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Field Colonization of the Melaleuca Snout Beetle (*Oxyops vitiosa*) in South Florida

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The Australian melaleuca snout beetle, *Oxyops vitiosa* Pascoe, a biological control agent of *Melaleuca quinquenervia* (Cav.) S. T. Blake, was first released in south Florida during spring 1997. Field-emerged adults appeared 3 months later, which affirmed survival of pupae despite occasional flooding. Releases at 12 other locations totaled more than 1500 adults and 6700 larvae by June 1998. Populations established at nine sites in Dade, Broward, Lee, Collier, Palm Beach, and Glades Counties. Habitats with short hydroperiods, intermediate stages of melaleuca invasion, and dry winter conditions engendered field colony development, whereas releases failed at aquatic sites. Even small releases (60 adults) produced populations at favorable sites. Abundant young foliage facilitated establishment, whereas soil type seemed unimportant: colonies developed at typical "glades" sites characterized by organic soils and at pineland sites with sandy soils. Larvae predominated during October to May, coincident with flushes of plant growth. In contrast, only adults remained during summer, except at a site that was periodically mowed. The new growth induced by mowing supported a persistent year-round larval population. This demonstrated that population trends are influenced more by plant phenology than by climatic conditions, possibly reflecting adaptation to the nonseasonal climatic oscillations (El Niño) of Australia. Transect sampling estimated the population at more than 2000 adults and 22,000 larvae, 1 year after release of 3300 larvae. Numbers swelled to almost 80,000 adults and over 15,000 larvae by June 1999 and to nearly 83,000 adults and 137,000 larvae by January 2000. Weevils dispersed throughout the 8.1-ha site but remained concentrated near release plots during the first 18 months. Ease of establishment and slow dispersal suggests an optimal strategy of many small releases at carefully selected but widely dispersed sites. Adults and larvae were subsequently relocated to other areas, and *O. vitiosa* is now widely established in southern Florida. © 2000 Academic Press

Key Words: **Everglades; invasive plants; Myrtaceae; bioagent establishment; classical biological control; weed control; dispersal; release strategies.**

INTRODUCTION

Melaleuca quinquenervia (Cav.) S. T. Blake (Myrtaceae), the broad-leaved paperbark (aka, melaleuca), is a large, native Australian tree (25–30 m tall). It occurs naturally within a 40-km-wide zone along the eastern coast of Queensland and New South Wales (11–34° S). It is the southernmost representative of the *M. leucadendra* complex composed of 15 closely related species with a center of diversity in tropical Queensland (Craven, 1999). It occurs in coastal wetlands, typically in freshwater "paperbark swamps" which often occupy sandy soils behind heath-dominated headlands. It also occurs along streams and in brackish water behind 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; Boland *et al.*, 1987).

M. quinquenervia is a serious weed in south Florida (Laroche and Ferriter, 1992). Melaleuca invasion 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 temporarily results in increased biodiversity, but diversity is drastically reduced during later stages of invasion after displacement of native vegetation (O'Hare and Dalrymple, 1997).

Economists and ecologists estimate the value of services provided by wetlands to be worth $14,785 per hectare per year (Costanza *et al.*, 1997). Assuming minimal losses comprising only 1% of these services arising from current melaleuca infestations (ca. 200,000 ha; Schmitz *et al.*, 1997), 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 over the 70 years since naturalization occured (F. A. Dray, unpublished data); so, melaleuca has increased at an average rate of 2850 ha per year or approximately 7.8 ha per day (assuming a continuous linear rate of change). If these infestations caused a 100% decrement of wetland functions, potential added losses could be as high as $42.1 million per year. The South Florida Water Management District alone spent nearly $11 million to control this tree from 1991 to 1997 (Laroche, 1998), and estimates of losses to the local economy range as high as $168.6 million per year (Diamond *et al.*, 1991). It is obviously very important to reduce the invasive potential of melaleuca while simultaneously eliminating existing stands. This requires an integrated management approach using biological, herbicidal, and mechanical methods (see Laroche, 1994, 1998).

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 subject to reinvasion and expansion is likely to continue. Hence, measures are needed to minimize seed production and survival of seedlings and saplings so as to provide sustainable management. Biological control offers the best hope for this. The melaleuca snout beetle, *Oxyops vitiosa* (Pascoe) (Coleoptera: Curculionidae), was proven to be host specific after prolonged study (Balciunas *et al.*, 1994; G. R. Buckingham, unpublished data) and it was released in Florida during spring 1997 at a site near the Everglades Conservation Area 3B in Broward County. This weevil damages young tissue growing at the tips of branches (Purcell and Balciunas, 1994). The resultant damage disrupts shoot elongation, causing gnarled, deformed growth. It also disables flower and seed production and reduces survival of saplings. The objectives of the work reported herein were (i) to determine an optimal release strategy for this weevil, (ii) to assess site characteristics that influence establishment of populations, and ultimately (iii) to establish self-sustaining field colonies throughout the south Florida range of melaleuca.

MATERIALS AND METHODS

Insect Identification

O. vitiosa specimens from Australia were identified by Dr. C. W. O'Brien (Florida A&M University, Tallahassee) at the beginning of the quarantine phase of the project and confirmed by him during the course of quarantine studies. Dr. O'Brien, along with Dr. M. Thomas (Florida Division of Plant Industries, Gainesville), examined all of the weevils that were transferred from quarantine to Fort Lauderdale on 24 March 1997. Thereafter, all weevils that were field-collected in Australia were examined by Dr. Thomas, as well as quarantine personnel, prior to transferral from quarantine to the Fort Lauderdale facilities to safeguard against the inadvertent release of an inappropriate species.

Pathogen Examinations

Field-collected weevils from Australia were dipped in 0.006% a.i. solution of benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] within 1 or 2 days after arrival into quarantine to prevent germination of external fungal spores (particularly *Beauveria bassiana* (Balsamo) Vuillemin). Samples of weevils (about 10% of each sex), taken 1 or 2 days prior to release from quarantine, were examined by USDA-ARS insect pathologists (Dr. A. Undeen, Dr. J. Becnel, and G. White, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville). A total of 72 field-collected adults, 27 laboratory-reared adults, and 60 laboratory-produced larvae were examined. No pathogens were ever found.

