BIOLOGY AND HOST SPECIFICITY OF *Tectococcus ovatus* (HEMIPTERA: ERIOCOCCIDAE), A POTENTIAL BIOLOGICAL CONTROL AGENT OF THE INVASIVE STRAWBERRY GUAVA, *Psidium cattleianum* (MYRTACEAE), IN FLORIDA

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2005

This document is dedicated to my parents, for their support and generosity throughout my educational career. Without them, this work would not have been possible.

ACKNOWLEDGMENTS

I would like to thank my major professor Dr. James P. Cuda for his invaluable guidance and help throughout my degree program. I also thank my other committee members, Dr. Kenneth A. Langeland and Dr. William A. Overholt, for their comments and suggestions on my research and this manuscript.

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December 2005

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Strawberry guava, *Psidium cattleianum* Sabine, is a woody tree or shrub native to coastal southeastern Brazil. Strawberry guava was introduced into Florida in the late 1800s as an ornamental species. The plant escaped cultivation and is invading natural areas throughout the southern half of the state. In addition to negative effects on Florida's native ecosystems, strawberry guava also is a preferred host of the Caribbean fruit fly, *Anastrepha suspensa* Loew (Diptera: Tephritidae).

The Caribbean fruit fly is a common agricultural pest that affects several important fruit crops. The Caribbean fruit fly can cause direct yield loss, and its presence can affect shipments to quarantine sensitive markets. In Florida, various control techniques have been used with limited success. A novel approach for reducing fruit fly populations is classical biological control of their preferred naturalized host plants, such as strawberry guava.

A survey of the entomofauna associated with strawberry guava identified five potential biocontrol agents. The most promising was a leaf-galling scale insect *Tectococcus ovatus* Hempel. Large infestations of *T. ovatus* cause premature leaf drop and inhibit fruiting, thereby reducing fruit fly breeding sites. It is essential to understand as much as possible about a potential biological control agent prior to release. An analysis of a series of leg measurements was conducted in order to determine the number of nymphal stages and discern them from the adult. Multivariate analyses of the measurements suggest the presence of two or possibly three instars. However, a greater sample size is necessary to determine if there truly are three nymphal stages or if the results are due to outlying data points.

Prior to the release of any classical biological control agent, the host specificity of the agent needs to be demonstrated. No choice tests were conducted because of the rigorous nature of the tests. In total, 57 species of plants representing 21 families were included in the host range tests. *Tectococcus ovatus* first instars fed on two closely related guava species, Brazilian guava (*Psidium friedrichsthalianum* O. Berg), and Costa Rican guava (*Psidium guineense* Sw.). Incomplete gall formation was observed on the Costa Rican guava. However, none of the *T. ovatus* nymphs completed their development. These non-target effects were determined to be negligible relative to the target, because the two species attacked were not native and are rarely cultivated in Florida. The results of the host specificity tests suggest that *T. ovatus* is a suitable candidate for biological control of strawberry guava in Florida.

CHAPTER 1
INTRODUCTION

Prior to the age of exploration, plant immigration to new habitats was primarily facilitated by seed dispersal adaptations. Seeds could be transported by natural elements such as wind and water or by animal movement and migration, either in the digestive tract or externally. Although these processes were relatively commonplace, large natural boundaries such as oceans and mountains limited the rate of long distance introductions. With the advent of human exploration and travel, these natural boundaries were readily crossed, and an explosion of plant introductions began. The majority of plant introductions have been intentional-for agricultural, industrial, or ornamental purposes (Pimentel et al. 2000). Most non-native introductions are sustained solely through cultivation, and do not persist on their own. However, a small percentage of non-native plants become established, or naturalized in their new habitat. Some of these naturalized plants flourish and readily disperse, posing a threat to native ecosystems. This is because the most important direct effect of non-native species is habitat modification (Simberloff 1997). Non-native species that alter native habitats through direct competition or hybridization are termed exotic invasive species (Florida Exotic Pest Plant Council [FLEPPC] 2003). Although the percentage of exotic invasive species is relatively low compared to the number of introduced species, their negative impacts are widespread and can be extremely costly (Gordon and Thomas 1997).

Florida's climate and geography make the state prone to non-indigenous species invasions (Invasive Species Working Group [ISWG] 2003). The high number of invasive species proliferating in the state is understandable considering that 85% of all foreign plants imported into the United States arrive through Miami International Airport (US Congress, Office of Technology Assessment [OTA] 1993). Because of this, approximately 31% of Florida's flora is of non-native origin (Wunderlin 1998). Of the 25,000 introduced species cultivated in the state, 925 have escaped cultivation and become naturalized in Florida's native ecosystems (Frank and McCoy 1995). A smaller number of these plants have expanded their range and are considered to be exotic invasive species in Florida. The exact number of these species varies depending on which source is cited. As previously mentioned, the majority of plant introductions are intentional, and Florida's large ornamental nursery industry may be responsible for the introduction of a large number of exotic invasive species. Not surprisingly, there has been a conflict of interest between environmental and commercial groups regarding which species are truly invasive, and which are not.

One organization that has been formed to inform resource managers of potential problems concerning exotic invasive plant species within the state is the Florida Exotic Pest Plant Council (Langeland 2002). To help resource managers make informed decisions about which plants to monitor and help set priorities for management, FLEPPC has compiled a list of 126 naturalized plant species to be which they consider to be exotic invasive species (FLEPPC 2003, Langeland 2002). The FLEPPC invasive species list is divided into two categories, Category I represents the most harmful species which have been shown to be altering native plant communities; Category II represents species that are increasing in abundance but are not affecting native communities to the extent of Category I species. Although the FLEPPC list provides detailed and readily accessible information; it has been criticized for being too liberal in its classification. This is due to the large discrepancy between the FLEPPC list and state and federal regulations (24 of the 67 Category I species and 6 out of 59 Category II species are regulated by the government) and the lack of supporting evidence and references (Fox et al. 2004a). Despite this controversy, the Florida Nursery, Growers, and Landscape Association (FNGLA) and FLEPPC asked FNGLA members to stop selling 45 potentially invasive species (Wirth et al. 2003). Although this was a good start, the organizations could not agree upon the status of 14 Category I exotic invasive species that are commonly sold as ornamentals. These 14 species represent only 2.8% of total nursery sales in Florida. However, considering the size of the ornamental nursery industry in the state, these species have a total combined economic impact of $59 million (Wirth et al. 2003). The FNGLA (2005) called for the creation of a single invasive plant list that distinguishes between Florida’s geographic regions and intended plant use, both of which are not taken into account by the FLEPPC list. A new list was created by the University of Florida, Institute of Food and Agricultural Sciences (IFAS) called the IFAS assessment of the status of non-native plants in Florida’s natural areas (Fox et al. 2004b). The primary objective of this assessment is to direct research and extension at the University of Florida to be focused in the proper direction (Fox et al. 2004b). Although, secondary objectives are to provide additional information to that available on other state invasive lists and identify gaps in knowledge of invasive species (Fox et al. 2004b). This assessment standardizes the classification of the status of selected plants in Florida based upon geographic location (north, central, or south Florida), economic importance, and effect on the environment. The conclusions of the IFAS assessment confirmed the invasive status of all 14 species designated invasive by FLEPPC (IFAS 2005). Although the IFAS assessment is not a regulatory list (Fox 2005), this list is considered to be the most complete and appropriate for invasive plant species in the state of Florida. Therefore, the IFAS assessment will be used for the purposes of defining the invasive status of a plant in Florida throughout this manuscript.

Although the environmental and economic threat exotic invasive plants pose to native ecosystems is great, invasive plants may also have indirect effects on their new habitats. One example is strawberry guava, *Psidium cattleianum* Sabine. This woody invasive species was brought to Florida from southeastern Brazil for the ornamental and fruit trade in the late 1800s (Gordon and Thomas 1997). Strawberry guava is one of the 14 controversial species that is still commonly sold as an ornamental throughout the state. In addition to the environmental problems caused by strawberry guava invading natural areas, the plant also serves as a major host of the Caribbean fruit fly, *Anastrepha suspensa* Loew (Swanson and Baranowski 1972). Swanson and Baranowski (1972) determined the host specificity of *A. suspensa* based on specimens reared from 84 field collected fruits in 23 families. They identified six major hosts of *A. suspensa*: loquat, *Eriobotrya japonica* (Thunb.) Lindl.; Surinam cherry, *Eugenia uniflora* L.; rose apple, *Syzygium jambos* (L.) Alst.; tropical almond, *Terminalia catappa* L.; common guava, *Psidium guajava* L.; and strawberry guava. Because of this study, Nguyen et al. (1992) counted the number of *A. suspensa* larvae present on the fruits of three major hosts; loquat, Surinam cherry, and strawberry guava. They found that the Caribbean fruit fly occurred on strawberry guava in larger numbers from July through October compared to loquat and Surinam cherry.

The Caribbean fruit fly is native to the West Indies; although it immigrated to Florida multiple times, finally becoming established in 1965 (Nguyen et al. 1992). The larval stage of the fly feeds on a wide variety of tropical and subtropical fruits. The Caribbean fruit fly is extremely polyphagous, with nearly 100 recorded hosts (Weems et al. 2005). Fruit fly damage resulting from larval feeding renders fruit unmarketable.

In addition to direct impacts, the Caribbean fruit fly has the potential to infest post harvest commercial fresh citrus shipments destined for quarantine sensitive domestic or foreign markets such as California, Texas, and Japan. For this reason, the Caribbean fruit fly is considered a major quarantine pest, and over the years multiple control strategies have been developed to reduce fly densities in Florida. Prior to 1983, shipments were fumigated with ethylene dibromide to eliminate the presence of fruit fly larvae (Nguyen et al. 1992). In 1983, the use of ethylene dibromide for this purpose was banned by the Environmental Protection Agency (Extension Toxicology Network [EXTOXNET] 2005). With the banning of this chemical, the Florida Department of Agriculture and Consumer Services (FDACS) developed the Caribbean fruit fly free protocol to certify citrus crops as fly free. This protocol is an integrated approach involving the use of monitoring traps, aerial pesticide sprays, and the removal of major hosts within 1.5 miles of designated groves (FDACS 2005). Removal of major host species such as strawberry guava costs citrus growers time and money. It is the grower’s responsibility to remove all major hosts, even if they are not present on the grower’s property. Because of this, developing alternative methods of controlling these weeds has become a priority, in order to help growers save money and prevent legal disputes over removing pests on neighboring properties.

Classical biological control of the major hosts would be preferential. Once released, classical biological control agents do not require any physical input aside from monitoring and they do not require landowner permission. In 1991, researchers with the U.S. National Parks Service and the University of Hawaii collaborated with the Federal University of Paraná in Curitiba, Brazil to investigate potential biological control agents for strawberry guava (Wikler et al. 2000). This project was undertaken because strawberry guava is a serious forest pest throughout the Hawaiian archipelago. The group discovered seven potential biocontrol agents. After preliminary testing, the most promising of these agents was determined to be *Tectococcus ovatus* Hempel, a leaf galling scale insect in the family Eriococcidae (Wikler et al. 2000). A colony of *T. ovatus* was established at the Institute of Pacific Islands Forestry in Volcano, Hawaii. At this laboratory, host specificity tests began to evaluate *T. ovatus* for release in Hawaii. In 2001, researchers at the University of Florida obtained funding from the USDA CSREES T-STAR program to investigate the biology and host specificity of *T. ovatus* in Florida. This manuscript is the result of this research. The data presented in the following chapters are the results of initial investigations of the biology of *T. ovatus* and the host specificity testing of this insect.

CHAPTER 2
LITERATURE REVIEW

***Psidium cattleianum* Sabine**

Taxonomy

The Myrtaceae is a large family including approximately 150 genera and 3600 species commonly found in tropical and subtropical climates worldwide, although some species are also established in temperate Australia (Cronquist 1981). The genus *Psidium* is one of the larger genera in the Myrtaceae containing approximately 100 species (Cronquist 1981). The genus was first described by Linnaeus, derived from the Greek word sidion, meaning pomegranate (*Punica granatum* L.) due to the similar shape of the fruits (Ellshoff et al. 1995). The complete classification of strawberry guava is as follows:

Class: Magnoliophyta

Subclass: Rosidae

Order: Myrtales

Family: Myrtaceae

Subfamily: Myrtoidea

Genus: *Psidium*

Species: *Psidium cattleianum* Sabine

Nomenclature

Scientific name

Psidium cattleianum was first described by Sabine (1821); it was named in honor of the botanist William Cattley (often misspelled *cattleyanum* in older literature). Cattley was the first person to successfully cultivate the plant in his conservatory in Britain, claiming that his plants were grown from seeds he received from China (Bretschneider 1898). However, some botanists consider the correct name to be *Psidium littorale* giving priority to the description by Raddi in 1823. This conclusion was reinforced by Fosberg (1941) and Merrill and Perry (1938). The confusion arises from the difference in the date of publication and the date in the section of Raddi's description of strawberry guava (Ellshoff et al. 1995). Schroeder (1946) noted that because of the confusion surrounding the actual date of publication, Raddi's description cannot be proven to be earlier than Sabine's. Since Sabine's description can be dated definitely, the currently accepted name should remain *Psidium cattleianum* while *Psidium littorale* is considered a junior synonym (Ellshoff et al. 1995, Schroeder 1946). Synonyms include (from Fosberg 1941, Wikler 1999):

Psidium littorale Raddi, Opusc. Sci. 4: 254. 1823 t. 7 f. 2.

Psidium variabile Berg, Fl. Bras. 14(1): 400. 1857.

Psidium coriaceum var. *obovatum* Berg, 1. c. 461 t. VI. 120.

Psidium coriaceum var. *grandifolium* Berg, 1. c. 401.

Psidium coriaceum var. *longipes* Berg, 1. c. 402.

Psidium cattleianum var. *coriaceum* (Berg) Kiaerskou, Enum. Myrt. Bras. 28. 1893.

Episyzygium oahuense Seuss. & A. Ludwig

Psidium cattleianum var. *cattleianum* f. *lucidum* Degener

Psidium cattleianum var. *littorale* (Raddi) Fosb.

Psidium littorale var. *lucidum* (Degener) Fosb.

There are two accepted varieties of strawberry guava, distinguished solely by the color of the fruit, the red-fruited variety *P. cattleianum* var. *cattleianum* and the yellow-fruited variety *P. cattleianum* var. *lucidum* (*P. littorale* var. *lucidum*) (Wikler 2000b). Both varieties are found within the native range of southeastern Brazil. The yellow variety is much more common, while the red is restricted to higher elevations (Hodges 1988). A similar situation occurs in Hawaii, with the yellow variety dominating lower elevations in Hawaii Volcanoes National Park and the red variety dominating higher elevations (Tunison 1991). In Florida, both varieties are present, despite the lack of a major elevation gradient (Langeland and Hall 2000).

In Hawaii, there are two different shapes of the fruit of the yellow variety of strawberry guava. One type is the typical round shape, while the other has ellipsoid-obconical fruit (Wagner et al. 1999). Wagner et al. (1999) consider these to be two separate varieties (*P. cattelianum* var. *lucidum* and *P. c.* var. *littorale*). The ellipsoid-obconical variety also is seen on Mauritius, although it has not been reported from Brazil or Florida.

Common names

Strawberry guava has been widely introduced throughout the world, and for this reason, there are a considerable number of common names for the species. In the US and other English speaking countries, *P. cattleianum* is known as strawberry guava (presumably due to the strawberry-like flavor and possibly the red color of *P. c.* var. *cattleianum*), purple guava in Jamaica, Chinese guava (due to Cattley's assumption of Chinese origin), Cattley guava, and pineapple guava. In Brazil, the plant is known as araçá da praia, araçazeiro coroa, araçá vermelho, and araçazeiro. Additional names are arazá (Uruguay), cas dulce (Costa Rica), guayaba japonesa (Guatemala), and guayaga peruana (Venezuela) (Gomes 1983, Morton 1987, Popenoe 1920). Other names include goiave de L'Afrique (Dominican Republic), araçá-saiyu, and guayabo amarillo (Argentina), Calcutta guava (India), goyavier of St. Martin, goyavier fraise (Guadeloupe), guayabita fresa (Cuba), and goyavier prune (Martinique) (Roig y Mesa 1953, Wikler 2000b). In Hawaii, the red variety is called waiawi ulaula, while the yellow variety is simply called waiawi (Morton 1987).

