MORTALITY OF THE LARVAL ROOT WEEVIL *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE) IN SIMULATED FLOODING

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ABSTRACT

Larvae of the weevil *Diaprepes abbreviatus* L. can cause substantial damage to sugarcane and citrus. To test the feasibility of managing *Diaprepes* populations by flooding canefields for extended periods of time, larval mortalities were recorded after submerging larvae under water in soil filled trays at temperatures from 18 to 27°C for up to 5 weeks. Mean mortality exceeded 90% by 3 weeks of submergence at 24 and 27°C and after 5 weeks at 21°C, but was only 46% after 5 weeks at 18°C. A model was derived by multiple regression analysis, describing the response of mortality to time and temperature. The model accounted for 84% of the variation in larval mortality. Levels of O_2 and pH were monitored in selected trays during the experiment; only pH correlated significantly with larval mortality but contributed only 20% of total variation.

Key Words: citrus root weevil, drowning, sugarcane, statistical modeling

RESUMEN

Las larvas de *Diaprepes abbreviatus* L. pueden causar daño sustancial a la caña de azúcar y los cítricos. Para probar la posibilidad de manejar poblaciones de *Diaprepes* mediante la inundación de campos de caña durante largos períodos, las mortalidades

larvales fueron registradas después de sumergir las larvas en agua en bandejas con suelo a temperaturas de 18-27°C hasta 5 semanas. La mortalidad promedio excedió el 90% antes de las 3 semanas de submersión a 24 y 27°C, y después de 5 semanas a 21°C, pero fue solamente del 46% después de 5 semanas a 18°C. Fue derivado un modelo que describe la respuesta de la mortalidad al tiempo y a la temperatura, mediante análisis de regresión múltiple. El modelo respondió por el 84% de la variación en la mortalidad larval. Los niveles de O_2 y pH fueron muestreados en bandejas seleccionadas durante el experimento; sólo el pH se correlacionó significativamente con la mortalidad larval pero contribuyó solamente en un 20% a la variación total.

Diaprepes abbreviatus has infested citrus and various ornamental and wild host plants in Florida since 1964 (Woodruff 1964, Beavers & Selhime 1975, Schroeder et al. 1979). In the Caribbean Basin, the weevil is called the West Indian sugarcane rootstock borer weevil and infests both sugarcane and citrus. The impact of *D. abbreviatus* on sugarcane has long been recognized in Puerto Rico, where it is the primary insect pest of citrus and sometimes a primary pest of sugarcane as well. Since its discovery in Florida, *D. abbreviatus* has spread from northwestern Orange County, Florida, into at least 18 counties, including some within the Florida sugarcane production area. The Florida sugarcane industry has been concerned for many years that *D. abbreviatus* could become a significant pest of sugarcane. Although no infestations have yet been documented in sugarcane, such infestations are imminent, especially since an introduced host plant, the Brazilian pepper tree (Schroeder et al. 1979), is widespread in the sugarcane growing areas.

The adult weevil lays its eggs on cane leaves, and neonate larvae fall to the soil and burrow down to begin feeding on roots. As a larva develops, it moves along a root toward the tree trunk or the crown of a cane stool. As larvae mature, they grow to at least 2 cm in length and sometimes tunnel into the base of cane stalks. Larval development may last 8 to 10 months or longer and results in extensive feeding damage to cane. Symptoms of damage to roots include the desiccation and death of leaves, stool lodging, and stunted stalk growth. Mechanical harvesting of infested cane is difficult, as damaged stalks can snap over.

Cane growers may be able to avoid or reduce the risk of weevil infestations by controlling or employing alternate host plants such as the Brazilian pepper tree (Cassani 1986). Adults are weak fliers, and consequently their spread may be limited by eliminating host plants that bridge infested and uninfested areas. The importance of host plants other than cane is indicated by the avoidance of some cane varieties in Puerto Rico except as foliar egg laying sites. Cane fields in Puerto Rico are sometimes infested primarily along edges of fields near alternate host plants. Such alternate host species are numerous (Simpson et al. 1996). If sugarcane in Florida becomes heavily infested, growers may have no choice but to disk and replant, since application of pesticides to soil is governmentally regulated. Control of populations by flooding might prove a useful alternative to replanting, which may do little for control, anyway. Depending on factors such as water availability, legal considerations, and environmental regulations, flooding is sometimes used as a pest management strategy in southern Florida sugarcane fields for insects such as grubs of the scarab *Ligyrus subtropicus* (Cherry 1984) and larvae of the wireworm *Melanotus communis* (Hall & Cherry 1993). This report presents results of laboratory research on the susceptibility of the larvae of *D. abbreviatus* to submergence when exposed to varying temperatures for varying periods of time in flooded soil.

