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RESIDUAL EFFECTS OF MICROMITE (DIFLUBENZURON) TREATED CITRUS LEAVES ON EGGS DEPOSITED BY *DIAPREPES ABBREVIATUS*

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Abstract. **Two different formulations of diflubenzuron (Micromite® 4L and 80WG) and FC 435-66 spray oil were applied to 'Redblush' grapefruit trees to determine the duration of residual activity on *Diaprepes abbreviatus* (L.) Coleoptera: Curculionidae egg development. Observations were made on the period to eclosion and the extent of egg mortality per week for each treatment. The effect of the chemical residue present on the leaf surface presented to the weevils as oviposition sites over an extended period of 13 weeks was emphasized. Ingestion of diflubenzuron by adult weevils was not a factor in this study. Based on Kruskal-Wallis ANOVA by ranks, percent mean mortality for the eggs deposited on treated leaves with diflubenzuron were significantly higher over time ($p < 0.05$) than the untreated control and spray oil treated leaves. Although the 80WG formulation caused greater egg mortality than the 4L formulation, the trend was not statistically significant. There was also a significant increase in the developmental time from oviposition to ecolsion for eggs deposited on leaves treated with both formulations of diflubenzuron.**

Diaprepes abbreviatus, also known as the sugarcane rootstock borer weevil, is an insidious pest of citrus, sugarcane, and other crops of economic importance in the West Indies, Florida, and recently Texas (Hall, 1995; Simpson et al., 1996; Skaria 2001). Since its discovery in Orange County, Fla. in 1964, *D. abbreviatus* has continued to spread and is currently estimated to infest 18%, or about 150,000 acres of commercial citrus in 20 Florida counties and about 100 ornamental plant nurseries (McCoy, 1999; Woodruff, 1964). All life stages of this insect are supported by 270 species in 157 genera, encompassing 59 plant families (Simpson et al., 1996). Annual economic losses (including lost production) from damage to citrus trees has been estimated to exceed $75 million (Faust, 1997). In citrus, adult weevils feed on tender young foliage and females deposit eggs between mature leaves, which hatch in 7 to 10 d. The neonates enter the soil and feed on roots, completing eight instars, undergo pupation, and adults emerge from the soil throughout the year (Fennah, 1942; Wolcott, 1936; Woodruff, 1968). A pathological interaction has been established between *D. abbreviatus* and *Phytophthora* spp. (Rogers et al., 1996). Specifically, larval feeding injury predisposes citrus roots to infection by *Phytophthora* spp., resulting in greater root mortality than would have resulted from larval feeding alone. The reproductive capability of *D. abbreviatus* is tremendous since females can oviposit approximately 60 egg masses containing 30 to 260 eggs each, or approximately 5,000 eggs during their arboreal lifetime of about 4 months (Wolcott, 1936).

Early attempts to eradicate *D. abbreviatus* with chemical insecticides were unsuccessful (Bullock et al., 1988). Currently, there are no approved chemical or biological control measures to control the destructive larval stages beyond the first instar and the potential for further spread of this pest is tremendous (Futch et al., 2001; McCoy, 1999). Historically, the use of adulticides as a control measure to decrease larval populations via reduced egg deposition, has shown to be unsuccessful due to several factors. They include the year round emergence of adults requiring costly multiple applications, the short residual activity of adulticide sprays (<4 weeks), the incompatibility of multiple adulticide applications with natural enemies, and the fact that young leaves (a strongly preferred food source by adults) emerging after foliar sprays would escape treatment (Adair, 1994; Bullock et al., 1988; McCoy and Simpson, 1994; Wolcott, 1936). Furthermore, efforts to establish egg parasitoids of *D. abbreviatus* have not yet been successful (Hall et al., 2001).

