposing to the organism a sequence of plants from those most closely related to the weed species, progressing to successively more and more distantly related plants until the host range has been adequately circumscribed." Researchers do not pursue release of biological control agents that do not demonstrate high host specificity to the target weed.

The test plant list used for *L. indentata* has been used for three previous melaleuca biocontrol insects and focuses on North American and Caribbean taxa. This list includes all Myrtaceous flora (exotic and native) in Florida and the northern Caribbean (the entire range of *M. quinquenervia*). The previous tests relied on taxonomic distinctions, rather than genetic relatedness, but to confirm the test plant list, genetic analyses were conducted using chloroplast genes. Though no species were omitted or added, the Wapshere categories were shifted based on Wilson et al. (2005). Based on analyses utilizing the matK chloroplast region, they split the Myrtaceae sensu lato into several tribes rather than subfamilies. Later work by Craven (2006) provided molecular and morphological evidence to absorb bottlebrushes (genus *Callistemon*) into *Melaleuca*. Though the Myrtaceae is cosmopolitan, the Melaleuceae tribe is restricted to Australia, New Guinea, and New Caledonia. Florida native members in the Syzygieae and Myrteae tribes, and cultivated, exotic or invasive members in the Eucalypteae and Leptospermeae tribes were included in testing, which do not have native members in the Florida or Caribbean flora. Non-related Florida native species and economic crops grown in Florida such as *Eriobotrya japonica* and *Citrus* x *aurantium* L, were also included in testing.

North American Test Plants by TAG Category (See Tables 1-2 through 1-7 for the complete species test list.)

Category 1: Genetic types of the target weed species.

Despite multiple introductions of *Melaleuca quinquenervia* to Florida during the early 20th century, it appears that the genotypic makeup remained relatively stable (Dray et al., 2006). Two chemotypes exist in Australia and Florida, where they occur in approximately equal admixture (Wheeler et al., 2007). Previous experiments with *L. trifida* showed no preference between these chemotypes (Pratt et al., 2013). Seeds were used to propagate seedlings that were sourced widely from the invaded range and presumably contained both chemotypes although the researchers did not explicitly test for this.

Category 2: Species in the same genus as the target weed, including environmentally and economically important species.

Melaleuca is a large genus containing nearly 350 species from Australia and New Caledonia (Edwards et al., 2010). Craven et al. (2014) provide reasoning and nomenclatural guidance suggesting that all genera within Melaleuceae should be incorporated into *Melaleuca* including those formerly within *Callistemon*. No other *Melaleuca* species are invasive in the United States, but *M. citrina* (crimson bottlebrush) is widely cultivated in Florida and Puerto Rico. *Melaleuca armillaris* and *M. linariifolia* var. *trichostachya* are both narrowleaved paper barks that are cultivated in California for horticulture.

Category 3: Species in other genera in the same family as the target weed, divided by tribes, including environmentally and economically important species.

The Myrtaceae is a large, mostly neotropical plant family, primarily distributed throughout the Southern Hemisphere (Grattapaglia et al., 2012). Within the Myrtaceae, there are 16 tribes containing over 5,650 species with the Myrteae containing about half of those described species. Of the Eucalypteae tribe, *Eucalyptus amplifolia, E. camaldulensis, E. cinerea, E. neglecta*, and *E. grandis* were tested, all of which are cultivated in either Florida, California, Hawai'i, and/or Puerto Rico. Within the Leptospermeae, *Leptospermum petersonii* was included, which is cultivated in California and Hawai'i as an ornamental tree and for essential oils.

All of the native Florida Myrtaceae species belong within the tribe Myrteae. Native and non-native members of the Myrteae were tested, including several species of *Eugenia* (Fig. 1-1), and two species of guava (*Psidium*). Another invasive myrtaceous species, *Rhodomyrtus tomentosa*, was also tested.

For the diverse tropical Syzygieae tribe, *Syzygium cumini* and *S. paniculatum* were tested. *Syzygium cumini* is listed as a Category 2 invasive species in Florida. Additionally, horticulturally and economically important Florida, California, and Caribbean species were tested: allspice (*Pimenta dioca*) and jabuticaba (*Myrciaria cauliflora*).