Morphology

Strawberry guava is an evergreen shrub or small tree between 2 and 6 m tall, although specimens of *P. cattleianum* var. *lucidum* have been reported growing up to 12 m (Morton 1987). Stems and branches are smooth, gray to reddish brown in color with bark that peels in thin sheets (Fig. 2-1A) (Langeland and Burks 1998). Young branches are round and sparsely pubescent. Leaves are alternate, obovate to elliptic-obovate between 3.5 and 13.5 cm long, petioles approximately 7-10 mm long (Wagner et al. 1999, Webb et al. 1988). Leaf surface is dark green glabrous and somewhat leathery in texture; lateral veins are slightly elevated but inconspicuous (Fig. 2-1B). Flowers are white, 1.5-6 cm in diameter with prominent stamens (Morton 1987); flowers are usually solitary and borne in almost all axils of upper leaves (Fig. 2-1C) (Langeland and Burks 1998, Webb et al. 1988). Fruits are sweet tasting, slightly acidic, round or elliptical, smooth and glabrous 2-3 cm in diameter. The fruits of *P. cattleianum* var. *lucidum* are yellow to white, while those of *P. cattleianum* var. *cattleianum* are reddish to purple in color (Fig. 2-1D). The red fruits also are reported to taste better, with a more subdued flavor (Dehgan 1998). Seeds are hard, flattened and triangular in shape approximately 2.5 mm long (Morton 1987).

Strawberry guava is a relatively hardy species that can grow in a wide variety of soils, although they perform best in rich sandy soils (Popenoe 1920). The red form seems to better withstand colder temperatures and can survive at temperatures as low as 22° F, whereas the yellow form is more susceptible to cold (Morton 1987). Both forms are drought resistant, although the yellow form also can withstand short periods of flooding (Morton 1987).

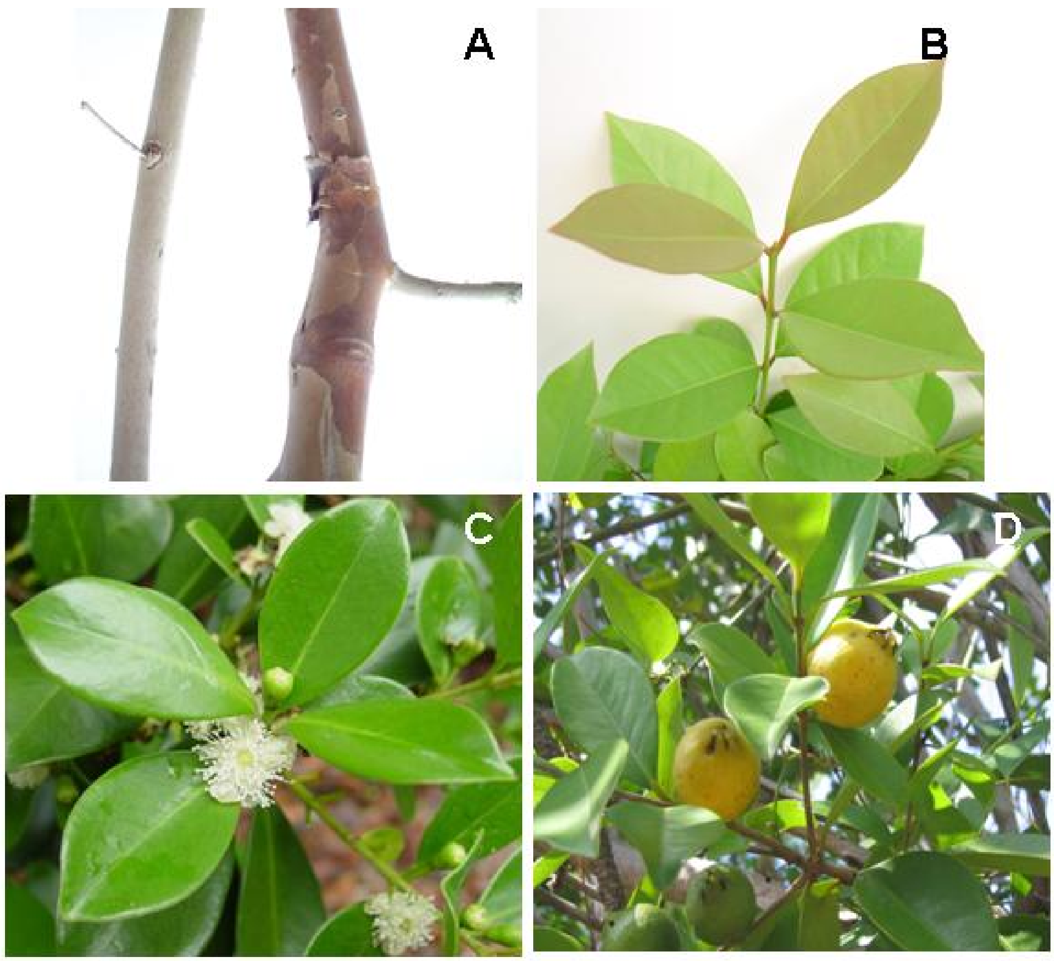

Figure 2-1. Morphology of *P. cattleianum*; A) characteristic bark; B) obovate leaves; C) flowers (Photo credit: Jeff Hutchinson); D) yellow fruits of *P. cattleianum* var. *lucidum*.

Distribution

Native distribution

In Brazil, where the Myrtaceae is a widespread and diverse family, the genus *Psidium* is represented by nine species (Wikler 2000a). Strawberry guava is located in the Atlantic forest ecosystem in the southeastern part of the country (Fig. 2-2). Within its native range, the plant is primarily a coastal species. Strawberry guava occurs in a coastal vegetation type known as “restinga”, although it can also be found in disturbed brush fields known as “capoeiras” (Hodges 1988). The northernmost range of strawberry guava in Brazil is in the state of Espirito Santo and extends south along the coast to the northern tip of Uruguay (Fig. 2-2) (Hodges 1988). Hodges (1988) noted the plant growing within an elevation range of 5 – 100 m; although except for cultivated plants, he did not notice the red fruited form growing wild.

Figure 2-2. Native distribution of strawberry guava in southeastern Brazil (adapted from Hodges 1988, Wikler 1995).

Worldwide distribution

Strawberry guava has been intentionally introduced in nearly all of the countries in which it is currently found. Its attractive fruit and leaves are generally desired more as an ornamental than a fruit crop. According to Popenoe (1920), strawberry guava was originally transported from its native range in Brazil to China at an “early period”, presumably by the Portuguese. Seeds were taken to Europe in 1818 by two Englishmen, Barr and Brookes, and described as originating from China (Bretschneider 1898). The only reference hinting to European introduction of this plant in Hawaii is from Degener (1932) mentioning that live plants of both forms of strawberry guava may have been brought to the archipelago on board the “Blonde” in 1825. Similar introductions may initially have been made by European or Portuguese ships resulting in the wide distribution of the plant.

Distribution information obtained from literature records and herbarium specimens have been compiled (Appendix A) in order to create a worldwide map of the distribution of strawberry guava (Fig. 2-3). However, the distribution of strawberry guava is most likely more extensive than mentioned in the literature. This plant is found in all 7 major world biogeographical regions; in the Afrotropical region it is present in multiple coastal countries of tropical Africa, and on Madagascar and surrounding islands. In the Australasian region, strawberry guava is found in Australia and New Zealand and surrounding island archipelagos. In the East Palearctic region it is found in southern China and Taiwan. Recorded distribution is most common in the Neotropical region where the plant is found throughout its native range of Brazil, surrounding South and Central American countries and throughout the Caribbean. In the Oriental region, strawberry guava has been reported on multiple islands in the Indian Ocean and the

Pacific and in Japan, Malaysia, and the Philippines. In the Western Palearctic region it is found on the islands of Madeira, the Azores, the British Isles, the Cape Verde Islands, various Mediterranean islands and, in central France. In the Nearctic region strawberry guava is found in the southern portion of the continental United States and on Bermuda.

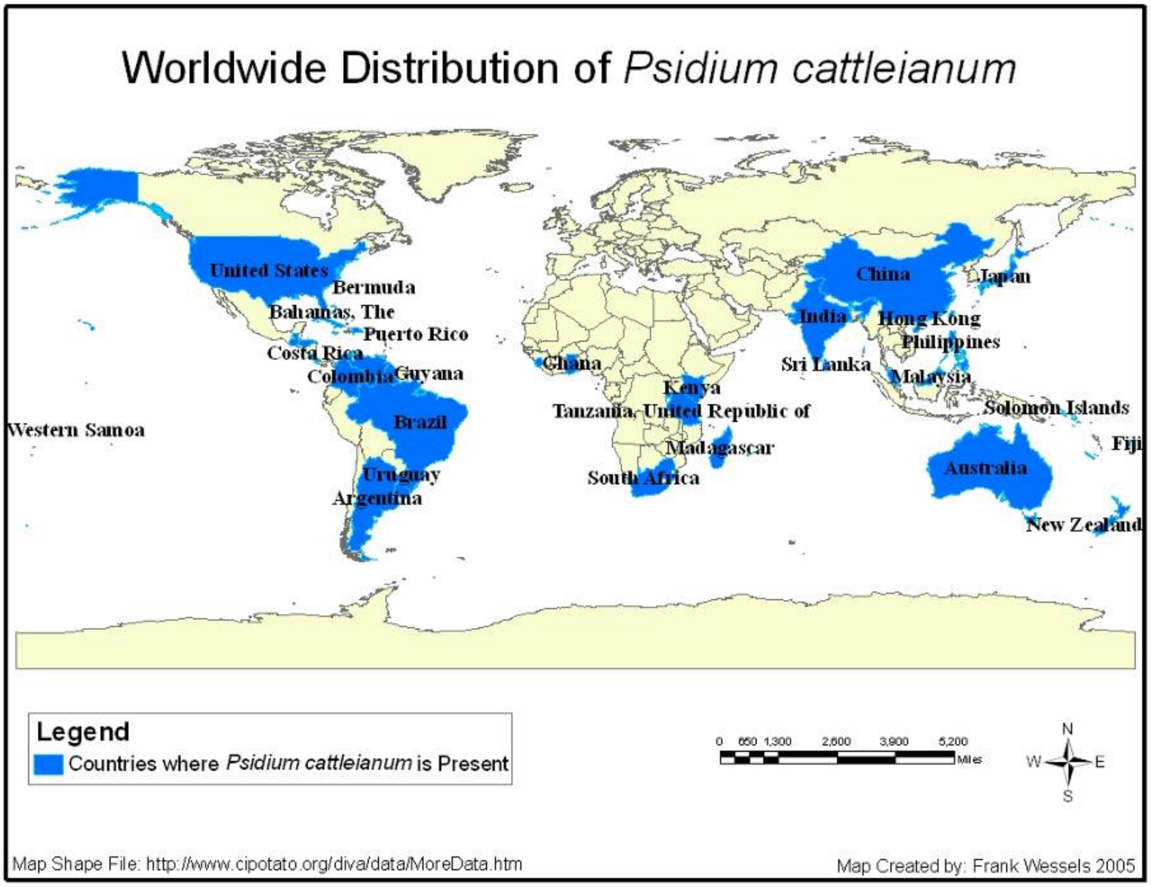

Figure 2-3. Countries where strawberry guava has been reported (based on literature references and herbarium specimens, see Appendix A).

Distribution in the United States

In 1884, approximately 3000 trees were planted as part of a commercial venture in La Mesa, California, and the trees were reported as producing heavily a half century later (Morton 1987). Additionally, a herbarium specimen was collected in 1995 from a cultivated specimen at the Quail Botanical Gardens in San Diego County, California (New York Botanical Garden [NYBG] 2005). Although strawberry guava was introduced into southern California, no records suggest that the species is considered invasive in the region. The California Invasive Plant Council (Cal-IPC) does not list the species as being invasive in any part of the state (Cal-IPC 2000).

Although there are a few references confirming the presence of strawberry guava in Texas, many books on the flora of Texas do not even include references to the family Myrtaceae. Strawberry guava is listed in a state checklist of vascular plants; however, there is no mention of locality or whether the specimen is cultivated or growing wild (Jones et al. 1997). Strawberry guava can grow in US Department of Agriculture plant zones 9-10, which suggests that the plant is capable of surviving in the southern parts of Texas and along the Gulf Coast.

Oddly, one herbarium specimen shows the presence of strawberry guava in Boone County, Missouri in 1974. This record is curious, because it is well out of the known geographic range of the species in the United States. The Boone county specimen was collected by D. B. Dunn at approximately at 39.02.00N, -92.20.00W at an elevation of 740 ft. (Missouri Botanical Garden [MBG] 2005). However, the collection data of the specimen are rather vague regarding whether or not this specimen was cultivated.

The presence of strawberry guava in Hawaii and its subsequent effects on the native ecosystems of the islands has been well documented in the literature. The weed is considered the worst invasive plant in Hawaii and is present on almost all of the major islands (Smith 1985). The plant forms dense stands in disturbed roadsides and in intact forests where it effectively out-competes native species. Although the fruits are attractive to birds and mammals, the feral pig (*Sus scrofa* L.) has been shown to be a major dispersal agent of the plant in Hawaii (Diong 1982).

In Florida, strawberry guava was first listed as an ornamental in the 1887-1888 Catalog and Price List for Royal Palm Nurseries (Langeland and Hall 2000). By 1956, it had escaped cultivation and was reported growing wild (Barrett 1956). Strawberry guava is currently naturalized in the central and southern parts of the state. Voucher specimens indicate the presence of the plant in 18 counties (Fig. 2-4) (Wunderlin and Hansen 2004). However, the actual distribution is most likely more extensive, and needs to be confirmed with additional voucher specimens.

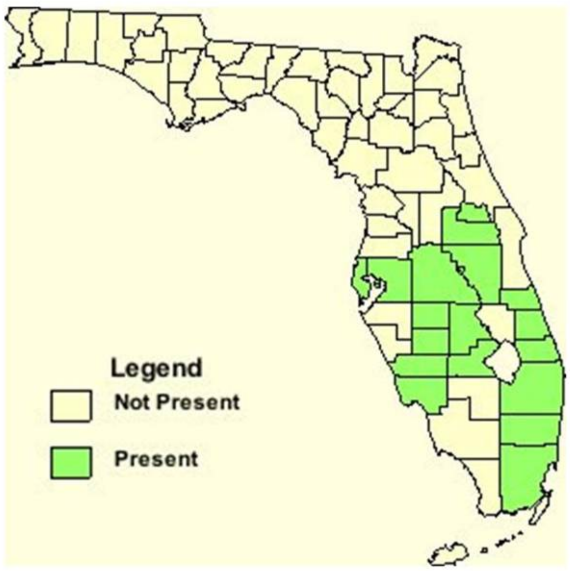

Figure 2-4. Florida counties where vouchered specimens of strawberry guava were collected (Wunderlin and Hansen 2004).

Beneficial Uses

The fruit of strawberry guava is said to be superior in taste to that of the common guava, *Psidium guajava* L., and is preferred by many (Popenoe 1920). The fruits can be eaten fresh, but are often contaminated by various fruit flies in Florida and Hawaii. Most often the fruit is made into jelly, jam, butter, paste, punch, and sherbet (Morton 1987). Although, the species is cultivated in many parts of the world, no distinct cultivars exist. In Florida, strawberry guava is primarily grown as an ornamental or in hedges around parking lots and other areas (Gilman and Watson 1994). In Brazil, the wood is sometimes collected for firewood (Hodges 1988, Wagner et al. 1999).

There is little promise of commercial fruit production due to the delicate skin of the ripe fruit, which would require careful shipping (Schroeder and Coit 1944). In addition, Schroeder and Coit (1944) note that the shelf life of the fresh fruit is too short for a commercial production, lasting only 3-4 days without refrigeration. The largest recorded commercial planting was in La Mesa, California. A local farmer planted approximately 3000 trees on a 5 acre plot. In 1943, production was approximately 30 tons. The majority of the fruit was processed into paste for sale; however, some fresh fruit was marketed locally (Schroeder and Coit 1944). Despite the attempts at commercial production, strawberry guava seems to have a more marketable potential as an ornamental hedge or dooryard tree.

