37 Release and Establishment of *Oxyops vitiosa* Pascoe for the Biological Control of Melaleuca in South Florida

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Abstract

Valuable wetland ecosystems are frequently degraded by various disturbances, one of which is invasive weeds. *Melaleuca quinquenervia* (Cav.) S. T. Blake, though experiencing declines in Australia, is naturalized and highly invasive in wetland habitats of south Florida where it infests about 200 000 ha. Many sedge-dominated wetlands, including such renowned areas as the Everglades, are rapidly becoming *M. quinquenervia* swamp forests, with major environmental and economic impacts. Current management methods include herbicides, mechanical or hand removal, flooding, and prescribed burning. Insufficient information, high costs, non-target impacts, and the resilience of *M. quinquenervia* constrain the effectiveness of these methods. Biological control offers sustainable management potential by lessening the spread and by reducing plant vitality and growth rates, rendering them more vulnerable to other environmental stresses and conventional control methods. The snout beetle *Oxyops vitiosa* Pascoe, a natural enemy of *M. quinquenervia* in Australia, was released in south Florida during spring 1997. Marked adults were released to enable recognition of field-produced offspring. Unmarked adults were found after about 3 months, signaling the successful completion of a full generation in the field. Releases continued at other locations and, during 1997, nearly 1400 adults and 6000 larvae had been liberated at 13 locations. Populations have established in Broward, Lee, Collier, Palm Beach, and Glades counties, and a major effort has been initiated to establish populations in Miami-Dade County, particularly within Everglades National Park. Short hydroperiod habitats with intermediate stages of melaleuca in-

vasion seemed most suitable and most likely to support vigorous *O. vitiosa* populations, whereas populations have generally failed to establish at permanently inundated sites. It's premature to conclude, however, that permanently wet sites are unsuitable. The quantity of insects needed to compensate for high larval mortality at these sites could be much greater than the amounts presently available. Releases of larger quantities and different stages, i.e., adults vs. larvae, may be more successful in the future, but the acquisition of sufficient stock will be contingent upon redistribution from established populations after field colonies have had time to attain adequate levels. Contrary to widespread rumors, these weevils have retained their host fidelity in the field and have not attacked native plant species.

Introduction

Melaleuca quinquenervia (Cav.) S. T. Blake (broad-leaved paperbark or melaleuca; Myrtaceae) is a large tree (25-30 m tall) native to Australia. It naturally occurs within a zone about 40 km wide along the eastern coast of Queensland and northern New South Wales (11-34° south latitude). It is the southernmost representative of the *M. leucadendra* complex, a group of 10-11 closely related species with a center of diversity in tropical northern Queensland. It occurs in coastal wetlands that are at least seasonally inundated, typically in freshwater "paperbark swamps" which often occur on nutrient-poor sandy soils behind heath-dominated headlands. It also occurs on stream sides and in brackish water at the back of mangrove swamps. Most melaleuca habitats are threatened by development in Australia, being located in highly desirable coastal areas of low topography, high rainfall, and mild climate (Turner et al. 1998).

In contrast, *M. quinquenervia* is a serious weed in southern Florida where it is rapidly increasing in abundance (Laroche and Ferriter 1992). It was introduced during the post-Civil War era (A. Dray, unpubl. data), but apparently didn't naturalize until the 1900s. The first *M. quinquenervia* to establish in southeastern Florida probably originated from seedlings planted along Biscayne Bay in 1906 (Gifford, 1937, 1945; Meskimen, 1962), although it was apparently being introduced many years earlier (A. Dray, pers. comm., 1997). It was widely used in ornamental landscapes for many years and eventually began to invade forested and non-forested wetland systems, most notably the sawgrass marshes comprising the Florida Everglades. By 1994, it had infested approximately 198 000 ha (A. Ferriter, pers. comm., 1997). Invasion by melaleuca has transformed graminoid-herbaceous wetlands into closed-canopy swamp forests. These melaleuca swamp forests typically form dense monocultures characterized by a sparse understory. Intermediate stages in this transformation include savannahs with scattered, individual trees and mature dense melaleuca heads surrounded by relatively pristine marshes that contain moderate to low levels of melaleuca (O'Hare and Dalrymple 1997). The increased structural diversity associated with these melaleuca savannahs actually results in increased species diversity, but diversity is drastically reduced during later stages of invasion (O'Hare and Dalrymple 1997). Currently, an inadequate $2.2 million are spent annually to control this tree; estimates of losses to the local economy range as high as $168.6 million/yr (Diamond et al. 1991).

Recently, a group of economists and ecologists, in a combined effort, estimated the value of services provided to humans by various ecosystems (Costanza et al. 1997). Wetland systems were deemed the most valuable by providing services worth $14 785 ha^{-1} yr^{-1}. Assuming that current melaleuca infestations result in minimal losses comprising only 1% of these services, the lost value would total nearly $30 million *per year*. Furthermore, melaleuca is continuing to invade new areas causing accelerated degradation of wetlands. Infestation levels reported in 1994 were attained in less than 88 years, so melaleuca increased at an average rate of 2250 ha/yr or approximately 6.2 ha/d. Assuming a continuous linear rate of change and 100% decrement of wetland functions due to infestation, potential added losses could be as high as $33.2 million/yr or $91 000 per day. It is obviously very important to reduce the invasive nature of melaleuca while simultaneously eliminating existing stands. This requires an integrated management approach using biological, herbicidal, and mechanical methods (see Laroche 1994).