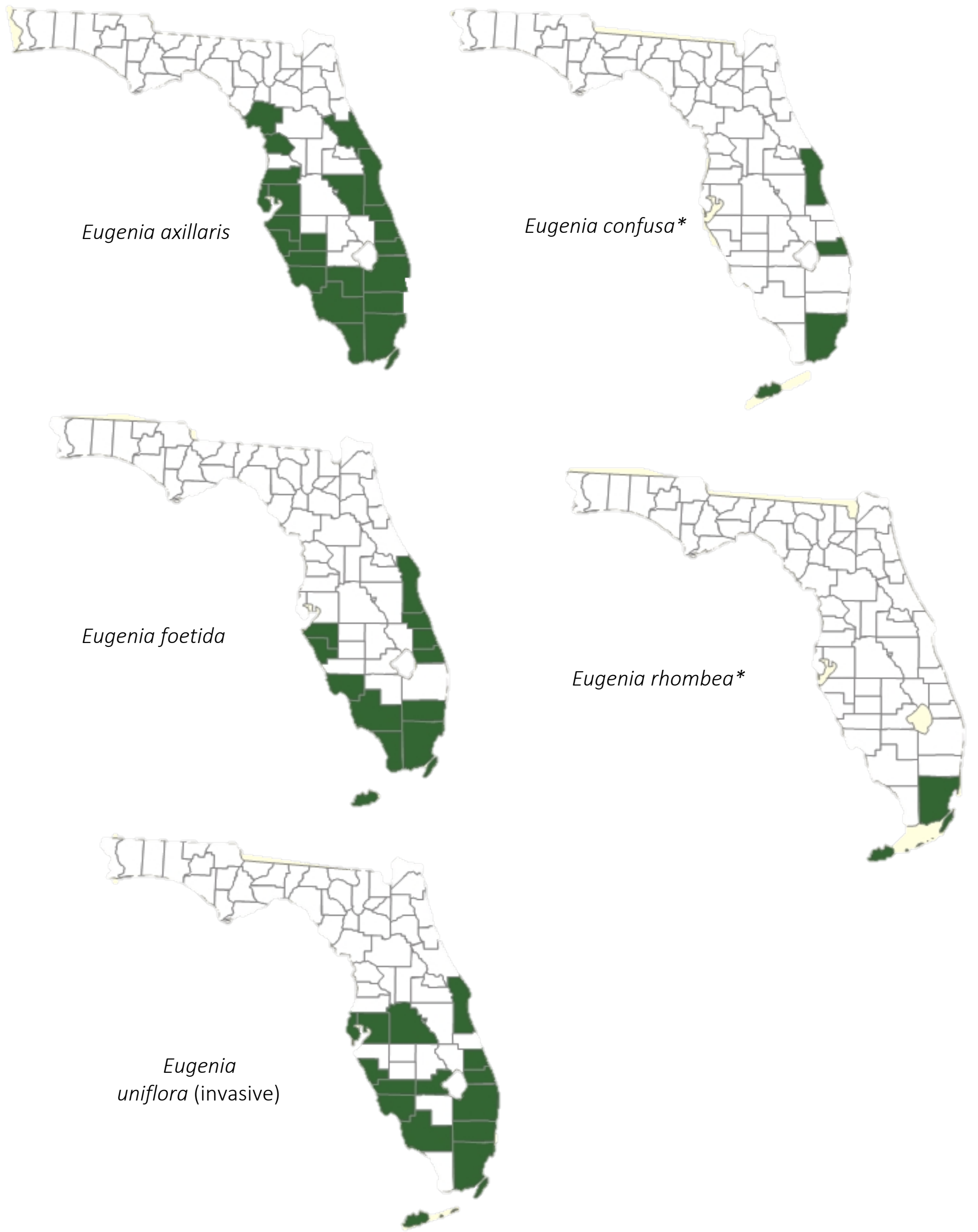

Figure 1-1. Distribution of native and invasive *Eugenia* species in Florida (Myrtaceae). Source: Atlas of Florida Plants (2020). An (*) denotes that species is listed within the state as threatened or endangered.

Category 4: Threatened and endangered species in the same family as the target weed.

In Florida, the Myrtaceae is represented by eight native species, six of which are state-listed as endangered or threatened – no species in the contiguous United States are federally listed. Most of the plants on this list are at the northernmost extent of their range in Florida and range throughout the West Indies. *Calyptranthes pallens* and *C. zuzygium* are threatened and endangered, respectively, in Florida, primarily owing to their limited distribution as hardwood hammock habitat specialists. Both species are at the northernmost extent of their ranges in the Florida Keys and Miami-Dade County. *Eugenia confusa* is a small coastal hammock tree along the Atlantic Ridge and extends as far north as Martin County. *Eugenia rhombea* is a very rare rockland hammock constituent that is under threat in Florida due to habitat loss. Another rocklands constituent facing habitat loss and plummeting populations is *Mosiera longipes*, a state listed threatened species. Whereas the other listed species are generally Keys, West Indies, or far south peninsular species, *Myrcianthes fragrans* is a hardwood hammock native that extends well into central and west Florida (Fig. 1-2).

