EFFECT OF ENZONE™ AS A SOIL FUMIGANT ON SURVIVAL OF VARIOUS DEVELOPMENTAL STAGES OF *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE) IN CONTAINER-GROWN CITRUS

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Diaprepes abbreviatus L., a root weevil native to the Caribbean region (Woodruff 1985), has become an important pest of citrus and ornamental plants in Florida since its introduction over 30 years ago (McCoy 1999). Both the adult and larval stages of *D. abbreviatus* are polyphagous, feeding on the leaves and roots of about 270 plant species (Simpson et al. 1996). Plants that support larval development to pupation, a period of 5-15 months, include citrus, sugarcane, various woody ornamentals, and several agronomic crops (Schroeder et al. 1979). Injury to citrus by the adult is characterized by a notching of the leaf margin while larval feeding destroys fibrous roots and the bark of the tap, lateral, and crown roots (Quintela et al. 1998). *D. abbreviatus* is a univoltine species with the adult, egg, and neonate stages appearing on the host plant aboveground, whereas all larval stages, pupae, and teneral adults reside belowground (Wolcott 1933).

The potential economic impact of *D. abbreviatus* on commercial growers of citrus and ornamental plants in nurseries and the field is significant. An estimated loss of $75 million annually has been reported from tree decline and lost production in open forum among citrus growers (Diaprepes Task Force 1997). About 100 commercial plant nurseries are infested throughout Florida. Sale of infested trees from nurseries offer one of many ways by which the weevil can be disseminated throughout the state. It is imperative that these nurseries do not sell liners infected with weevil larvae. All weevil-infested nurseries operate under a compliance agreement with the Florida Department of Agriculture and Consumer Services that regulates the movement of nursery stock (McCoy 1999). Larval control in infested nurseries must be performed using an approved chemical or mechanical treatment of potting media in containers. Currently, bifenthrin, formulated as Talstar® 10 WP and Talstar T & O granular are the only approved regulatory chemical treatments (McCoy et al. 1995). Talstar® is a soil barrier treatment applied to prevent neonate invasion of the soil. It is, however, ineffective against later instars already infesting the plant roots.

To find a soil treatment effective against all developmental stages of *D. abbreviatus*, two greenhouse studies were initiated to determine the effect of sodium tetrathiocarbonate (Enzone™ 31.8%) as a soil fumigant on all developmental stages of *D. abbreviatus*. The active ingredient decomposes in the soil environment to release carbon disulphide, a broad spectrum biocide of plant parasitic nematodes, grape phylloxera and some soil fungi (Hinds 1902, Young 1990, Weber et al. 1996). The formulation exhibits very low phytotoxicity and is environmentally benign.

In both tests, 3-year-old Marsh grapefruit (*Citrus* × *paradisi* Macfad) trees grafted to Swingle Citrumelo rootstocks (× *Citron cirus* 'Swingle') were bare rooted and pruned lightly for transplanting into 15 liter plastic containers. Each tree was planted in sieved Candler soil (Entisol type; 92% sand, 2.9% clay, 2.0% silt) at a maximum soil depth of 26.7 cm (soil volume/pot = 0.0179 m^3, soil surface area = 670 cm^2). Trees were placed on a bench in an air-conditioned greenhouse maintained at 25.5-26.5°C, where they received regular watering and 60 ml of liquid fertilizer (8:4:8) per tree every 2 weeks. Any weeds growing on the soil surface were removed by hand. In test 1, 100 neonate *D. abbreviatus* (48 hours old) were scattered on the soil surface next to the trunk of each containerized tree each week for 6 consecutive weeks prior to treatment. In addition, five, 6th instars, were placed in each pot on four different occasions beginning at the 6th week after neonate inoculations were begun. In test 2, 100 neonates of the above age were added to the containerized trees twice at 5 and 6 weeks prior to treatment. Ten, 7th instar larvae, 3 pupae and 3 teneral adults were buried in the soil at a depth of about 7.6 cm of each pot 1 week prior to treatment.

Prior to treatment, infested and non-infested containerized trees were randomized according to treatment and replicate and removed from the greenhouse to a shaded out-of-doors site for treatment. After 20 hours, they were returned to the greenhouse. In test 1, Enzone™ was applied as a drench at rates of 500 (4.6 ml in 3.78 liters H_2O) and 1000 ppm. Initially, all containers were watered using a sprinkling can to achieve soil saturation (3.78 liters/unit).

The entomopathogenic nematodes, *Heterorhabditis bacteriophora* Poinar and *H. indicus* Poinar, Karunaker, & David supplied on a sponge (Integrated BioControl Systems, Aurora, IN), were

applied in equal number to each container as a standard at rates of 22 and 54 infective juveniles (IJ's)/cm^2 or 14,740 and 36,180 IJ's/unit. For application, the required number of IJ's were pipetted into one liter of water and sprinkled on the soil with a watering can. Prior to inoculation, nematode viability was estimated microscopically by counting the number of motile and dead IJ's in 10 fields of view at 60× mag. Both species had viabilities of 82%. Treatments including an infested and non-infested control were replicated 6 times. In test 2, Enzone™ was applied in the manner described for test 1, except five rates were tested ranging from 100 to 2000 ppm and nematodes were not applied. Treatments included an infested and non-infested control and were replicated 5 times.