Removal of existing paperbark stands is contingent upon felling of mature trees, hand removal of saplings, prescribed burning, and herbicidal treatment. Without further impediments, however, cleared areas are likely to be reinvaded and spread is likely to continue. Hence, measures are needed to minimize seed production and the resultant survival of seedlings and saplings in order to provide sustainable management. Biological control offers the best hope for this. The melaleuca snout beetle, *Oxyops vitiosa* (Pascoe), damages young tissue growing at the tips of branches (Purcell and Balciunas 1994). This damage disrupts the process of shoot elongation and causes gnarled, deformed growth. This disables flower and seed production and reduces survival of saplings. This insect was proven to be host-specific after prolonged study (Balciunas et al. 1994; G. Buckingham, pers. comm.) and was finally released in Florida during spring 1997 at a site near Water Conservation Area 3B in Broward County. Releases continued thereafter in an attempt to colonize this insect throughout the south Florida range of melaleuca. This report summarizes those releases and the results to date.

Methods

Insect identification

Oxyops vitiosa was identified at the beginning of the quarantine phase of the project and confirmed during the course of quarantine studies. All of the weevils that were transferred from quarantine to Fort Lauderdale on 24 March 1997 were examined. All weevils that were field-collected in Australia were examined prior to transferal from quarantine to the Fort Lauderdale facilities to safe-guard against the inadvertent release of an inappropriate species.

Pathogen examinations

All field-collected weevils were dipped in a Benomyl solution within a day or two after arrival into quarantine from Australia to prevent germination of external fungal spores (particularly *Beauveria bassiana*). A sample of weevils (about 10% of each sex) was taken a day or two prior to their release from quarantine and examined by insect pathologists of the U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS). Specimens were also examined periodically during the course of quarantine testing. A total of 72 field-collected adults, 27 laboratory-reared adults, and 60 laboratory-produced larvae were examined during the period when weevils were being transferred to Fort Lauderdale. No pathogens were ever found.

Release procedures

Insects were either released directly at field sites after their receipt in Fort Lauderdale or released into a screen house onto container-grown melaleuca plants. Figure 1 shows the location of the release sites in south Florida in relation to the distribution of melaleuca and to primary natural areas. A summary of release information is provided in Table 1. Table 2 summarizes release site characteristics.

Results

Everglades Buffer Strip (Sites 1, 2 & 3)

Site 1 was first examined on 27 March 1997, the day after the first release. Adults were found feeding on young tips, some had already laid eggs. Larvae had moved to new foliage at the tips of the saplings in the first section. By 15 April 1997, young larvae were present in the second section (in the middle of the ATV trail), where only adults had been released. Marked adults were consistently found during the ensuing weeks but the first unmarked adult was not found until 19 May, 53 days after the first release. It was unclear whether this represented the parental stock or the F_1 generation, as it could have been produced from released larvae. However, the time interval was sufficient for the production of a new generation. Nonetheless, the presence of this individual proved that the pupae could survive at this site, despite the relatively saturated soil conditions. As new adults were found they were marked and released. Unmarked adults continued to appear during the next few weeks; by 10 August, a total of 10 had been found. Adults and larvae continued to be present at the site during the remainder of the year. Larvae were sufficiently abundant during late February 1998 to allow for redistribution to another site (Site 9). The site was examined about one year after the first release, on 25 March 1998. Larvae were found 200 m to the north and at the western limit of the stand, 60 m from the nearest release point. Seven adults were also noted, even though we did not intensively search for them. The site was last examined on 25