Release Procedures

Eggs, larvae, and adults were transferred from the Gainesville quarantine facility to the Fort Lauderdale laboratory during the period between February and October 1997. Twenty-three consignments, which included an estimated 5434 (± 100) eggs, 1740 (± 175) larvae, and 823 adults, were received at Fort Lauderdale. Most of the eggs and larvae comprised F_1 progeny of field-collected insects. However, most of the 823 adults received after late March 1997 were field-collected in Australia and held briefly in quarantine to confirm identifications. A portion were sacrificed to check for pathogens, and the remainder were consigned to Fort Lauderdale. Insects were either released directly at field sites after their receipt or released into a screen house onto container-grown plants. Progeny of those released into the screen house provided stock for later releases. Adults were marked on their ventral sternites with a small dot of paint using a fine-tipped paint pen (Sanford Silver Coat; Sanford Corp., Bellwood, IL) prior to release to facilitate later recognition of new individuals.

Adults and larvae were transported to field sites on melaleuca cuttings (stem tips ca. 10–15 cm in length) held in polyurethane boxes lined with moistened paper towels. Adults were removed from this plant material and placed directly onto melaleuca branch tips. At first, cut stems bearing larvae were tied to branches of the field plants and positioned so as to intermingle the leaves of the cutting with the apical foliage of the trees. However, these cuttings desiccated too quickly, sometimes before the larvae were able to move, which caused some mortality. This was remedied by inserting the lower stems of cuttings through a plastic cap into 15-ml water-filled tubes (florists' "Aqua-pics") to create a bouquet. Larvae were transferred to these bouquets, which were then transported to the site in the manner described above. The tubes containing larval-infested bouquets were tied to the trees by wrapping one or more wire ties ("twist-ties") around both the tube and a branch of the tree. This system retarded dessication of the bouquet, thus allowing the larvae more time to move onto the live foliage. Larvae that remained in the box were carefully removed with a soft, fine-tipped brush and deposited directly onto branch tips of the field plants.

Table 1 provides a summary of the numbers released at the original 13 sites. Adults were released at most sites. However, only larvae were released (initially) at the Loxhatchee National Wildlife Refuge and at the Estero site (Sites 11 and 12). The releases at the Loxahatchee Refuge were done to determine whether larvae could find pupation sites in an aquatic habitat. The releases of larvae at the Estero site were made to assess the impact of larval feeding on the plants as part of a related study.

Selection of Release Sites

Figure 1 depicts the location of the release sites. Sites were classified using O'Hare and Dalyrmple's (1997) scheme as 75–100% dense mature melaleuca (DMM), 75–100% sapling dense melaleuca (SDM), 50–75% melaleuca coverage (P75), 10–50% melaleuca coverage (P50), or marsh (MAR: 0–10% melaleuca coverage). Additionally, some sites had previously been clear-cut but coppices (clumps of shoots from a common stump) had formed from the cut stumps. These were classified as coppice growth (COP). Sites were chosen on the basis of the requisites needed by the weevils, a primary consideration being the availability of young foliage, which provides oviposition and larval feeding sites (Purcell and Balciunas, 1994). This type of plant material was most abundant on saplings, coppices, or trees growing in the open or at the edge of dense stands.

Determination of Establishment

Sites were visited on an irregular basis during the 12–16 months following the initial release of *O. vitiosa.* Insects were generally too rare and sporadic to enable quantitative assessment of abundance, so a semiquantitative approach was used. Generally, this involved one or more observers searching for signs of damage to the foliage and then searching the damaged foliage and counting the number of larvae and/or adults encountered. This continued, with no time limit, until no additional insects could be found. This was modified somewhat in June 1998, when we instituted monthly site visits and imposed a 30-min time limit on the counts. The counts were averaged across observers and then doubled to provide the number of adults or larvae encountered per observer-hour. A population was considered established if insects persisted 1 year after the last release. It should be noted that adults are very cryptic and easily overlooked unless accompanied by their feeding damage, which is more obvious. Larvae, on the other hand, are generally conspicuous and thus more easily counted.

Factors Affecting Establishment Success

Most of the sites (Table 1) consisted of sawgrass ("glades") habitat characterized by organic (muck) soils (Sites 1–5, 8, 10, 11, and 13). Some, however, were invaded pinelands characterized by sandy soils (Sites 6, 7, 9, and 12). Because larvae enter the soil to pupate (Purcell and Balciunas, 1994), we assumed that they would not survive saturated conditions and would therefore require permanently dry sites. To test this assumption, we released weevils in permanently wet (Sites 4 and 11), seasonally wet (Sites 1, 2, 5, 6, 8–10, and 13), and dry (Sites 3, 7, and 12) sites. Three of these sites (one of each type) were selected for direct comparison using uniform release procedures. Sixty adult weevils were released at each selected site (Sites 3–5) during an 8-day period (29 April–6 May 1997).

Plant samples were collected beginning in September 1998 by grabbing branches within arm's reach while walking through each site in as random a manner as possible. At first, this was done by grasping branches that were contacted by one's outstretched arms while looking away. However, the purpose of this sampling was to quantify plant phenology, so it was desirable to sample undamaged branches. This became increasingly difficult as weevil populations increased and necessitated a biased search for undamaged branches. Only the apical 25 cm of each branch was collected. The branches were stripped of leaves and the leaves were individually classified as young or old foliage and counted. The young and old leaves were weighed fresh, dried at 70°C, and then weighed again. When the point of demarcation between the young and the old leaves on a branch was not obvious, the hardness of the leaves was assessed using a gram-gauge modified to a penetrometer as described by Wheeler and Center (1996). Young leaves were then defined as

TABLE 1

Summary of the Physical and Biological Characteristics of the Sites and the Release and Establishment of *Oxyops vitiosa*