Invasive Properties

Plants that are invasive have a variety of characteristics that aid in their dominance of new habitats. Many authors have tried to generalize these characteristics in order to help predict which species may be potential problems (Noble 1989, Reichard and Hamilton 1997). Although there are characteristics that many invasive plants share, generally these are poor predictors of whether or not a species will be invasive. Determining the invasiveness of a species based on biological attributes is probably too simplistic. Sakai et al. (2001) argues that the invasiveness of a plant depends on a combination of ecological, genetic, and evolutionary factors. A combination of factors most likely aids strawberry guava in its ability to out compete native plants in areas where it is introduced.

Strawberry guava has a high tolerance to environmental heterogeneity, it is tolerant of moderate to highly acidic soils, and can tolerate the heavy litter fall present in Hawaiian rainforests (Huenneke and Vitousek 1990). The plant also can grow in a wide variety of soils including: rocky soil, clay, sandy loam, and wet forest soils (Diong 1983). Strawberry guava is more cold tolerant than the common guava, and has been reported withstanding temperatures as low as 22 degrees (Schroeder and Coit 1944). Strawberry guava also is highly shade tolerant, a trait which can help seedlings grow up through the understory and overtake native vegetation (Tunison 1991).

Another trait aiding to the invasiveness of strawberry guava is its ability to reproduce both sexually and asexually. Huenneke and Vitousek (1990) found that asexual plants produced by clonal root suckering had larger leaves and produced a greater total leaf area than seedlings. This clonal behavior most likely contributes to the formation of dense monotypic stands of strawberry guava which are common in invaded habitats. These dense stands exclude native vegetation and may significantly alter native plant and animal communities. Strawberry guava is a prolific fruiter with a high seed count. Germination rates also are very high; Huenneke and Vitousek (1990) recorded laboratory germination rates between 60-80% and field germination rates of approximately 56%.

The fruits of strawberry guava are attractive to birds and other frugiverous animals, which aid in the dispersal of the plant. In Hawaii, an interesting mutualistic relationship between two invasive species occurs between strawberry guava and the feral pig, *Sus scrofa* L. (Diong 1983). Diong (1983) found that strawberry guava was a preferred food of the feral pig; during the fruiting season, strawberry guava represented approximately 78.3% of the pig's stomach contents. Strawberry guava benefits from the relationship by being carried to new habitats and deposited in nutrient rich pig droppings. Frequently, the pig droppings were deposited on soil disturbed by the rooting activity of the feral pig. Although seed viability was not affected by passage through the pig's gut, gut treatment did result in earlier germination time compared to untreated seeds (Diong 1983).

In Hawaii, introduced agricultural livestock, such as goats, sheep, and cattle, also have been reported feeding on strawberry guava (MacCaughey 1917). In addition, a variety of birds have been reported as feeding on the fruits of strawberry guava, e.g. the laced-necked dove (*Streptopelia chinensis* (Scopoli), mynah bird (*Acridotheres tristis* L.), rice bird (*Lonchura punctulata* L.), house sparrow (*Passer domesticus* L.), the melodius laughing thrush (*Garrulax canorus* Hwamei (Cheng), Japanese white eye (*Zosterops japonicus* Temminck and Schlegel), and the red-billed leiothrix (*Leiothrix lutea* Maier & Bowmaker), in addition to many others (Diong 1983, MacCaughey 1917). Other animals reported to consume strawberry guava fruits include mongooses, bats (Diong 1983), and squirrels (B. Overholt, University of Florida, IRREC – pers. comm.).

Control Methods

Mechanical control

It is important for public land managers in charge of parks and preserves to remove invasive species from their properties. In many cases, the most cost effective method of removing small infestations is mechanical control (Tunison 1991). Young plants and saplings originating from seed can be uprooted either by hand or with a weed wrench (Tunison 1991). The uprooted plants must be disposed of, because they may re-root if left on the ground. In extreme cases, mechanical control has been used to control large infestations. In Florida, at Jonathan Dickinson State Park, the strawberry guava infestation was so dense that it was necessary to bulldoze and restore a three acre portion of the park (Langeland and Hall 2000).

Chemical control

The most common method of controlling woody weed species is by using chemical herbicides, or a combination of mechanical and chemical control (cut stump, basal frill treatments). Strawberry guava is sensitive to picloram (Tordon, Grazon, Dow AgroSciences), dicamba (Banvel, Velsicol Chemical Corp.), glyphosate (Roundup, Monsanto Co.; Accord, Rodeo, Dow AgroSciences), and triclopyr (Garlon, Dow AgroSciences) (Tunison 1991). Pratt et al. (1994) tested the efficacy of 4 herbicides for managing strawberry guava in Haleakala National Park, Hawaii. They found that soil treatments with Velpar (hexazinone, 90% active ingredient, DuPont) and Spike 20P (tebuthiuron, 20% active ingredient, Elanco now Dow AgroSciences) were ineffective. The two most effective herbicides were 2 formulations of triclopyr produced by Dow AgroSciences, Garlon 3A (0.36 kg/l) and Garlon 4 (0.48 kg/l) applied with the cut stump method. These chemical control measures work reasonably well for managing strawberry guava infestations within the maintained borders of parks and preserves. However, chemical control methods are often non-selective, can be expensive, because re-treatment is often necessary, and are not a long-term solution to the problem.

Biological control

A feasible long term control strategy for strawberry guava is biological control (Smith 1985). Biological control is defined as the use of natural enemies to reduce the numbers of pest organisms. Biological control is not an eradication technique but rather a management strategy. There are many types of biological control, ranging from augmentative releases of natural enemies to the traditional approach of classical biological control. Classical biological control refers to the introduction of specialist natural enemies from the native range of an adventive pest, with the intention of reducing the pest population density. For the remainder of this manuscript, unless otherwise mentioned, the terms biological control and biocontrol will be considered synonymous with classical biological control.

The tropical climate and geographically isolated nature of the Hawaiian archipelago has made it an ideal habitat for introduced exotic plant species to proliferate. In the early 1980s, the National Park Service and the University of Hawaii undertook a project to prioritize the potential of invasive plant species for biological control (Gardner and Davis 1982). Initially, it was thought that strawberry guava was a poor candidate due to its close relationship to the common guava, for which a small commercial market exists in Florida and Hawaii (Gardner and Davis 1982). In addition, the family Myrtaceae contains various genera which are commercially or ecologically important in both states; for example *Eucalyptus*, *Eugenia*, *Pimenta*, and *Syzygium*. Prior to 1988, the only mention of predators or potential pathogens for strawberry guava was a parasitic alga of the genus *Cephaleuros*, which also attacks common guava (Marlatt 1980, Marlatt and Alfieri 1981). In the mid 1980s, the National Park Service and the University of Hawaii funded initial explorations for potential biological control agents of strawberry guava in Brazil (Hodges 1988). The objective of this study was to determine the native distribution of strawberry guava, to make local contacts for future collaboration, and to determine kinds and relative impacts of predators and pathogens on strawberry guava. No promising pathogens were discovered, although a wide variety of insects were found to attack strawberry guava (Hodges 1988). Due to the wide distribution of the species in Brazil, and the numerous insects found associated with the plant, it was recommended that local entomologists be contracted to perform the initial survey of biological control agents. As a result, the National Park Service and the University of Hawaii formed a collaboration with the Paraná Forestry Foundation and the Federal University of Paraná, Brazil. The Brazilian entomologists undertook the initial exploration for insects that attacked strawberry guava within its native range.

Table 2-1. Potential biological control agents for strawberry guava (Wikler et al. 2000).

AGENT	TAXONOMY	CONTROL POTENTIAL
Dasineura gigantea Angelo and Maia	(Diptera: Cecidomyiidae)	Good– bud galling species
Lamprosoma azureum Germar	(Coleoptera: Chrysomelidae)	Poor- non-target effects
Unidentified Psyllid	(Hemiptera: Psyllidae)	Good- leaf galling species
Tectococcus ovatus Hempel	(Hemiptera: Eriococcidae)	Good- leaf galling species
Haplostegus epimelas Konow	(Hymenoptera: Pergidae)	Poor- non-target effects
Sycophilia sp.	(Hymenoptera: Eurytomidae)	Good- seed galling species
Eurytoma sp.	(Hymenoptera: Eurytomidae)	Good- stem galling species

The Brazilian researchers identified seven potential biological control agents (Table 2-1) (Wikler et al. 2000). Two of these species were determined unsuitable because of non-target effects. In initial studies, the sawfly *Haplostegus epimelas* Konow attacked common guava, and the chrysomelid *Lamprosoma azureum* Germar attacked common guava and other myrtaceous species (Wikler et al. 2000). Of the remaining five agents, it was determined that the leaf galling eriococcid *Tectococcus ovatus* was the most promising agent because of the type of damage inflicted and the ease of handling (Wikler et al. 2000). A shipment of *T. ovatus* was then sent to the Institute of Pacific Islands Forestry in Volcano, Hawaii to establish a laboratory colony for host specificity testing.

In 2001, the University of Florida initiated a biocontrol program against strawberry guava in the state. Shipments of *T. ovatus* were sent from the Hawaii colony to the FDACS Division of Plant Industry, Florida Biological Control Laboratory in Gainesville, Florida, to establish a colony for host specificity testing.

***Tectococcus ovatus* Hempel**

Higher Classification

The higher taxonomic classification of the Order Hemiptera has historically been one of great debate. The term higher classification is in reference to the ordinal and subordinal taxonomic level. The Order Hemiptera often has been divided into two separate orders: the Hemiptera and the Homoptera (Borror et al. 1989). This earlier classification scheme was based solely on morphological characteristics. With the advent of molecular systematics, many authors suggested that Homoptera was not a monophyletic group (Campbell et al. 1995, Schuh and Slater 1995, Sorensen et al. 1995). Recent molecular data supports this, showing that the order Homoptera is paraphyletic. Currently, most taxonomists agree and combine the traditional Orders Homoptera and Hemiptera into one order, the Hemiptera (*sensu lato*). This can be confusing, therefore unless otherwise mentioned in this manuscript; Hemiptera will be used in the broad sense (*s.l.*).

A debate also exists regarding the organization of the suborders. The Order Hemiptera is usually split into 4 or 5 suborders; and different authors often suggest different names for the suborders which further confuse the issue. To date, the most complete treatment of the higher classification of the Hemiptera has been compiled by Bourgoin and Campbell (2002). Bourgoin and Campbell (2002) built their phylogeny, using a combination of morphological, molecular, and fossil data. They divide the

Hemiptera into 5 suborders; the Sternorrhyncha, Fulgoromorpha, Cicadomorpha, Coleorrhyncha, and the Heteroptera (Fig. 2-5). *Tectococcus ovatus* belongs to the family Eriococcidae. This family is grouped under the suborder Sternorrhyncha, which also contains the psyllids and aphids. Most authors agree on the placement of the scales, psyllids, and aphids into the Sternorrhyncha (Bourgoin and Campbell 2002, Campbell et al. 1995, Schuh and Slater 1995, Sorensen et al. 1995).

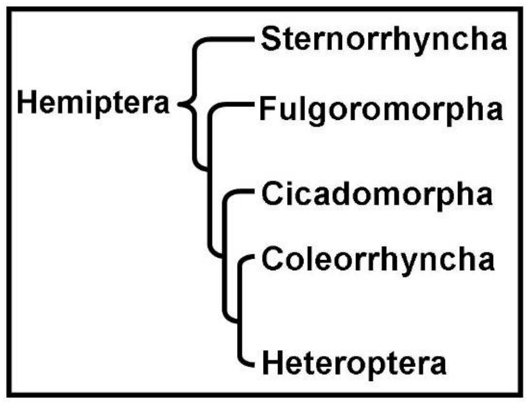

Figure 2-5. Phylogeny proposed by Bourgoin and Campbell (2002) for the higher classification of the Hemiptera based on morphological, molecular, and fossil data.

Taxonomy

Members of the family Eriococcidae are commonly called felt scales or felted scales. The family contains approximately 50 genera and 350 species (Hoy 1963). Aside from the absence of paired anal plates, there are very few defining characteristics of the family. In fact, the family seems to be comprised of a collection of unrelated groups (Rung et al. 2005). Recent molecular data using small subunit rDNA supports this conclusion, rendering the family paraphyletic (Cook et al. 2002). Although these findings have serious nomenclatural implications, Cook et al. (2002) regard their data as preliminary and suggest that the group remain intact until more extensive studies are conducted. The currently accepted classification scheme for *T. ovatus* is as follows:

Class: Insecta

Order: Hemiptera

Suborder: Sternorrhyncha

Superfamily: Coccoidea

Family: Eriococcidae

Genus: *Tectococcus*

Species: Tectococcus ovatus Hempel

Life History

Gall description

Leaf galls of *T. ovatus* each contain one insect and are visible on both sides of the leaf. There is one opening per gall and this is at the apical portion of the gall. The opening of the gall is formed on the side of the leaf upon which the insect originally initiates feeding (Vitorino et al. 2000). The galls are generally acuminate on both sides of the leaf; occasionally the gall may be acuminate only on the side of the leaf with the opening and convex on the other side. The inside of the gall is flat and covered with a fine powdery wax (Vitorino et al. 2000). The size of the gall is variable and depends on the developmental stage and sex of the insect. The galls are sexually dimorphic; the base of the female gall is much wider than that of the male (Fig. 2-6).

Morphology

The genus *Tectococcus* is monotypic. *Tectococcus ovatus* was originally described by the Brazilian entomologist Hempel in 1900. Ferris (1957) described and illustrated the adult female and first instar “crawler” stage (Fig. 2-7). Adult males are typically not described in detail because they are rarely collected in the field and are not used for species identification. *Tectococcus ovatus* is a small species approximately 1.5 mm long, ovate in form with the caudal end acuminate (Fig. 2-8). Derm is membranous and pink to brown in color, dusted in a fine white powder (Vitorino et al. 2000). Legs are present and well developed although the adult female is relatively sessile, spending her entire life within the confines of a leaf gall. The antennae are six segmented with the first joint being the longest. The major distinguishing feature of the species is the small, slightly sclerotized and hairless anal plates (Ferris 1957, Hempel 1901). The males are slight and narrow, either pink or light brown. Antennae are long and slender, approximately half of the length of the body. Males have narrow legs and one pair of wings; they are capable of weak flight.

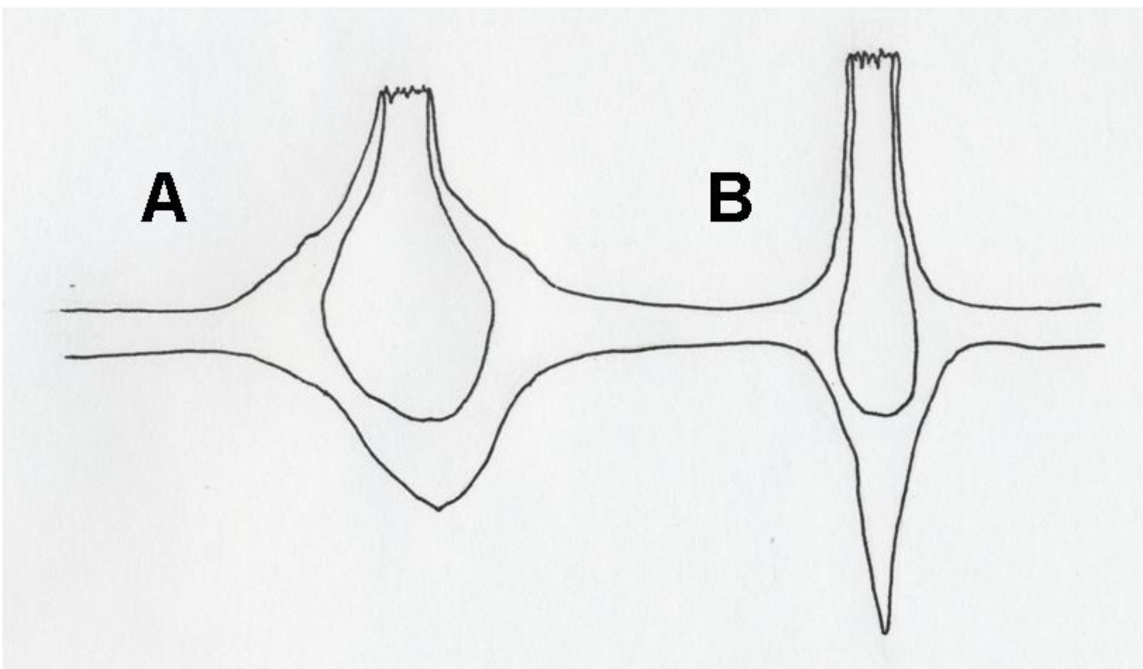

Figure 2-6. Cross sectional view of the galls of *T. ovatus* A) Female B) Male.