Application of FC 435-66 spray oil to citrus was shown to interfere with the attachment of *D. abbreviatus* egg masses to leaves (Schroeder et al., 1977) but its effect diminished after 4 weeks. It has been demonstrated that *D. abbreviatus* adults confined on citrus foliage treated with the insect growth regulator (IGR) diflubenzuron [1-(4-chlorophenyl)-3-(2,6-diflurorbenzoyl)urea] suffer reduced egg hatch for 4 weeks post treatment while no harm occurs to populations of non-target citrus pests and other beneficial species (Schroeder et al., 1976; Schroeder and Sutton, 1978; Schroeder et al., 1980). In a subsequent study, Schroeder (1996) investigated the effect of citrus leaf residues from a commercial product containing diflubenzuron (Micromite® 25W, Uniroyal Chemical Co., Inc., Middlebury, Conn.) on egg hatch by eliminating ingestion and found significant reductions in egg hatch for 46 days after treatment (DAT). Recently, the University of Florida has recommended a new formulation of diflubenzuron, Micromite® 80WG, for use on Florida citrus to control citrus root weevils such as *D. abbreviatus* (Knapp, 2000).

In view of the lack of adulticides with long residual activity that are compatible with an integrated pest management (IPM) program and the relatively short evaluation periods (46 d) for diflubenzuron residues in prior studies, we conducted a trial to determine the duration of ovicidal effects of two formulations and rates of diflubenzuron (Micromite® 4L and 80WG) and FC 435- 66 spray oil on *D. abbreviatus* egg mortality over a 13-week period. Emphasis of this investigation was on the effect of the chemical residue on the leaf surface serving as oviposition sites.

Materials and Methods

The study site is a 10-acre citrus grove planted in 1982 with 'Redblush' grapefruit trees (*Citrus paradisi* Macf.) on 'Swingle' citrumelo [*Citrus paradisi* Macf. × *Poncirus trifoliata* (L.)

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Raf.] rootstock located 0.4 mile east of the Kerr Center's Vero Beach Research Station (VBRS) in Indian River County, Fla. Typical horticultural practices were provided to the grove site with the exception that no chemical sprays were applied to the foliage for 7 months prior to the study period. Five single tree plots were randomly selected for each of five treatments henceforth referred to as Control, Spray Oil, 4L, LR 80WG, and HR 80WG (Table 1). Treatments were prepared as indicated in Table 1 and were applied on 15 Feb. 2000 by means of a tractor-powered hydraulic sprayer equipped with a handgun calibrated to deliver 1 gal per tree. This rate per tree was based on an assumed spray volume of 150 gal per acre applied to 150 trees per acre and was deemed to provide good coverage to the foliage. Rainfall was recorded daily at the VBRS.

Once a week, 10 healthy, mature (>9 months old) leaves were randomly selected from each of the designated trees for each treatment. The leaves were clipped with petioles intact using latex gloves and placed into separate bags according to treatment and returned to the laboratory. Oviposition sites were fashioned by attaching a pair of leaves together using a paperclip and placing them in a 25-ml Erlenmeyer flask filled with 25 ml of deionized water with the petioles immersed. Five leaf pairs for each treatment were then placed in 12 in × 12 in × 12 in aluminum screen cages (BioQuip Inc., Gardena, Calif.) along with five gravid female *D. abbreviatus*. Adult weevils were collected weekly from Tedders traps (Diaprepes Task Force, 1996) at the VBRS groves. Weevils were fed a diet of untreated fresh, young 'Redblush' grapefruit leaves along with a water source and maintained in a humidity-controlled (75-80% R.H.) insectiary prior to use.

After 30 h female *D. abbreviatus* were removed and leaf pairs with egg masses were placed in labeled, 1 qt clear-plastic containers and sealed with a plastic lid. The number of eggs per egg mass was determined 6 d after oviposition by the average of three counts per mass. Each egg mass was considered an experimental replication. Egg masses were examined under a stereomicroscope every other day (excluding weekends), until all eggs either hatched or died. Eggs exhibiting no embryo movement were considered dead. Percent mortality was determined for each egg mass by dividing the number of dead eggs by the number of eggs deposited times 100. The duration from oviposition to eclosion was defined as the number of days after oviposition until the last day eclosion was observed. The experiments were conducted in a laboratory maintained at 76%F and using an 11/13 h light/dark photoperiod illuminated with ceiling mounted fluorescent lights equipped with a timer.

