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EVALUATING ACEPHATE FOR INSECTICIDE EXCLUSION OF *OXYOPS VITIOSA* (COLEOPTERA: CURCULIONIDAE) FROM *MELALEUCA QUINQUENERVIA*

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ABSTRACT

One method of evaluating the impact of insect weed biological control agents is to exclude them from their host with insecticides, thereby enabling comparisons of host fitness between infested and non-infested plants. However, the insecticide must not positively or negatively affect the plant being protected. The insecticide acephate was tested for its effects on *Oxyops vitiosa* Pascoe and *Melaleuca quinquenervia* (Cav.) S. T. Blake. Saplings of *M. quinquenervia* were sprayed with concentrations of 0, 0.073, 0.36, and 0.73% a.i. acephate every 7, 14, and 21 days. A bioassay using leaves from sprayed plants and third instars of *O. vitiosa* found reduced defoliation up to 21 days after treatment at the 0.36 and 0.73% concentrations of acephate. There were minor phytotoxic effects on younger, more tender leaves at the 0.73% concentration of acephate which reduced leaf biomass. Acephate can protect *M. quinquenervia* foliage from *O. vitiosa* larvae at the 0.36% concentration and spraying every 14 days will not affect the plant.

Key Words: Biological Control, weeds, bioassay, phytotoxicity

RESUMEN

Un método para evaluar el impacto de las agentes de control biológico de malezas es excluirlos de su hospedero con insecticidas, en esta manera se permite las comparaciones del la adaptabilidad óptima reproductiva del hospedero en plantas infestadas y no infestadas. Sin embargo, la insecticida no debe afectar positivamente o negativamente la planta que esta siendo protegida. La insecticida acephate fué probado por sus efectos sobre *Oxyops vitiosa* Pascoe y *Melaleuca quinquenervia* (Cav.) S. T. Blake. Vástagos de *M. quinquenervia* fueron rociadas con concentraciones de 0, 0.073, 0.36 y 0.73% i. a. acephate cada 7, 14, y 21 dias. Se encontraron que un bioensayo utilizando hojas de plantas rocidas y la estadia tercera de *O. vitiosa* redujó la defoliación hasta 21 días después del tratamiento a las concentraciones de 0.36 y 0.73% de acephate. Hubian efectos menores fitotóxicos sobre las hojas más jovenes y tiernas a la concentración de 0.73% de acephate que reducieron la masa biológico de las hojas. Acephate puede proteger el follaje de *M. quinquenervia* de larvas de *O. vitiosa* a la concentración de 0.36% y aplicandolo cada 14 dias sin afectar la planta.

Evaluation of the impact of introduced biological control agents on their weed targets is an essential component of any classical biological control project (Smith & DeBach 1942). Objective analyses can provide insights or evidence as to why agents succeed or fail, present justification for future projects, and may contribute to decisions affecting the future direction of the project (Farrell & Lonsdale 1997).

There are a number of techniques for conducting field evaluations, each with their own advantages and disadvantages. They include: exclusion of the biological control agent using cages, hand removal, or insecticides; inclusion of agents using cages; correlation of agent numbers with host fitness; pre- and post-release comparisons of host fitness; and selective releases of the agent (Adair & Holtkamp 1999).

Oxyops vitiosa (Coleoptera: Curculionidae) was released in south Florida during 1997 as part of a classical biological control project for the invasive Australian tree *Melaleuca quinquenervia*, which infests 200,000 ha in the Everglades and surrounding conservation areas (Center et al. 2000). Adults prefer to feed on fresh buds and leaves that have not fully expanded. Eggs are laid singly or in groups on stems and leaves and the larvae typically defoliate entire tips of new vegetation. Adults actively oviposit and larvae are present year round but are most abundant October through March, coincident with the annual flush of foliage produced by the trees.

Evaluating the impact of *O. vitiosa* in this system by excluding them with cages would be difficult given the rapid rate of growth of the trees, their large size, and shading effects. Hand removal of insects is similarly impractical. Populations of *O. vitiosa* are now widespread and increasingly fewer suitable control sites are now available for comparing infested versus non-infested areas. A more practical evaluation method for this system would be insecticide exclusion be-

cause of the aforementioned problems and the fact that *O. vitiosa* is the only significant herbivore feeding on the plant, thereby avoiding any interpretation errors caused by generalist herbivores (Annecke et al. 1969). Potential disadvantages of this approach include negative effects of the insecticides on the plant such as phytotoxicity and interference with pollination, or positive effects such as stimulation of growth (Jones et al. 1986).