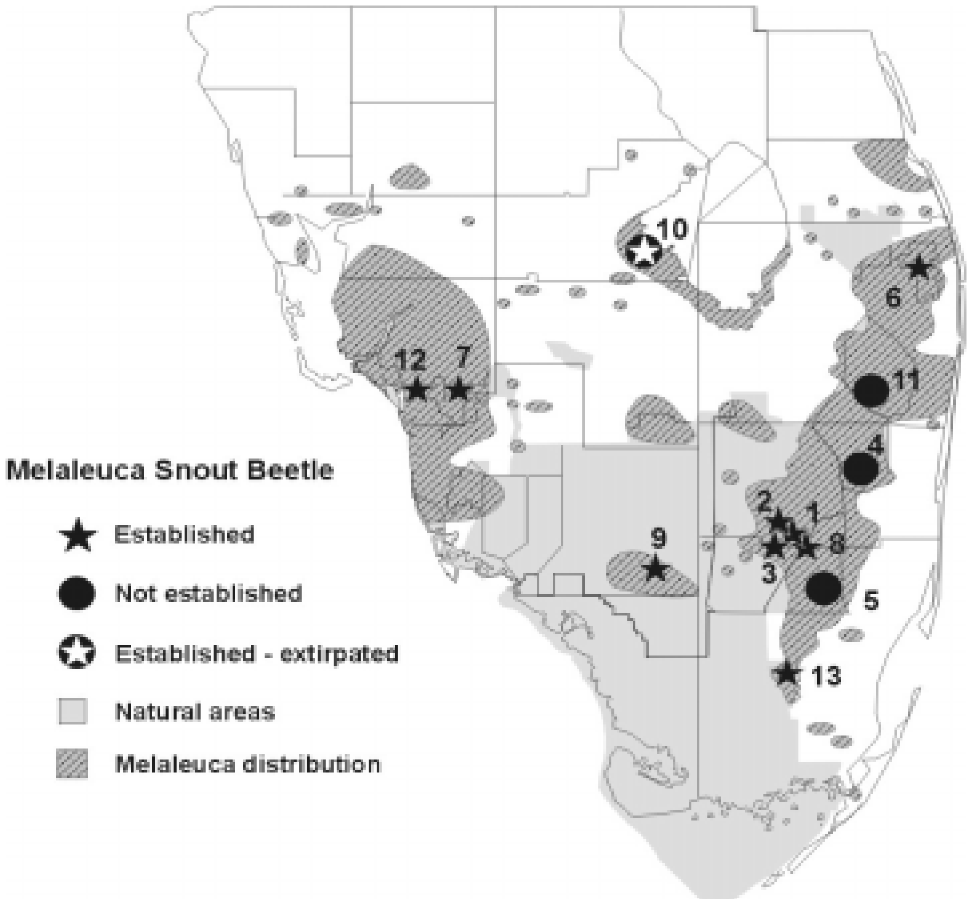

Figure 1. The distribution of *Oxyops vitiosa* release sites in Florida.

May 1998 when very few larvae were noted; however, damage produced by adult feeding was abundant and, in some cases, severe on small plants. Two observers were able to find 50 adults were found within 25 minutes. Most of the trees in the clump within the middle of the road had been knocked over, presumably by ATVs, but six larvae (mid-instars) were found on one of the few that remained. These were the only larvae found at any of the sites during May.

All of the adults that could be found at Site 2 were collected and moved to other sites. The area was checked periodically during the ensuing months but no additional insects were found. This led us to believe that the combination of vandalism and high water levels had resulted in the failure to establish a population at this location. Then, during March and again in April 1998, one late instar larva was found, suggesting that a small population had persisted.

Table 1. A summary of the releases of *Oxyops vitiosa* made from March 1997 to June 1998.

Locality information				Release information						
Site No.	County	Site name	N. Latitude W. Longitude	First/last release dates	Number of releases	Duration (days)	Adults	Larvae	First F_1 adults	Result (as of 11- Mar-98)
1[1]	Broward	Everglades Buffer Strip	26°03.427' 80°26.536	26-Mar-97/ 23-Jun-97	4	91	143	312	19-May-97	Established
2[1]	Broward	Everglades Buffer Strip	26°03.550' 80°26.364'	26-Apr-97	1	1	300	-	none found	Established
3	Broward	Everglades Buffer Strip	26°03.380' 80°26.410'	29-Apr-97	1	1	60	-	26-Aug-97	Established
4	Broward	Conservation Area 2B	26°09.420' 80°21.950'	01-May-97	1	1	60	-	none found	Not established
5[1]	Dade	Krome Avenue	25°55.830' 80°27.030'	06-May-97	1	1	60	-	none found	Not established
6	Palm Beach	Palm Beach Catchment Area	26°44.028' 80°09.005'	22-May-97/ 6-Nov-97	2	168	40	240	28-Jan-98	Established
7	Lee	Corkscrew Well Field	26°27.715' 81°42.147'	6-Jun-97/ 3-Dec-97	5	180	120	931	late Oct. 1997	Established
8	Broward	Andytown Substation	26°02.129' 80°26.097'	18-Jun-97/ 5-Sep-97	3	79	451	-	08-Sep-97	Established
9	Collier	Big Cypress National Preserve	25°56.247' 81°00.527'	9-Jul-97/ 26-Feb-98	3	232	105	258	17-Sep-97	Established
10	Glades	Hoover Dike, nr. SR78	26°53.644' 81°07.297'	01-Aug-97	1	1	100	-	31-Oct-97	Established
11	Palm Beach	Loxahatchee NWR	26°29.620' 80°16.360'	12-Sep-97/ 25-Feb-98	3	20	12	342	none found	Not established
12	Lee	Estero	26°25.530' 81°48.620'	8-Oct-97/ 12-Mar-98	13	155	-	3900	03-Dec-97	Established
13	Dade	Everglades NP	25°41'19.8" 80°29'49.6"	4-Dec-97/ 6-Jun-98	5	184	131	730	11-May-98	Established
Totals					43		1510	6713		

[1]20 weevils moved from Site 2 to Site 1 on 28 April, and 23 moved on 30 April; 13 weevils moved from Site 1 and 16 moved from Site 2 to Site 5 on 6 May 1997.

Evidence of adult feeding was observed on trees within and adjacent to the plots at Site 3 during the first month, then disappeared. No additional damage was observed until late August. Then, on 26 August 1997, over 100 larvae and many eggs were observed on trees as far away as 30 m south of the release area. This area had a few melaleuca trees scattered amongst sawgrass and wax myrtle. The released adults apparently had moved from the plots into this more open area to lay eggs as evidenced by the high numbers of larvae on small trees west of the plots. A single unmarked adult was later found during first week of September in the open area. Then, in October, we observed many eggs and more than 100 new larvae on both previously infested as well as new trees. Larvae were found throughout an area extending about 100 m south and southwest of the plots. Fewer eggs and larvae were observed during the last week of December as compared to the numbers present during September through November. The low numbers continued into January and February 1998. However, during February, larvae and eggs were recorded on 11 of 42 trees examined. As of April 1998, *O. vitiosa* was well established but the population remained low and none were evident in the plots.

Water Conservation Area 2B (Site 4)

As at site 3, some adult damage was observed on the trees within the plots during the first month. Afterwards, the damage subsided and no further evidence of their presence was found. The trees that the adults had been placed on lacked the young plant tissue needed to provide oviposition sites. Therefore, we assume that released adults dispersed away from the site probably in search of young foliage. No insects or further signs of damage had been seen as of the last week of April 1998, almost one year after the release.