Table 1. Treatments and rates of two formulations of diflubenzuron applied by handgun to 'Redblush' grapefruit trees on 15 Feb. 2000 to evaluate the effects of diflubenzuron residue on eggs deposited by *Diaprepes abbreviatus*.

Treatment	Material	Rate/tree[z]	Rate/acre[y]
Control	Untreated	—	—
Spray Oil	FC 435-66 Spray Oil	1.28 fl oz	1.5 gal
4L	Label Rate Micromite® 4L + FC 435-66 Spray Oil	0.067 fl oz 1.28 fl oz	10 fl oz 1.5 gal
LR 80WG	Label Rate Micromite® 80WG + FC 435-66 Spray Oil	0.042 oz 1.28 fl oz	6.25 oz 1.5 gal
HR 80WG	High Rate Micromite® 80WG + FC 435-66 Spray Oil	0.067 oz 1.28 fl oz	10 oz 1.5 gal

[z]Represents amount of material in 1 gal applied by handgun per tree for each treatment.
[y]Assumes 150 trees per acre receiving 1 gal per tree or 150 gal/acre.

Oviposition data were expressed as total eggs, number of eggs hatched, percent hatch, mean percent mortality, and number of days to eclosion. Appropriate transformations were used to improve the fit of the raw data to the Gaussian distribution, but the recommendation of Bartlett (1947) was followed in which a 0 proportion was counted as $1/(4n)$ and a proportion of unity was treated as $(n\text{-}1/4)/n$ before the angular transformation. Data were preliminarily analyzed using ANOVA (randomized complete block design) to assess overall inter-treatment variation with time as the smallest experimental unit (Gomez and Gomez, 1984). Tukey's multiple comparison test was used due to the relatively large number of comparisons. However, even the transformed data appeared nonnormal on further inspection, so the nonparametric Kruskal-Wallis ANOVA by ranks was applied, followed by Dunn's mean rank test (Daniels, 1990; Hollander and Wolfe, 1973). In almost all cases, there was direct correspondence between the two ANOVAs and post hoc tests: when the ANOVA or the Kruskal-Wallis test detected (or failed to detect) significant variation, the associated post hoc test also identified (or failed to identify) significant inter-group differences. Therefore, only the results of the Kruskal-Wallis and Dunn's tests are presented, as they are less dependent on assumptions of normality. Statistical analyses were performed using the Systat 10 statistical analysis package for Windows (SPSS, Inc., Chicago, Ill.), but Dunn's multiple comparison analysis was performed using GraphPad Prism 3.02 (GraphPad Software, Inc., San Diego, Calif.).

Another measure of the magnitude of the difference between treatments is the area under the temporal curves for each quantified parameter. Data were grouped by treatment, averaged according to DAT and plotted vs. time. The area under each curve was calculated using trapezoidal integration and the results expressed as a single number representing the relative magnitude of the treatment effects. Curve area integrations were performed using GraphPad Prism 3.02 (GraphPad Software, Inc., San Diego, Calif.).

Results

The duration of residual activity of diflubenzuron based on egg hatch was evaluated for 91 DAT from 15 Feb. 2000 through 16 May 2000. The total number of egg masses harvested ranged from 55 for LR 80WG to 65 for control, whereas the total number of eggs per treatment varied from 4,398 for spray oil to 5,155 eggs for control (Table 2). Based on the Kruskal-Wallis ANOVA by ranks, there were no significant differences among the mean numbers of eggs per mass for any of the five treatments (adjusted $H = 2.46$, $\alpha = 0.05$, df = 4, P = 0.6512) (Table 3). While both the number of egg masses and eggs oviposited was uniform among all treatments (Table 2), the number of eggs that hatched per mass varied considerably (Kruskal-Wallis adjusted $H = 101.49$, $\alpha = 0.05$, df = 4, $P < 0.0001$) (Table 3). All three diflubenzuron treatments exhibited significantly reduced hatch rates compared to both control and spray oil treatments (Table 3). There were no significant differences between the control and spray oil treatments (Table 3). Although no statistical differences between the 4L and 80WG formulations were identified through Dunn's test, there were identifiable trends that the 80WG was