Identifying and evaluating an insecticide to exclude feeding by *O. vitiosa* was the objective of this study. We considered factors like cost, availability of systemic formulations for large tree studies, environmental fate, and mammalian toxicity. On the basis of these criteria we selected acephate, an organophosphate compound first introduced in 1969 and used in many crops (Thomson 1982). It is readily available, inexpensive, with relatively low mammalian toxicity and an antidote (Spencer 1981). Acephate has a half life of <3 days and 6 days in aerobic and anaerobic soils, respectively (Thomson 1982). It is also rapidly absorbed into leaf tissue when applied foliarly. For example less than 25% of the acephate remained on the surface of cotton leaves 24 h after application (Bouchard and Lavy 1982). Finally, there is a commercially available formulation for protecting large trees using implantable cartridges of acephate (AceCaps™, Creative Sales, Inc., Fremont, NE).

Materials and Methods

The experimental design was a randomized complete block with 12 treatments and four blocks (replications). Acephate (9.4% a.i.) was applied at four concentrations, 0, 0.073% a.i., 0.367% a.i., and 0.73 a.i.% to the plant leaves until runoff. Water was applied to controls. Applications were made with a hand-pressurized garden sprayer with approximately 0.1 liter of finished product applied to each plant. Application frequencies were once every 7, 14, or 21 days. The same plants were sprayed up to five times (7 day frequency) under the same regime of acephate concentration and application frequency.

Test *M. quinquenervia* trees originated from greenhouse-grown plants with similar trunk widths that were cut back to equalize heights, number of branches, and root mass. Plants were then placed in water for three weeks before repotting. The following data from each randomly numbered sapling was recorded before transplantation to pots: stem diameter at mid-height, height, number of buds, and fresh weight. Once planted in pots, plants were fertilized with 52 g of 13-13-13 controlled release fertilizer and randomly assigned to a treatment.

Twelve potted plants were placed randomly into each of four 950 liter concrete tanks (blocks) equipped with a continual water exchange system that completely replaced the volume of each tank about 4 times per day. This was designed to flush away any pesticide residues which might contaminate adjacent plants. Distance between pots was maximized. Plants were removed from the tanks, treated with the appropriate concentration of insecticide, and allowed to dry. The outside of the pots was washed with water to remove any residues, allowed to dry, then placed back into the tank.

Table 1. Means (±SE) of bioassay responses of *Oxyops vitiosa* larvae to leaves of *Melaleuca quinquenervia* sprayed with acephate.

Variable	DAT[1]	Acephate concentration (% a.i.)			
		0	0.073	0.367	0.73
		%			
Defoliation (24 h)	1	66.5 ± 6.6	39.0 ± 5.6	11.7 ± 1.4	11.0 ± 1.3
	7	57.5 ± 6.6	24.6 ± 4.2	20.6 ± 3.1	14.8 ± 1.8
	14	68.3 ± 4.3	58.3 ± 4.4	25.3 ± 5.1	28.3 ± 4.3
	21	75.0 ± 8.6	68.7 ± 11.7	45.0 ± 10.6	35.0 ± 9.3
Defoliation (48 h)	1	76.0 ± 5.9	42.2 ± 5.2	13.5 ± 1.7	11.0 ± 1.6
	7	67.7 ± 6.2	32.2 ± 4.9	22.5 ± 3.2	14.9 ± 1.8
	14	78.7 ± 4.4	68.7 ± 4.6	47.9 ± 6.1	33.7 ± 5.8
	21	90.0 ± 0.1	85.0 ± 2.0	61.2 ± 9.6	42.5 ± 13.6
		number dead after 48 h			
Larval Mortality	1	2.1 ± 0.3	2.0 ± 0.2	3.2 ± 0.3	3.3 ± 0.3
	7	2.3 ± 0.4	2.8 ± 0.2	2.9 ± 0.3	3.3 ± 0.3
	14	3.1 ± 0.2	3.5 ± 0.2	3.1 ± 0.3	3.4 ± 0.3
	21	1.2 ± 0.2	3.0 ± 0.0	2.0 ± 0.1	3.7 ± 0.1

[1]Days after treatment.