Site No.	Sites	GPS coordinates	Hydroperiod[a]	Surface physiography/ soil type	Melaleuca invasion stage/ surrounding area cover type[b]	First/last release dates	Number of releases	Adults	Larvae	First F_1 adults	Result (May 1999)
1	Everglades buffer strip	26°03.427′ 80°26.536′	Short	Uneven/muck	Advanced and scattered/ sawgrass–waxmyrtle (DMM-edge)	26-Mar-97 23-Jun-97	4	143	312	19-May-97	Established
2	Everglades buffer strip	26°03.550′ 80°26.364′	Short	Uneven/muck	Advanced and scattered/ sawgrass–waxmyrtle (DMM-P50)	26-Apr-97	1	300	—	None found	Secondarily established
3	Everglades buffer strip	26°03.380′ 80°26.410′	Short	Uneven/muck	Advanced and scattered/ sawgrass–waxmyrtle (DMM-P50)	29-Apr-97	1	60	—	26-Aug-97	Established
4	Conservation Area 2B	26°09.420′ 80°21.950′	Long	Variable water depth up to 1.2 m/muck	Advanced and scattered/ sawgrass (P75)	01-May-97	1	60	—	None found	Not established
5	Krome Ave.	25°55.830′ 80°27.030′	Long (moderate)	Variable water depth up to 1.3 m/muck	Advanced and scattered/ sawgrass (DMM)	06-May-97	1	60	—	None found	Not established
6	Palm Beach catchment area	26°44.028′ 80°09.005′	Short	Uneven/muck	Advanced and scattered/ pine–other hardwood (P75)	22-May-97 6-Nov-97	2	40	240	28-Jan-98	Established
7	Corkscrew well-field	26°27.715′ 81°42.147′	Dry	Flat/sand	Advanced and codominant/ pine–palmetto (DMM-edge)	6-Jun-97 3-Dec-97	5	120	931	Late Oct. 1997	Established
8	Andytown substation	26°02.129′ 80°26.097′	Short	Uneven/muck	Coppice regrowth/ sawgrass–waxmyrtle	18-Jun-97 5-Sep-97	3	451	—	08-Sep-97	Established
9	Big Cypress National Preserve	25°56.247′ 81°00.527′	Short	Relatively flat surface/marl	Advanced melaleuca–pine association/pond cypress strands (P50–P75)	9-Jul-97 26-Feb-98	3	105	258	17-Sep-97	Established
10	Hoover Dyke Moorehaven	26°53.644′ 81°07.297′	Moderate	Slightly elevated/ sand	Dense melaleuca saplings/ willow–palmetto tree islands (DMM-edge)	01-Aug-97	1	100	—	31-Oct-97	Established
11	Loxahatchee National Wildlife Refuge	26°29.620′ 80°16.360′	Long	Variable depth with floating peat/muck	Advanced and scattered/ waxmyrtle–willow islands (P50–P75)	12-Sep-97 25-Feb-98	3	12	342	None found	Not established
12	Estero	26°25.530′ 81°48.620′	Short (dry)	Uneven/sand	Coppice regrowth/pine–palmetto	8-Oct-97 12-Mar-98	13	—	3900	03-Dec-97	Established
13	Everglades National Park	25°41.33′ 80°29.83	Short	Uneven/muck and limestone	Advanced and scattered/ sawgrass (P50)	4-Dec-97 6-Jun-98	5	131	730	11-May-98	Established

[a] Dry, never inundated; short, inundated <6 months; moderate, 6–9 months; long, 12 months (Ewel, 1990). Actual hydroperiod at some sites differed from expected. In those cases, expected values are enclosed in parentheses.

[b] Codes within parenthesis are adapted from O'Hare and Dalrymple (1997) system of melaleuca stand classification that approximates our system of invasion stage description.

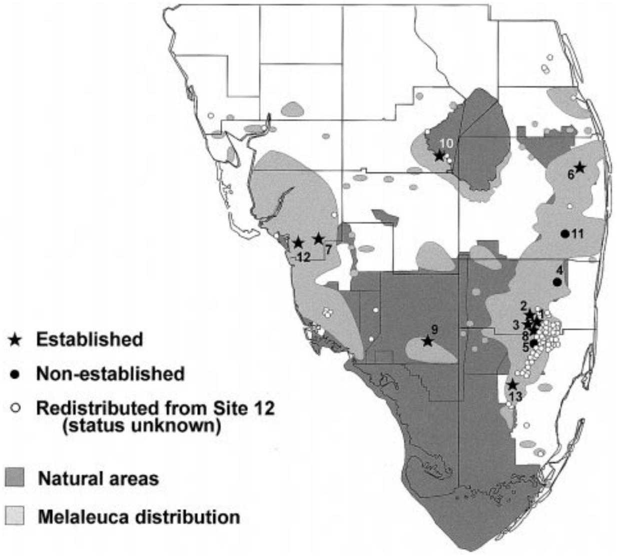

FIG. 1. A map of south Florida (excluding the Florida Keys) showing the location of sites where *Oxyops vitiosa* was released. The primary research sites are numbered. The small white circles designate later releases that have not been monitored.

those producing a penetration measurement of less than 65 g on the gauge, which calibrated to 53 g by depression of the pan on a digital balance (Ohaus Galaxy 4000D; Ohaus Corp., Florham Park, NJ).

In addition to habitat type, we were interested in knowing whether release effort influenced establishment success. We thus analyzed whether the probability of establishment was independent of (1) number of releases per site, (2) number of weevils released, (3) stages of weevils released, and (4) habitat type, using G tests of independence (Sokal and Rohlf, 1981). Sample sizes ($n = 13$ sites) were small, so the resulting G values were adjusted using Williams' correction, as recommended by Sokal and Rohlf (1981). The relationship between plant phenology and weevil abundance was examined by regressing the number of larvae encountered per observer-hour (averaged across sites) against the availability of young foliage, measured as a proportion (also averaged across sites) of the total number of leaves per branch. However, proportions are binomially distributed and therefore have variances that are not independent of their means. To overcome this, the regression was conducted on arcsine transformed proportions (Sokal and Rohlf, 1981); the analysis was carried out using SigmaStat software (SPSS, 1997).

Within-Site Distribution of a Weevil Population

Larvae were released within three 5-m^2 plots (1.50×3.33 m) placed near the center of a pasture near Estero, Lee County, Florida (Site 12). Weekly releases of 300 larvae were made on the same trees (20 larvae per tree; five trees per plot) beginning on 8 October 1997 and continuing until 17 December 1997, with supplemental releases made on 20 February and 12 March 1998 (Table 1). Our original intent was to simulate and assess the impact of sustained larval feeding on small trees (about 3 m tall). However, this study was terminated when the land owner unexpectedly mowed the pasture during June 1998. This proved fortuitous because the resultant regrowth provided an abundant resource for the remaining insects during the summer when young foliage is usually sparse. As a result, a larger population was present earlier than at other sites during the following fall.

We mapped the Estero population during 12–28 October 1998 (1 year after the intial release) to determine the distribution of weevils. The cut melaleuca stumps had coppiced by this time, producing clumps of young shoots. Observers lined up 10 abreast at arms length from one another at the north end of the field facing the shortest distance (toward the west). Two ropes were stretched across the width of the field perpendicular to and at each end of the line of observers to demarcate a search area. The observers then traversed this area while examining each melaleuca clump for signs of damage. Each damaged clump was marked with a surveyor's flag. When one sweep was completed, the observers lined up on the opposite side of the field, the northernmost rope was moved to the south end of the line, and another sweep was made. This continued until the entire pasture was covered. Each marked clump was then reexamined and classified as to degree of damage (severe, moderate, or light), and its location (coordinate system: NAD83 Florida State Plane, West Zone) was registered at submeter accuracy using a real-time differential GPS system (Trimble Pathfinder Pro XR; Trimble Navigation Limited, Sunnyvale, CA). The perimeter of the field was also mapped using this system by walking the fence line with the GPS unit operating in continuous mode. A map of the field showing the location of each damaged plant encoded with a damage level attribute was then created using GIS software (ESRI, 1996).