Table 2. Summary of residual effects of two formulations and rates of diflubenzuron on eggs deposited by female *Diaprepes abbreviatus* on treated 'Redblush' grapefruit leaves collected weekly from 16 Feb. 2000 thru 16 May 2000.

	Treatment				
	Control	Spray Oil	4L	LR 80WG	HR 80WG
Total no. egg masses	65	59	60	55	56
Total no. eggs	5155	4398	4609	4489	4691
Total no. eggs hatched	4940	3890	2269	1259	989

a more effective formulation. Based on integrated area under the curve analyses, the 4L formulation showed lower effectiveness based on the number of eggs hatched, percent mortality and days to eclosion. That is, the total area under the temporal treatment curve was lower for the 4L formulation than for either rate of the 80WG formulation (Table 3).

Percent mortality for each treatment displayed erratic fluctuations over time for all treatments except the control (Fig. 1). The initial percent mortality observed for the 4L formulation of diflubenzuron at 1 DAT was 1%, whereas both rates of the 80WG formulation of diflubenzuron were 64% (LR 80WG) and 60% (HR 80WG) for the same time period. Similarly, a lower than expected mortality of 13% was observed at 49 DAT for the 4L treatment. This unexpected deviation was likely due to little or no diflubenzuron being deposited on the selected leaves during treatment, since subsequent leaf collections from the same 4L treated tree all exhibited greater mortalities corresponding to the established trend.

The LT_{70} (the number of days elapsed in which mortality was above 70%) for the 4L formulation occurred around day 42 (Fig. 1). The LT_{70} could not be established for the HR 80WG, since mortality did not fall below 70% before the conclusion of the experiment. However, an interpolated LT_{70} for the LR 80WG rate of diflubenzuron was determined to occur at about 84 d. Therefore, there was a clear, but statistically insignificant trend that both rates of the 80WG formulation exhibited greater mortalities than the 4L throughout the 91-d investigation. Assayed mortalities for individual egg masses ranged from 4.7% to 100% for the LR 80WG and 7.7% to 100% for the HR80WG (Table 3). Higher than expected mortalities were observed for Spray Oil at 28 DAT (44.1%) and 56 DAT (47.8%) (Fig. 1). On both of these occasions, entomopathogenic fungi were observed on two of the eight egg masses harvested.

When weekly rainfall was superimposed over percent mortality for the same time period, there was no relationship observed (linear regression, r^2 ranging from 0.00 to 0.07, $p < 0.05$) between rainfall and percent mean weekly mortality for any treatment (Fig. 2). The average rainfall occurring during the extent of this study was 1.43 in, while the average rainfall for the past 30 years for this same time period was 3.07 in (Fig. 2). The low rainfall may have possibly allowed for a longer period of residual activity of diflubenzuron due to reduced wash off.

The residual effects of diflubenzuron on mortality throughout the entire 91-d study are evident in Fig. 1. The HR 80WG

Table 3. Summary of residual effects of two formulations and rates of diflubenzuron on eggs deposited by female *Diaprepes abbreviatus* on treated 'Redblush' grapefruit leaves collected weekly from 16 Feb. 2000 thru 16 May 2000.