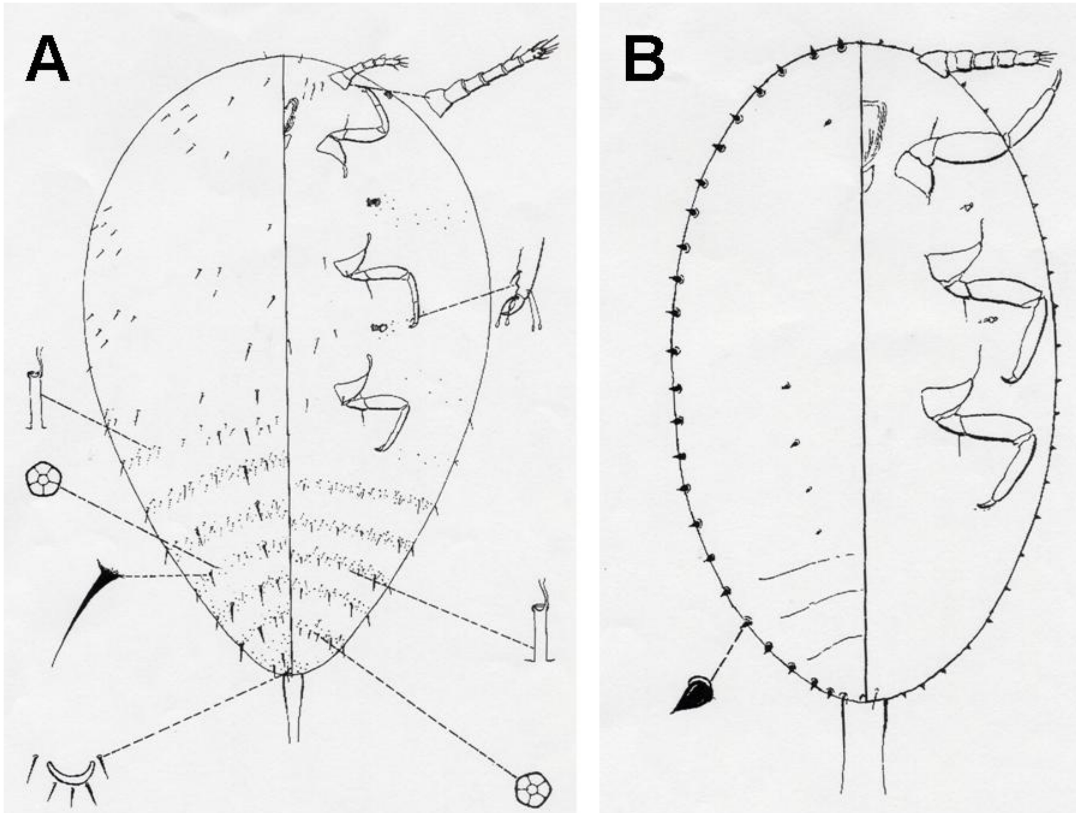

Figure 2-7. Ferris (1957) drawings of *T. ovatus* A) Adult female B) First instar crawler.

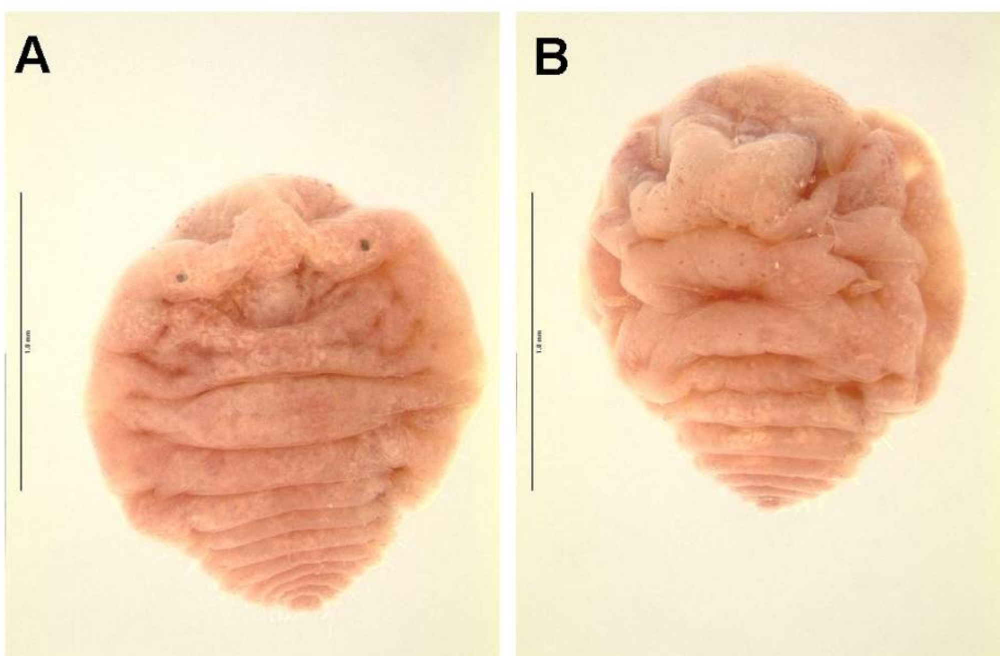

Figure 2-8. *T. ovatus* adult female A) Dorsal B) Ventral (scale bars represent 1 mm).

Biology

The first instar of *T. ovatus* is the mobile stage of the insect and they are commonly called crawlers. Upon hatching, *T. ovatus* crawlers search the plant for suitable feeding sites, the ideal site usually being new flush. Once a suitable site is found, the insect begins feeding and becomes sessile. The plant responds by forming a gall around the insect (Fig. 2-9 A, B, and C). Galls are typically formed on leaves, although they may also form on floral buds, young branches and developing fruit (Vitorino et al. 2000). Gall formation always begins on the same side of the leaf where the insect begins feeding. Females are facultatively parthenogenetic, although there is at least one alternation of generations per year (Vitorino et al. 2000). The female remains in the gall for her entire life, whereas males are mobile in their adult stage. Once a female reaches the adult stage, she begins producing eggs within the gall. The eggs are then extruded in a cottony wax through the opening in the gall (Fig. 2-9 D). This wax probably aids in the dispersal of the eggs via wind.

Nutritional Ecology

The majority of phytophogous insects consume large amounts of plant tissues or fluids. To fulfill their nutritional requirements, these insects must move about their host, feeding at multiple sites. Gall forming insects are unique in that they remain sessile, and feed only on specialized nutritive cells that line the gall chamber (Dreger-Jauffret and Shorthouse 1992). The gall former stimulates an abnormal growth process in its host, from which it receives both nutrition and protection (Abrahamson and Weis 1987). Because of this highly specialized relationship between the herbivore and its host, most gall-inducing insects are highly host specific. The reduced risk of non-target effects makes gall forming insect's ideal weed biological control agents (Harris and Shorthouse 1996).

Figure 2-9. Life stages of *T. ovatus*; A) gall initiation around first instar crawlers; B) close up of leaf galls; C) view of galls on multiple leaves; D) eggs extruded in cotton-like wax. (Photo credit: M. Tracy Johnson).

Plant galls can be divided into two basic groups, organoid and histioid (Rohfritsch 1992). Organoid galls differ slightly from the normal plant growth pattern while histioid galls alter the basic growth patterns of the host. The galls of *T. ovatus* are histioid, or more specifically they are categorized as prosoplasmic. Prosoplasmic refers to a histioid gall which is highly organized and displays tissue differentiation (Rohfritsch 1992). Galls of the Eriococcidae, however, typically have a less characteristic nutritive tissue (Rohfritsch 1992).

The induction of abnormal cell growth by gall forming insects results in a metabolic nutrient sink (Harris and Shorthouse 1996). In addition to depriving normal plant cells of nutrients, high densities of *T. ovatus* leaf galls may inhibit photosynthesis. Heavy infestations of *T. ovatus* result in premature leaf drop and in some cases complete defoliation (Vitorino et al. 2000). There is anecdotal evidence that *T. ovatus* infestations also may reduce fruit production, reducing both the seed bank and potential fruit fly breeding sites (Vitorino et al. 2000).

Recorded Host Range

When first described by Hempel (1900), the only reference to the host plant was that it belonged to the family Myrtaceae. The majority of the older literature also is vague regarding host species, mentioning only the family Myrtaceae (Da Costa Lima 1927, Hempel 1912, Lepage 1938, MacGillivray 1921). In a catalogue of Brazilian insects published in 1936, the host records are more specific, although only common names are cited (Da Costa Lima 1936). Da Costa Lima (1936) lists hosts as araçazeiro (strawberry guava) and a plant known as “embira”. Subsequent investigation revealed that the name “embira” refers to two different species, in two different families, *Daphnopsis racemosa* Griseb. (Thymelaeceae) and *Rollinia salicifolia* Schltdl. (Annonaceae). This reference most likely led to the inclusion of *D. racemosa* as a host by Hoy (1963) in his catalogue of the Eriococcidae of the world. This reference is probably erroneous and may be due to confusion between *T. ovatus* and its relative *Pseudotectococcus anonae* Hempel (Johnson 2005). Whether the host record is erroneous or not, members of the genera *Daphnopsis* and *Rollinia* from Florida and the Caribbean would be appropriate for inclusion in a host specificity test plant list.

In field observations, *T. ovatus* has been reported on other members of the genus *Psidium* in Brazil. *Psidium longipetiolatum* Legrand and *Psidium spathulatum* Mattos both have been reported as being attacked by *T. ovatus* (Vitorino et al. 2000). These two species are not present in the continental United States and therefore are in no danger of being attacked by *T. ovatus,* should it be approved for release in Florida.

CHAPTER 3
LABORATORY BIOLOGY OF *Tectococcus ovatus*

Introduction

Tectococcus ovatus Hempel is a leaf galling scale insect (Eriococcidae) native to the coastal regions of southeastern Brazil. The distribution of the insect is closely correlated with the native range of its primary host plant, strawberry guava, *Psidium cattleianum* Sabine (Myrtaceae); ranging from the Brazilian state of Espirito Santo in the north to northern Uruguay in the south (Wikler 1995). Strawberry guava was originally imported into Florida for the ornamental fruit trade in the late 1800s (Langeland and Hall 2000). However, it escaped cultivation and is now considered a highly invasive natural areas weed in the state (IFAS 2005). In addition to invading native plant communities and altering the natural ecological balance of plant and animal communities in Florida, strawberry guava also is considered one of the major hosts of the Caribbean fruit fly, *Anastrepha suspensa* Loew (Tephritidae) (Nguyen et al. 1992, Swanson and Baranowski 1972).

Native to the West Indies, the Caribbean fruit fly is a highly polyphagous pest species with nearly 100 recorded hosts (Weems et al. 2005). Populations of the Caribbean fruit fly eventually became established in central and southern Florida in 1965 (Swanson and Baranowski 1972). This establishment was largely ignored until 1968, when the fly was discovered in commercial grapefruit, *Citrus* x *paradisi* Macfad. (Greany and Riherd 1993). To eliminate the spread of this pest, many domestic and foreign markets initiated quarantines on fresh citrus shipments from Florida. Multiple management strategies are currently being used to combat Caribbean fruit fly infestations in the state. These strategies include the sterile insect technique, classical biological control, and the development of the fruit fly-free protocol, an integrated approach to certify citrus crops as fly-free (Baranowski et al. 1993). The fruit fly-free protocol involves a combination of trapping, baiting, spraying, and the removal of major hosts (including strawberry guava) from surrounding areas (FDACS 2005). Compliance with this protocol can be problematic because the citrus grower is responsible for removal of major hosts from adjacent properties, even if they do not own the property (FDACS 2005).

Controlling strawberry guava infestations mechanically and chemically are viable options for easily accessible plants. However, these methods are not practical in environmentally sensitive areas (e.g., in natural areas and state parks and preserves). Classical biological control of strawberry guava is ideal because once released, biological control agents are self sustaining, can locate less accessible plants, and do not require landowner permission.

A colony of *T. ovatus* was established at the Florida Department of Agricultural and Consumer Services (FDACS), Division of Plant Industry (DPI) Florida Biological Control Laboratory (FBCL) in Gainesville, FL. Currently, this insect is under investigation for potential release as a biological control agent for strawberry guava in Florida. High infestations of *T. ovatus* act as a nutrient sink. Diverting nutrients from plant growth and reproduction can cause premature leaf drop, may reduce photosynthesis, and inhibit fruit production, ultimately reducing fruit fly breeding sites (Vitorino et al. 2000).

Prior to the release of any classical biological control agent, it is necessary to understand the biology and ecology of the agent. The purpose of this study was to make detailed biological observations on *T. ovatus* in order to expand on the limited information available in the literature regarding this species. An important factor in understanding the developmental biology of an insect is determining the number of instars and distinguishing these from the adult. The soft bodied nature of this insect makes it difficult to determine the number of instars by traditional means. Because of this, multivariate analyses of multiple leg measurements were conducted. The legs were selected for these analyses because they are sclerotized and readily identifiable. The majority of the life cycle of this species occurs inside of a protective plant gall. Therefore, in order to better understand the life history of *T. ovatus*, a linear regression was constructed to determine if a correlation exists between the size of the leaf gall and the life stage of the insect (excluding the egg). This information may be useful for studies where destructive sampling of specimens is not a feasible option, for example, the construction of age-specific life tables.

Materials and Methods

Tectococcus ovatus specimens used in this study were obtained from the laboratory colony maintained at the FDACS DPI Florida Biological Control Laboratory in Gainesville, FL. All biological observations were made from insects reared in this colony. The colony was maintained in acrylic cylinders, 46 cm tall and 15 cm in diameter. These cylinders were placed over the host plants and the bottom was partially buried in the soil to prevent *T. ovatus* from escaping. The acrylic cylinders were ventilated by six holes 6 cm in diameter. The ventilation holes and the top of the cylinder were covered with a fine mesh, with a screen size of 150 μ x 150 μ (Green.tek Inc., Edgerton, WI). The colony was maintained under natural light conditions in a quarantine greenhouse, supplemented with fluorescent light (40 Watts) set on a 14:10 light: dark photoperiod. Average colony temperature inside of the cylinders was 28.88 ± 1.61 °C and the average humidity was 66.13 ± 6.95 %. Insects were reared on the yellow fruiting variety of strawberry guava (*P. cattleianum* var. *lucidum*) either grown from seeds collected in the field or purchased from nurseries within Florida. The yellow variety was selected because preliminary studies indicated that *T. ovatus* preferred this to the red fruiting variety of strawberry guava (*P. cattleianum* var. *cattleianum*).

Five specimens of *T. ovatus* were dissected from leaf galls every other day for 30 days during development, resulting in a total of 75 specimens. This method of sampling was chosen to ensure that every developmental stage was included in the analysis. During the slide mounting process, 13 specimens were lost or damaged. All 13 specimens that had missing data were eliminated from this study, leaving a total of 62 specimens to be analyzed.