MATERIALS AND METHODS

Insects

Neonate *D. abbreviatus* larvae were obtained from a laboratory colony collected from Florida field populations and maintained in Orlando for over 4 yr (approximately 8 generations) in isolation. Larvae were collected within 2 days of hatching and about 10 larvae per cup were added to 30-ml cups containing 20 ml of a commercial citrus root weevil diet (Bio-Serve, Frenchtown, NJ). Rearing was as described by Beavers (1982). Larvae were reared on diet for approximately 6 weeks, then transferred to individual cups with fresh diet and reared approximately 10 more months before the test was begun. The mean weight ± SD of all larvae used in the tests (n = 1,296) was 427 ± 125 mg. Larvae of *D. abbreviatus* molt indeterminately and asynchronously, so the stage of development of these large larvae could not be accurately determined.

Soil

Soil was collected from a sugarcane field located about 0.4 km from a *D. abbreviatus* infestation in citrus in Glades County near Moore Haven, Florida. Analysis identified the soil as an Immokalee sand characterized by 7% organic content, 4% mineral content, and 89% silica content. Soil analysis using a 0.7 N NH_4OAc test at pH 4.8 indicated extractable contents of 45.4 kg/acre phosphorous, 88.0 kg/acre potassium, 3,995 kg/acre calcium, and 177.4 kg/acre magnesium. The soil averaged 11.9% moisture by weight prior to submergence of soil and larvae.

Polyethylene trays with hinged lids were used in the study. Each tray contained 18 individual compartments, each 5.1 × 5.1 × 5.1 cm. One or two 3-mm holes were drilled into the floor and ceiling of each compartment to allow influx of water; they remained open through the study. Each compartment of each tray (1 tray = 1 replicate) was filled with the field-collected soil, one larva per compartment was placed into a 1.5-cm χ 1-cm diam depression made in the soil with a wooden dowel, and larvae were covered with soil. Each tray of 18 larvae was closed and submerged in water purified by reverse osmosis and deionization, tilted, and tapped to completely fill all larval compartments and eliminate air pockets. A few small bubbles remained in some compartments beneath the lid, a situation comparable to that in the field, where air bubbles may persist around roots and crown of a stool. Two of the three closed replicate trays were placed together in a single water-filled 22.9 × 35.6 × 7.6-cm polystyrene storage box and one replicate tray was placed alone in the water-filled box. The two polystyrene boxes were covered by loose-fitting lids and placed into controlled-temperature cabinets with three unsubmerged (control) polyethylene trays.

The following durations and temperatures of submergence were studied: non-submerged control larvae (in soil with no water added) were maintained at 18, 21, 24, and 27°C; submerged larvae were maintained for 1, 2, 3, 4, or 5 weeks at 18, 21, 24, and 27°C. Three replicate trays of each duration and temperature of submergence plus three control trays at each temperature were included, with 18 larvae per replicate tray. Submergence of all replicates was initiated simultaneously. The dissolved oxygen contents of water in each of two boxes (but outside the submerged trays) at each temperature were measured weekly with an oxygen meter; the pH was measured in each of three boxes at each temperature (except at week 5, when only two boxes remained) with a pH meter.

One set of three replicate trays at each temperature was removed and examined at the end of each week. The soil and larva were removed from each compartment, the

box was dried, and larvae were replaced in their respective cells and checked for survival 24 hours later. Larvae in soil within the three unsubmerged trays at each temperature were spot-checked for survival and returned to the controlled temperature chamber. Soil from all compartments in all control trays was removed and mortalities were recorded at the end of 5 weeks.

Statistical Analysis

To define a model correlating temperature and time with mortality, multiple regression was employed using the Multiple Regression module of Statistica (StatSoft 1995). Values of the dependent variable, Mortality, were arcsine-transformed (arcsin $\sqrt{\text{mortality}}$). The independent variables Time and Temperature were progressively included in their linear, quadratic, cubic, and combined linear forms until further addition of terms yielded no appreciable increase in r^2. To test the significance of changes in pH and O_2 with time and the effects of pH and O_2 on mortality, regressions followed by ANOVA of the regression were performed using the Multiple Regression module of Statistica (StatSoft 1995).