	Treatment[z]				
	Control	Spray Oil	4L	LR 80WG	HR 80WG
No. eggs/mass					
Mean	79.3 (± 5.5) a	74.5 (± 4.8) a	76.8 (± 5.6) a	81.6 (± 5.0) a	83.8 (± 5.9) a
Minimum	3	19	14	19	11
Maximum	168	170	227	179	235
Area under curve	7283	7067	7307	8351	7695
No. eggs hatched/mass					
Mean	76.0 (± 5.3) a	65.9 (± 5.3) a	37.8 (± 5.7) b	22.9 (± 4.1) b	17.7 (± 2.5) b
Minimum	2	0	0	0	0
Maximum	168	166	225	121	70
Area under curve	6976	6401	4005	2112	1431
Percent mortality					
Mean	4.2 (± 0.9) a	11.3 (± 3.5) a	57.7 (± 4.5) b	72.9 (± 3.6) b	79.4 (± 2.8) b
Minimum	0.00	0.00	0.00	4.72	7.69
Maximum	33.33	100.0	100.0	100.0	100.0
Area under curve	396.8	961.0	4525	6754	7240
Days to eclosion					
Mean	9.8 (± 0.3) a	9.8 (± 0.3) a	17.3 (± 1.0) b	17.8 (± 0.9) b	18.7 (± 0.9) b
Minimum	7	8	8	8	9
Maximum	15	20	32	32	28
Area under curve	870.1	895.5	1499	1557	1622

[z]Differences between treatments were analyzed using the Kruskal-Wallis One-way ANOVA by ranks ($\alpha = 0.05$, df = 4). Means (± SE) followed by the same letter in rows are not significantly different based on Dunn's nonparametric multiple comparison test at the 5% level.

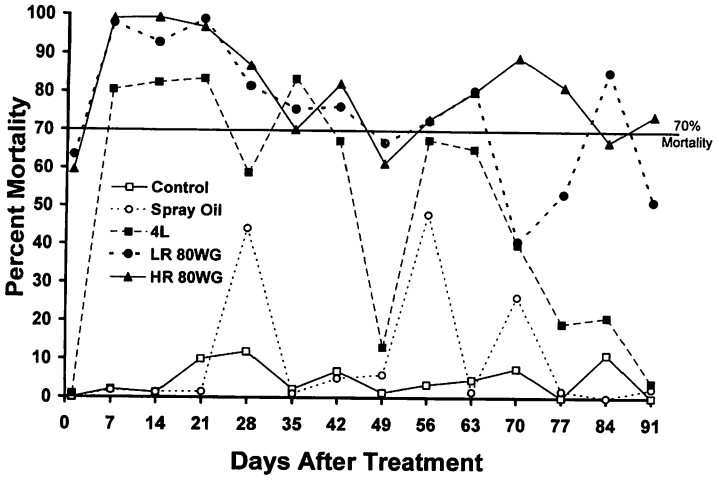

Figure 1. Degradation of residual effect of diflubenzuron based on percent mortality for eggs deposited by female *Diaprepes abbreviatus* on treated 'Redblush' grapefruit leaves collected weekly from 16 Feb. 2000 through 16 July 2000.

treatment exhibited the greatest mortality with an overall mean mortality of 79.4% (Table 3). However, there was no significant difference for the two rates of the 80WG or 4L formulations based on the Kruskal-Wallis ANOVA by ranks (Table 3). Accordingly, the highest weekly mortalities observed for both rates of the 80WG formulation, 99.1% for HR 80WG and 98.9% for LR 80WG, were notably higher than the 83.4% mortality exhibited by the 4L formulation.