Before specimens were dissected from their protective plant galls, the width of the insect gall was measured using micrometer calipers. These measurements were taken in order to determine if a correlation exists between the gall size of *T. ovatus* and the various life stages after hatching. Due to variation between galls, the portion of the gall measured was the diameter at the base of the apical portion (Fig. 3-1). Preliminary investigation indicated that this portion of the gall had the least amount of variation. The specimens were then slide mounted according to a protocol modified from Wilkey (1992). Prior to slide mounting the specimens were cleared in 10% KOH for 24 to 48 h. Specimens were then stained with #6379 double stain (BioQuip Products, Inc. Rancho

Dominguez, CA), and transferred into 95% EtOH (15 min) and then submerged in clove oil (30 min) (Ward's Natural Science, Rochester, NY). Canada balsam (Fisher Scientific Co., Pittsburgh, PA) was used as the slide mounting medium. The slides used in this study were 75 x 25 mm Fisherbrand® plain pre-cleaned slides (Fisher Scientific Co., Pittsburgh, PA) used with Fisherbrand® (Fisher Scientific Co., Pittsburgh, PA) 12 mm circular cover glass.

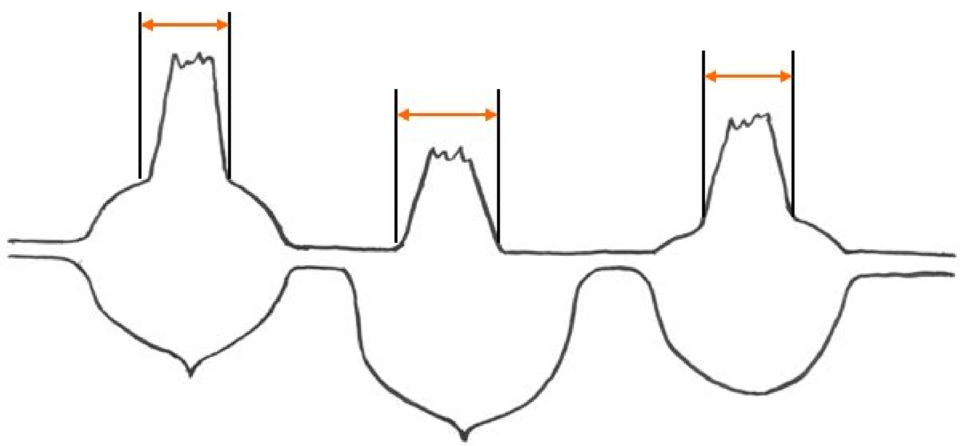

Figure 3-1. Cross section depicting variation in female leaf galls of *T. ovatus*. Arrows indicate the diameter of the apical base of the gall where measurements were taken.

In order to determine the number of nymphal stages and distinguish them from the adult, multiple leg measurements were recorded for each insect. The legs were selected as the identifying feature because they are heavily sclerotized and readily identifiable; *T. ovatus* is a soft bodied insect and slide mounting can alter the dimension of non-sclerotized parts. Due to the discontinuous nature of insect development, the number of instars and the adult can be determined by comparing variation of multiple leg measurements. The length of the fused trochanter/femur segment, the tibia, and the tarsus was recorded for one the pairs of each leg (prothorcacic, mesothoracic, and metathoracic). In addition, the width of the fused trochanter/femur and tibia was recorded at their widest points on each leg. This procedure resulted in a total of 15 measurements for each specimen. The slide mounted specimens were digitally photographed with a JVC® model KY-F70B 3-CCD digital camera (JVC Americas Corp.) mated to a Leica DMLB compound microscope (Leica Microsystems AG) with a Diagnostic Instruments T-49C 0.45x c-mount coupler (Diagnostic Instruments, Sterling Heights, MI). The Syncroscopy Auto-Montage software (Synoptics Ltd., Frederick, MD) was used to measure the images at a resolution of 1360 x 1024 pixels. This system also was used to measure the eggs of *T. ovatus.* Measurements were recorded digitally in order to reduce error (the microscope only had to be calibrated once); this method also is less time consuming than using an ocular micrometer.

Data were analyzed using the SAS® statistical software (SAS Institute Inc., Cary, NC). Due to the number of dimensions measured (15 observations per insect; n = 62), a principal components analysis was performed using PROC PRINCOMP. This procedure reduced the dimensions of the data by deriving a small number of linear combinations (in this case 2 principal components) from the data. Next, a cluster analysis was performed on the results of the principal components analysis using PROC FASTCLUS in order to delineate distinct clusters of observations. Due to the discontinuous nature of insect growth, these data clusters were used to differentiate the number of instars and separate them from the adult stage. PROC FASTCLUS also calculated the mean and standard deviation for all 15 morphometric parameters in each cluster. Finally, to obtain a better graphical representation of the clusters, a canonical analysis was performed using PROC CANDISC. This analysis transformed the data from the FASTCLUS analysis into two canonical variables (Can1 and Can2).

In order to correlate gall size to a particular life stage (excluding the egg), a regression analysis was performed using Microsoft Excel (Microsoft Corporation, Redmond, WA). The diameter at the base of the apical portion of the gall was correlated with the length of the fused prothoracic trochanter/femur. The developmental instar can then be determined by comparing the prothoracic trochanter/femur length measurement with the mean length provided by the FASTCLUS analysis (see Table.3-2 in Results and Discussion).

Results and Discussion

Biology

Tectococcus ovatus has a simple life cycle which has been previously described by Vitorino et al. (2000). Eggs are deposited inside the gall of the female, and are then extruded from the gall opening in a filamentous waxy secretion which may aid in their dispersal by wind. The eggs are oval in shape and range in color from nearly white to a light yellow (Fig. 3-2). Average egg length is 0.216 ± 0.008 mm and average width is 0.115 ± 0.006 mm (n = 20). Upon hatching, the mobile first instar or “crawler” disperses on the plant in search of a suitable feeding site. Ideal feeding sites are young flushes of leaf growth. Vitorino et al. (2000), however, mentions that galls also can form on floral buds, young branches, or developing fruit. Once a suitable feeding site is found, feeding elicits a plant response to form a gall around the sessile insect. The female will spend the rest of her life within the confines of this protective gall. However, the winged male is mobile as an adult (Fig. 3-3).

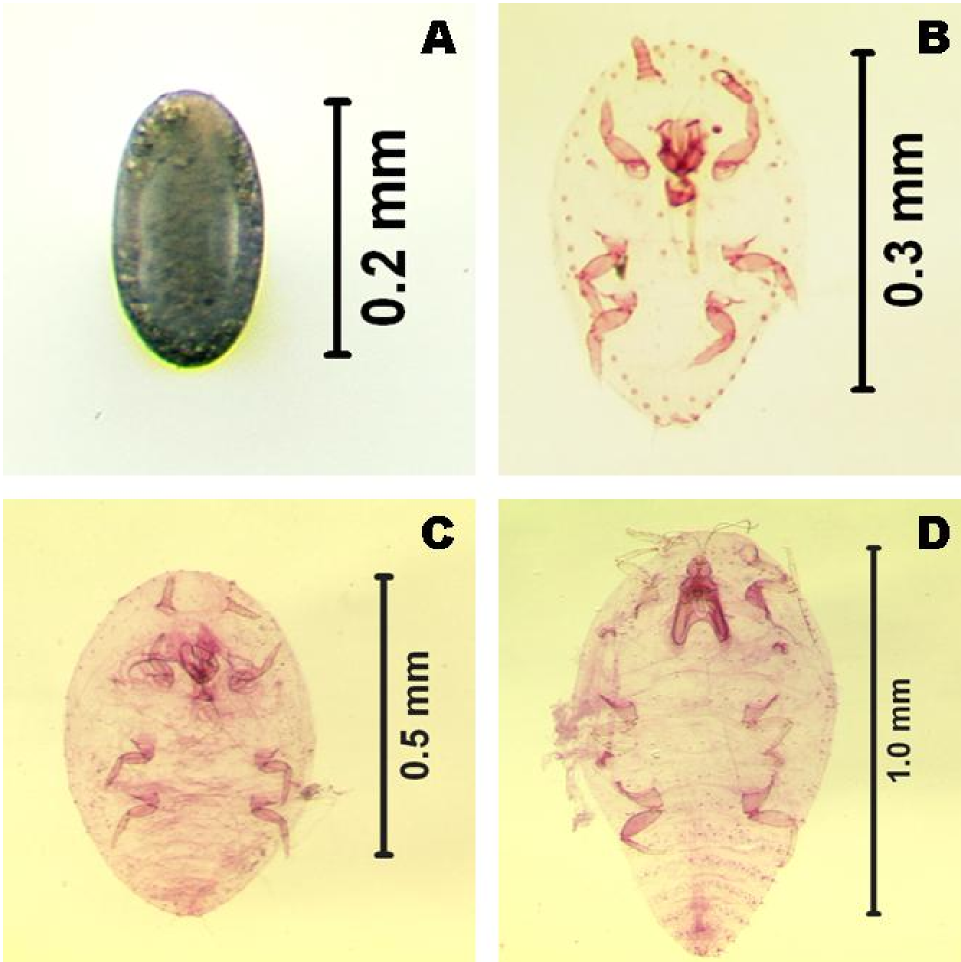

Figure 3-2. Developmental stages of female *T. ovatus*; A) egg (abnormal coloration due to preservation in 70% EtOH); B) first instar “crawler”; C) second instar; D) adult (possible third instar not depicted).

Figure 3-3. *T. ovatus* adult male (scale bar represents 0.5 mm).

Vitorino et al. (2000) describes reproduction as facultatively parthenogenetic with at least one alternation of generations per year. Prior to this study, copulation has never been observed. In this study, copulation was observed only once in the laboratory. Prior to copulation, the male inspected the opening of the gall with his antennae, presumably looking for a receptive female. The receptive female partially exposed her posterior end from the gall. The male turned around and rubbed the posterior portion of his abdomen around the anal area of the female in an apparently random writhing motion. Copulation lasted for approximately 40 seconds. After copulation, the male continued searching the leaf, possibly for other females, and the mated female re-entered her gall.

Separation of Nymphal and Adult Stages of *Tectococcus ovatus*

A traditional method of determining the number of instars of an insect is by constructing a frequency histogram of a particular body measurement (Kishi 1971). Typical measurements for this type of study are inter-ocular distance or some other head capsule measurement; instars are identified as distinct peaks in the histogram (Daly 1985). This method works reasonably well in cases where the data produce discrete non-overlapping peaks. To check if any instars were missed during visual inspection of the histogram, the logarithms of the means for each mode are plotted against the presumed number of instars. If all instars are included, a straight line should be observed (Daly 1985). This analysis is based on the prediction of Dyar (1890) that the head capsule size increases by a constant geometric progression every molt, commonly known as Dyar's law. However, this type of analysis only looks at one dimension of growth, and it has been shown that the limited amount of dimensions may result in the misinterpretation of the number of instars, particularly if the peaks are not discrete (Kishi 1971; Schmidt et al.

1977). In addition, Dyar's law does not necessarily hold true for every insect, or even within a single family (Daly 1985, Gaines and Campbell 1935).

Describing morphological variation in biological organisms using a multivariate morphometric analysis is a relatively old technique that has been widely applied (Daly 1985). The term multivariate morphometric analysis is used to describe any multivariate statistical procedure used to describe relationships between measurements of a biological organism. Blair et al. (1964) indicates that multivariate morphometric analyses have potential for describing morphological variation in difficult groups such as the Coccoidea. Blair et al. (1964) looked at the variation in a homogenous population of *Coccus hesperidum* L. based upon the analysis of multiple measurements of the legs and antennae. Similarly, Boratynski and Davies (1971) analyzed multiple morphometric characters to describe taxonomic variation in male coccids. Insects do not grow continuously but rather in discontinuous steps or instars. Therefore, it should be possible to measure the average size increase of each instar of a particular species by using this type of analysis.

By analyzing the 15 separate leg measurements per specimen, it was possible to determine the number of life stages (excluding the egg). The results of the FASTCLUS cluster analysis of the principal components indicate that there are three distinct clusters. These clusters may represent two instars and the adult. However, the analysis also identified a weak fourth cluster. This may indicate the presence of a supernumerary instar in addition to the adult. The transformation produced from the canonical analysis provides a better display of these results (Fig. 3-4). The first three clusters are clearly visible; while the fourth cluster is shown by the five star shaped points on the right of the graph. Typically, females in the family Eriococcidae have two instars in addition to the adult (Stehr 1991). There are a few possible explanations for the potential presence of a fourth cluster. There may be a wide range of size variation in the size of the adult of *T. ovatus*. In this case, the weak fourth cluster could be the result of outlying data from measurements of extremely large adults. Outlying data are more difficult to identify when comparing data sets with several dimensions such as this (15 observations per specimen). The presence of a fourth cluster could also be due to variation in the initial measurements. The measurements taken from the Auto-montage image were in two dimensions; slight errors in the measurements could have occurred if the leg segment of the slide mounted specimen was not perfectly horizontal. Both of these problems could be solved by taking a larger sample size ($n > 62$). Another explanation is that there is a supernumerary instar; this could also be elucidated by analyzing a greater sample size. The occurrence of supernumerary molts in laboratory reared colonies is not an uncommon observation (Chapman 1998).

Correlation of Gall Size to Nymphal and Adult Stages of *Tectococcus ovatus*

By comparing r^2 values for multiple regressions of gall size vs. insect measurements, the length of the prothoracic trochanter/femur segment was determined to have the closest relationship with the gall width (Table 3-1). Figure 3-5 illustrates the close correlation between these two variables; the best fit equation is $y = 44.603x + 16.233$ ($r^2 = 0.7126$; df = 1, 65; $p < 0.001$).

Table 3-1. r^2 values for multiple regressions of separate leg measurements of *T. ovatus* vs. the gall size. Gall size is determined by the diameter of the base of the apical portion of the gall.

Insect Measurement (Length)	r^2 Value
Prothoracic Troch/Fem	0.7126
Mesothoracic Troch/Fem	0.7070
Metathoracic Troch/Fem	0.7114
Prothoracic Tibia	0.6718
Mesothoracic Tibia	0.6809
Metathoracic Tibia	0.7095

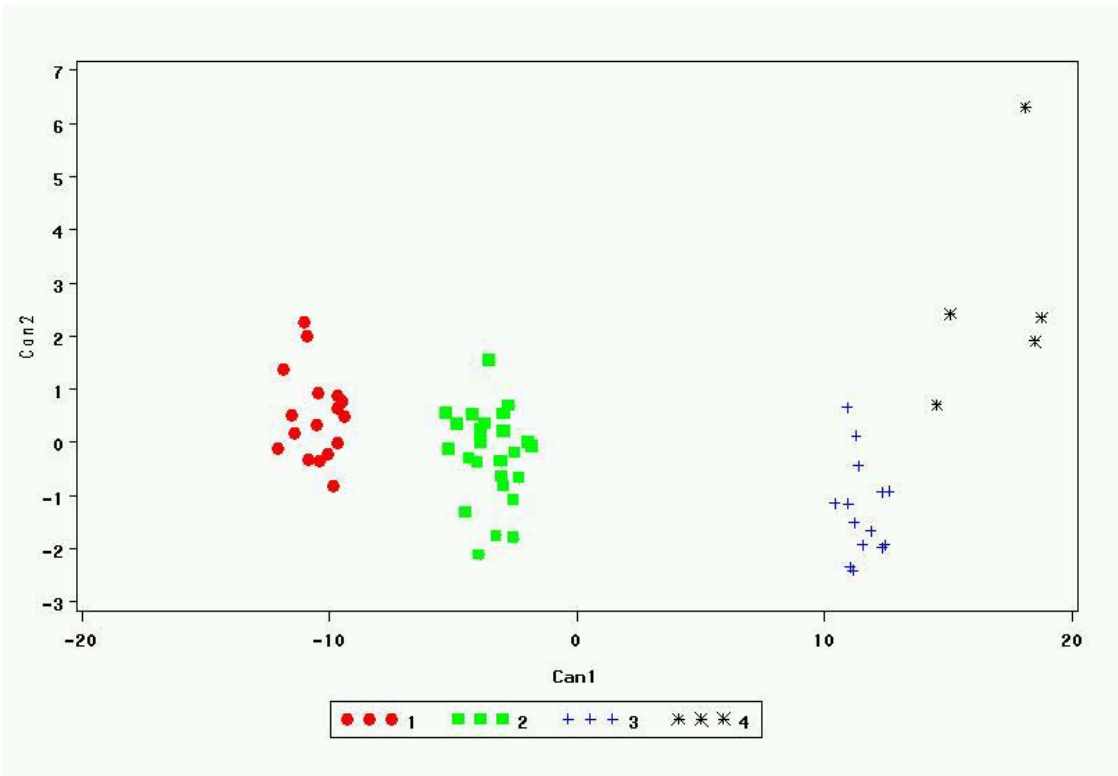

Figure 3-4. Two dimensional representation of the FASTCLUS cluster analysis. PROC CANDISC was used to generate two canonical variables (Can1 and Can 2) for graphing the results of the cluster analysis.