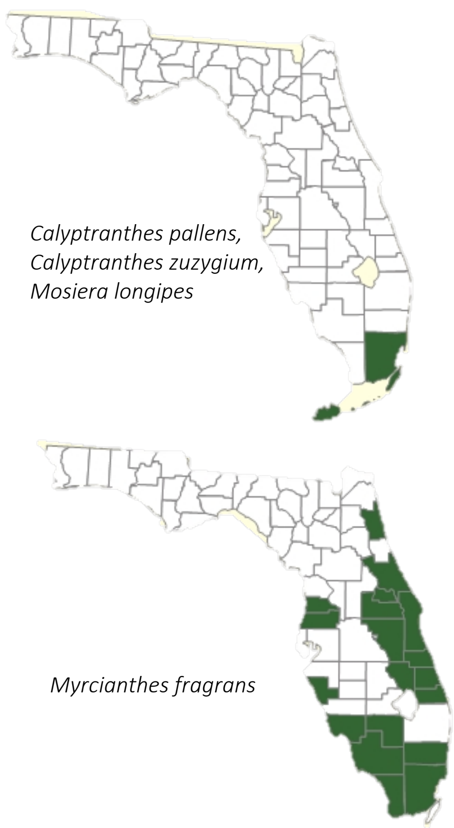

Figure 1-2. State-listed threatened and endangered members of Myrtaceae in Florida.

Category 5: Species in the same order (Myrtales) as the target weed.

The Myrtales underwent diversification largely in the southern hemisphere with ancestries in South America (Lythraceae, Melastomataceae, Onagraceae, Vochysiaceae) or Africa and Australia (the [Crypteroniaceae [Alzateaceae + Penaeaceae]] (CAP) clade, Myrtaceae) (Berger et al., 2016). The Myrtaceae and Vochysiaceae form a clade that is sister to another larger clade containing the Melastomataceae and the CAP clade (Berger et al., 2016). Representatives from several of these families were tested to gauge order-level specificity, including *Bucida buceras* (Combretaceae), a large ornamental tree native to the Caribbean, Mexico, and Central America, *Lagerstroemia indica* (Lythraceae), or crepe myrtle, a small flowering tree from Asia used as a street tree, and *Tibouchina granulosa* (Melastomataceae) another small, woody tree used in the ornamental plant trade. This group was based on the previous responses of *L. trifida* with the caveat that more species could be added if responses necessitated (i.e., use of the plant by the proposed agent).

Category 6: North American species (native or introduced) in other orders that have a phylogenetic, morphological, or biochemical relationship to the target weed. North American species (native or introduced) of economic and environmental importance in the same habitats as the target weed.

Melaleuca infests several native communities in Florida. This section was focused on testing of dominant species and species that are ecologically or economically important within these communities. The prominence of these plants in invaded areas exposes them to potential spillover events by biological control agents. *Taxodium distichus* is the dominant constituent of cypress swamps and can be displaced by melaleuca invasions. *Ilex cassine* and *Hypericum fasciculatum* are both understory constituents in cypress swamps and sawgrass prairies but are also culturally important to Native American groups in the area. Finally, *Citrus* x *aurantium* (orange) and *Saccharum officinarum* (sugar cane) were tested, which are both important crop species in south Florida, although they are not related to melaleuca.

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/reproduce.

Lophodiplosis species have only been collected and described from *Melaleuca* species in Australia. These have already been encompassed into the testing strategy under Category 1.

Table 1-2. Category 1: Genetic types of the target weed.

Species	Family	Significance
1 *Melaleuca quinquenervia*	Myrtaceae	Both chemotypes present in source seed collections.

Table 1-3. Category 2: Species in the same genus as the target weed.

Species	Family	Significance
2 *Melaleuca alternifolia*	Myrtaceae	All *Melaleuca* species are native to Australia
3 *Melaleuca armillaris*	Myrtaceae	All *Melaleuca* species are native to Australia
4 *Melaleuca citrina*	Myrtaceae	All *Melaleuca* species are native to Australia

Table 1-4. Category 3. Species in different genera in the same family as the target weed (Myrtaceae).

Species	Family	Significance
5 *Acca sellowiana*	Myrtaceae	South America Native, Cultivated in Florida
6 *Eucalyptus amplifolia*	Myrtaceae	Australia
7 *Eucalyptus cinerea*	Myrtaceae	Australia, cultivated in W. North America
8 *Eucalyptus neglecta*	Myrtaceae	Australia, cultivated in W. North America
9 *Eucalytpus grandis*	Myrtaceae	Australia, cultivated in W. North America
10 *Eugenia axillaris*	Myrtaceae	Florida Native
11 *Eugenia confusa*	Myrtaceae	Florida Native, state-listed endangered
12 *Eugenia foetida*	Myrtaceae	Florida Native
13 *Eugenia reinwarditiana*	Myrtaceae	Australia, cultivated outside of US.
14 *Eugenia rhombea*	Myrtaceae	Florida Native, state-listed endangered
15 *Eugenia uniflora*	Myrtaceae	South America Native, Florida Invasive
16 *Leptospermum petersonii*	Myrtaceae	Australia, cultivated in CA and HI
17 *Myrciaria cauliflora*	Myrtaceae	Central America native, cultivated in the Caribbean
18 *Pimenta dioca*	Myrtaceae	Central America native, cultivated in the Caribbean
19 *Psidium cattleianum*	Myrtaceae	Native to Brazil, Invasive in Pacific Islands and weedy in FL
20 *Psidium friedrichsthalianum*	Myrtaceae	Native and Cultivated in Central America. Cultivated in FL
21 *Rhodomyrtus tomentosa*	Myrtaceae	Native throughout Asia, invasive in Florida
22 *Syzygium cumini*	Myrtaceae	Australia, Florida Invasive
23 *Syzygium paniculatum*	Myrtaceae	Australia, cultivated in W. North America