The mean percent mortalities observed in the control and spray oil treatments were 4.2% and 11.3% respectively. Moreover, the time from oviposition to eclosion for the Control and Spray Oil treatments both averaged 9.8 DAT. Both these findings were not significantly different based on Dunn's multiple comparison test (Table 3). Together, they suggest that the FC 435-66 spray oil in the diflubenzuron treatments was not appreciably affecting egg mortality at the rates used here. In contrast, all diflubenzuron treatments yielded significantly increased periods to eclosion when compared with the Control and Spray Oil treatments (Kruskal-Wallis adjusted $H = 138.92$, $\alpha = 0.05$, $df = 4$, $P < 0.0001$). However, there was no significant delay in eclosion time between any of the diflubenzuron formulations. The 4L treatment averaged 17.3 d to eclosion and ranged from 8 to 32 DAT while the LR 80WG averaged 17.8 d and also ranged from 8 to 32 DAT. The HR 80WG averaged 18.7 d and ranged from 9 to 28 DAT (Table 3). It should be pointed out that there is an intentional absence of time data for the occasions in which egg masses failed to hatch at all. This occurred exclusively with the three diflubenzuron treatments. Therefore, the number of days to eclosion for the diflubenzuron data in these instances would be underestimated.

The initial percent mortality and number of days to eclosion at 1 DAT for all three diflubenzuron treatments was observed to be lower than subsequent observations (Figs. 1 and 3). This was especially evident for the 4L treatment. The number of days to eclosion for the diflubenzuron treatments also was consistently longer than either control or spray oil treatments throughout the 91-d study except at 21 and 35 DAT for the HR 80WG and 4L treatments respectively (Fig. 3).

Microscopic observations of embryo movement within the egg revealed that if eclosion did not occur after 25 d from oviposition for the diflubenzuron treatments, seldom did these eggs hatch (Fig. 3). Moreover, microscopic inspection of the neonate larvae that did hatch from eggs oviposited on diflubenzuron treated leaves frequently appeared to be lethargic, exhibited depleted yolk reserves, and usually died within a few days. Interestingly, deformed mandible incisors were sometimes observed on neonate larvae hatched from diflubenzuron treatments, presumably due to the IGR activity of diflubenzuron on chitin formation (Christiansen, 1980). In contrast, the neonate larvae collected from the Control and Spray Oil treatments were generally active, had full yolk reserves, and exhibited normal mandibles.

Discussion

Based on the percent egg mortality, the 80WG formulation of Micromite® appeared to perform better and its residues persisted longer than the 4L formulation when both

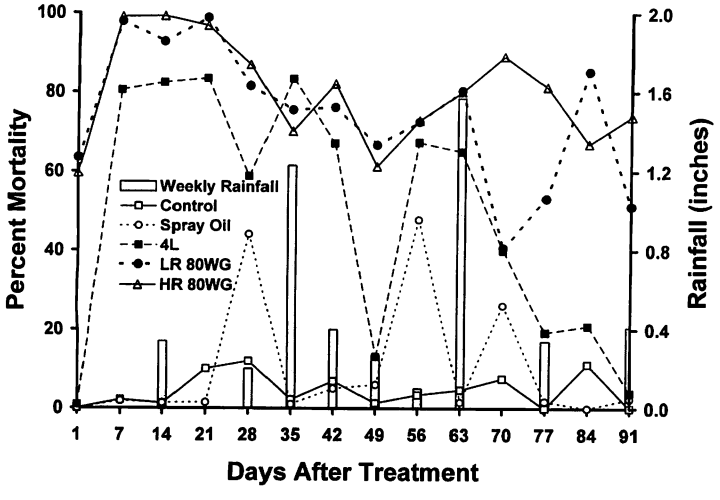

Figure 2. Weekly rainfall and degradation of residual effect of diflubenzuron based on percent mortality for eggs deposited by female *Diaprepes abbreviatus* on treated 'Redblush' grapefruit leaves collected weekly from 16 Feb. 2000 through 16 July 2000.