From 3 to 5 days post-treatment, each tree from the infested control and Enzone treatments was removed from its container and the soil carefully washed from the roots. Soil from the container and from the roots was wet sieved (2.0 mm mesh) to recover surviving and dead larvae detectable to the naked eye. Dead larvae were diagnosed as having no movement and color change. In test 1, trees treated with nematodes and the uninfested control were processed at 14 days post-treatment in the manner described above. Many dead larvae exhibited red color typical of nematode infection or partial cadaver disintegration upon diagnosis. All live and dead larvae recovered from the soil in the Enzone treatments and the infested control had head capsule measurements performed microscopically to determine approximate instar at the termination of the test. In both tests, fibrous roots were examined for symptoms of Enzone™ phytotoxicity. None was observed.

In test 1, 203 live and dead larvae recovered from soil of the infested control had completed numerous molts according to head capsule measurements; 8.2% were categorized as 4th instar, 40.4% as 5th instar, 36.9% as 6th instar and 14.5% as 7th instar. These findings suggest that larval development from neonate to 7th instar occurred within 60 days, which agree with Quintela et al. (1998). By comparison to the uninfested control, larval injury to infested trees was uniform, with virtually no fibrous root survival and excessive bark loss (Fig. 1). Obviously, root injury occurred before Enzone™ treatment were applied to containers.

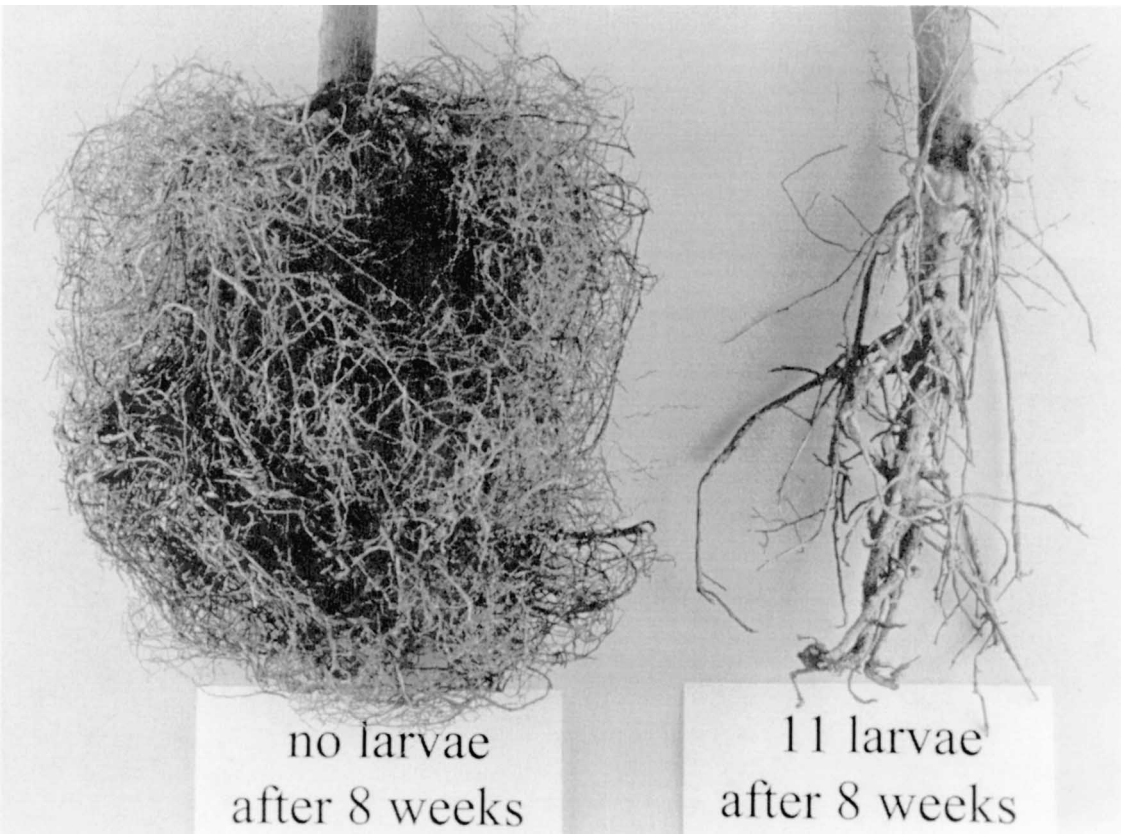

Fig. 1. A comparison of Swingle citrumelo root masses with and without larval feeding of *Diaprepes abbreviatus* after 8 weeks exposure.

