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EFERENCES CITED

Recommended methods for the detection
of resistance of agricultural pests to pes-
hod no. 12. Tentative methods for adults
potato beetle, *Leptinotarsa decemlineata*
Prot. Bull. 22: 112–116.
4 Tech. Bull. Mobay Chemical Corp. 3

nd E. B. Radcliffe. 1980. Effect of de
es. J. Econ. Entomol. 73: 131–134.
J. Gijswijt. 1973. The laboratory eval-
mising new insecticides which interfere
ition. Pestic. Sci. 4: 737–745.
E. Cancelado, and W. S. Cranshaw.
action thresholds for insect pests. Pap.
r. Minn. Agric. Exp. Stn. 2 pp.
A. Butt. 1978. Impact of *Perillus bio-*
lorado potato beetle and plant damage.
Bull. 1581. 11 pp.
mann, and W. Sirrenberg. 1980. BAY
hitin synthesis inhibitor. Z. Angew En-
7.

Study of First-Instar *Diaprepes abbreviatus* (Coleoptera: Curculionidae) Activity for Control Purposes[1]

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ABSTRACT Activity of 1st-instar *Diaprepes abbreviatus* (L.) was studied to determine their susceptibility to biological and cultural control and to obtain the minimum amount of information needed to develop a bioassay for soil insecticides against the larvae. Results showed that eggs in egg masses between leaves hatched (144 to 224 h after oviposition) before the larvae dropped to the ground (235 to 248 h after oviposition). Larvae remained on the soil surface no longer than 3 h, between 1100 and 2400 h, and penetrated soil optimally ($P = 0.05$) when its moisture was between 7 and 16.7%. They did not penetrate dry soil. Larvae that were 64 h old penetrated soil most effectively ($P = 0.05$). A prototype soil-insecticide bioassay chamber developed on the basis of these data showed that 84% of larvae penetrated 10 cm of soil in 13 days.

Diaprepes abbreviatus (L.) is a West Indian root weevil which has become established in the United States (Woodruff 1964). Under natural conditions, the weevil oviposits between leaves of various plants. First instars drop to the ground, burrow into the soil, and feed on woody root systems until they emerge as adults (ca. 1 year) (Wolcott 1936). In citrus, root feeding can kill the tree.

Since the introduction of *D. abbreviatus* into this country, the primary goals of research have been to keep the population at low levels, to limit population spread, and to eradicate the weevil if possible. First instars have been the target of control with insecticides for several reasons. Insecticides applied aerially to control adults would destroy beneficial species and, even when applied on the soil, would not reach subterranean larvae. Use of soil insecticides to prevent larvae from surviving to attack citrus roots is the best means of control. Since the ban of cyclodiene insecticides, considerable effort has been directed toward evaluating potential replacements. First instars have also been the subject of studies on biological control, primarily by predators (Whitcomb et al. 1982), because of the vulnerability of the larvae on the soil's surface.

The research reported here was undertaken to obtain specific information about the activities of 1st instars from egg hatch to soil penetration, to develop an effective bioassay for candidate insecticides in soil against such larvae, and to obtain information useful for biological and cultural control. The desired information included the optimum soil moisture for larvae to penetrate soil, penetration depth over time, and the effect of larval age on penetrating efficiency. For biological and cultural control, information on the following is important: the influence of ground cover (e.g., weeds or surface litter), light or darkness, and soil moisture on larval soil penetration; the time of day larvae drop to the soil surface; and the duration that larvae remain on the surface before burrowing into soil. Experiments presented here were designed to provide this information.

Materials and Methods

D. abbreviatus adults were collected in the field and placed in holding cages with *Citrus paradisi* Macf. foliage as food. In the laboratory, folded strips of wax paper provided a surface for oviposition. Egg masses were placed in containers at 100% relative humidity (RH) and left to hatch (ca. 7 days). Larvae were segregated into age groups (hours after eclosion) and used in tests 3 through 9.

In test 1, to determine the postoviposition age at which larvae drop to the ground, female weevils were caged on potted *Citrus paradisi* Macf. (1.0 m high) in the laboratory (26 ± 3°C and 40 to 50% RH) until egg masses were laid. The deposition date of each egg mass was noted, and funnels were placed beneath each egg mass with their tips directed to the face of a modified clock. A disc (18.5 cm in diameter) with 12 equal sectors (representing hour divisions) drawn on its surface was coated with Tack-trap and affixed to the mechanical drive of the hour hand. Larvae emerging from each egg mass dropped to different concentric portions of the disc, and their numbers were recorded each hour. Five egg masses were evaluated.