Table 1-5. Category 4: Threatened and endangered species in the same family as the target weed

Species	Family	Significance
24 *Calyptranthes pallens*	Myrtaceae	Florida Native, state-listed threatened
25 *Calyptranthes zuzygium*	Myrtaceae	Florida Native, state-listed endangered
26 *Eugenia confusa*	Myrtaceae	Florida Native, state-listed endangered
27 *Eugenia rhombea*	Myrtaceae	Florida Native, state-listed endangered
28 *Mosiera longipes*	Myrtaceae	Florida Native, state-listed threatened
29 *Myrcianthes fragrans*	Myrtaceae	Florida Native, state-listed threatened

Table 1-6. Category 5: Species in the same order (Myrtales) as the target weed.

Species	Family	Significance
30 *Lagerstroemia indica*	Lythraceae	Cultivated throughout North America
31 *Tibouchina granulosa*	Melastomataceae	Cultivated throughout North America

Species	Family	Significance
32 *Bucida buceras*	Combretaceae	Native, cultivated throughout Florida and Caribbean

Table 1-7. Category 6: North American species (native or introduced) of economic, cultural, and environmental importance in the same habitats as the target weed.

Species	Family	Significance
33 *Citrus x aurantium*	Rutaceae	Citrus - Economically important to Florida
34 *Eriobotrya japonica*	Rosaceae	Loquat - Landscape and fruit plant in Florida.
35 *Ficus aurea*	Moraceae	Native. Occurs in the same habitat
36 *Hypericum fasciculatum*	Clusiaceae	Native. Culturally important. Occurs in the same habitat
37 *Myrica cerifera*	Myricaceae	Native. Occurs in the same habitat
38 *Persea americana*	Lauraceae	Native. Cultivated. Occurs in the same habitat
39 *Prunus caroliniana*	Rosaceae	Native. Occurs in the same habitat
40 *Quercus virginiana*	Fagaceae	Native. Occurs in the same habitat
41 *Rapanea punctata*	Myrsinaceae	Native. Occurs in the same habitat
42 *Saccharum officinarum*	Poaceae	Sugarcane - Economically important in Florida
43 *Salix caroliniana*	Salicaceae	Native. Occurs in the same habitat
44 *Sambucus nigra*	Adoxaceae	Native. Culturally important. Occurs in the same habitat
45 *Sideroxylon reclinatum*	Sapotaceae	Native. Occurs in the same habitat
46 *Taxodium distichum*	Cupressaceae	Native. Occurs in the same habitat

4. Design of Host Specificity Tests

Experiments are described below that detail larval testing in no-choice feeding trials.

Plant parts and growth stages tested – *Lophodiplosis indentata* requires whole plants with flushing new growth. All tests were performed on whole plants that were pruned and fertilized to produce optimal new foliar growth. Pests were controlled through mechanical methods (e.g., hand pulling) or organic, non-residual insecticides, or insecticidal soaps.

Source populations of the test plants – Most of the test plants we utilized were purchased from Silent Native Nursery in Miami, Florida. Melaleuca, downy rose myrtle, and *Eugenia uniflora* plants were cultivated from seeds collected from several nearby locations. Citrus, sugar cane, avocado, crepe myrtle and black olive were purchased from Flamingo Road Nursery in Davie, Florida.

Numbers of replicates – Five individual plants were used for each host range test. Within each plant, six branches were selected on which mesh sleeve cages were placed to isolate individual branches.

Number, stage and age of individuals – Adults were introduced to mesh sleeve cages that fully encompassed a plant branch. Individual females with bloated abdomens were aspirated from colony cages and confirmed by microscopic inspection. The females were then added to large mesh sleeves on the branches of each sapling. If there was concern that females may not have had adequate time to mate before being added to the sleeves, a male was also added to the sleeve cage. All adults were allowed to comingle until they died 7–8 days later. All experiments were conducted in quarantine greenhouses at 27 ± 5°C at 55–70% relative humidity (RH) under ambient photoperiod. Melaleuca plants were kept in water-saturated conditions and other test plants were watered as needed throughout the study. The number of eggs laid on leaves and number of galls produced with emergence holes were counted.