Krome Avenue study area (Site 5)

Adult damage was observed on trees in the plots during the first month, but none thereafter. The adults apparently dispersed to other areas. As a result, no signs that a population had persisted were evident as of the last week of April 1998. We assume that *O. vitiosa* has not established at this site.

Loxahatchee Slough, West Palm Beach Catchment Area (Site 6)

A few released adults remained on trees on which they had been placed during the first month where their damage was readily apparent. Later, however, no adults could be found and the feeding damage gradually diminished suggesting that the adults had left the site without laying eggs. No additional insects were released until 6 November 1997 when larvae were placed on trees growing on the berm of a dirt road at the western edge of the original stand. The site was examined one month later with negative results but two unmarked adults were found at the road berm location on 28 January 1998, 83 days after the last release. Numerous larvae, ranging from early to late instars, were on the same trees suggesting that adults had

Table 2. Summary of the physical and biological characteristics of the *Oxyops vitiosa* release sites.

Site No.	Site	Hydro-period[1]	Soil order[2]	Surface soil	Surface physiography	Ground surface cover	Historic habitat[3]	Current stage of invasion by melaleuca
1	Everglades Buffer Strip	Short	Mostly Histosol (Saprist)	Everglades peat	Localized mounds	Forb/melaleuca litter	Sawgrass marsh	Advanced and scattered centrifugally in sawgrass-wax myrtle cover type
2	Everglades Buffer Strip	Short	Mostly Histosol (Saprist)	Everglades peat	Localized mounds	Forb/melaleuca litter	Sawgrass marsh	Advanced and scattered centrifugally in sawgrass-wax myrtle cover type
3	Everglades Buffer Strip	Short	Mostly Histosol (Saprist)	Everglades peat	Localized mounds	Forb/melaleuca litter	Sawgrass marsh	Advanced and scattered centrifugally in sawgrass-wax myrtle cover type
4	Conservation Area 2B	Long	Mostly Histosol (Hemist)	Everglades peat	Variable water depth up to 1.2 m	Water/ sawgrass/ periphyton	Sawgrass marsh	Prominent heads surrounded by trees scattered in sawgrass marsh
5	Krome Ave.	Moderate	Mostly Histosol (Saprist)	Everglades peat	Variable water depth up to 1.3 m	Melaleuca roots and litter/ sawgrass	Sawgrass marsh	Advanced and scattered centrifugally forming thickets in sawgrass marsh
6	Palm Beach Catchment Area	Short	Mostly Entisols	Sandy	Slightly undulating	Forb/melaleuca litter/bare sand	Wet prairie with drier tree islands	Prominent heads surrounded by trees scattered in sawgrass marsh
7	Corkscrew Well Field	Dry	Mostly Entisols	Sandy	Flat	Forb/melaleuca litter/bare sand	Pine-palmetto flatwoods	Advanced and codominant forming second canopy underneath pine

[1] Based on Ewel (1991): short <6 mos., mod. 6-9 mos., long 12 mos; [2] Based on Brown (1991); [3] Based on Davis et al. (1994) and Abrahamson & Hartnett (1991).

Table 2. *concluded*

Site No.	Site	Hydro-period[1]	Soil order[2]	Surface soil	Surface physiography	Ground surface cover	Historic habitat[3]	Current stage of invasion by melaleuca
8	Andytown Substation	Short	Mostly Histosol (Saprist)	Everglades peat	Localized mounds	Grass, melaleuca litter	Sawgrass marsh	Advanced and scattered centrifugally in sawgrass-wax myrtle cover type
9	Big Cypress NP	Short	Mostly Histosol (Saprist)	Calcitic mud (marl)	Relatively flat surface	Forb/melaleuca litter/bare marl	Cypress strand	Melaleuca-pine association, surrounded by pond cypress, grass, and periphyton
10	Hoover Dyke	Short	Mostly Histosol (Saprist)	Sandy	Slightly elevated	Thin layer of melaleuca litter/ grass on sand	Swamp forest tree island type	Melaleuca regrowth at mature sapling stage
11	Loxahatchee NWR	Short	Mostly Histosol (Saprist)	Loxahatchee peat	Variable depth with floating peat	Water and sawgrass/ periphyton	Tree island marsh	Prominent heads surrounded by trees scattered in sawgrass marsh
12	Estero	Dry	Entisols ?	Sandy	Undulating surface	Thin grass cover on sand	Pine-palmetto flatwoods	Open field with melaleuca saplings spotted with pine trees
13	Everglades NP	Short	Mostly Histosol (Hemist), exposed limstone	Everglades peat	Localized mounds	Grass, melaleuca litter or exposed limestone bed	Sawgrass marsh	Prominent heads surrounded by trees scattered in sawgrass marsh

[1] Based on Ewel (1991): short <6 mos., mod. 6-9 mos., long 12 mos; [2] Based on Brown (1991); [3] Based on Davis et al. (1994) and Abrahamson & Hartnett (1991).

been present and laying eggs 3 to 4 weeks earlier. Then, on 16 April 1998, a total of 47 larvae were found while examining 63 trees. About 36% of the trees examined showed evidence of present or past feeding damage. One unmarked adult was found as were eggs. Larvae were predominately late instars although some early instars were present. Hence, a well structured population seemed to be present at the site by spring 1998. More evidence of establishment was obtained on 24 April when more adults were found and about 25% of the trees inspected had damage. Feeding activity was observed 200 m to the east and 200 m to the west of the release area.