The status of the weevil population was then assessed by more detailed sampling done during 15–22 October 1998. Thirteen transects were established in an east–west direction across the field. These were spaced 100 ft (30.5 m) apart, beginning about 10 m from the north end of the field. Sampling points were positioned at 4-m intervals along each transect and the closest melaleuca clump to each point was identified. The location of the clump sampled was determined using the differential GPS system as described above. Data taken from each plant included the number of weevil adults, small (early instar) larvae, large (late instar) larvae, and the presence or absence of eggs. In addition, the number of plants touching the transect line on each line segment between sampling points was recorded to determine clump density.

Although the clumps sampled were flagged to enable later inspection of the same trees, the flags were destroyed when the field was again mowed during December 1998. However, the weevil population recovered quickly. Hence, new transects were established in June 1999 and January 2000. These were set up in a grid pattern with 20 transects oriented east to west at 20-m intervals with points on each transect spaced 20 m apart. This arrangement of sampling transects differed from the one used in October 1998. However, we wanted to compare insect life stages and feeding damage across the two dates, so we converted transect points into relative positions to facilitate graphical analysis of these data.

Four plants were sampled at each point during 3–9 June using the quarter method of vegetation sampling (Smith, 1966). The area was divided into four quarters at each sampling point based on the four cardinal compass points. The nearest clump in each quarter was then sampled and the numbers of *O. vitiosa* adults and larvae (again separated into early and late instars) were counted. The presence or absence of eggs was also noted. Plant density was determined by measuring the distance of each clump from the transect point and then dividing the sum of the distances by four (the number of clumps). The resultant average distance (in meters) was squared and then divided into 10,000 to determine the number of plants per hectare (as per Smith, 1966).

RESULTS AND DISCUSSION

Determination of Establishment

Eggs were found on plants on the day after the first release (Site 1, 26 March 1997), and young larvae were present within 19 days. The first unmarked adult was found after 53 days and several more had been found by late summer. Standing water was present during much of this period. Despite this, the population persisted and ultimately established. In fact, weevil populations persisted for more than 1 year (and are thus considered established) at 9 of the original 13 release sites (Table 1, Fig. 1; note that Site 2 only became established secondarily, probably via immigration from Site 1). Populations at 8 sites thrived and increased substantially during the ensuing 3 years.

Effect of Release Effort on Establishment Success

The release effort varied considerably among sites (Table 1), but level of effort had little influence on whether or not a population established. For example, release of only 60 weevils resulted in population establishment at Site 3, whereas identical releases at Sites 4 and 5 failed. Larger (>300 weevils) releases failed at some sites (e.g., Site 2), but succeeded at others (e.g., Site 8). Likewise, multiple releases at Site 13 produced an established *O. vitiosa* population, but failed to do so at Site 11. Similarly, single releases produced populations at Sites 3 and 10, but not at Sites 4 and 5. Thus, establishment success was unaffected by the number of insects released ($G_{adj} = 2.025$, $P = 0.421$), and the influence of number of release events was equivocal (single vs multiple: $G_{adj} = 2.854$, $P = 0.074$).

Effect of Weevil Life Stage Released on Establishment Success

Populations also established regardless of which *O. vitiosa* life stage was used to inoculate a site ($G_{adj} = 1.310$, $P = 0.526$). Exclusive release of adults produced viable populations at Sites 3 and 8, but not at Sites 2 and 5. Only one melaleuca stand (Site 12) was inoculated exclusively with larvae and that effort succeeded. Site 11 was inoculated primarily with larvae, however, and that effort failed. Populations generally established at sites receiving a mixture of larvae and adults (e.g., Sites 1 and 7), but this relationship is not necessarily as straightforward as it might appear. For example, 40 adults were originally released in the interior of an open stand (occupying about 200 m^2) on trees 6–10 m tall at the West Palm Beach site on 22 May 1997. A few marked adults remained present during the 1st month. By late summer, however, no adults could be found. Later (6 November 1997), 240 larvae were released on small saplings (ca. 2–3 m) a few meters west of the first release. These trees supported a persistent population. Thus, we credit the release of larvae, rather than the earlier release of adults, for establishment of the resultant population at this location (Site 6).

Effect of Habitat Type on Establishment Success

The Krome Avenue site was dry when we first inoculated the melaleuca stand with weevils, but flooded soon thereafter and remained that way throughout the study. This precluded our comparison of sites by hydroperiod (as defined by Ewel, 1990) because it eliminated the moderate hydroperiod site. However, *O. vitiosa* populations consistently established at sites that were generally dry or seasonally wet, but never at permanently wet (aquatic) sites. Thus, weevil establishment success was closely associated with habitat type ($G_{adj} = 6.495$, $P = 0.032$).

This finding corresponds well with Purcell and Balciunas' (1994) observation that *O. vitiosa* requires soil for pupation. We were somewhat surprised that population establishment occurred at seasonally wet sites (e.g., Sites 8 and 9) even during the rainy season, when dry soil for pupation was rare. Soil accretion associated with litter accumulation and adventitious root growth

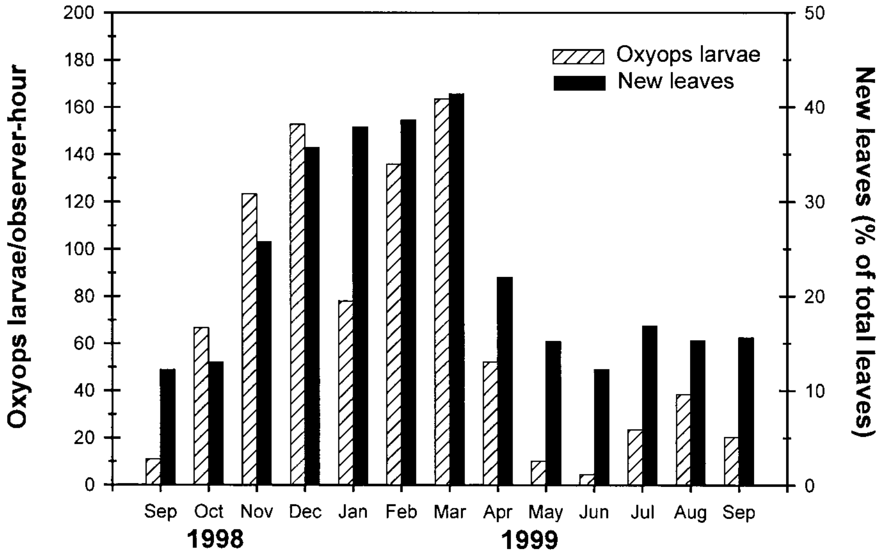

FIG. 2. Seasonal comparison of mean number of larvae counted during 30-min periods with mean proportion of young foliage available at *Oxyops vitiosa* release sites.

raised soil levels at the bases of many trees above the water surface during the rainy season at seasonally wet locales, which perhaps provided dry sites for larvae to successfully pupate. It is also possible that some larvae pupated in the crotches of tree branches or under peeling bark. This aspect of the weevil's biology needs more investigation.