RESULTS

Mortality of submerged larvae increased with both time and temperature (Fig. 1). Since sampling was destructive (individual trays were dismantled when examined), mortalities were not cumulative with time, but represent replicates of trays unique to each individual time point and temperature. Mortality was therefore sometimes lower than the week before (as from 4 to 5 weeks at 18°C), due to variability. By week

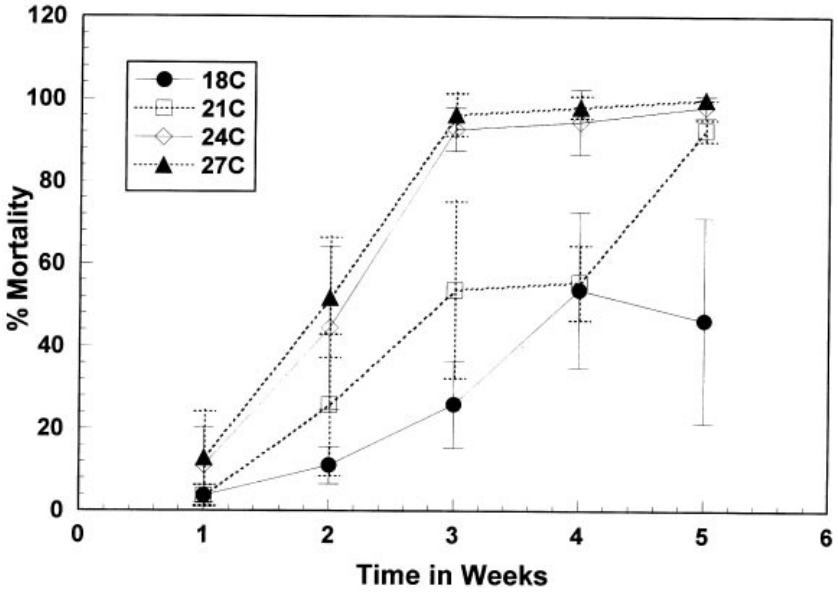

Fig. 1. Mortalities of larvae submerged 1-5 weeks at four temperatures. Standard deviations are indicated by vertical error bars.

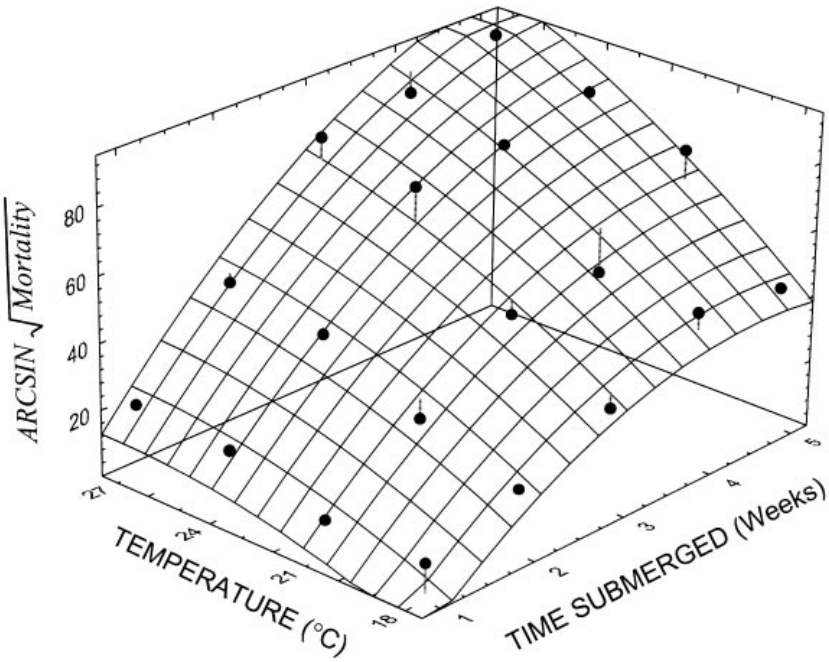

Fig. 2. Response surface derived from regression model. Mean mortalities (•) are also shown. Equation: $z = -2.725 + 0.2258x + 0.1935y - 0.0413x^2 - 0.0038y^2 + 0.0097xy$, where z = Mortality (proportion), χ = Time (weeks), and y = Temperature (°C). Circles represent the transformed means (N = 3) of actual measurements.