<u>Details of experimental setup</u> – No-choice adult tests were initially planned, and choice tests were to be conducted if development occurred on non-target plant species. No-choice starvation tests are the most conservative and are used to define a species' fundamental host range (Van Klinken and Heard, 2000; Schaffner, 2001). Choice tests provide a more realistic scenario for most species and would have been implemented if development had occurred on non-target species (Schaffner, 2001). Based on previous experience with *L. trifida*, oviposition was not necessarily indicative of host affinity. Egg deposition was recorded but was not used as a means of confirming host affinity because like *L. trifida*, *L. indentata* is a non-discriminating ovipositor, although not to the same extent as *L. trifida*. Eggs that were deposited were allowed to develop into larvae within galls, which indicates feeding. If no galls formed, larvae were presumed to have starved and did not feed or develop. This was confirmed for several species by inspecting leaves with oviposition for feeding scars. Each cage on each plant was scored for immature galls, galls with windows (indicating larval development), abnormal growths, and galls with emergence holes.

5. Results of Host Specificity Testing

No-choice tests

Like *L. trifida, L. indentata* has a somewhat non-discriminating oviposition behavior and will deposit eggs on multiple hosts (and surfaces) in caged scenarios. Oviposition occurred on *Melaleuca citrina, Calyptranthes pallens, Eugenia axillaris, Pittosporum tobira, Persea americana, Mosiera longipes, Myrcianthes fragrans, Tibouchina semidecandra, Illicium parviflorum, Eucalyptus camaldulensis,* and *Rhodomyrtus tomentosa* (Table 1-3). Though oviposition was somewhat broad, no gall formation, tissue swelling, development, or adult emergence occurred on any non-target species. Adults only emerged from *M. quinquenervia.* Though oviposition occurred on a number of non-target hosts, the metric is frequently not indicative of host affinity or even host suitability. Larval diet rather than egg position determines adult fitness. Furthermore, gall-induction is likely caused by larval secretions and is not influenced by oviposition (Gagné, 1989; Wright and Center, 2008). Under artificial conditions such as those in a caged no-choice test, it is not uncommon for otherwise fastidious cecidomyiid flies to oviposit indiscriminately or "dump" eggs on multiple surfaces including the cage and the non-target host plant (Larsson and Ekbom, 1995). This behavior clearly indicates that eggs may be deposited for physiological reasons outside of host suitability. In the closely related species, *L. trifida,* similar behavior was observed with the same outcome: no larvae survived to gall on any species except *M. quinquenervia* (Wright and Center, 2008).

Table 1-8. Host specificity test outcomes for L. indentata as measured by gall formation on plants. Values presented for *M. quinquenervia* in the first row is the global average ± standard error (SE) of all controls. The columns represent the total eggs laid on the non-target plants and the number of galls produced on the single control plant for each non-target. Egg deposition on non target species is given as a sum of all trials. No development or emergence occurred on any of the non-target test species so no data on gall formation was presented for non-targets. Twenty-five emerged adults from the control was set as a threshold to accept the positive control. Native species are denoted with ([a]), unrelated native species in adjacent/the same habitats are denoted with ([†]), cultivated species (for agriculture or horticultural) are denoted with (*), state-listed threatened or endangered species are denoted with ([v]), and invasive species are denoted with ([‡]).

Species	Family	Number of plants tested	# eggs deposited on test plant	Galls on Control (*Melaleuca*)
Melaleuca quinquenervia[‡]	Myrtaceae	42	359.98 ± 37.97	225.36 ± 36.87
Sambucus nigra[a†]	Adoxaceae	5	0	230
Hypericum fasciculatum[a†]	Clusiaceae	5	0	243
Bucida buceras[a*]	Combretatceae	5	0	73
Taxodium distichum[a†]	Cupressaceae	5	0	80
Quercus virginiana [a†]	Fagaceae	5	0	335
Persea americana[a*]	Lauraceae	5	324	26
Lagerstroemia indica[*]	Lythraceae	5	0	63
Tibouchina granulosa[*]	Melastomataceae	5	40	68
Ficus aurea[†]	Moraceae	5	0	36
Myrica cerifera[a]	Myriaceae	5	0	76
Rapanea punctata[a]	Myrsinaceae	5	0	39
Acca sellowiana[*]	Myrtaceae	5	14	534
Calyptranthes pallens[av]	Myrtaceae	5	162	646
Calyptranthes zuzygium [av]	Myrtaceae	5	0	83
Eucalyptus amplifolia	Myrtaceae	5	0	51
Eucalyptus cinerea	Myrtaceae	5	0	460
Eucalyptus neglecta	Myrtaceae	5	0	704
Eucalytpus grandis	Myrtaceae	5	0	303
Eugenia axillaris[a]	Myrtaceae	5	3	25
Eugenia confusa[av]	Myrtaceae	5	0	161
Eugenia foetida[a]	Myrtaceae	5	0	329
Eugenia reinwarditiana	Myrtaceae	5	0	44
Eugenia rhombea[av]	Myrtaceae	5	0	360
Eugenia uniflora[‡]	Myrtaceae	5	0	99
Leptospermum petersonii	Myrtaceae	5	0	74
Melaleuca alternifolia	Myrtaceae	5	7	1146
Melaleuca armillaris	Myrtaceae	5	0	57
Melaleuca citrina	Myrtaceae	5	498	92
Mosiera longipes[av]	Myrtaceae	5	0	147
Myciaria cauliflora[a]	Myrtaceae	5	0	338
Myrcianthes fragrans[av]	Myrtaceae	5	186	67