The first acephate application was done on Feb. 21, 2001 when at least five fully expanded new leaves were present on all plants. Plants were fertilized as before on March 1, 2001. Bioassays for the toxicity of treatments to 3rd instar *Oxyops vitiosa* were conducted weekly by removing four suitable leaves from each plant, including those plants whose leaves were not going to be tested that week, in order to equalize the total number of leaves removed from all test plants. Fine-bladed scissors were used to cut through the petioles of selected leaves. Suitable leaves, preferred by larvae, were those that were fully formed but still supple and soft. Two of the four leaves were used to assess leaf toughness using a penetrometer (Halda Gram Gauge, Stockholm, Sweden). Leaves were held on a plexiglass stage that prevented movement and provided a guide

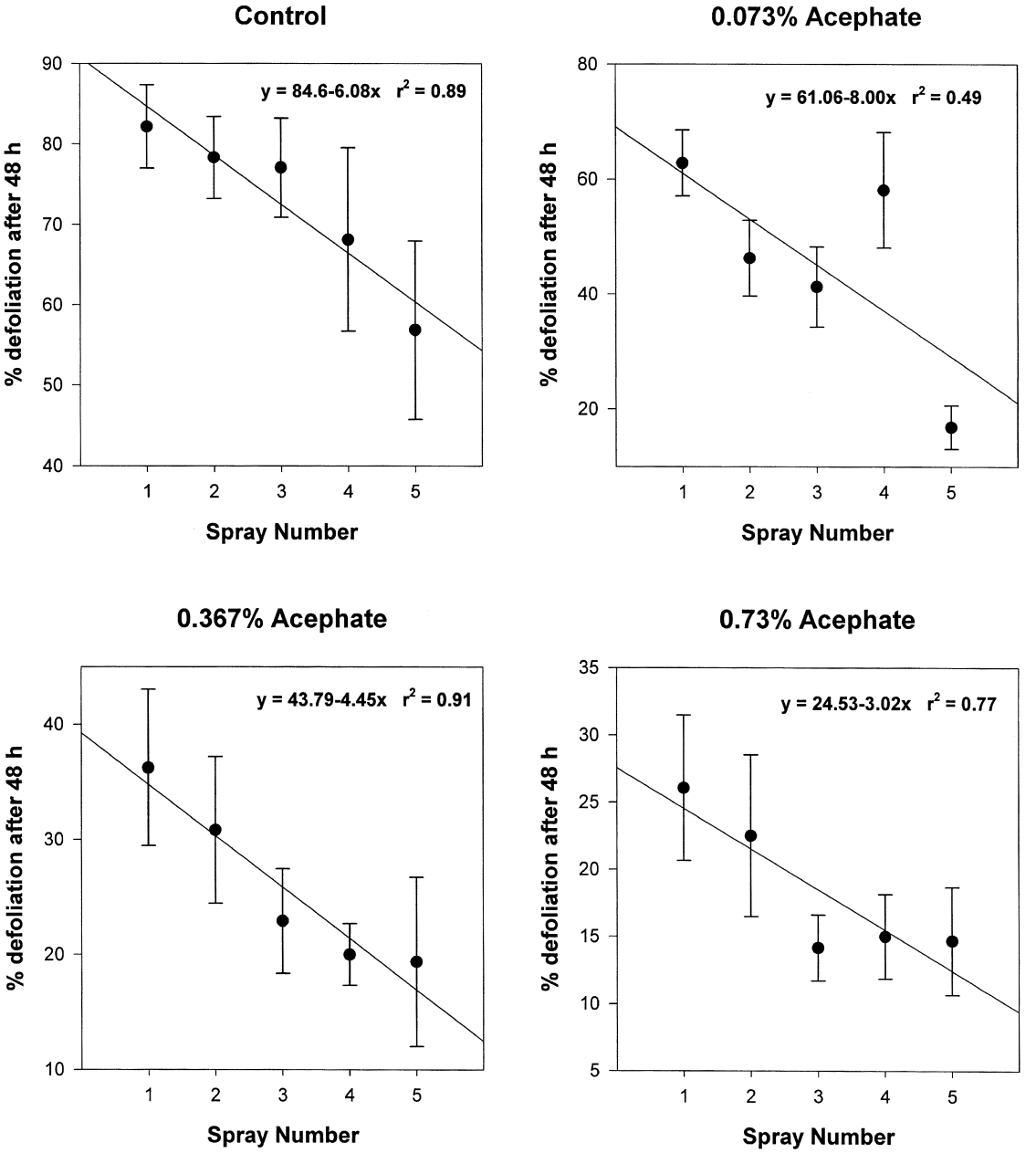

Fig. 1. Average defoliation by *Oxyops vitiosa* after 48 h of *Melaleuca quinquenervia* leaves treated with multiple applications of four concentrations of acephate. Vertical lines denote standard error of the mean.

hole for the stylus of the penetrometer (Wheeler and Center 1996). Readings were taken at the mid-point along the long axis of the leaf, immediately adjacent to the midrib.