Corkscrew Well Field (Site 7)

The first adults were found in mid-October 1997 in the area where only larvae were released. The site was again examined on 5 November 1997 and quite a bit of larval damage was evident within the plots. However, this damage was old and no associated larvae were found. Larvae were found outside of the plots on roadside trees. At least 6 larvae were found on one tree north of the plots. This same tree also showed evidence of adult feeding. Another late-stage larva was found in the same area but overall, damage was sparse. A few additional larvae were found on the east side of the road, but the greatest concentration appeared to be north of the plots near the intersection of the two roads. Nothing was found in the area where larvae were released, despite the fact that an adult had been found a few weeks earlier, but there was abundant evidence of past larval activity.

The population seemed to have established a tenuous foothold in the area by early November and about 50% of the trees along the roadsides exhibited recently initiated (within 2 weeks) new growth that appeared suitable for the insects. We decided to make additional releases to supplement the existing population. We returned to the site on 13 November 1997 and released 300 larvae. The site was again checked prior to making the release, however, and ample evidence of adult activity (adult feeding damage and eggs) was found in one of the plots (plot #1), as were several larvae. Adult damage, eggs, and an second instar larva were also found in the larval release area. Larvae were again released on 3 December 1997 when a cursory check revealed the presence of several third and fourth instar larvae.

The plots were again checked on 10 December 1997 when larval damage was found on 25 trees within the plots in the adult release area and larvae were found on 16 trees. Larvae continued to be present on trees lining the road near the larval release area on 17 December 1997. The site was not checked again until 20 February 1998 when several larvae were found east of the larval release area on roadside trees near the intersection of the two dirt roads. Numerous larvae were again found on 12 March 1998 on roadside trees in the proximity of the larval release area three adults were found at the edge of the forest near the plots. We conclude that *O. vitiosa* has established a permanent population at the Corkscrew Well Field.

On 24 April 1998, 28 adults were found along the roadside near the adult release area and 2 adults were found near the larval release area. Only late instar larvae were found, none in the larval release area and 12 in the adult release area.

Andytown Substation (Site 8)

The site was checked on 24 June 1997, 6 days after releasing 40 weevils into plot #2. Only 3 adults were found in the plot, but 2 were found between plot #2 and plot #3, and one was found in a control plot (plot #4). Larval presence was detected on 9 July 1997 when about 15 damaged plants were found. Most of these were not in the plots. Trees that had been damaged by larvae were noticed in two control plots (#4 and #5) on 23 July, the day after the second release. These were obviously progeny of the adults released a month earlier. On 24 July, adults were found outside of the plots and in the control plots. They seemed quite active, readily moving about within the stand. For example, one weevil found about 10 m east of plot #1 quickly took flight when disturbed. Two adults were found on one small flower in plot #4 (a control plot). These, too, quickly flew when disturbed. At least 32 larvae were present on one small coppice of 11 stems located at the southern edge of the stand, outside of the plots. Nonetheless, many adults remained present in the plots so we continued the experiment by periodically checking the controls then transferring any insects found to the release plots. No adults were found in the control plots on 5 August 1997, but on 2 September, 3 days before the last release, 19% of the trees in a control plot (plot #1) were damaged, whereas only 6% were damaged in the first release plot (plot #2). About 23% of the trees were damaged in one release plot (plot #3) but no damage was evident in the other (plot #6). Six unmarked adults were found a few days later (8 September) on the aforementioned clump of 11 stems. These were probably progeny of adults released in June and July. Additional clumps at the southern edge of the stand were checked on 27 September and numerous larvae were found as well as several marked and a few unmarked adults. We estimated that 95% of the apices were damaged on the clump with 11 stems by 10 October and 21 larvae were found on another clump near the southeastern corner of the site. At this point, it seemed apparent that a vigorous population had become established at the site but had shifted from the release plots in the middle of the stand to the southern edge. Thus, despite earlier observations to the contrary, it became evident that they would not remain in the plots, thus rendering this method of evaluation unsatisfactory. Nonetheless, a population remained at the site on 16 April 1998. This site was last observed on 27 May 1997. No larvae were seen and most of the damage was old; however, 9 adults were found, most of which were in the interior portion of the stand.

Big Cypress National Preserve (Site 9)

The site was first checked on 17 September 1997, about two months after the first release. A total of 26 trees were selectively (non-randomly) examined, 11 exhibited

O. vitiosa damage. Two adults were found, one was unmarked. The tree that the unmarked weevil was on was heavily damaged with all of the tips destroyed. In contrast, less than 50% of the tips were destroyed on the other damaged trees. The site was next visited about 2 months later (19 November) when 45 adult weevils and 258 larvae were released. This was merely a supplemental release, as it was apparent that a population was already established by this time. Thirty trees were examined on the west side of the site. About half exhibited damage with eggs and/or larvae on about 35%. All stages were present but eggs and early instars were predominant. Biologists at the refuge examined the site again during late January and counted over 250 plants with damage (T. Pernas, pers. comm., 1998). They noted that some plants were severely defoliated and that larvae (all stages) were abundant. These observations prompted us to conduct a thorough search of the site on 26 February 1998. We found that most of insects were concentrated in the southeastern corner of the site (37 trees damaged of 187 examined), near one of the release areas (Plot #1), but larvae could be found throughout the site all the way to the northeastern corner of the island. Another supplemental release was made consisting of 138 larvae. This included 48 larvae moved from Site #1, as well as 90 larvae reared in screenhouses. About 3 weeks later (20 March 1998), an unmarked adult was found where some of these larvae had been placed. This was some distance (ca. 25 m) from the main population, so we suspect that it was derived from those larvae. In addition, numerous eggs and early instars were found on a small adjacent tree, suggesting that not only had adults emerged but reproduction had occurred within an unexpectedly short period of time.