Effect of Plant Phenology on Establishment Success

The presence of young foliage on the trees was an important requisite for establishment of *O. vitiosa*. Larval abundance was closely associated with active plant growth (as measured by the relative proportion of new foliage on a branch; $R^2 = 0.768$, $F = 36.407$, $P < 0.001$), which generally occurred from October to April (Fig. 2). This was predictable from observations of *O. vitiosa* in Australia, where eggs and larvae are usually found on the young foliage produced after flowering during the dry season (Purcell and Balciunas, 1994). Adults predominated during the summer, except where circumstances caused an unseasonable abundance of young foliage. For example, at the Everglades Griffin Road site (Site 1) very few larvae were present by late May 1999, but adults were abundant (Fig. 3).

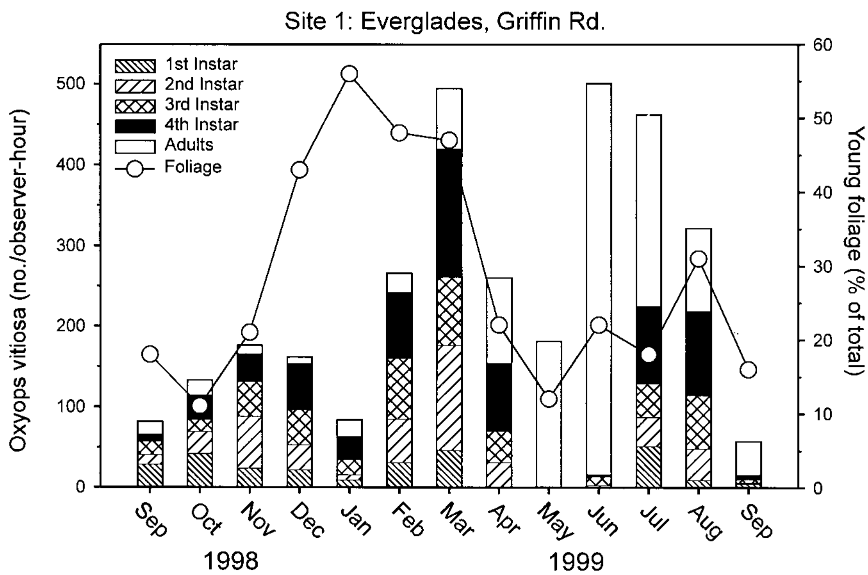

FIG. 3. Comparison of numbers of *Oxyops vitiosa* adults and larvae counted during 30-min periods with proportion of young foliage available at Site 1.

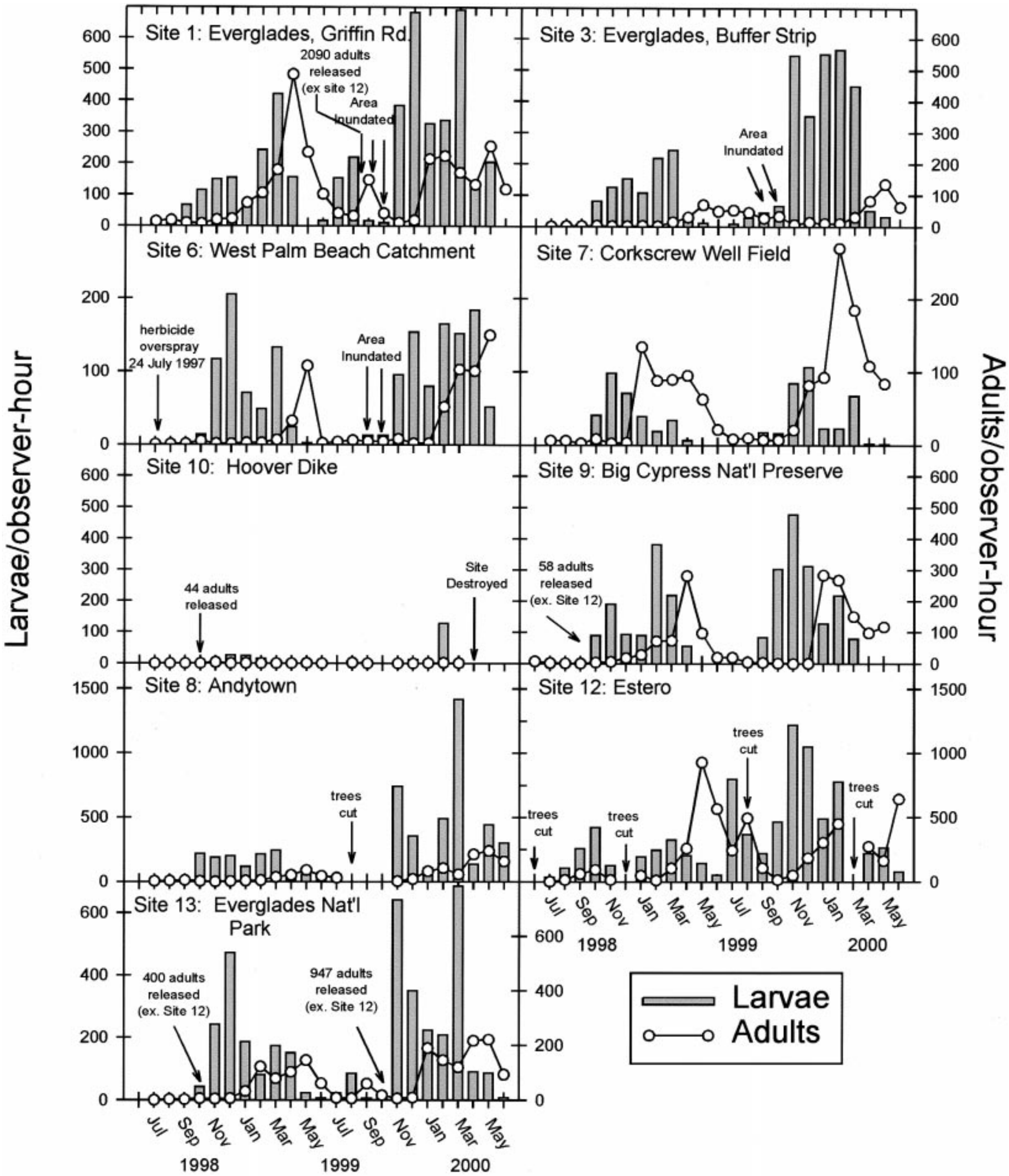

FIG. 4. Data for numbers of adults and larvae counted during 30-min periods at 9 of the 13 original *Oxyops vitiosa* release sites. Supplemental releases indicated on the graphs are not included in Table 1.