The other two leaves were used for bioassays and placed side-by-side on moistened filter paper in a standard 15 cm petri dish. The length and width of each leaf was recorded to estimate leaf area which was calculated by the formula length × width × 0.8. Five field collected 3rd instars of *O. vitiosa* were placed on the leaves and the dishes were sealed with parafilm and held in an environmental chamber at 27°C and 12:12 (L:D) photoperiod. After 24 and 48 h, the number of larvae on plants, the number of dead larvae, and percent defoliation to the nearest 10% was recorded.

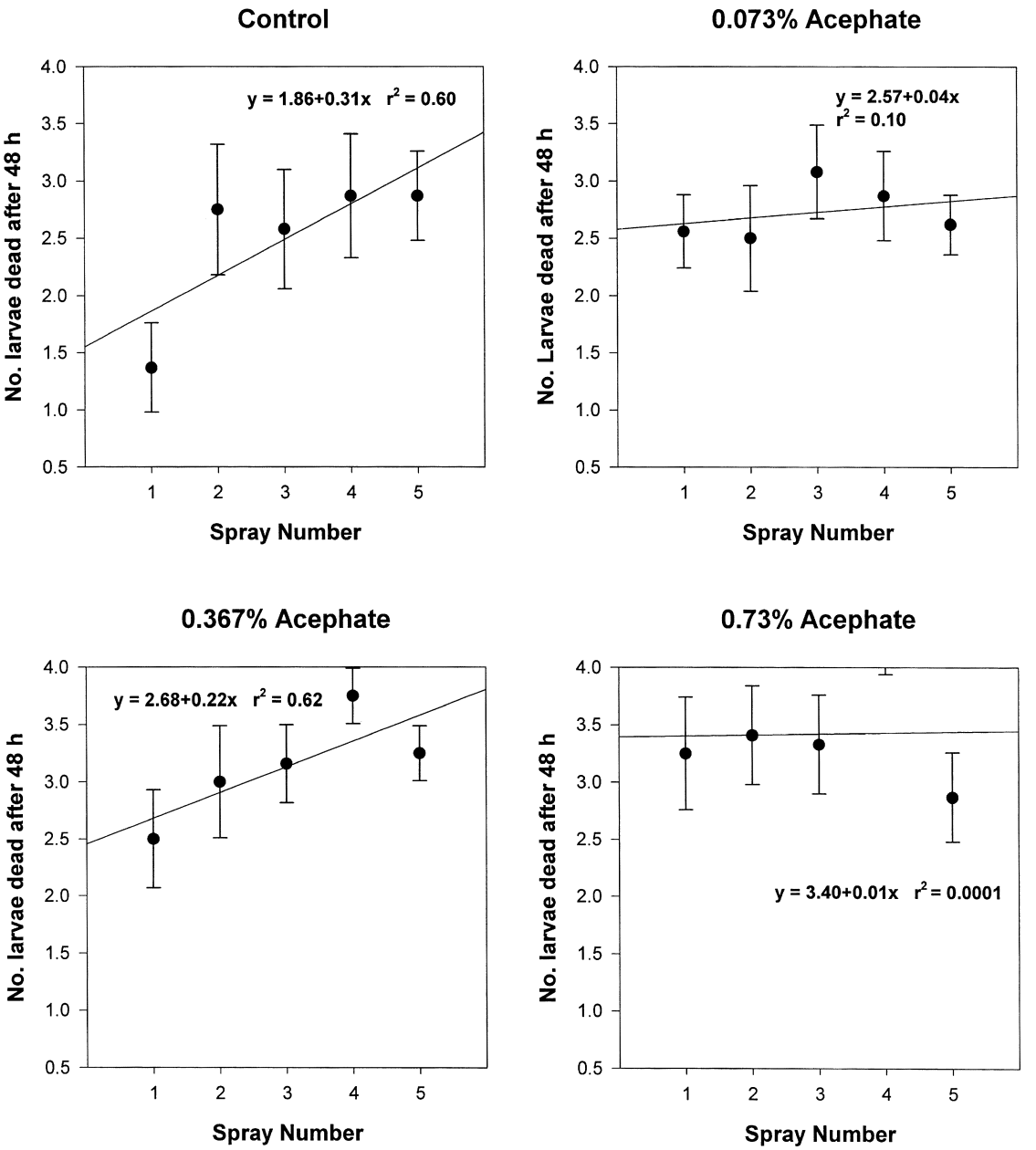

Fig. 2. Average number of dead larvae of *Oxyops vitiosa* 48 h after exposure to *Melaleuca quinquenervia* leaves treated with multiple applications of four concentrations of acephate. Vertical lines denote standard error of the mean.

Defoliation was estimated visually by two evaluators and the mean estimate was recorded. Larvae were considered dead if they did not move after gentle prodding with a brush. At the end of the test, the larvae were removed and leaves were dried at 50°C for 48 h and weighed.