Lake Okeechobee, Moorehaven (Site 10)

Only one release consisting of 100 adult weevils was made at this site. This release was made in early August 1997, and by late October many larvae (all stages) were present as well as several unmarked adult weevils. Larval feeding was observed within the release area extending to the edge of the dike, then about 50 m southward. Twenty five trees were examined on 23 December 1997 and *O. vitiosa* was found on 20 of them. All instars were present, including eggs. Thus, a population appeared to be well established by the end of the year. This was despite the fact that rising water levels in Lake Okeechobee had flooded the site to a depth of about 20 cm and the lake had risen to an elevation of 16.5 ft above mean sea level (msl) by this time. An unmarked adult was again found on 28 January 1998, and larvae were present on 7 of 21 trees examined. Water depth had increased to about 45 cm (17.51 ft msl). Nonetheless, all instars were found along with numerous eggs. Lake levels continued to increase during the remainder of the winter and reached maximal levels during late February to mid-March (peaking at 18.39 ft msl on 24 February 1998). The site was examined again on 27 April 1998 and only old damage was found. No adults or larvae were found. By this time, the lake level had receded to 16.78 ft msl, but the site remained flooded to a depth of about 15 cm. When last examined (21 May 1998), the lake level had receded to about 15.7 ft msl and the site was dry. Nonetheless, no insects were found. We've concluded that the prolonged presence of deep standing water at the site caused the loss of the population after it had successfully established.

Loxahatchee National Wildlife Refuge (Site 11)

Initially, only larvae were placed at this site to determine if they could survive permanently wet conditions. Some damage was observed soon after release, although no insects were found No adult or larval damage was observed as of February 1998. Twelve marked adults were released on 25 February 1998. The site was visited on 19 May 1998 when old damage caused by adult feeding was found on 5 trees inside the plot where adults had been released. However, no insects were found. It is doubtful that a population has established at this site.

Estero (Site 12)

Twenty larvae were placed on the five marked saplings in each of the three plot in the release area at weekly intervals from 8 October 1997 to 17 December 1997, then on 20 February and 12 March 1998. This was by far the largest number of insects released at any one site. By continually releasing insects on the same plants, we had hoped to constantly stress them and thereby induce an early impact. This would allow us to subsequently recognize symptoms of stress when it occurred in more natural situations. Unfortunately, just as we were beginning to see some slight differences between these trees and those in the control plots, our supply of larvae dwindled and we were forced to discontinue the release pattern. Also, during the spring all of the larger plants at the site became infested with a rust fungus rendering the new growth unsuitable for the larvae, so they failed to persist on those plants. Instead, the population shifted from the tall, uncut plants in our study area to the surrounding plants that had been cut a few months earlier. They, in fact, seemed to thrive on the abundant new foliage that the cut stumps were producing.

The first evidence of the presence of adults was found in mid-November, 36 days after the initial release. A small amount of characteristic leaf damage was found, but no adult weevils were detected on more than 200 trees examined. The first weevils were found about 20 days later (3 December 1997), when 100 trees were examined and 3 adults were found. Four adults were also found 2 weeks later (17 December 1997), despite the fact that heavy rains had inundated the surrounding area to a depth of about 30 cm. Fortunately, because the plots were on a ridge, they remained above water although the soil was quite wet. By 13 February 1998, very little insect activity was evident within the taller trees of the study area, but numerous larvae were found on trees at the edge of the "island" and on the small clumps of regrowth, some as far as 40 m from the nearest point of release. These seemed restricted to the east and southeast side of the uncut tree island and were on shoots that ranged from 46 to 61 cm tall. A total of 40 larvae were on just three of these clumps, and lesser amounts occurred on many others. Only one larvae was found in the plots on 20 February prior to a release, and the rust fungus had infested many of the tips of the taller plants. Water levels were high and one plot was partially inundated, although the other two remained dry. By 12 March, the damage to the tops of the uncut trees in the island caused by the rust was severe, and no insects were found within the study area. However, many small shoots regrowing from the cut stumps were severely damaged, and larvae were still present to the east and southeast of the island. In fact, one plant with obvious larval damage was found 100 m east of the nearest release point, so the population appeared to be spreading in that direction. The distribution also appeared to be expanding to the south, towards the control plots, although fewer larvae were apparent. Twenty-five larvae were found, mostly late instars, and no adults. Thus, we suspected that these were between generations, with most of the larvae found earlier having pupated. On 24 April 1998, 13 adults were found near the south end of the release area and larvae were found at the north end of the control site, about 100 m from the nearest point of release.