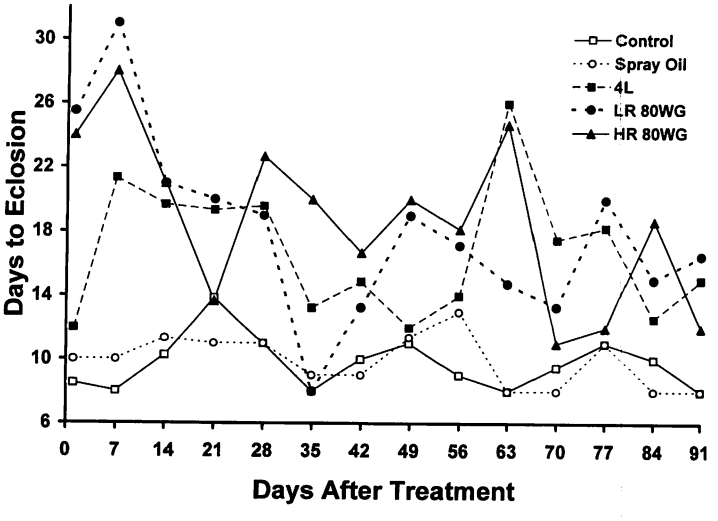

Figure 3. Degradation of residual effect of diflubenzuron, based on time from oviposition to eclosion, for eggs deposited by female *Diaprepes abbreviatus* on treated 'Redblush' grapefruit leaves for the 91 day period from 16 Feb. 2000 through 16 July 2000.

were applied at label rates even though no statistically significant differences were observed. Since the label rates for use on citrus root weevils for all three formulations of diflubenzuron (25WS, 4L, and 80WG product labels, Uniroyal Chemical Co., Middlebury, Conn.) contain identical amounts of diflubenzuron, comparisons with previous studies are warranted. Schroeder (1996) demonstrated that diflubenzuron (Micromite® 25WS)-treated citrus leaves produced egg mortalities of 77% and 86% for the label and 2× label rates respectively. This suppression is similar to the 72.9% and 79.4% egg mortality for the label and 1.6× label rate for the 80WG formulation in this study (Table 3). Assuming a minimum threshold of 70% egg mortality as an effective ovicide for the control of *D. abreviatus*, the 4L treatment was efficacious for 42 DAT (LT_{70} = 42 d), similar to the time period observed by Schroeder (1996) using the label rate of 25WS (Fig. 1). However, our study demonstrated the ovidical effects of LR 80WG to persist for at least 84 DAT (LT_{70} = 84 d) before beginning to decay in contrast to 44 DAT for the 25WS formulation reported in Schroeder's (1996) study (Fig. 1). These differences could be due to at least two factors: environmental (e.g., rainfall) and chemical (formulation chemistry and particle size). Rainfall, which would affect wash-off of the active ingredient on the leaf surface, failed to demonstrate a significant relationship (Fig. 2). Further, Mulder and Gijswijt (1973) reported that the effectiveness of diflubenzuron decreased rapidly with increasing particle size. Therefore, it seems plausible that the variations in ovicidal activity for the different formulations are due to the 80WG formulation having a smaller particle size.

Although neonate survival was not quantified in this study, visual observations of the neonates emerging from the diflubenzuron treatments, revealed reduced egg sac contents, lethargic movement, and mortality within days after eclosion. Thus, it is possible to postulate that the effect of diflubenzuron-treated foliage on neonate mortality extends beyond that of simply a reduction in egg hatch. The likelihood of survival for neonates exposed to diflubenzuron would be low due in part to reduced nutrient reserves for the embryo resulting from the extended period to eclosion of 17.3 to18.7 d versus the 9.8 DAT for control (Table 3). Based on these observations, it is likely that the ensuing neonate mortality due to LR 80WG would extend beyond just the 72.9% egg mortality demonstrated here.

The ovicidal effect of diflubenzuron on female, adult boll weevils, *Anthonomus grandis* Boheman, through ingestion, dipping, and tarsal contact has been documented (McLaughlin, 1976; Moore et al., 1978; Moore and Taft, 1975; Taft and Hopkins, 1975). The possibility of casual contact or ingestion of diflubenzuron by adult *D. abbreviatus* females affecting the eggs prior to oviposition in our study seems unlikely due to the limited exposure time of 30 h and to the absence of evidence of feeding on the mature leaves. Therefore, the primary avenue for diflubenzuron activity on *D. abbreviatus* eggs in this study was likely limited to direct contact of the eggs with leaf residue. This contact with the eggs presumably resulted in the translocation of diflubenzuron through the chorion and into the eggs, thereby initiating IGR activity on the chitin formation of the developing embryo.