However, larvae (mid-instars) were later found on saplings that had been damaged (during June) by vehicles and had subsequently sprouted new shoots from points of injury. The only larvae found at Site 1 during the summer months were on these trees. Eggs and larvae can also be found during summer in Australia when trees produce an unseasonable abundance of young foliage following defoliation by the melaleuca sawfly, *Lophyrotoma zonalis* Rohwer (Hymenoptera: Pergidae) (Purcell and Balciunas, 1994).

This phenomenon was further illustrated at Site 12 (Estero, Lee County), which consisted of 8.1 ha of former pineland that had been converted to pasture. It was once heavily infested with melaleuca but the field had been cleared, leaving numerous cut stumps. Coppices had regrown from the stumps but periodic mowing (September 1997; July and December 1998; August and December 1999; and March 2000) maintained the presence of young foliage. Although the pasture was mowed 1 month before the first release, the intended release area was left unmowed, which created an "island" of taller trees surrounded by thousands of cut stumps.

Adult presence was detected 36 days after the initial release of larvae. By 13 February 1998, very little activity was evident within the taller (2–3 m) trees prevalent in the release area, but numerous larvae were at the edge of the “island” and on regrowth arising from the cut stumps, some as far as 40 m from the nearest point of release.

Each time that the pasture was mowed, the weevil population declined temporarily. The cut stumps then coppiced profusely, producing abundant new foliage, and the insect population quickly recovered and increased to unprecedented levels (Fig. 4). As a result, we were able to collect 2745 adults and 1019 larvae for release at other areas during 1998, 19,074 adults during 1999, and 13,698 adults and 2895 larvae during the first 3 months of 2000. This was, by far, the most productive site for the weevil populations and we ascribe this productivity to the proliferation of young shoots after each mowing.

During May 1999 we counted nearly 1000 adults per observer-hour or 1 weevil every 3 s. These counts were over 12 times higher than the peak count (76 adults per observer-hour) recorded during similar surveys conducted in Australia (M. F. Purcell *et al.*, unpublished report). Similarly, larval counts were nearly triple (November 1999) the highest count observed in Australia (1220 vs 426 larvae per observer-hour). Several factors account for these differences. *O. vitiosa* larvae suffer heavy parasitism in Australia (Purcell and Balciunas, 1994), mostly by *Anagonia* sp. (Diptera: Tachinidae). In contrast, parasites have yet to be observed in Florida. This lack of density-dependent regulatory mechanisms, when coupled with abundant resource availability, likely contributes to the much larger densities present in Florida. Furthermore, nonannual climatic change associated with the El Niño Southern Oscillation makes resource availability less predictable in Australia (Flannery, 1995). Hence, populations must persist through long, unfavorable periods during which the trees produce very little new foliage (M. F. Purcell, unpublished data). The insects must then respond quickly when new foliage briefly becomes available. The characteristics that enable *O. vitosa* to overcome unpredictable resource availability enable it to flourish in Florida, where the climate is more predictable, resources are more constant, and unfavorable periods are relatively brief.

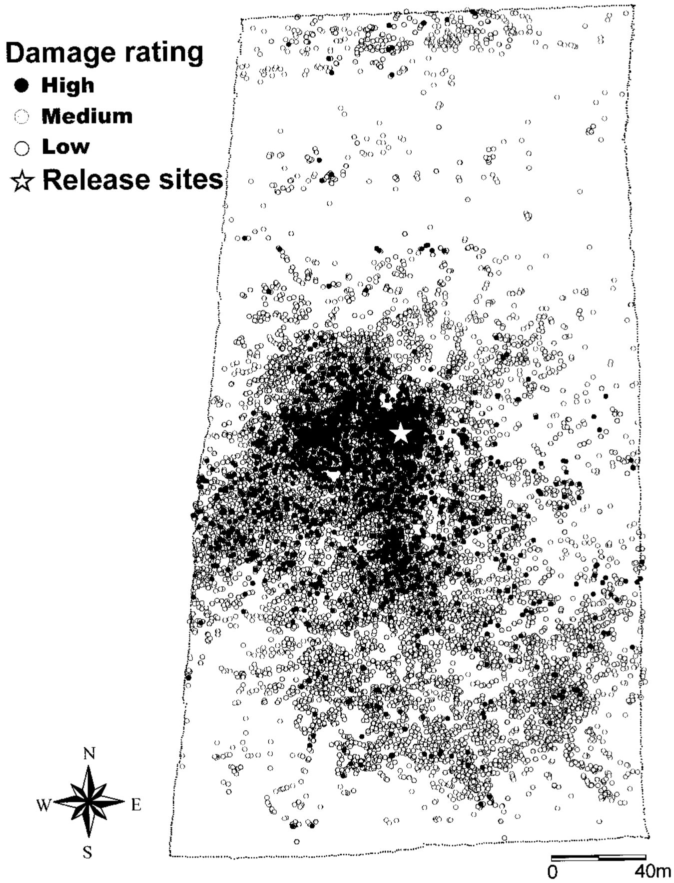

FIG. 5. The distribution and relative severity of damaged melaleuca clumps at the Estero pasture (Site 12) during October 1998. The white star near the center of the site map denotes the location of the three plots into which all insects were released.

Phenology of Established Weevil Populations

We made more consistent observations beginning in June 1998 by conducting timed counts (Fig. 4). These counts were comparable among sampling dates within a site but not among sites due to structural differences in the melaleuca infestations. Counts in mature forests, for example, were made at the forest edge, generally on lower branches adjacent to road cuts but not in the tops of tall trees where large weevil populations may have resided. This may account for increases in the adult population at some sites despite the lack of high larval counts during the preceding periods (e.g., Site 7 in Fig. 4). Unobserved larvae probably resided in the tree tops. These possibly dropped to the ground to pupate and the resultant adults remained, at least for a while, on the lower branches so as to become temporarily observable during ground-level counts. This might also account for the unexpected scarcity of adults following periods of larval abundance (e.g., Sites 3, 6, and 8) if the adults dispersed to higher strata soon after emerging.