Everglades National Park (Site 13)

Eggs were found one week (11 December 1997) after the first release at this site even though only two adults were included in the release. Additional larvae were released at that time. The site was not checked again until 13 February 1998 when about 100 trees were examined. Some old damage was noticeable on 18 of these trees but no larvae or adults were detected. An additional 60 adults were then released. The site was checked again on 6 March 1998. About 16% of 122 trees examined showed characteristic signs of *O. vitiosa* damage. Most was larval damage but feeding scars of adults were found on 12 trees (10%). Numerous larvae were present, mostly young instars (mainly first instars with a few second instars). Eggs were found on at least two of the trees examined. Park biologists examined the site two weeks later (19 March) and reported that 50% of the 50 trees they examined exhibited damage, and larvae were found on 7 trees (14%). They noted that most of the larvae were young, but they found at least 6 late instars. However, they didn't find any adults or larvae. We examined the site on 21 April 1998 when we inspected 160 trees. Only 20 of these (12%) appeared to be damaged and live larvae were found only on two of them. None of the larvae were young, most appeared to be fourth instars. As of that date, population establishment seemed tentative. An additional 27 adults were, therefore, released. We collected 10 of these from trees growing on the grounds of our research center. These were apparently progeny of weevils that had escaped from our cultures and had naturally colonized these trees. We last visited the site on 11 May 1998 and found four adults on smaller trees (up to 6 m) and 18 trees damaged out of 128 inspected (14%). Two mid-instar larvae were also found and eggs were present on in the general area of the previous release.

Discussion

Our primary objective for this first year was to establish self-perpetuating field populations of the melaleuca snout beetle, *O. vitiosa*. This has been accomplished. Although we would normally wait about two years before making such a proclamation, the populations seem firmly entrenched and, barring any unforeseen catastrophes, should persist indefinitely. *Oxyops vitiosa* proved relatively easy to establish with success usually apparent at a given site within 60-90 days after release, provided that the site was satisfactory. We had also hoped to strategically establish populations to initiate colonies in all of the major melaleuca infestations in southern Florida, and thereby facilitate dispersal of *O. vitiosa* throughout these infestations. This, too, has largely been accomplished. Populations are now established in Broward, Lee, Collier, Palm Beach, and Glades counties, and a major effort has been initiated to establish populations in Miami-Dade County, particularly along the eastern edge of Everglades National Park.

Population establishment was extremely dependent upon site conditions. For example, the insects seemed to prefer open grown trees, those typical of intermediate stages of invasion (see O'Hare and Dalyrmple 1997). Trees that were growing in the open or at the edge of a head tended to be smaller and bushier with more stem apices. As a result, they provided more sites for egg deposition and were more likely to provide young foliage for larval development. Results at some sites, e.g., Andytown, also seemed to suggest that they favored a southern exposure. Perhaps plants at the southern edge of extensive stands received more direct sunlight and produced more new growth. Although the weevils seem to shun dense stands, i.e., 75-100% mature and sapling dense melaleuca coverage classes used by O'Hare and Dalyrmple (1997), this might be a biased observation. We were able to easily inspect smaller trees whereas the canopies of large trees were virtually inaccessible. Nonetheless, at both Andytown and Estero where all of the plants were small enough to be easily inspected, the insects moved away from release sites within the stands to the southern peripheries and onto more isolated trees.

Our initial assumption, based upon our knowledge of the biology of *O. vitiosa* (Purcell and Balciunas 1994), was that it would be restricted to the driest sites due to its requirement to pupate in the soil. However, it proved to be more tolerant of wet conditions than we presumed. Saturated soil conditions prevailed at many of the sites for prolonged periods, yet the weevils established and persisted at those sites. Irregularities of the ground surface at sites inundated with shallow water perhaps provided sufficient emergent ground for pupation, thereby enabling the populations to persist. Or, perhaps, alternate pupation sites are used, e.g., elevated boles of the trees, stumps, branch crotches containing accumulated litter, exfoliating bits of bark, etc., and they don't need to burrow into the soil at all. As populations become larger and pupae easier to find, we'll be more able to answer this question.

Weevils failed to establish only at long-hydroperiod sites where the ground surface and the bases of the trees were completely submerged under deep water. We can't yet safely deem even those sites unsuitable, though, because of our hesitancy to commit large quantities of insects to less-than-ideal locations. Survival is probably lower at such sites and we might compensate for low survival by releasing large numbers of insects. However, at this early stage, the supply of insects has been too limited to risk wasting them in this manner. We hope to more fully address this question after large field populations become available for redistribution to these more marginal locations.

With the possible exception of the cases mentioned above, the numbers of insects released or the frequency of release seemed to have little bearing on the ultimate outcome. A vigorous population established from a single release of only 60 adults at Site #3 in the Everglades buffer strip and the initial establishment at Big Cypress also resulted from 60 adults. The stage released also didn't seem to matter, although egg-laying adults seemed to be best. Nonetheless, the initial release of adults at the Jog Road Catchment failed, whereas the later release of only 240 larvae succeeded. Again, the choice of where and when to release seemed to make the difference, rather than the stage released.

The date of release also seemed unimportant, although plant phenology and foliage quality which may be under seasonal influence, were clearly important. The dry period experienced in south Florida during April and May 1998 would not have been favorable for the survival of larvae due to the paucity of young foliage. In fact, during this period, there were virtually no larvae present in the field even though adults were abundant. Thus, releases during drought periods (or periods of low shoot growth) should probably be restricted to adults. Release of larvae was effective provided that appropriate food sources were available, i.e., during fall through early winter, but generally larger numbers and multiple releases were necessary. Data from Australia revealed that larvae are present there mainly during the winter (May-September), which coincides with the availability of young foliage that is produced after the flowering period (December-June) (M. Purcell and Galway, pers. comm.). Although adults are present all yea round, they tend to be most abundant in Australia during the warmer months (October-March). It is unclear whether this pattern will be similar in Florida since melaleuca tends to flower more frequently here and (we think) produces new growth more or less continuously. We are presently studying several melaleuca populations to acquire a better understanding of these phenological patterns.