We believe that the causes for egg mortality observed in this study can be attributed primarily to three factors acting singly or in concert: 1) the IGR activity of diflubenzuron on chitin formation that delayed the development of the eggs; 2) diflubenzuron affecting the ability of the neonate to emerge through the chorion of the egg; and, 3) the presence of pathogenic fungi on egg masses that resulted in embryo mortality while still inside the egg capsules. The mechanism by which diflubenzuron prevents egg hatch is presumably due to its effect on the cuticle deposition in the embryo where the muscle attachments and strength of the exoskeleton are not adequate to rupture the chorion (Mulder and Gijswijt, 1973). Such an effect would make eclosion in *D. abbreviatus* unlikely due to the impaired ability of the neonate to use its muscles to tear the chorion. As described earlier, microscopic examinations revealed deformed mandibles on the neonates hatched from diflubenzuron-treated eggs, confirming its IGR effects on chitin formation.

One possible source of experimental error in this study was egg mortality due to entomopathogenic fungi observed in both the spray oil and diflubenzuron treatments. In the case of the spray oil treatment, the high percent mortality at 28 and 56 DAT was possibly due to the presence of a pathogenic fungus on some of the egg masses (Fig. 1). This observation is supported by findings of Mayer and Doostdar (USDA-ARS, Ft. Pierce, pers. comm.) on field-collected egg masses of *D. abbreviatus* exhibiting mortality due to entomopathogenic fungi. Based on our observations, enhanced egg mortality could be expected from entomopathogenic fungi infecting egg masses deposited on diflubenzuron-treated leaves as a result of the delayed development time.

Another possible source of experimental error could have been due to variable spray coverage, which produced higher than expected egg hatches for the diflubenzuron treatments on individual leaves that escaped treatment. The ovicidal activity of diflubenzuron spray residues targeted for *D. abbreviatus* are predicated by direct contact with the eggs oviposited between mature leaves making optimum spray coverage of citrus foliage a requirement (Adair et al., 1997; Adair et al., 1999; Fennah, 1942). Consequently variable spray coverage represents a major limitation in product performance where grower applications are performed for the control of *D. abbreviatus*. Furthermore, since alternate hosts for *D. abbreviatus* are extensive, including two exotic species frequently found in Florida citrus, Guinea Grass *(Panicum maximum* Jacq.) and Brazilian Pepper-tree *(Schinus terebinthifolius* Raddi), effective control of *D. abbreviatus* with diflubenzuron sprays will remain a challenge for growers (Simpson et al., 1996).

The present study not only confirmed the finding of Schroeder (1996) that diflubenzuron inhibits the reproductive potential of *D. abbreviatus*, but also revealed that the residue of the new formulation Micromite® 80WG at the label rate may persist up to 3 months. The fact that our study included the application of diflubenzuron to mature trees in a typical citrus grove, exposed to the natural elements over a long period of time, contributed helpful insights on the persistence and practicality of such an approach in suppressing the reproductive potential of *D. abbreviatus* and confirmed that it should be considered as part of an IPM program for this insect pest. Further studies such as the present one, in conjunction with a detailed study of the mycology of the fungal invasions, may go a long way in finding a sustainable way of combating *D. abbreviatus*. Finally, non-parametric statistical analyses, such as the Kruskal-Wallis ANOVA by ranks applied here, may fail to detect statistically significant differences that might otherwise be detected using more rigorous parametric analyses of variance. Therefore, future studies will also more completely characterize the statistical distributions and test the underlying assumptions of the analyses in order to improve the reliability of the experimental design.

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