In contrast, entire plants were searched at sites where the trees were smaller and more accessible. In these instances, large larval populations almost always presaged (by about 2 months) peaks in adult populations. Thus, the proportion of the population sampled depends upon the accessibility of immature foliage, which differs considerably depending upon the size and density of the trees. This limitation must be borne in mind when comparing among sites. Nonetheless, the most successful weevil populations, based on the highest adult counts, became established at Sites 1, 9, 12, and 13. All were characterized by short hydro-

FIG. 6. An example of the damage (right) caused by *Oxyops vitiosa* feeding on melaleuca.

periods and relatively open conditions (i.e., numerous small plants, discontinuous canopies, etc.).

Within-Site Distribution of Weevil Populations

Dispersal away from release areas was slow. For example, larvae were found about 200 m north and about 60 m west of the release point at Site 1 about 1 year after the first release (25 March 1998). The distribution remained at this limit 1 year later (2 April 1999) despite the fact that the population had increased tremendously (Fig. 4). This was also true at Site 7, which comprised a mature stand of tall trees. It was bisected by a dirt road which provided a cleared area along the edge of the forest. The weevils were consistently found on new growth along this edge, but they had not moved appreciably from release points even after $1\frac{1}{2}$ years.

This phenomenon is further illustrated by results at the Estero site (Site 12). Figure 5 shows the distribution of damaged plants by severity rating during October 1998. The map encompasses an area of 8.1 ha which contained an estimated 51,360 cut melaleuca stumps that had coppiced profusely. Nearly 13,000 (25%) of the coppices had been fed upon by *O. vitiosa*. Feeding damage on about half (53%) of these plants was insignificant ("low," generally consisting of nibbling on one or a few tips), whereas that on about a third (31%) was extensive ("moderate," extensive damage to several stem tips). The remaining one-sixth (16%) were rated as "high" with almost all of the young foliage destroyed (Fig. 6). Transect sampling estimated that the site contained over 2000 adults and more than 22,000 larvae, with eggs present on over 3600 clumps.

Even though the weevil population had spread throughout the pasture, the greatest concentrations persisted nearest the release points, as indicated by the distribution of clumps with the most severe damage. Transect data presented in Fig. 7 illustrate this point. The transects revealed that during October 1998 adult weevils as well as eggs and larvae were most numerous on relative transect position 50 (i.e., at 50% of the length of the field as measured north to south;

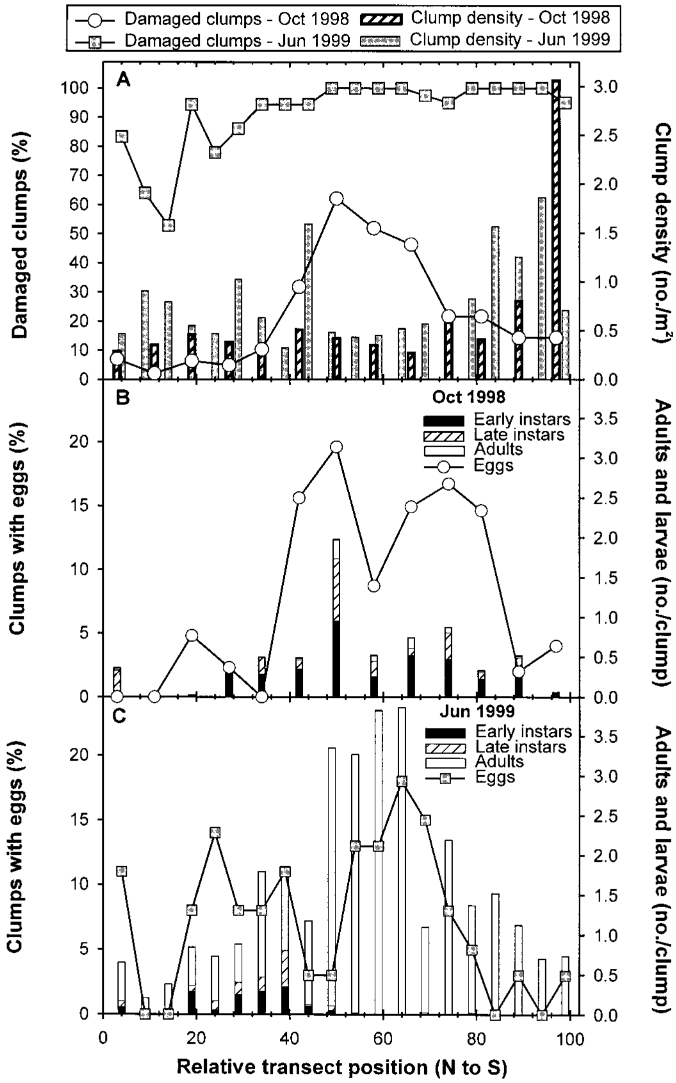

FIG. 7. Transect data taken during October 1998 and June 1999 at the Estero pasture showing numbers of insects found per clump (adults and larvae), percentage of clumps with eggs, and percentage of the clumps within each transect that had been damaged by feeding. The data for each transect represent summaries of 41 to 50 plants.

Fig. 7B), which intersected the release area, and on transects immediately to the south. Also, this transect contained the highest percentage of damaged clumps, with adjacent southern transects only somewhat lower (Fig. 7A).

Surprisingly, not much movement was detected to the north, as evidenced by the low frequency of eggs and the low densities of larvae, adults, and damaged clumps on the five most northerly transects (Figs. 7A and 7B). Hence, it seemed that the population was dispersing mainly toward the south. Reasons for this biased movement are unclear, but the prevailing winds seemed to come from the south, so they might have been orienting upwind. Alternatively, this area provided slightly higher ground, so the infestation might have been following the drier "ridge." The heaviest damage occurred along this ridge for a distance of at least 100 m south of the release plots.

This dispersal pattern persisted the following June even though the population increased to an estimated 79,704 adults and 15,424 larvae on 54,523 clumps after the clumps recovered from being mowed. Transects intersecting the release area averaged 3 to 4 adults per clump, whereas those to the north and south generally averaged less than 2 adults per clump, with the lowest numbers at the northern end of the field (Fig. 7C). Highest numbers of larvae, however, were immediately north of the release area, with very few at the southern end of the field. This reflected the distribution of the healthy stem tips available to the larvae. Most clumps within the release area and to the south were heavily damaged (>50% of the tips destroyed), whereas those to the north showed light damage. The population was estimated to contain 82,948 adults and 137,304 larvae by 12–13 January 2000, when transects were last inspected. All but 2 of the 640 plants examined exhibited damage. However, by this time the largest concentrations of adults and larvae were at the east side of the field next to a ditch bordered by taller trees, rather than near the release plots.