TABLE 1. EFFECT OF TWO RATES OF ENZONE™ COMPARED TO ENTOMOPATHOGENIC NEMATODES ON THE SURVIVAL OF LARVAL INSTARS OF *DIAPREPES ABBREVIATUS* INFESTING CONTAINERIZED CITRUS TREES IN THE GREENHOUSE.

Treatment	Rate	No. live and dead larvae recovered	Mean % larval mortality ± SE[b]
Enzone™ (31.8%)	500 ppm	247	88.2 ± 14.0 a
Enzone™ (31.8%)	1000 ppm	202	94.3 ± 8.1 a
Mixed nematodes	22/cm^2	83	19.3 ± 8.9 b
Mixed nematodes[a]	54/cm^2	64	28.1 ± 7.8 b
Infested control	—	203	11.5 ± 6.1 b

[a]Represents mixed population of Heterorhabditis bacteriophora and H. indicus infective juveniles of equal density.
[b]Non-infested control free of larvae. Treatments replicated 6 times. Means followed by the same letter are not significantly different at the 5% level of probability via Tukey's Studentized Range (HSD) Test.

Observations made on larval distribution in soil upon tree removal showed that about 80% were within the tree rhizosphere, usually along tap root. Other larvae were found throughout the soil but not in the upper 2.5 to 5.0 cm. Saturated soil found at the bottom of the container had no apparent effect on larval distribution. Treatment means were compared using the Tukey's studentized range (HSD) test after correction by Abbott's formula (SAS Institute 1990).

As shown in Table 1, Enzone at 500 and 1000 ppm killed 88.2 and 94.3% of the 4-7th instar larvae of *D. abbreviatus*, respectively, after 72 hours and had significantly higher mortality than the mixed nematode standard and the infested control. Larval mortality from nematode parasitism was not significantly different from control mortality. Larvae were no doubt missed during the soil sieving process in all treatments; however, some nematode-infected cadavers appeared to have decomposed during the 14 days after treatment based on number recovered. If so, larval mortality is likely to have been more in the order of 60%.

In test 2, 990 live and dead larvae were recovered from all treatments. Although head capsule measurements were not made, observation suggests that most were 4th instar or older. Many pupae buried in the soil within 5 days of recovery transformed to the adult stage and teneral adult recovery was good (Table 2). The root system of all infested trees were severely damaged by *D. abbreviatus* larvae.

Adult mortality for the range of dosages tested, was not affected by Enzone™ rate and all rates were significantly higher than the infested control ($F = 21.54$, $P = 0.001$) (Table 2). Enzone™ concentrations of 500 ppm or greater killed 100% of the adults recovered from soil. Though the low number tests prevents statistical analysis, pupae were also highly susceptible to Enzone™ with 100% kill at all concentrations. Larval mortality in relationship to rate was positively linear ($R^2 = 0.671$). Mean mortality for all treatments was significantly different from the control ($F = 75.84$, $P = 0.0001$).

Data presented herein suggest that Enzone is an effective fumigant against all developmental stages of *D. abbreviatus* in infesting containerized citrus. It is imperative that the chemical fully saturate the containers from top to bottom at the time the soil is near saturation to assure complete volatization (Young 1990). This requirement could limit any field use of the fumigant; however, it has

TABLE 2. EFFECT OF DIFFERENT RATES OF ENZONE™ ON THE SURVIVAL OF VARIOUS DEVELOPMENTAL STAGES OF *DIAPREPES ABBREVIATUS* INFESTING CONTAINERIZED CITRUS TREES SWINGLE CITRUMELO ROOTSTOCK IN THE GREENHOUSE.

Treatment	Rate (ppm)	Recovery/treatment			Mean % mortality ± SE[a]		
		Larvae	Pupae	Adults	Larvae	Pupae	Adults
Enzone™ (31.8%)	2000	101	4	18	99.0 ± 0.01 a	100.0 ± 0.0	100.0 ± 0.0 a
Enzone™	1000	192	0	18	98.7 ± 0.01 a	—	100.0 ± 0.0 a
Enzone™	500	183	4	14	94.4 ± 0.02 ab	100.0 ± 0.0	100.0 ± 0.0 a
Enzone™	250	182	1	22	90.9 ± 0.02 ab	100.0 ± 0.0	95.5 ± 0.04 a
Enzone™	100	195	2	19	71.8 ± 0.05 b	100	94.7 ± 0.07 a
Infested control	—	137	0	17	12.9 ± 0.05 c	—	47.1 ± 0.08 b
Non-infested control	—	0	0	0	—	—	—

[a]Arcsin transformed means ± standard errors within a column followed by the same letter are not significantly different by ANOVA followed by Tukey's Studentized Range (HSD) Test ($P \geq 0.05$). Values based on 5 replications.

potential for quarantine use in citrus and ornamental nurseries if phototoxicity does not pose a problem.

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SUMMARY

Enzone™ at rates of 250 ppm or greater was highly effective as a soil fumigant for the near eradication of larvae, pupae and adult *D. abbreviatus* infesting container-grown citrus with no phytotoxic effect.

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