The mean and standard deviation of the lengths for the prothoracic trochanter/femur segments of each cluster were provided by the FASTCLUS cluster analysis (Table 3-2). The mean and standard deviation are provided for each of the four clusters because the possibility of a supernumerary instar could not be ruled out. In Table 3-2, the first and second clusters represent the first and second instar respectively. Further sampling is necessary, but the third cluster may represent either a supernumerary third instar or the adult stage. The fourth cluster represents the adults with three instars or outlying data from measurements of extremely large adults.

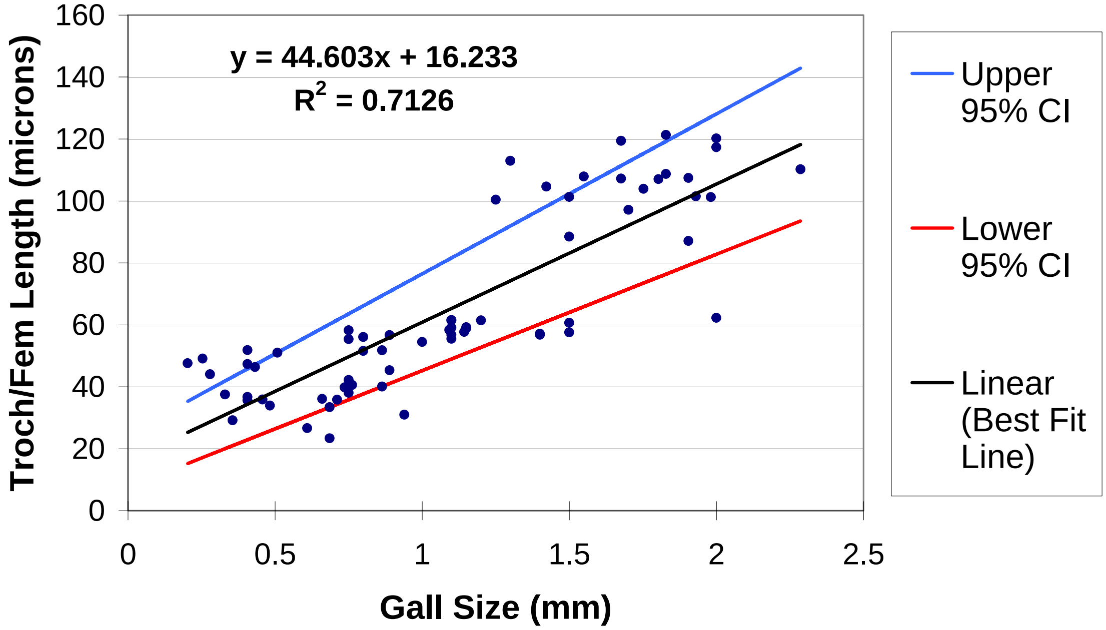

Figure 3-5. Linear regression analysis of the relationship between the diameter of the apical base of the plant gall and the fused prothoracic trochanter/femur length of T. ovatus ($r2 = 0.7126$; df = 1, 65; $p < 0.001$).

Table 3-2. Mean and standard deviation of the length of the fused prothoracic trochanter/femur segment for each developmental instar.

Cluster	Mean Prothoracic Trochanter/ Femur Length (μ)	Standard Deviation
1 (First Instar)	35.748	5.706
2 (Second Instar)	55.315	5.118
3 (Third Instar or Adult)	101.706	6.823
4 (Adult)	116.914	4.390

Acknowledgements

The author would like to thank Greg Hodges and Yen Dao for instruction in preparing the slide mounted specimens. The multivariate statistical analyses conducted for this study would not have been possible without the professional assistance of Meghan Brennan and Kenneth Portier. In addition, thanks are due to Alejandro Arevalo for his help with analyzing the regression data. This research was funded by the USDA CSREES Tropical/Subtropical Agriculture Research program (T-STAR-Caribbean) grant No. 01062227.

CHAPTER 4
HOST SPECIFICITY OF *Tectococcus ovatus*

Introduction

Strawberry guava, *Psidium cattleianum* Sabine, is a woody evergreen tree or shrub native to the coastal regions of southeastern Brazil. Closely related to common guava *Psidium guajava* L., strawberry guava was introduced to numerous countries worldwide because of its small edible fruit, attractive foliage, and broad environmental tolerances (Morton 1987). Strawberry guava was first introduced into Florida by the horticultural trade in the late 1800s (Langeland and Hall 2000). The delicate nature and short shelf life of the fresh fruit has inhibited the commercial potential of the plant (Schroeder and Coit 1944). There are no horticultural cultivars of strawberry guava, although there are two, possibly three varieties that are distinguished solely by the color and shape of the fruit. Two varieties are present in Florida, a yellow fruiting variety, *P. cattleianum* var. *lucidum*, and a red fruiting variety, *P. cattleianum* var. *cattleianum* (Wikler 2000b).

Strawberry guava is still commonly sold as an ornamental hedge and fruit tree in the state. This is despite the fact that the plant has escaped cultivation and is invading natural areas within the southern and central parts of Florida (Langeland and Hall 2000). Factors that aid in the spread of strawberry guava are its ability to grow in low light conditions, reproduce vegetatively, produce large amounts of fruit which are attractive to birds and other animals, and lack of natural enemies (Vitorino et al. 2000, Huenneke and Vitousek 1990). Because of this, the Florida Exotic Pest Plant Council and the University of Florida, Institute of Food and Agricultural Sciences assessment of the status of non-native plants in Florida's natural areas (IFAS Assesment) has categorized strawberry guava as an exotic invasive species (FLEPPC 2003, IFAS 2005). In natural areas, strawberry guava can out-compete native plant species and form dense mono-specific stands, which alter native plant and animal assemblages (Tunison 1991).

In addition to the threat to native ecosystems, strawberry guava also is a major host of the adventive Caribbean fruit fly, *Anastrepha suspensa* Loew (Nguyen et al. 1992). The Caribbean fruit fly, a native of the West Indies, is an extremely polyphagous species with almost 100 recorded hosts (Weems et al. 2005). In 1968, the Caribbean fruit fly was discovered in commercial grapefruit, which was previously thought not to be a host (Greany and Riherd 1993). To eliminate the potential spread of Caribbean fruit fly in fresh citrus shipments, the shipments were fumigated with ethylene dibromide. However, ethylene dibromide was banned by the Environmental Protection Agency for this purpose in 1984 (Nguyen et al. 1992). This led to the development of alternative methods to control Caribbean fruit fly populations, such as the sterile insect technique, classical biological control, and the Caribbean fruit fly-free protocol. Participation in the Caribbean fruit fly-free protocol is necessary if a grower intends to export fresh fruit to quarantine sensitive domestic and foreign markets (FDACS 2005). The protocol involves a combination of trapping, baiting, spraying, and the establishment of a buffer zone around the designated grove. This buffer zone consists of an area free of major hosts extending 1.5 miles from the perimeter of the designated grove; major hosts are common guava, strawberry guava, Surinam cherry (*Eugenia uniflora* L.), rose apple (*Syzygium jambos* L.), and loquat (*Eriobotrya japonica* (Thunb.) Lindl.). The citrus grower is responsible for removal of major hosts from the buffer zone, and it is their responsibility to negotiate with property owners regarding removal (FDACS 2005). This can lead to disputes that can hinder citrus growers from exporting their product. Controlling major host plants with classical biological control may help solve this problem by reducing plant populations without active involvement from the citrus grower or property owners.

The classical biological control program against strawberry guava began in Hawaii, where the plant is considered the worst invasive weed in the archipelago (Smith 1985). In 1991, the U.S. National Park Service and the University of Hawaii formed a collaboration with the Federal University of Paraná, Brazil to identify and evaluate potential biological control agents (Wikler et al. 2000). Five potential agents were identified and the leaf galling erioccocid, *Tectococcus ovatus* Hempel was determined to be the most promising based on the type of damage inflicted and the ease of handling (Wikler et al. 2000). *Tectococcus ovatus* is a scale insect that forms galls on the leaves, stems, and fruit of strawberry guava. Feeding and subsequent gall formation act as a nutrient sink depriving the plant of nutrients used for growth and reproduction. High infestations of *T. ovatus* can cause premature leaf drop, may inhibit fruit production, and may reduce photosynthesis.

The damage caused by *T. ovatus* directly effects the growth and sexual reproduction of strawberry guava, which may ultimately reduce fruit fly breeding sites (Vitorino et al. 2000). In addition, weakened plants will make removal easier in state parks and preserves. However, prior to the release of any biological control agent, the host range must be evaluated in order to ensure that the biocontrol agent will not harm non-target species. The purpose of this study was to determine if *T. ovatus* is suitably host specific in order to evaluate the potential for release in Florida.

Materials and Methods

The test plant list for this study was developed in accordance with the U.S. Dept. of Agriculture, Animal and Plant Health Inspection Services Technical Advisory Group for Biological Control Agents of Weeds (TAG) guidelines. The TAG guidelines are based on the centrifugal-phylogenetic method developed by Wapshere (1974). The test plant list is divided into 8 categories based on TAG guidelines and agricultural and economic concerns in Florida. These 8 categories and the division of the test plant list into the categories, as well as justification for inclusion are available in Appendix B. Alterations made to the original test plant list are available in Appendix C.

Specimens for establishing the *T. ovatus* colony used for host specificity testing in Florida were shipped to the Division of Plant Industry quarantine facility in Gainesville from the Hawaii Volcanoes National Park Quarantine Facility in Hawaii (APHIS PPQ 526 permit 54024). Transport between the two states is more reliable and faster than receiving shipments from Brazil. In addition, the colony of *T. ovatus* in Hawaii was already free of predators and parasitoids. The insects reared at the Hawaii quarantine facility were obtained from an outdoor nursery colony established at the Federal University of Paraná (APHIS PPQ 526 permits 47452, 69049) (Johnson 2005). This outdoor colony was obtained from field collected populations east of the city of Curitiba (in the municipal districts of Piraquara, São José dos Pinhais, and Colombo) (Lat -25.5167°, Long -49.1667) (Johnson 2005).

To transport the insects, strawberry guava leaves containing mature *T. ovatus* galls were shipped in individual containers. The leaves were then placed on uninfested caged strawberry guava plants, to allow emerging crawlers to establish on new plants.

Tectococcus ovatus colony production and host specificity experiments were conducted at the Florida Dept. of Agriculture and Consumer Services, Division of Plant Industry, Florida Biological Control Laboratory in Gainesville, Florida. Voucher specimens of *T. ovatus* were deposited in the Florida State Collection of Arthropods, Gainesville, Florida.

Strawberry guava plants used to maintain the colony and as experimental controls as well as all other test plants were maintained at the University of Florida, Department of Entomology and Nematology, Gainesville, Florida. Plants used for these experiments were either grown from seed, purchased, or collected from the field. If there was any question regarding plant taxonomy or identification, a qualified botanist was consulted. Test plants were not treated with systemic insecticides to eliminate the chance of these chemicals altering the results. All control and colony plants were potted with Fafard® middleweight mix # 4 potting soil (Conrad Fafard Inc., Agawam, MA), test plants were potted with soil mixtures appropriate for each species. Plants that required fertilization were fertilized with Dynamite® Plant Food (Florikan E.S.A. Corp., Sarasota, FL) 6 month time-release pellets with a 13:13:13 (N:P:K).

No-choice host specificity tests were conducted because this type of test is rigorous in nature (Heard 1997). Tests were replicated three times and the yellow fruiting form of strawberry guava was used as a control (Heard 1997). When testing *Rhexia lutea* (Melastomataceae) and *Punica granatum* (Punicaceae), only 2 replications were conducted due to death of plants prior to testing. Control plants were set up at the same time as the test plants. Being a leaf gall former, *T. ovatus* requires new flush to produce a gall. Therefore, prior to testing, test plants were pruned to induce the growth of new flush. To break plant dormancy, *Prunus angustifolia* and *P. persica* were maintained in a growth chamber with a 12:12 light: dark photoperiod at -1.11 °C for one month and then placed outside under ambient conditions.

Figure 4-1. Test arena for host specificity experiments.

Twenty first instar nymphs or "crawlers" were placed on the new growth of each test plant; no more than 5 insects were placed on one individual leaf. Test plants ranged in height from 25 – 45 cm, and were planted in 3.8, 7.6, or 11.4 L pots. Insects were transferred individually from a colony plant using fine forceps. Once the insects were transferred onto the test plant, an acrylic cylinder 46 cm tall and 15 cm in diameter was placed over the plant and the bottom was partially buried in the soil to prevent the crawlers from escaping. The acrylic cylinders were ventilated by six holes 6 cm in diameter. The ventilation holes and the top of the cylinder were covered with a fine mesh, with a screen size of 150 µ x 150 µ (Green.tek Inc., Edgerton, WI) (Fig 4-1). Once the test arenas were assembled, the plants were placed in a quarantine greenhouse. Test plants were exposed to both natural and artificial light conditions. Supplemental fluorescent lighting in the greenhouse was set on a 14:10 light:dark photoperiod. The average temperature inside test cylinders was 28.88 ± 1.61 °C and the average humidity was 66.13 ± 6.95 %. Tests were conducted for a duration of 2 weeks, after which the plants were inspected for the presence of *T. ovatus* or gall development. If surviving *T. ovatus* were found, then the tests were extended for another 2 weeks and subsequently re-examined.

Results

In total, 57 species representing 21 families were tested. *Tectococcus ovatus* only survived and formed viable galls on strawberry guava (Table 4-1). However, *T. ovatus* survived longer than the 2 week test period on three species; strawberry guava, Brazilian guava (*Psidium friedrichsthalianum* O. Berg), and Costa Rican guava (*Psidium guineense* Sw.). Because of this, the Brazilian guava and Costa Rican guava tests were extended for an additional 2 weeks. *Tectococcus ovatus* fed on Brazilian guava although no gall was formed and all insects died within 4 weeks. Feeding on Costa Rican guava induced a weak gall, which was poorly formed; the galls were cuplike in shape and did not fully cover the insect, as a normal gall would. In total, 5 of these galls were formed and the insects did not survive longer than 4 weeks on Costa Rican guava.

Table 4-1. Results of *T. ovatus* host specificity testing. A "+" indicates feeding damage and gall development, whereas a "-" indicates a lack of feeding damage and gall development.

Test Plant	Family	Results	Replications
Psidium cattleianum var. *lucidum* Sabine	Myrtaceae	+	50
Psidium cattleianum var. *cattleianum* Sabine	Myrtaceae	+	3
Psidium friedrichsthalianum O. Berg	Myrtaceae	-[a]	3
Psidium guineense Sw.	Myrtaceae	+[b]	3
Psidium guajava L.	Myrtaceae	-	3
Acca sellowiana (O. Berg) Burret	Myrtaceae	-	3
Eugenia axillaris (Sw.) Willd.	Myrtaceae	-	3
Eugenia foetida Pers.	Myrtaceae	-	3
Eugenia uniflora L.	Myrtaceae	-	3
Myrciaria cauliflora (C. Martius) O. Berg	Myrtaceae	-	3
Pimenta dioica (L.) Merr.	Myrtaceae	-	3
Pimenta racemosa (P. Mill.) J.W. Moore	Myrtaceae	-	3
Syzygium malaccense (L.) Merr. & Perry	Myrtaceae	-	3
Syzygium paniculatum Gaertner	Myrtaceae	-	3
Callistemon citrinus (Curtis) Staph	Myrtaceae	-	3
Callistemon viminale (Gaertn.) G.Don ex Loudon	Myrtaceae	-	3
Eucalyptus camaldulensis Dehnhardt	Myrtaceae	-	3
Leptospermum scoparium J.R. & G. Forst.	Myrtaceae	-	3
Melaleuca quinquenervia (Cav.) Blake	Myrtaceae	-	3
Calyptranthes pallens Griseb.	Myrtaceae	-	3
Calyptranthes zuzygium (L.) Sw.	Myrtaceae	-	3
Eugenia confusa DC.	Myrtaceae	-	3
Eugenia rhombea Krug & Urban	Myrtaceae	-	3
Mosiera longipes (Berg) McVaugh	Myrtaceae	-	3
Myrcianthes fragrans (Sw.) McVaugh	Myrtaceae	-	3
Ammannia coccinea Rottb.	Lythraceae	-	3
Cuphea hyssopifolia Kunth	Lythraceae	-	3
Cuphea micropetala Humb., Bonpl. & Kunth	Lythraceae	-	3
Decodon verticillatus (L.) Ell.	Lythraceae	-	3
Lagerstroemia indica L.	Lythraceae	-	3
Lythrum alatum Pursh	Lythraceae	-	3
Rhexia lutea Walt.	Melastomataceae	-	2
Rhexia mariana L.	Melastomataceae	-	3
Rhexia nashii Small	Melastomataceae	-	3

Table 4-1. Continued.