Dispersal occurred most rapidly where the trees were scattered savannah-like in open areas. The release at Site 3, made during April 1997 in the understory of a dense stand of mature trees, seemed to have failed at first. Four months later (October 1997), however, larvae and eggs were found 30 m south of the release point, but not at the actual point of release. This adjacent area was very open, with several outlying melaleuca trees scattered among sawgrass and wax myrtle. Two months later, the weevils were found 100 m southwest of the release point. By March 1999 they occurred several hundred meters to the north. This reflected dispersal from unsuitable trees (tall, dense stands with a paucity of young foliage) onto trees which provided acceptable foliage (smaller, bushier, open-grown trees with an abundance of young foliage). Hence, even though the actual release site was classified as dense mature melaleuca (DMM), establishment actually occurred in an area that would have been classified as P50. Nonetheless, the dispersal rate within this area was relatively slow, considering the time and distances involved.

CONCLUSIONS

The melaleuca weevil *O. vitiosa* is now well established in southern Florida, with populations thriving and increasing in abundance. They readily established when site conditions were suitable. Short hydroperiod habitats with intermediate stages of melaleuca invasion (P50) and dry winter conditions enhanced success, whereas populations failed at fully aquatic sites. Even relatively small releases (60 adults) produced nascent populations when site conditions were favorable. Larvae predominated during October to May, coincident with abundant young foliage, whereas generally only adults were present during summer when young foliage was scarce. Dispersal has been slow, with most populations remaining concentrated at or near release points during the first 2 years. Ease of establishment and slow dispersal suggests an optimal strategy of many small releases at carefully selected but widely dispersed sites. We are therefore continuing to make numerous small releases over the entire south Florida range of melaleuca to ensure maximum distribution of weevil populations. Future studies will be aimed at evaluating the ability of this insect to influence the dynamics of melaleuca populations.

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REFERENCES

Balciunas, J. K., Burrows, D. W., and Purcell, M. F. 1994. Field and laboratory host ranges of the Australian weevil, *Oxyops vitiosa* (Coleoptera: Curculionidae), a potential biological control agent for the paperbark tree, *Melaleuca quinquenervia*. *Biol. Control* **4,** 351–360.

Boland, D. J., Brooker, M. I. H., Chippendale, G. M., Hall, N., Hyland, B. P. M., Johnston, R. D., Kleinig, D. A., and Turner, J. D. 1987. "Forest Trees of Australia." Nelson Wadsworth, Melbourne, Australia.

Costanza, R., d'Arge, R., de Groots, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R. V., Paruelo, J., Raskin, G. G., Sutton, P., and van den Belt, M. 1997. The value of the world's ecosystem services and natural capital. *Nature* **387,** 253–260.

Craven, L. A. 1999. Behind the names: The botany of tea tree, cajeput and niaouli. *In* "Tea Tree: The Genus *Melaleuca*" (I. Southwell and R. Lowe, Eds.), pp. 11–28. Harwood Academic, Amsterdam.

Diamond, C., Davis, D., and Schmitz, D. C. 1991. Economic impact statement: The addition of *Melaleuca quinquenervia* to the Florida prohibited aquatic plant list. *In* "Proceedings of the Symposium on Exotic Pest Plants" (T. D. Center, R. F. Doren, R. L. Hofstetter, R. L. Myers, and L. D. Whiteaker, Eds.), pp. 87–110. Tech. Rpt. NPS/NREVER/NRTR-91/06, U.S. Dept. Interior/National Park Service, Washington, DC.

ESRI. 1996. "ArcView GIS, ver. 3.0a." Environmental Systems Research Institute Inc., Redlands, CA.

Ewel, K. C. 1990. Swamps. *In* "Ecosystems of Florida" (R. L. Myers and J. J. Ewel, Eds.), pp. 281–323. Univ. of Central Florida Press, Orlando.

Flannery, T. F. 1995. "The Future Eaters. An Ecological History of the Australasian Lands and People." Braziller, New York.

Laroche, F. B. (Ed.). 1994. "Melaleuca Management Plan for Florida—Recommendations from the Melaleuca Task Force, Exotic Pest Plant Council." South Florida Water Management Dist., West Palm Beach, FL.

Laroche, F. B. 1998. Managing melaleuca (*Melaleuca quinquenervia*) in the Everglades. *Weed Technol.* **12,** 726–732.

Laroche, F. B., and Ferriter, A. P. 1992. The rate of expansion of melaleuca in south Florida. *J. Aquat. Plant Manage.* **30,** 62–65.

O'Hare, N. K., and Dalrymple, G. H. 1997. Wildlife in southern Everglades wetlands invaded by melaleuca (*Melaleuca quinquenervia*). *Bull. Fla. Mus. Nat. Hist.* **41,** 1–68.

Purcell, M. F., and Balciunas, J. K. 1994. Life-history and distribution of the Australian weevil *Oxyops vitiosa* (Coleoptera: Curculionidae), a potential biological control agent for *Melaleuca quinquenervia* (Myrtaceae). *Ann. Entomol. Soc. Am.* **87,** 867–873.

Schmitz, D. C., Simberloff, D., Hofstetter, R. H., Haller, W., and Sutton, D. 1997. The ecological impact of nonindigenous plants. *In* "Strangers in Paradise: Impact and Management of Nonindigenous Species in Florida" (D. Simberloff, D. C. Schmitz, and T. C. Brown, Eds.), pp. 39–61. Island Press, Washington, DC.

Smith, R. L. 1966. "Ecology and Field Biology." Harper & Row, New York.

Sokal, R. R., and Rohlf, F. J. 1981. "Biometry," 2nd ed. Freeman, New York.

SPSS. 1997. "SigmaStat Statistical Software, ver. 2.03" SPSS Inc., Richmond, CA.

Turner, C. E., Center, T. D., Burrows, D. W., and Buckingham, G. R. 1998. Ecology and management of *Melaleuca quinquenervia,* an invader of wetlands in Florida, U.S.A. *Wetl. Ecol. Manage.* **5,** 165–178.

Wheeler, G. S., and Center, T. D. 1996. The influence of hydrilla leaf quality on larval growth and development of the biological control agent *Hydrellia pakistanae* (Diptera: Ephydridae). *Biol. Control* **7,** 1–9.