Test 2, designed to determine the time after oviposition when egg-larval eclosion occurs (within 12 h), utilized 20 egg masses deposited on wax paper within the same 24-h period. The wax paper was peeled apart so that each larva, upon hatching, dropped from the egg mass. Hatched larvae were removed and counted each day.

Test 3 was designed to obtain an estimate of the length of time larvae spend on the soil surface. Twenty larvae per replication (10 replications) for each age group (9 ± 9 and 72 ± 9 h old) were placed on the surface of Lakeland-type soil (thermic, coated Typic Quartzipsamments) packed to a density of 1.63 g/cm^3. Soil moisture was 9.1% water (wt/wt). Larvae which had not burrowed under the soil were counted every 15 min.

Tests 4 to 8 were designed to test the effects of different conditions on the tendency of 1st instars to penetrate soil (Lakeland type in all tests). Tests were

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conducted in Plexiglas chambers (9 by 9 by 5 cm^3) at 25 ± 2°C with constant light (unless otherwise noted) for 24 h per replication. Each chamber had five receptacles 1.88 cm in diameter and 1.17 cm deep. The bottom opening of each receptacle contained an 80-mesh brass screen, and below each receptacle was a removable vial partly filled with water. Soil was placed in all receptacles, larvae (100 per chamber) were placed in the chambers, and the chamber tops were covered with glass plates. Test evaluations were based on the number of larvae in the water under each receptacle.

Test 4, to establish the optimum soil moisture for larval penetration, utilized six soil moistures (0, 3.2, 7.4, 10.7, 16.7, and 23.0% saturated, wt/wt, with water). Oven-dried soil was used and brought to the desired moisture with distilled water. Soil samples of different moisture were placed in chamber receptacles so that no given moisture appeared more than once at the same time in a chamber. Chambers were run five at a time, and no given soil moisture was in the same receptacle position relative to the other chambers during a replication (to preclude positional effects). Each soil moisture was replicated five times with 100 larvae (48 ± 24 h old) per replication. Test 4 established a range of soil moistures that were optimal for larval soil penetration. A single moisture within this range, 9.1% (wt/wt) water, was selected for tests 3 and 6 through 9.

In test 5, all chamber receptacles contained dry soil (0% water), and five replications were made, with 100 larvae per replication.

Test 6 was designed to indicate any preference by larvae to penetrate soil near grass stems or underneath surface cover. Grass stems (ca. 2 mm in diameter, four per receptacle) or leaf discs (2 cm in diameter, green citrus) were placed vertically in soil or horizontally on the soil surface, respectively. Chambers with soil and surface litter were paired with chambers with soil only (5 replicates, 100 larvae per replication per treatment).

Test 7 was conducted to determine the effects of darkness and light on soil penetration. Chambers were paired in the dark and in light (five replications, 100 larvae per replication per treatment).

Test 8 was done to see if age (hours after eclosion) influenced larval penetration of soil. Larvae 12 ± 12, 64 ± 24, 112 ± 24, and 160 ± 24 h old were tested for soil penetration (five replications, 100 larvae per replication per age group).

In test 9, a prototype soil-insecticide bioassay chamber was constructed consisting of a cylindrical, plastic 185-ml vial (5 cm in diameter by 11 cm high), with an 80-mesh brass-screen bottom and a plastic snap-top closure. Each chamber was filled with 242 g of soil compacted to a depth of 10 cm. The chamber bottom was placed in the mouth of a plastic funnel (5.5 cm in diameter) with its tip in a 185-ml vial partly filled with water. Larvae (24 ± 12 h old) were placed on the soil, and the chamber was capped (five replications, 100 larvae per replication). Larvae penetrating through the soil fell into the water, where they were counted and removed each day. The test was run for 20 days.

Test 10 was performed over a period of 5 months. The bioassays were performed as described in test 9, and 100 chambers were run in groups of 10 for 14 days each (100 larvae per chamber).