Species	Family	Number of plants tested	# eggs deposited on test plant	Galls on Control (*Melaleuca*)
Myrtus communis[a]	Myrtaceae	5	0	43
Pimenta dioica[*]	Myrtaceae	5	0	120
Psidium cattleianum[‡]	Myrtaceae	5	0	51
Psidium friedrichsthalianum[*]	Myrtaceae	5	41	224
Rhodomyrtus tomentosa[‡]	Myrtaceae	5	0	289
Syzygium cumini[*]	Myrtaceae	5	0	535
Syzygium paniculatum	Myrtaceae	5	0	106
Saccharum officinarum[*]	Poaceae	5	0	312
Eriobotrya japonica[*]	Roseaceae	5	0	26
Prunus caroliniana[a†]	Rosaceae	5	3	25
Citrus x *aurantium*[*]	Rutaceae	5	0	72
Salix caroliniana[a†]	Salicaceae	5	0	25
Sideroxylon reclinatum[a†]	Sapotaceae	5	0	165

Impacts of *L. indentata* on Melaleuca

Field examples of the sole impact of *L. indentata* are essentially impossible due to the ubiquitous nature of gall formers on *Melaleuca* in Australia. Kumaran et al. (in prep) set out to determine the additive and sole impact of *L. indentata* on melaleuca seedlings and saplings in the presence and absence and before and after the introduction of *L. trifida*. A randomized complete block design was employed including the following treatments: an insect-free control, *L. indentata* only (LI), *L. trifida* only (LT), *L. indentata* and *L. trifida* (LI+LT) released at the same time, *L. indentata* released 1 week before *L. trifida* (LI-LT) and *L. indentata* released one week after *L. trifida* (LT-LI). Individual plants were measured for basal diameter and height and then placed into cloth screen cages over a two-week inoculation period.

For single-species treatments, 100 pairs of insects were introduced into cages. For LI+LT, 50 pairs of each species were added to the cages; 100 pairs of LI were added one week before 100 pairs of LT were added for the LI-LT treatment and 100 pairs LT were added one week before 100 pairs of LI were added. All plants were held in the treatment cage for one week after the two-week inoculation period during which all oviposition and then death occurred. Plants were maintained for 20 weeks in a greenhouse (22–25°C, 40–70% RH) and then harvested for the following parameters: plant height, basal diameter, number of tips and root:shoot ratio. Growth parameters were analyzed using a two factor ANOVA with plant stage (seedling, sapling) as fixed factors.

In seedlings, *L. indentata* alone significantly reduces the basal stem diameter. Additionally, plants allocate resources to *L. indentata* galls when fed upon. They show that galling from *L. indentata*, along with *L. trifida*, significantly reduces height growth rate in seedlings (Fig. 1-3). Compared to controls and individual impacts, *L. indentata* and *L. trifida* impact sapling height growth rate more together than alone (Fig. 1-4). No strong evidence was found to suggest that these insects in combination would act in an antagonistic fashion. In this experiment, *L. trifida* had a greater effect on seedlings than saplings (Height: $F = 526.92$, $P < 0.0001$; Basal stem diameter: $F = 153.73$, $P < 0.001$; root:shoot ratio: $F = 17.11$, $p < 0.0001$; tips growth: $F = 73.24$, $p < 0.0001$). However, when released together, herbivory reduced growth height in saplings.

Observations from the native range indicate that spatial niche differentiation occurs in field settings that was not tested (nor could be tested) in these studies. *Lophodiplosis trifida* is found in apical meristematic tissue close to the ground (i.e., seedlings and saplings), whereas *L. indentata* generally galls foliar tissue higher up in the canopy. Melaleuca channels significant amounts of resources towards these galls which reduces sapling height when both insects are present but could have further implications for seeding and flowering. Foliar feeding by other biological control insects on melaleuca produced dramatic reductions in seed production, flowering, seed rain and seedling survival, among other metrics (Pratt et al., 2006; Rayamajhi et al., 2007; 2008; Center et al., 2012; Tipping et al., 2016; 2018). Because this study focused on more readily measurable metrics in early life stages (root:shoot, biomass, etc.), the full weight of the impact of the midges cannot be fully gauged. Center et al. (2012) found that tip damage from *O. vitiosa* prevented sapling regeneration and reinvasion by new seedlings. *Oxyops vitiosa* does not establish in areas with persistent standing water or wet soils – areas in which melaleuca thrives. The studies by Kumaran et al. only investigated impacts on seedlings and saplings. *Lophodiplosis indentata* could have a significant impact on recruitment by diverting plant resources away from reproductive structures.