3, mortality exceeded 90% at 24°C and 27°C, while only 44% and 33% had died at 21°C and 18°C, respectively. At 21°C, 94% had died by the fifth week. Larvae at 18°C exhibited low, variable mortality rates (46 ± 24%) after 5 weeks of flooding. Control mortalities (% ± SD; N = 3) at the end of 5 weeks were 0 at 18°C; 11.1 ± 5.6 at 21°C; 16.7 ± 14.7 at 24°C; and 18.5 ± 3.2 at 27°C.

A model was developed by multiple regression, yielding excellent correlation of Time and Temperature (independent variables) with Mortality (dependent variable). The best fit, shown graphically in Fig. 2, was obtained using the linear and quadratic forms of the independent terms Time and Temperature, plus their linear interaction, in the form of the equation:

$$z = -151.766 + 12.696x + 10.772y - 3.338x^2 - 0.214y^2 + 1.004xy$$

where × = time (weeks), y = temperature (°C), and $z = \arcsin\sqrt{\text{mortality}}$, with mortality as a proportion. The model strongly correlated with the observed results ($P < 10^{-6}$; $r^2 = 0.843$; $F(5,54) = 57.82$), as can be seen from comparisons between observed mortality and mortality calculated from the model (Table 1).

The biophysical microenvironments in the boxes that contained sample trays varied with time, based on representative sampling. Perhaps due to variation and small sample size, changes in O_2 concentration with time at specific temperatures (Fig. 3) were not significant. Mortality also did not significantly correlate with oxygen levels.

However, pH did increase significantly with time at all temperatures (Fig. 4; $P < 0.007$ in all cases), especially at higher temperatures, up nearly one pH unit at 27°C

(Fig. 4). When a regression of mortality vs. pH was run, mortality significantly correlated with pH ($P = 0.002$, $r^2 = 0.163$) if temperature was not considered as a variable. When mortality at individual temperatures was considered, however, correlation between mortality and pH was significant only at 18°C ($P = 0.003$, $r^2 = 0.538$) and 24°C ($P = 0.006$, $r^2 = 0.433$).

Discussion

The regression model of larval mortality with time and temperature accurately described the results (Table 1). Eighty-two percent of observed variation in mortality was explained. The model does not address all factors that could govern the success of flooding as a control strategy. Larval behavior of grubs may modify susceptibility to environmental conditions (Villani & Wright 1990). For example, in cane fields larvae may move to avoid moisture, but such behavior was precluded in the laboratory. Since our experimental matrix did not include plant material, interactions of larvae with plants—e.g. crown stem tunneling activity—were also precluded. It may be difficult to entirely flood all areas inhabited by larvae. Developmental state can also have a significant effect. Larval size or age could have a dramatic effect on the success of flooding. In fact, general information on behavioral and biochemical interactions of root-

Table 1. Comparison of observed mortalities with mortalities calculated from the arcsin-transformed model.

Time (Wks.)	Temp (°C)	Mortality, from Model	Mortality, Observed
1	18	0.00	0.04
2	18	0.13	0.11
3	18	0.33	0.26
4	18	0.46	0.54
5	18	0.47	0.46
1	21	0.03	0.04
2	21	0.32	0.22
3	21	0.61	0.54
4	21	0.78	0.56
5	21	0.83	0.93
1	24	0.09	0.11
2	24	0.48	0.44
3	24	0.81	0.93
4	24	0.95	0.94
5	24	0.99	0.98
1	27	0.11	0.13
2	27	0.58	0.52
3	27	0.91	0.96
4	27	1.00	0.98
5	27	0.98	1.00