Results and Discussion

Test 1 indicated that 1st instars dropped from egg mass-containing leaf envelopes between 235 ± 31 and 248 ± 31 h after oviposition (1,100 to 2,400 h on a 24-h clock). An average of 75.2 ± 50.1 larvae per egg mass was recovered. Test 2 showed that larvae hatched from egg masses beginning 144 ± 24 h and ending 224 ± 24 after oviposition. An average of 65.1 ± 30.8 larvae per egg mass was recovered. Test 3 showed that half of the groups comprising 9- and 72-h-old larvae required 80 and 105 min, respectively, to disappear from the surface by burrowing into the soil. All larvae in both groups had disappeared in 180 min.

A comparison between age of eggs at time of hatch (test 2) and age of larvae at time of drop (test 1) indicated that hatch was completed by the time escape from the leaf envelope started, and that the average larval age was about 48 h. The finding that larvae on the ground required no more than 3 h (test 3) to disappear into the soil suggests that predators must be active during the drop period (1100 to 2400 h) to contribute to control.

Test 4 showed the following mean numbers of larvae penetrating soil of different moisture percentages: 0 in 0%; 52.0 ± 29.7 in 3.2%; 113.4 ± 27.3 in 7.4%; 93.4 ± 42.4 in 10.7%; 69.2 ± 20.0 in 16.7%; 6.2 ± 3.7 in 23.0%. The moisture percentages rated statistically (analysis of variance [ANOVA] and Duncan's multiple range test [$P = 0.05$]), based on preference by larvae for penetration were $7.4 = 10.7 = 16.7 > 3.2 > 23.0 > 0.0$. Test 5 also showed no penetration through dry soil. In test 6, larval soil pentration near grass stems or under surface cover was not significantly ($P = 0.05$) different from that in checks (paired t test). Tests 4 through 6 indicated that the moisture content of soil is the principal factor affecting larval penetration. Certain citrus grove practices, such as regular discing, would help maintain a dry surface.

Test 7 was conducted because 1st instars are positively phototrophic. No significant difference was evident, however, between soil penetration in light and darkness ($P = 0.05$, paired t test). Therefore, the presence or absence of light did not affect the tendency of larvae to penetrate soil.

Test 8 showed the following relationships between mean number of soil penetrations and larval age in hours: 28 ± 7.3 for 12 h; 82.2 ± 8.6 for 64 h; 40.6 ± 14.4 for 112 h; 53.3 ± 9.4 for 160 h. ANOVA and Duncan's multiple range test showed that soil penetration at different larval ages was significant ($P = 0.05$) for these age groups as follows: $64 > 112 = 160 > 12$. In test 9, a mean of 84 ± 6.14 larvae required 13 days to penetrate through 10 cm of soil, with 78% of these larvae requiring only 3 days. No additional penetration occurred between days 13 and 20. Tests 4, 8, and 9 provided information necessary to establish optimum conditions to bioassay candidate insecticides in soil. Test

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2 showed that a range of soil moistures from 7 to 17% would serve equally well, although 9.1% was selected for uniformity. Test 8 suggested that larval ability to penetrate soil, at least to 7 days, does not decline with age. Test 9 indicated that the dimensions selected for the bioassay chamber would be acceptable if the assay is continued for 13 days or more. A soil depth of 10 cm was necessary to approximate the depth of insecticide incorporation into citrus grove soil. The bioassay time interval (13 days) would be necessary for an 80+% survival rate (as measured by penetration through soil). Abbott's formula is valid for correcting check mortalities of <20%, unless the number of checks is quite large (Finney 1962). Test 10 was a large-scale test and confirmed the results of test 9. A mean penetration or 80.92 ± 11.12 was obtained for 10,000 larvae in a test period of 14 days.

These results establish a basis for future control efforts against *D. abbreviatus* 1st instars, which are presently considered the best target for control.

REFERENCES CITED

Finney, D. J. 1962. Probit analysis. Cambridge University Press, 2nd ed. Cambridge, England. 318 pp.

Whitcomb, W. H., T. D. Gowan, and W. Buren. 1982. Predators of *Diaprepes abbreviatus* larvae. Fla. Entomol. (in press)

Wolcott, G. N. 1936. The life history of *Diaprepes abbreviatus* at Rio Piedras, Puerto Rico. J. Agric. Univ. P.R. 20: 883–914.

Woodruff, R. E. 1964. A Puerto Rican weevil new to the United States (Coleoptera: Curculionidae). Fla. Dep. Agric. Entomol. Circ. 30: 1–2.