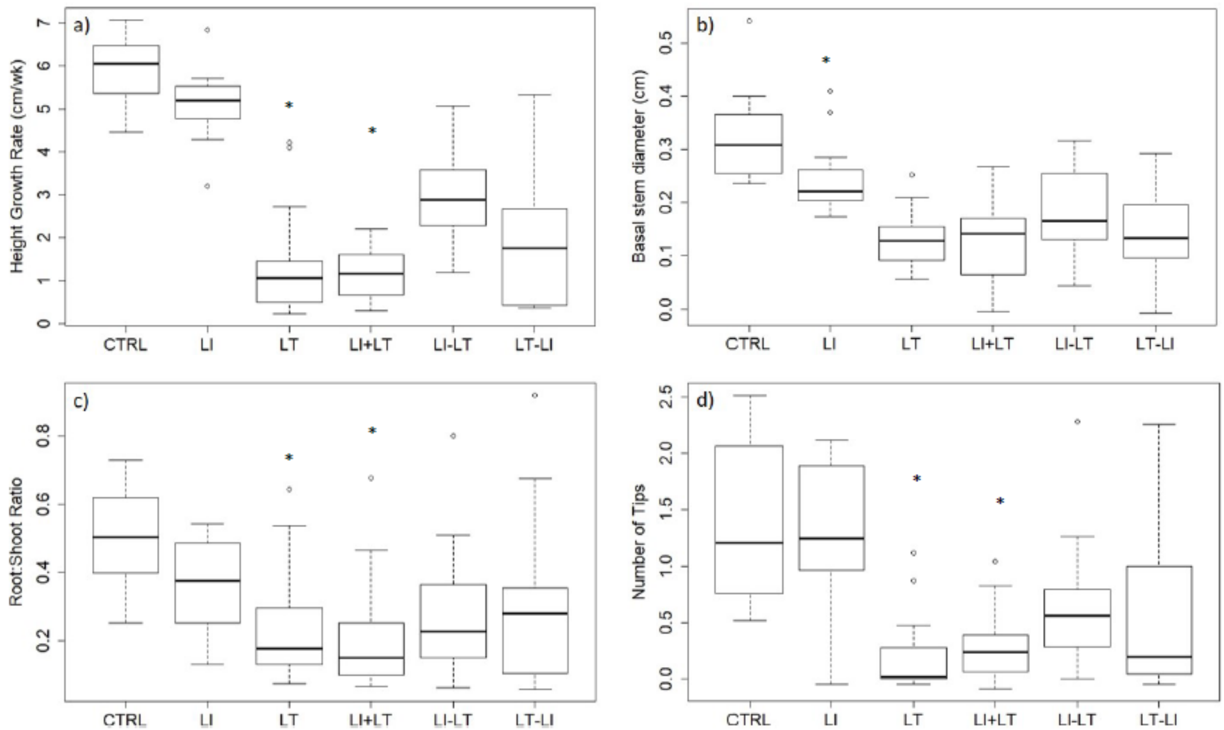

Figure 1-3. Effect of interaction between *Lophodiplosis trifida* and *L. indentata* on growth of *Melaleuca quinquenervia* seedlings. A) Height growth rate; B) Basal diameter growth rate; C) Root: Shoot ratio and D) Tips growth rate. Significant differences indicated by (*) ($P <$ 0.05) in growth rate. CTRL – Control, LI – *Lophodiplosis indentata* alone, LT – *L. trifida* alone, LI+LT – *L. indentata* and *L. trifida* released together, LI-LT – *L. indentata* released prior to *L. trifida*, LT-LI – *L. trifida* released prior to *L. indentata*. (From Kumaran et al., in prep).