Bioassays were conducted 1, 7, 14, and 21 d after treatment (DAT). The experiment was terminated on April 3, 2001 and the plants were removed from the pots and the soil washed from the roots. Leaves were removed and counted, and above and below ground portions of the plants were dried and weighed separately.

Data on plant effects were analyzed with repeated measures analysis of covariance with initial plant height, width, and the number of buds as the covariates (SAS Institute 1990). Main factors in the analysis were insecticide treatment, application frequency, and the number of applications. Data on insect effects were analyzed with repeated measures analysis of variance with insecticide treatment, application frequency, DAT, and the number of applications as main effects. Values were transformed using square root transformation when variances were heterogeneous. Simple linear regression was used to examine relationships among plant and insect responses to application frequency, number of treatments, and insecticide concentrations.

Results and Discussion

Acephate protected *M. quinquenervia* foliage from feeding by *O. vitiosa* larvae. Defoliation after 24 and 48 h was affected by both the treatment and the number of days after treatment (F = 102.8; df = 3, 189; $P < 0.0001$ and F = 31.8; df = 3, 189; $P < 0.0001$ for treatment and DAT, respectively, after 48 h) (Table 1). The number of larvae found dead after 48 h was not a useful indicator of protection because the larvae in the non-sprayed control consumed most of the plant tissue after 24 h and may have suffered stress from a lack of food after then. As a result, it's difficult to discern whether larval mortality was caused by the insecticide treatment or the lack of food. The same was true of the number of larvae found on leaves after 24 and 48 h (data not shown). It did not appear that larvae were repelled by the insecticide before feeding because most of the leaves showed some feeding scars.

In addition to the acephate concentration, increasing the number of applications affected defoliation and larval death (F = 3.34; df = 4, 189; P = 0.01 and F = 4.01; df = 4, 189; P = 0.003 for defoliation and the number of dead larvae after 48 h, respectively). Defoliation declined with increasing application number (Fig. 1). Larval death increased as the number of applications increased in two of four treatments, most notably the control, indicating that factors other than insecticide toxicity were involved (Fig. 2). These results may be explained by the increased toughness of the leaves as the experiment progressed (F = 7.54; df = 2, 49; P = 0.001) (Fig. 3). As the number of sample dates increased and plants were repeatedly sampled, a larger percentage of the leaves used in bioassays were from more proximal positions on the branches, with concomitant increases in leaf toughness. This effect was magnified in the higher concentration treatments which caused some phytotoxic effects on the tips, resulting in burned and mis-shapened leaves, thereby forcing the selection of more proximal leaves for the bioassay. Although the leaves were more proximal, they were still suitable for normal larval growth and development.

Leaf biomass was the only plant factor affected by acephate. The highest concentration of acephate caused reduced leaf biomass, primarily through the previously mentioned phytotoxic effects (F = 4.2; df = 3, 46; P = 0.01). Stem and root biomass, leaf number, leaf toughness, and trunk width were unaffected by the concentrations of acephate used in this study.

The general use insecticide acephate proved to be lethal against larger larvae of *O. vitiosa* and was persistent for several weeks in the foliage of *M. quinquenervia* saplings. Applications of this compound at the 0.36% concentration every 14 d should protect the foliage of *M. quinquenervia* from *O. vitiosa* without damaging the plant. A similar period of insect protection was found by Azarbayjani et al. (1999) for *M. linariifolia* using permethrin, a non-systemic synthetic pyrethroid. Significant larval populations are present on *M. quinquenervia* in south Florida for about 6 months. Therefore, excluding *O. vitiosa* larvae from saplings and small trees for evaluation purposes using acephate may require up to twelve applications a year.

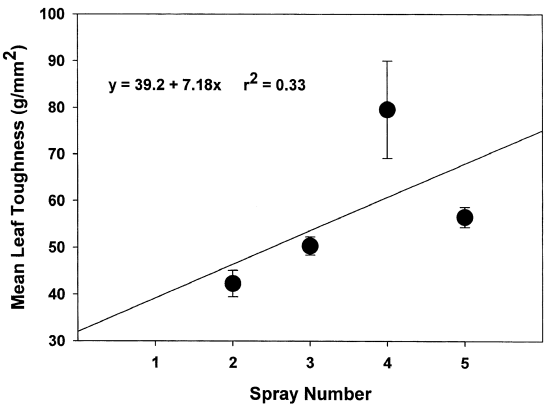

Fig. 3. Mean leaf toughness of *Melaleuca quinquenervia* leaves after multiple applications of acephate.

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