Test Plant	Family	Results	Replications
Tetrazygia bicolor (P. Mill.) Cogn.	Melastomataceae	-	3
Rollinia mucosa (Jacq.) Baill.	Annonaceae	-	3
Punica granatum L.	Punicaceae	-	2
Conocarpus erectus L.	Combretaceae	-	3
Chrysobalanus icaco L.	Chrysobalanaceae	-	3
Nyssa sylvatica var. *biflora* Walt.	Nyssaceae	-	3
Daphnopsis americana (P. Mill.) J.R.	Thymelaeaceae	-	3
Ilex cassine L.	Aquifoliaceae	-	3
Ilex x *attenuata* Ashe	Aquifoliaceae	-	3
Delonix regia (Bojer ex Hook) Raf.	Fabaceae	-	3
Quercus hemisphaerica Bartr. ex Willd.	Fagaceae	-	3
Persea americana P. Mill.	Lauraceae	-	3
Ficus aurea Nutt.	Moraceae	-	3
Myrica cerifera (L.) Small	Myricaceae	-	3
Saccharum officinarum L.	Poaceae	-	3
Eriobotrya japonica (Thunb.) Lindl.	Rosaceae	-	3
Prunus angustifolia Marsh.	Rosaceae	-	3
Prunus persica (L.) Batsch	Rosaceae	-	3
Pyrus x *lecontei* ‘Hood’	Rosaceae	-	3
Citrus limon (K.) Burm. F.	Rutaceae	-	3
Citrus x *paradisi* Macfad.	Rutaceae	-	3
Citrus sinensis (L.) Osbeck	Rutaceae	-	3
Taxodium distichum (L.) L.C.	Cupressaceae	-	3
Pinus elliottii Engelm.	Pinaceae	-	3
Podocarpus macrophyllus (Thunb.) Sweet	Podocarpaceae	-	3

[a] *T. ovatus* survived longer than the 2 week test period; test was extended to 4 weeks, but no damage or gall formation was observed.
[b] *T. ovatus* survived longer than the 2 week test period; test was extended to 4 weeks, weak leaf gall formation was observed.

Discussion

The results of the host specificity tests show that *T. ovatus* is highly host specific; feeding and weak gall formation were only observed on two species closely related to the target weed, Costa Rican guava and Brazilian guava. These results are not surprising because *T. ovatus* has been reported to attack a close relative, *Psidium spathulatum*

Mattos in Brazil (Vitorino et al. 2000). This species is not native to North America or the Caribbean and therefore is in no danger, should *T. ovatus* be released.

Costa Rican guava is found throughout South and Central America (Morton 1987). There is no commercial production but the fruit can be used to make jellies or fruit drinks (Morton 1987). This guava is occasionally grown as a minor ornamental species in Florida, although it is not commonly listed by most nurseries. *Tectococcus ovatus* was able to feed on Costa Rican guava, and weak gall formation was observed. This interaction may be explained by the conservative nature of no-choice testing because this association has never been reported to occur in the wild. However, Costa Rican guava is not commonly grown as an ornamental or fruit crop within the United States, therefore damage inflicted by *T. ovatus* may not be of concern. *Tectococcus ovatus* also was observed feeding on Brazilian guava, although feeding damage was not noticeable and no gall formation was observed. Because feeding by *T. ovatus* did not appear to have any noticeable adverse effect on the plant, and all insects died within 4 weeks, this behavior should not pose a risk to Brazilian guava if the insect were approved for release in Florida.

Most importantly, *T. ovatus* did not attack common guava, which is grown as a fruit crop in south Florida. Originally, the biological control of strawberry guava was thought to be impossible because of the close relationship between common guava and strawberry guava (Wikler et al. 2000). These results have been confirmed in Brazil, where the two species are found within the same range, and in host specificity tests in Hawaii (Vitorino et al. 2000; Johnson 2005).

In a literature search of additional hosts of *T. ovatus, Daphnopsis racemosa* Griseb. (Thymelaceae) was listed as a host in a worldwide catalog of the family Eriococcidae (Hoy 1963). The references from this catalog were obtained, and the reference to this host plant association was traced back to a catalog of Brazilian insects (Da Costa Lima 1936). Da Costa Lima (1936) lists *T. ovatus* as producing galls on the leaves of strawberry guava and another plant called “embira”. Subsequent investigation revealed that the common name embira refers to two plants in two different families *D. racemosa* and *Rollinia salicifolia* Schltdl. (Annonaceae). These particular species do not occur in North America or the Caribbean and therefore are in no danger of being attacked by *T. ovatus*. However, there are members of the two genera present in the Caribbean, including some endangered members of the genus *Daphnopsis*. Although this host association is most likely erroneous, one representative of each genus (*D. americana* and *R. mucosa*) was tested and were found not to be attacked by *T. ovatus.* An advantage of choosing a gall forming insect as a biological control agent is they tend to have narrow host ranges (Harris and Shorthouse, 1996). This is due to the complex co-evolutionary relationship that gall forming insects have with their host plants. The results of this study support this observation.

The most pressing issue that will need to be addressed prior to release of this insect is the continued sale of strawberry guava as an ornamental in Florida. Despite evidence that this species is invasive; the ornamental industry is reluctant to phase out strawberry guava because of its economic value. The FDACS, DPI is the state agency charged with implementing and enforcing laws regarding invasive plants. Their focus has historically been on agricultural threats, most of which are not yet present in the state (FDACS 2004).

However, the IFAS assessment focuses on environmentally relevant invasive species. The IFAS assessment is not a regulatory list; the primary goal of this assessment is to direct research and extension at the University of Florida. A possible solution for nurseries would be to substitute invasive species with native plants that have similar desirable characteristics. A native plant substitution guide for Florida was developed by FLEPPC (Ferriter 2003). Recommended substitutions were based on the aesthetic values of the plants, and similarity of fruit characteristics. Three plants recommended as substitutions for strawberry guava are Simpson's stopper, *Myrcianthes fragrans* (Sw.) McVaugh, Guianese colicwood, *Rapanea punctata* (Lam.) Lundell, and Jamaican caper, *Capparis cynophallophora* L.

Based on the results of this study, *T. ovatus* is highly host specific and would make a suitable biological control agent for the control of strawberry guava in Florida. The non-target effects observed on Costa Rican guava and Brazilain guava were minimal, and the insect was unable to complete its development on these guavas. However, the continuing conflict with the nursery industry regarding the sale of guavas as ornamentals in Florida needs to be resolved prior to the release of this organism.

Acknowledgments

The author would like to thank the botanists Mark A. Garland and Richard Weaver for their professional assistance with nomenclature, identification, and collection of many specimens used throughout this project. Generous plant donations were made by the Chicago Botanical Garden and Ornamental Plants & Trees Inc. Thanks are also due to Judy Gillmore for her assistance in procuring and maintaining test plants. This research was funded by the USDA CSREES Tropical/Subtropical Agriculture Research program (T-STAR-Caribbean) grant No. 01062227.

CHAPTER 5
DISCUSSION AND CONCLUSIONS

Prior to this study, little was known about the biology of *T. ovatus*. Literature references were limited to mainly taxonomic descriptions (Ferris 1957) and catalog citations (Hoy 1963). This is most likely due to the fact that *T. ovatus* is not an economically important species and is limited in its geographical and host range. Therefore, it is not surprising that prior to an outbreak of strawberry guava, the primary host plant of this species, the main scientific value of *T. ovatus* was taxonomic in nature.

The identification of strawberry guava as a major natural areas weed in Hawaii and its association with another invasive species, the feral pig attracted attention to strawberry guava and its natural enemies (Wikler et al. 2000). Initial investigations for biological control were directed towards plant pathogens in hopes of developing a bioherbicide. These initial explorations were not successful and the focus shifted to highly specific phytophagous insects (Hodges 1988, Wikler 2000b, Wikler et al. 2000).

Previous studies on the biology of *T. ovatus* were preliminary in nature and published in non-refereed proceedings (Wikler et al. 2000, Vitorino et al. 2000). These two papers were the first published biological studies on *T. ovatus*. Wikler et al. (2000) studied seven different natural enemies of strawberry guava. They identified *T. ovatus* as being the most promising agent for biological control. Additionally, the paper included general descriptions of the leaf gall, the male and female *T. ovatus*, a note on distribution, records of two parasitoids, *Metaphycus flavus* Howard (Hymenoptera: Encyrtidae) and *Aprostocetus* sp. Westwood (Hymenoptera: Eulophidae), and one predator, *Hyperaspis* *delicate* Massuti and Vitorino (Wikler et al. 2000). Wikler et al. (2000) reported that *T. ovatus* is found more frequently on the red fruited variety of strawberry guava, whereas Vitorino et al. (2000) stated that the coccid is more common on the yellow fruiting variety.

Vitorino et al. (2000) provides a much more detailed account of biological observations than Wikler et al. (2000). Vitorino et al. (2000) recorded mean diameters on both sides of the leaf gall and height measurements. The gall sizes were divided into three groups, based on the median for all of the measurements and one standard deviation from the mean in both directions. Vitorino et al. (2000) also conducted tests to determine the best method for transferring the first instar crawlers, and conducted preliminary host specificity tests. Most biological observations were morphological in nature with a brief description of life cycle. However, these two papers provided the basis for further investigations into the biology of *T. ovatus*.

Investigating the developmental biology of the female *T. ovatus* was chosen for a couple of reasons. Female coccids are typically used in taxonomic descriptions of species (Ferris 1957). This is because they are more persistent and usually sessile; therefore, they are encountered more frequently in nature. Additionally, the life history and physiology of female *T. ovatus* makes them more important for biological control. Females are much larger because of their ability to produce progeny. Unlike males, they must continue to feed in the adult stage in order to produce eggs. According to Stehr (1991), female members of the Family Eriococcidae typically have two instars and one adult stage. However, preliminary molecular investigations by Cook et al. (2002) suggest that the family is not monophyletic. Therefore, researchers should be cautious of making any general assumptions about the family.

Females of *T. ovatus* spend the majority of their life inside a protective plant gall. This makes investigating the development of the insect difficult without dissecting the gall and disrupting the normal growth process. Therefore, an attempt was made to correlate gall size to a particular nymphal stage or the adult, in order to make assumptions about development without destructive sampling. However, before this could be accomplished, the number of life stages (excluding the egg) had to be confirmed. This turned out to be more difficult than anticipated. Typical methods of determining the number of instars are by constructing a frequency histogram of a particular body measurement (typically a head capsule measurement) (Daly 1985). However, this was not possible for *T. ovatus* because it is a soft bodied insect and slide mounting can alter the shape of the soft integument, resulting in excessive variation in the measurements. Measurements needed to be made of sclerotized structures that would not be altered by the slide mounting process. The two sclerotized portions of *T. ovatus* are the mouthparts and the legs; the legs were chosen because they are more distinct and easily identified and measured. Multivariate morphometric analyses (principal components analysis, cluster analysis, and canonical analysis) were conducted on 15 measurements of female *T. ovatus* leg. The results indicated the presence of two or possibly three instars. The presence of supernumerary instars is not uncommon in laboratory colonies; however, the possible third instar also could have been produced by outlying data points. It was determined that increasing the sample size will be necessary to definitively determine the correct number of developmental instars.

A regression analysis was performed to satisfy the original concept of correlating instar to gall size. A significant correlation was found between the length of the fused prothoracic trochanter/femur segment and the diameter at the base of the apical portion of the leaf gall ($r^2 = 0.7126$; doff. = 1, 65; $p < 0.001$). The length of the fused prothoracic trochanter/femur can be used to correlate the life stage (excluding egg) with the gall size (based on the morphometric analysis).

The original purpose of studying *T. ovatus* was to evaluate the potential of the insect as a classical biological control agent of strawberry guava in Florida. The primary concern of researchers attempting to evaluate classical biological control agents is the safety of cultivated or socially important plants from attack by the agent (Wapshere 1979). The test plant list for this study was developed in accordance with the Technical Advisory Group for Biological Control Agents of Weeds guidelines. These guidelines are based on the centrifugal-phylogenetic method developed by Wapshere (1974). The analysis of 57 species of plants representing 21 families resulted in only two relatively minimal non-target effects. *Tectococcus ovatus* fed on two closely related *Psidium* species, Brazilian guava and Costa Rican guava. Additionally, incomplete gall formation was observed on the Costa Rican guava. Both of these tests were extended for 2 additional weeks, and no *T. ovatus* specimens survived on either plant species. Due to the limited value of these two guavas in Florida, these non-target effects were determined to be negligible and not a concern if *T. ovatus* were approved for release in Florida.

The most important result of the host range test was the lack of damage to common guava, which is closely related to the target weed and commercially produced on a small scale in Florida. *Tectococcus ovatus* did not attack common guava in this experiment and these results have been confirmed by observations in Brazil and host specificity tests in Hawaii (Vitorino et al. 2000, Johnson 2005).

These studies have elucidated a portion of the biology of *T. ovatus*, although the continued evaluation of this insect in Florida is important. Further research on the developmental biology of the insect is needed to establish the length or duration of each instar. Additionally, data from these studies could be used to further correlate the gall size of *T. ovatus* with instar number. This correlation would make it possible to follow multiple cohorts of individuals throughout their life cycle without disrupting the integrity of the gall of the insect. The cumulative data would be useful for the constructing of multiple age-specific life tables. These life tables could help researchers better anticipate peaks in natural populations of *T. ovatus,* should the insect be released in Florida. These tables also may assist in timing subsequent releases to coincide with natural population spikes, increasing the chances of a successful release.

If *T. ovatus* is approved for released in Florida, studies also could be conducted on the distribution rates of mobile life stages. This may aid researchers in predicting the spread of *T. ovatus* under field conditions. The efficacy of *T. ovatus* as a biological control agent also could be better analyzed in the field. This could help researchers determine if further studies on additional biological control agents for strawberry guava should be pursued. This brings up the importance of evaluating the effect of generalist predators and parasitioids on populations of *T. ovatus* in the field. Vitorino et al. (2000) recorded a high parasitism rate of 49% within the native range of *T. ovatus*. Understanding how a biological control agent interacts within the new habitat is fundamental to the continued progress of weed biological control as a science.

An important aspect of this study was the aim of reducing Caribbean fruit fly populations by reducing breeding sites in strawberry guava. If *T. ovatus* is approved for released in Florida, studies should be conducted on the subsequent effect on populations of the Caribbean fruit fly. Field studies could be analyzed along with state fruit fly trapping data collected before and after field releases of *T. ovatus*. A reduction of Caribbean fruit fly populations that is correlated with reduction of breeding areas in strawberry guava could justify the implementation of biological control programs against other invasive hosts such as Surinam cherry (IFAS 2005).

APPENDIX A
WORLDWIDE DISTRIBUTION OF *Psidium cattleianum*

The following table, lists the worldwide distribution of *P. cattleianum*. The table is divided into seven sections based on biogeographic regions. The records are organized by country or island chain and locations such as cities or particular islands are provided.