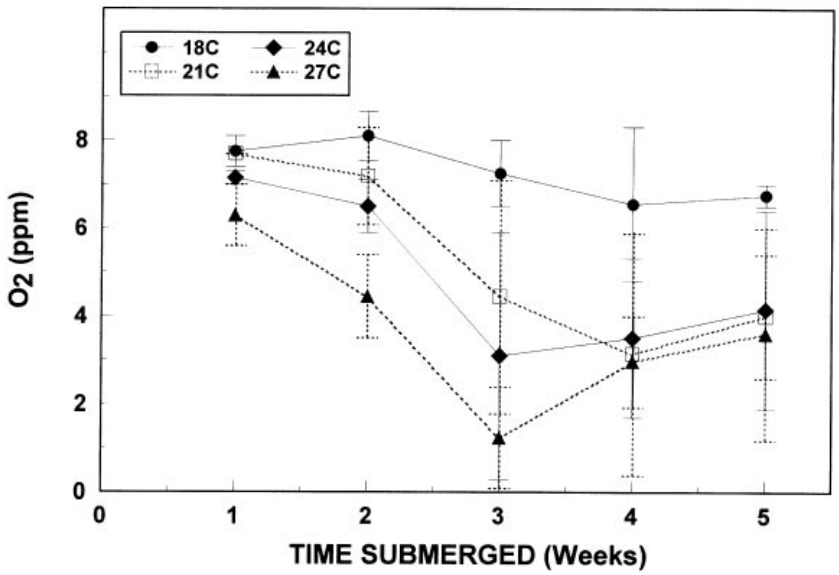

Fig. 3. Oxygen content (ppm) of water in representative containers at each temperature.

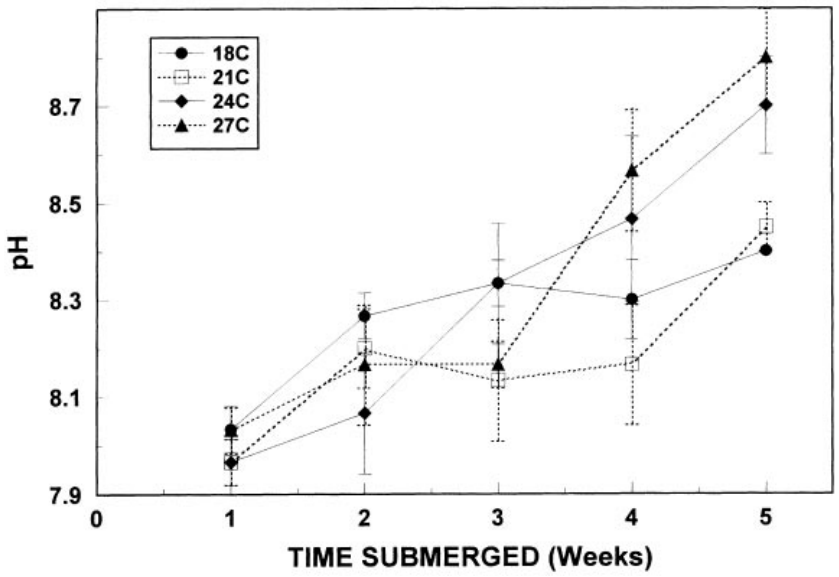

Fig. 4. pH of water in representative containers at each temperature.

feeding insects with hosts, host-derived phytochemicals, and the abiotic environment is extremely limited due to the difficulty of study of subterranean root-feeders (Shapiro 1991, Shapiro & Gottwald 1995, Villani & Wright 1990). In short, the success of flooding as an emergency control tactic for *Diaprepes abbreviatus* in sugarcane needs to be evaluated under field conditions.

In comparison to our findings on *Diaprepes*, wireworms (*Melanotus communis*), sustained less than 80% mortality after six weeks of submergence, even at 27°C (Hall & Cherry 1993). At the other extreme, first through third instar grubs of the scarab *Ligyrus subtropicus* sustained 100% mortalities after 5-10 days of submergence (Cherry 1984).

The cause(s) of observed mortality of submerged *Diaprepes* larvae is not clear. Mortality may have been due to drowning or suffocation (asphyxiation) due to decreasing oxygen and increasing carbon dioxide levels, or to sepsis from the growth of microorganisms in the stagnant water. The two possibilities are difficult to differentiate, since temperature could interact with either. If increased temperature results in increased metabolic rate, lower oxygen content may very well cause suffocation. However, carcasses of dead larvae were found to have deteriorated substantially. In many cases, only some thin outer cuticle remained intact, the internal organs entirely removed, apparently by microbial growth. Thus, sepsis may have been at least partly responsible for mortality, offering possible ramifications for the biological control of larval *Diaprepes*.

Results presented here suggest that flooding of sugarcane fields may be useful in the control of larval *Diaprepes*, though only during the summer or fall months when temperatures of water in flooded fields reach their reported maximum of 27°C (Hall & Cherry 1993).

Acknowledgments

The authors thank Thomas Moyer, USDA-ARS, for his excellent technical assistance; Victor Chew, USDA-ARS, for review of statistical procedures; and Mabry McCray, soil scientist with U.S. Sugar Corporation, for soil analyses.

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