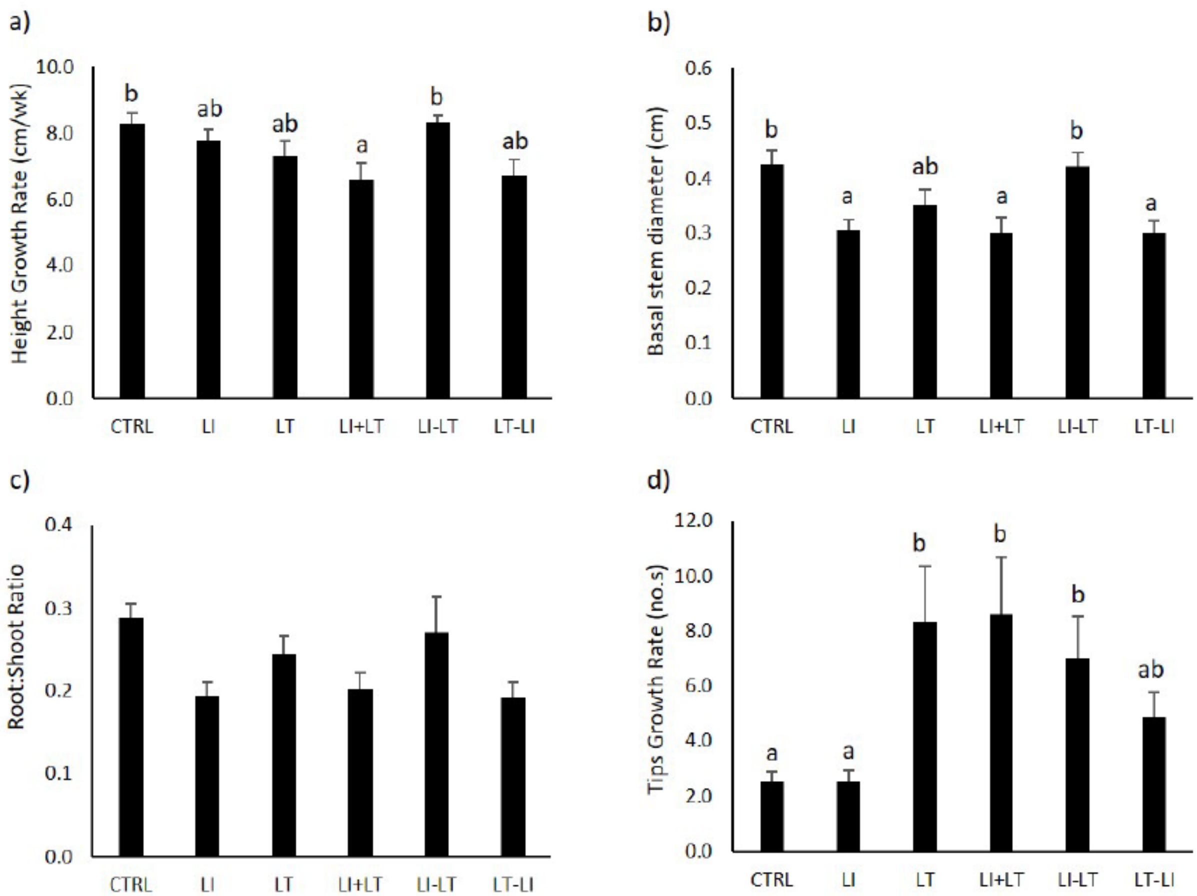

Figure 1-4. (a-d). Effect of interaction between *Lophodiplosis trifida* and *L. indentata* on growth of *Melaleuca quinquenervia* saplings. A) Height growth rate; B) Basal diameter growth rate; C) Root:Shoot ratio and D) Tips growth rate. Different lowercase letters on adjacent bars indicate significant difference (P < 0.05) in growth rate. CTRL – Control, LI – *Lophodiplosis indentata* alone, LT – *L. trifida* alone, LI+LT – *L. indentata* and *L. trifida* released together, LI-LT – *L. indentata* released prior to *L. trifida*, LT-LI – *L. trifida* released prior to *L. indentata*. (From Kumaran et al., in prep).

Appendix 2. Release Protocol and Post-Release Monitoring Plan for Lophodiplosis indentata (Smith et al. 2019).

Release Protocol

The insect will be mass reared for releases by personnel at USDA-ARS. The network of land managers and extension agents that have long made melaleuca control and biological control a success will be utilized again for this release. Established long-term field sites in a variety of habitat types will be used for post-release monitoring of establishment and impact. Previous experience with *L. trifida* indicates dispersal and establishment will happen rapidly regardless of founding population size (Pratt et al., 2013). Initial releases will inlcude both adult releases and the placement of galled plants. Pratt et al. (2013) demonstrated that small releases of 100 adult *L. trifida* were able to establish and disperse as well as large (>1,000). To maximize resources, several small releases will be conducted in targeted persistent melaleuca populations (e.g., Picayune Strand, Arthur R. Marshall Loxahatchee National Wildlife Refuge).

Post-Release Monitoring

The permittee and colleagues at the USDA Invasive Plant Research Lab will conduct monitoring with cooperation from local land managers. APHIS, the permitting agency, does not have any involvement in post-release monitoring.

Post-release studies carried out by the researchers will utilize a multi-faceted approach including demography, common garden studies, and long-term monitoring plots. Because *L. indentata* will join three established insects in Florida, its individual impact will likely not be isolated from the impact it has in concert with *O. vitiosa*, *B. melaleucae,* and *L. trifida*. The researchers will conduct all impact and post-release monitoring and effects studies in field settings with stands of varying ages and size distribution. A large garden with melaleuca interspersed amongst non-target native relative species (*Myrcianthes fragrans, Mosiera longipes, Calyptranthes pallens, Calyptranthes zuzygium, Eugenia rhombea, Eugenia confusa, Eugenia amplifolia*) has been established for determining field host specificity. Large plots in various habitat types will be used to determine impact. The background level of melaleuca herbivory on these has already been measured. The researchers also plan to work with land managers in several places to design studies that test how *L. indentata* integrates with mechanical, chemical and cultural removal. One of the reasons biological control of melaleuca has been successful is that it integrates in tandem with other removal methods.