Although soils have not yet been fully analyzed, soil type didn't seem to preclude establishment. Weevil populations are now doing well at sites with both sandy and mucky soils. However, the best results to date seem to be at sites with sandy soils, i.e., historically pine-palmetto habitats such as Jog Road Catchment Area, Corkscrew Well Field, and Big Cypress National Preserve.

Biological control projects are often criticized when the effectiveness of the agents is not evaluated. We have therefore made a strong commitment to thoroughly evaluate the melaleuca project. Our early attempts, however, have been futile. We've set up long term studies at paired sites in three different habitat types and released weevils into one of each pair of sites within each habitat, only to have the insects move outside of the study areas. We established plots at Estero in two areas of a pasture that was completely covered with small melaleuca trees. One of the areas was designated a weevil release area while an area about 100 m the south of the first area was to serve as a control. The land owner subsequently mowed the pasture, but he left the trees surrounding our plots. We counted and measured every tree in three 5 m^2 plots in each area, then measured the length and position of every branch, and counted all of the leaves on five trees in each plot. These five trees were marked and 20 larvae were placed on each one every week for 11 weeks. Although we knew that the weevils would not stay put, we felt that we could induce an effect by continuously loading enough new insects onto the trees to destroy any new growth as it was being produced. We, in fact, began to see an effect by mid-December when colony production dropped making it impossible to maintain the release schedule. Subsequently, the insects moved out of our plots and onto the new sprouts on cut stumps in the surrounding pasture, seemingly preferring this mowed area to the taller trees in our experimental area. As they built up on the south side of the release plots, they begin to disperse towards the control plots. By April 1997, larvae occurred more or less continuously between the release and the control areas. A similar attempt was made at the Andytown site where we released adult weevils into three of six plots in each of two areas. The weevils rapidly moved to the southern edge of the stand, where they severely damaged many of the trees, but the trees in the plots remained unscathed. We intend to continue to try to evaluate the performance of *O. vitiosa*, but as the above examples illustrate, a proper field experiment with appropriate controls will be difficult to set up. As we learn more about the biology and behavior of these insects, we will be better able to design appropriate studies but, as the weevils become more widespread, true controls may be unattainable so we may have to rely on correlational observations.

Quarantine studies proved *O. vitiosa* to be quite specific to melaleuca, but some results indicated that it could feed and perhaps partially develop on wax myrtle (*Myrica cerifera* L.). This required an abundance of young foliage, however, and sufficient quantities were seldom available on a single plant to sustain larval development, so the risk to wax myrtle seemed small. We confirmed this after quarantine. Larvae were reared at Fort Lauderdale on wax myrtle fairly readily, but all died before they could pupate, even though they were provisioned with the best plant material obtainable, i.e., soft, young shoots. Nonetheless, we remain cognizant of the potential for collateral damage to wax myrtle and will be vigilant during the course of site examinations. Next to melaleuca, in fact, wax myrtle is the most abundant plant species at many of our sites, so ample opportunity exists for them to move to it.

Despite large *O. vitiosa* infestations on melaleuca, sometimes adjacent to or even with branches intermingled with wax myrtle, we never found any evidence that *O. vitiosa* would eat it until recently. While visiting Site #1 on 26 May 1998, when adult weevils were abundant and adult feeding damage was prolific and widespread, we noticed extensive insect damage to some of the wax myrtle bushes. Upon checking one, we found a single adult *O. vitiosa* on a leaf. However, we checked more closely and noticed that a heavily damaged melaleuca branch protruded into the wax myrtle very near to the weevil. Closer inspection indicated that the weevil probably had, in fact, gouged the wax myrtle leaves, as the typical feeding pattern was present on two or three leaves near the weevil. However, these gouges were shorter than normal and atypically serpentine in shape rather than linear. The other damage was quite different, consisting of notches in the leaf edges or close cropping of the new growth. Closer scrutiny revealed that the notching was caused by another weevil, *Diaprepes abbreviatus*, which feeds on a wide variety of plant species (including melaleuca). We also found several leaf beetles (Chrysomelidae, as yet unidentified but apparently two species) that were clipping the new growth. So, even though *Oxyops* had nibbled wax myrtle, the damage was insignificant, especially when compared to the amount of *Oxyops* damage on melaleuca or the damage caused by the other insects to wax myrtle. The *Oxyops* weevil had apparently been feeding on the melaleuca leaves when it wandered onto the intertwined wax myrtle foliage. It probably then "tested" the wax myrtle and found it to be unsuitable, because it wasn't present the next morning and the amount of feeding hadn't increased. Normally, when these weevils encounter a good food source, they'll remain on it, sometimes for several days. The fact that this one didn't stay indicates that the wax myrtle wasn't acceptable. Nonetheless, it is possible that some spillover to wax myrtle will occur, as was predicted from quarantine studies, especially when it occurs amongst *Oxyops*-infested melaleuca. It is doubtful though, that *O. vitiosa* will ever "switch" to wax myrtle and we have never found any evidence of larvae utilization or oviposition on it. We intend to continue to intensively check wax myrtle as weevils become more abundant. Meanwhile, we are concerned that the obvious insect feeding damage evident on wax myrtle could be mistakenly attributed to *O. vitiosa* by persons less familiar with these various herbivorous insects.

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