COUNTRY/ ISLAND CHAIN	LOCATION	SOURCE[a]	REFERENCE[b]	STATUS
AFROTROPICAL				
Gana		HS	Wikler 1999	
Kenya	Nairobi	HS	Wikler 1999	
Madagascar	Antananarivo	HS	MBG	
	Fianarantsoa	HS	MBG	
	Toamasina	HS	MBG	
	Toliara	HS	MBG	
	Tamatave	HS	Wikler 1999	
Mascarenes Islands		LR	Weber 2003	Invasive
Mauritius		HS	MBG, Wikler 1999	Invasive
		LR	Wyse-Jackson 1990	Invasive
Reunion		HS	Wikler 1999	
Rwanda	Butare	HS	Wikler 1999	
Seychelles		HS	Wikler 1999	Invasive
	Mahé	LR	Gerlach 2004	Invasive
	Silhouette Island	LR	Gerlach 2004	Invasive
Sierra Leone		HS	Wikler 1999	
South Africa	Natal, Durban	HS	Wikler 1999	Invasive
	Natal, Durban	LR	Henderson 1989	Invasive
Tanzania	Amani	HS	Wikler 1999	
	Lushoto	HS	Wikler 1999	
AUSTRALASIAN				
Australia	Lord Howe Island	HS	Wikler 1999	
	Queensland	HS	Wikler 1999	
Lord Howe Island		HS	Wikler 1999	
Micronesia		LR	Weber 2003	Introduced
Melanesia		LR	Weber 2003	

COUNTRY/ ISLAND CHAIN	LOCATION	SOURCE[a]	REFERENCE[b]	STATUS
New Caledonia	St. Louis	HS	MBG, Wikler 1999	
New Zealand		LR	Webb et al. 1988	
Norfolk Island		HS	Wikler 1999	Invasive
New Hebrides	Pentecost Island	HS	Wikler 1999	
Polynesia		LR	Weber 2003	Invasive
Samoa	Upolu	HS	NYBG	
Solomon Islands		HS	Wikler 1999	
EAST PALEARCTIC				
China		LR	Bretschneider 1898	Cultivated/ Possibly Naturalized
Taiwan		LR	Li and Huan 1979	Cultivated/ Possibly Naturalized
NEARCTIC				
Bermuda		HS	Wikler 1999	Naturalized
		LR	Britton 1918	Naturalized
Mexico		LR	Weber 2003	
United States	Arizona	HS	ASU Herbarium	
	California	HS	NYBG	Cultivated/ Possibly Naturalized
	Florida	HS	MBG, NYBG, Wikler 1999	Invasive
	Missouri	HS	MBG	
	Texas	LR	Jones et al. 1997	
NEOTROPICAL				
Argentina		LR	Wikler 2000	
Bahamas		LR	Morton 1987	
Brazil	Bahia	HS	NYBG, Wikler 1999	Native

COUNTRY/ ISLAND CHAIN	LOCATION	SOURCE[a]	REFERENCE[b]	STATUS
Brazil	Espirito Santo	HS	NYBG	Native
	Parana	HS	NYBG	Native
	Rio de Janeiro	HS	NYBG, Wikler 1999	Native
	Rio Grande do Sul	HS	NYBG, Wikler 1999	Native
	Santa Catarina	HS	MBG, NYBG, Wikler 1999	Native
	Sao Paulo	HS	MBG, NYBG, Wikler 1999	Native
Chile		LR	Weber 2003	
Colombia	Antioquia	HS	MBG	
Costa Rica		LR	McVaugh 1963	
	Cartago	LR	Standley 1937	Cultivated/ Possibly Naturalized
	San Jose	LR	Standley 1937	Cultivated/ Possibly Naturalized
Cuba		LR	Roig and Mesa 1953	
Dominican Republic		LR	Wikler 2000	
Galapagos Islands		LR	Weber 2003	
Guatemala		LR	McVaugh 1963	
Guayana		LR	McVaugh 1969	
Honduras	Francisco Morazan	HS	MBG	
Jamaica	Clarendon	HS	MBG, NYBG	Naturalized
		LR	Fawcett and Rendle 1926	Naturalized
Lesser Antilles		LR	Wikler 2000	Cultivated/ Possibly Naturalized
	Guadeloupe	LR	Howard 1989	
	Martinique	LR	Howard 1989	
	Montserrat	LR	Howard 1989	
	Nevis	LR	Howard 1989	

COUNTRY/ ISLAND CHAIN	LOCATION	SOURCE[a]	REFERENCE[b]	STATUS
Puerto Rico		LR	Liogier and Martorell 1982	Naturalized
Uruguay		LR	Lombardo 1964	Native
Venezuela		LR	Pittier 1926	
ORIENTAL				
Christmas Island		HS	Wikler 1999	
Fiji	Taunovo	HS	Wikler 1999	Invasive
	Viti Levu	HS	Wikler 1999	Invasive
	Viti Levu	LR	Greenwood 1944, 1949	Invasive
Hong Kong		HS	Wikler 1999	
India		LR	Morton 1987	
Japan	Bonin Islands	HS	Wikler 1999	
Malaysia	Sabah	HS	Wikler 1999	
	Selangor	HS	Wikler 1999	
Philippines		LR	Morton 1987	
Singapore		LR	Morton 1987	
Sri Lanka	Seethaganguala	HS	Wikler 1999	
		LR	Fosberg 1971	
Tahiti		HS	NYBG, Wikler 1999	
United States	Hawaii	HS	MBG, NYBG, Wikler 1999	Invasive
WESTERN PALEARCTIC				
Azores		LR	Weber 2003	
				Introduced
British Isles		LR	Weber 2003	
Cape Verde Islands		LR	Weber 2003	
France, Central		LR	Weber 2003	
Mediterranean Isl.		LR	Weber 2003	

COUNTRY/ ISLAND CHAIN	LOCATION	SOURCE[a]	REFERENCE[b]	STATUS
Madeira		LR	Lowe 1868	

[a]HS denotes that the source is a herbarium specimen, while LR denotes a literature reference.

[b]MBG refers to the Missouri Botanical Garden and NYBG refers to the New York Botanical Garden

APPENDIX B
FINAL TEST PLANT LIST FOR HOST SPECIFICITY TESTING OF *Tectococcus ovatus*

- **Category 1 -** Genetic types of the target weed species (varieties, races, forms, genotypes, apomicts, etc.) found in North America.

- **Category 2 –** Species present in North America in the same genus as the target weed, divided by subfamily (if applicable).

- **Category 3 -** North American species in other genera in the same family as the target weed, divided by subfamily (if applicable).

- **Category 4** - Threatened and endangered species in the same family as the target weed divided by subgenus, genus, and subfamily

- **Category 5 -** North American species in other families in the same order that have some phylogenetic, morphological or biochemical similarities to the target weed.

- **Category 6 -** North American species in other orders that have some morphological, or biochemical similarities to the target weed

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

- **Category 8 -** Plants not closely related to weed, which have economic significance and are grown in the same range as the weed in North America

PLANTS	AUTHOR	COMMON NAME	REASON FOR INCLUSION
CATEGORY 1			
Family Myrtaceae			
Subfamily Myrtoideae			
Psidium cattleianum var. *cattleianum*	Sabine	strawberry guava	target weed
Psidium cattleianum var. *lucidium*	Sabine	strawberry guava	target weed
CATEGORY 2			
Family Myrtaceae			
Subfamily Myrtoideae			
Psidium friedrichsthalianum	O. Berg	Costa Rican guava	closey related to target, grown as an minor ornamental in FL
Psidium guajava	L.	common guava	closey related to target, grown as a minor fruit cropl in south FL
Psidium guineense	Sw.	Brazilian guava	closely related to target, possibly grown as an ornamental in FL
CATEGORY 3			
Family Myrtaceae			
Subfamily Myrtoideae			
Acca sellowiana	(O. Berg) O. Berg	feijoa, pineapple guava	commonly grown as an ornamental in FL
Eugenia axillaris	(Sw.) Willd.	white stopper	native Eugenia, also cultivated as an ornamental
Eugenia foetida	Pers.	Spanish Stopper	native Eugenia, also cultivated as an ornamental
Eugenia uniflora	L.	Surinam cherry	not native but commonly sold as ornamental, preferred host of Caribfly
Myrciaria cauliflora	(C. Martius) O. Berg	jaboticaba	not native, ornamental fruit in south FL

PLANTS	AUTHOR	COMMON NAME	REASON FOR INCLUSION
Pimenta dioica	(L.) Merr	allspice	not native, invasive in HI, minor ornamental in FL
Pimenta racemosa	(P. Mill) J.W. Moore	bay-rum tree	native in Caribbean, minor ornamental in FL, extracts used in perfumes
Syzygium malaccense	(L.) Merr. & Perry	malay apple	common ornamental in FL
Syzygium paniculatum	Gaertner	Australian brush cherry	ornamental in FL
Subfamily Leptospermoidiae			
Callistemon citrinus	(Curtis) Staph	crimson bottlebrush	not native, but cultivated as an ornamental in FL
Callistemon viminale	(Gaertn.) G. Don ex Loudon	weeping bottlebrush	not native, but cultivated as an ornamental in FL
Eucalyptus camaldulensis	Dehnhardt	red river gum	ornamental in US
Leptospermum scoparium	J.R. & G. Forst.	broom teatree	invasive in HI, cultivated in FL
Melaleuca quinquenervia	(Cav.) Blake	melaleuca	invasive in FL, had on hand
CATEGORY 4			
Family Myrtaceae			
Subfamily Myrtoideae			
Calyptranthes pallens	Griseb.	spicewood	native threatened species
Calyptranthes zuzygium	(L.) Sw.	myrtle of the river	native endangered species
Eugenia confusa	DC.	redberry stopper	native endangered species
Eugenia rhombea	Krug & Urban	spiceberry eugenia	native endangered species
Mosiera longipes	(Berg) McVaugh	mangroveberry	native threatened species
Myrcianthes fragrans	(Sw.) McVaugh	Simpson's stopper	native threateded species
CATEGORY 5			
Family Lythraceae			
Ammannia coccinea	Rottb	valley redstem	native species

PLANTS	AUTHOR	COMMON NAME	REASON FOR INCLUSION
Cuphea hyssopifolia	Kunth	Mexican false heather	introduced species, common ornamental
Cuphea micropetala	Humb., Bonpl. & Kunth	tall cigar plant	cultivated species
Decodon verticillatus	(L.) Ell.	swamp loosestrife	native endangered species
Lagerstroemia indica	L.	crapemyrtle	introduced species, commercially important
Lythrum alatum	Pursh	winged Lythrum	native species
Family Melastomataceae			
Rhexia lutea	Walt.	yellow meadowbeauty	native species
Rhexia mariana	L.	Maryland meadowbeauty	native species
Rhexia nashii	Small	maid Marian	native species
Tetrazygia bicolor	(P. Mill.) Cogn	flordia cover ash	native threatened species
Family Punicaceae			
Punica granatum	L.	Pomegranite	introduced, commercially important, minor fruit crop
Family Combretaceae			
Conocarpus erectus	L.	button mangrove	native, economically and environmentally important
CATEGORY 6			
Family Chrysobalanaceae			
Chrysobalanus icaco	L.	icaco coco plum	native species also sold as ornamental
Family Nyssaceae			
Nyssa sylvatica v. *biflora*	Walt.	swamp tupelo	native species also sold as ornamental

PLANTS	AUTHOR	COMMON NAME	REASON FOR INCLUSION
CATEGORY 7			
Family Annonaceae			
Rollinia mucosa	(Jacq.) Baill	wild sugar apple	closely related to recorded host of *T. ovatus*
Family Thymelaeaceae			
Daphnopsis americana	(P. Mill.) J.R.	burn nose	closely related to recorded host of *T. ovatus*
CATEGORY 8			
Agriculturally Important Plants			
Aquifoliaceae			
Ilex cassine	L.	dahoon holly	native, and common ornamental, as are many Illex species
Ilex x *attenuata*	Ashe	topal holly	native, and common ornamental, as are many Illex species
Fabaceae			
Delonix regia	(Bojer ex Hook) Raf.	royal poinciana	ornamental tree in S Florida
Fagaceae			
Quercus hemisphaerica	Bartr. Ex Willd.	darlington oak	native, common hardwood tree
Lauraceae			
Persea americana	P. Mill	avocado	introduced crop tree, common in S Florida
Moraceae			
Ficus aurea	Nutt.	Florida strangler fig	native, common ornamental in S Florida
Myricaceae			
Myrica cerifera	(L.) Small	wax myrtle	native ornamental
Poaceae			
Saccharum officinarum	L.	sugarcane	introduced, common crop

PLANTS	AUTHOR	COMMON NAME	REASON FOR INCLUSION
Rosaceae			
Eriobotrya japonica	(Thunb.) Lindl.	loquat	introduced, common ornamental/ fruit tree
Prunus angustifolia	Marsh.	chicksaw plum	native, ornamental
Prunus persica	(L.) Batsch	peach	introduced crop tree
Pyrus x *lecontei* 'Hood'	Rehd.	Hood pear	introduced, cultivated crop tree
Rutaceae			
Citrus limon	(L.) Burm. F.	lemon	introduced crop tree
Citrus x *paradisi*	Macfad.	grapefruit	introduced crop tree
Citrus sinensis	(L.) Osbeck	sweet orange	introduced crop tree
Cupressaceae			
Taxodium distichum	(L.) L.C.	cypress	native, common ornamental species
Pinaceae			
Pinus elliottii	Engelm.	slash pine	native, common ornamental species
Podocarpaceae			
Podocarpus macrophyllus	(Thunb.) Sweet	southern yew	introduced, common ornamental species

APPENDIX C
CHANGES TO THE FINAL TEST PLANT LIST FOR HOST SPECIFICITY TESTING OF *Tectococcus ovatus*

Plants Removed From the Original Test Plant List

SCIENTIFIC NAME	AUTHOR	COMMON NAME	REASON FOR INCLUSION
Albizia julibrissin	Durazz	silktree	Invasive according to IFAS Assessment
Calyptranthes thomasiana	Griseb.	Thomas. Lidflower	endangered in PR, unable to obtain, tested 2 native threatened Calypthranthes species
Daphnopsis helleriana	Urban	Heller's cieneguillo	native to PR, could not obtain, tested *D. americana*
Daphnopsis philippiana	Krug & Urban	emajagua de sierra	native to PR, could not obtain, tested *D. americana*
Dirca palustris	L.	eastern leatherwood	native to PR, could not obtain
Eucalyptus cinera	F. Muell. Ex Benth	silver dollar tree	introduced to Hawaii
Eucalyptus grandis	W. Hill ex Maid	grand eucalyptus	introduced in FL, not common as ornamental
Eugenia aggregata	(Vell.) Kiaersk	aggregate eugenia	not native, plenty of Eugenias represented in test plant list
Eugenia brasiliensis	Lam	Brazil cherry	not native, plenty of Eugenias represented in test plant list
Eugenia haematocarpa	Alain.	luquillo mountain stopper	endangered in PR, unable to obtain, tested many native Eugenias
Eugenia koolauensis	O. Deg.	koolau eugenia	endangered in HI, in no danger from proposed agent
Eugenia reinwardtiana	Blume	mountian stopper	native to Hawaii, in no danger from proposed agent
Eugenia woodburyana	Alain.	Woodbury's stopper	endangered in PR, unable to obtain, tested many native Eugenias
Pseudanamomis umbellulifera	(Kunth) Kausel	monos plum	introduced, not common as ornamental
Senna pendula	(Humb. & Bonpl. ex Willd.) Irwin & Barneby	valamuerto	invasive in south Florida according to IFAS Assessment
Syzygium cumini	(L.) Skeels	java plum	invasive, tested 2 Syzygium species, none native to continental US

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BIOGRAPHICAL SKETCH

Frank Wessels was born in 1980 in Indianapolis, IN, and moved to Minnesota before finally settling in Columbus, OH, where he grew up. From a young age Frank developed an interest in biology by exploring the inhabitants of a creek and small woods near his house. This eventually led him to Tampa, FL, where he pursued a double major of marine science and biology at the University of Tampa. In May of 2002, Frank received his BS degree. Internships at the Department of Environmental Protection and with Dow AgroSciences in Tampa piqued his interest in entomology. Frank decided to pursue his master's degree in entomology at The University of Florida and has spent the last two and a half years in Gainesville, FL.