EFFECT OF DIETARY COPPER ON LARVAL DEVELOPMENT OF DIAPREPES ABBREVIATUS (COLEOPTERA: CURCULIONIDAE)

Authors: Lapointe, Stephen L., Weathersbee, Albert A., Doostdar, Hamed, and Mayer, Richard T.

Source: Florida Entomologist, 87(1) : 25-29

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/0015-4040(2004)087[0025:EODCOL]2.0.CO;2

EFFECT OF DIETARY COPPER ON LARVAL DEVELOPMENT OF *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE)

Stephen L. Lapointe[1], Albert A. Weathersbee III[1], Hamed Doostdar[2] and Richard T. Mayer[3]
[1]USDA-ARS, U.S. Horticultural Research Laboratory, 2001 South Rock Road, Ft. Pierce, FL 34945

[2]Morse Enterprises Limited, Inc., 151 SE 15 Road, Miami, FL 33129

[3]USDA-ARS, Arthropod-Borne Animal Diseases Research Laboratory, P.O. Box 3965
University Station, Laramie, WY 82072

Abstract

Larvae of the Diaprepes root weevil, *Diaprepes abbreviatus* (L.), were reared from hatching on an artificial diet containing four concentrations of two copper compounds, cupric sulfate ($CuSO_4$) or cupric hydroxide [$Cu(OH)_2$]. Negative effects of copper on insect development were observed only for early instars. Survival of larvae from hatching to 4 weeks of age was significantly affected by the copper compounds compared with the artificial diet alone, and greater mortality was associated with $CuSO_4$ compared with $Cu(OH)_2$. The two compounds had equivalent effects on larval weight gain of early instars. Weight gain was negatively correlated with increasing copper concentration. No effect of copper was observed on late instars maintained on these diets beyond the initial 4 weeks. Larval and pupal period, weight gain, and survival of late instars were statistically similar. No effect on larval survival or weight gain was observed when copper solutions were applied at nonphytotoxic levels to two varieties of citrus rootstock. The potential for manipulating citrus tree copper content to control this pest is discussed.

Key Words: Citrus, heavy metal, artificial diet

Resumen

Se criaron larvas del cucurlionido *Diaprepes abbreviatus* (L.), sobre una dieta artificial con cuatro concentraciones de dos compuestos de cobre, sulfato de cobre ($CuSO_4$) o hidroxido de cobre [$Cu(OH_2)$]. Los efectos negativos del cobre sobre el desarrollo del insecto fueron observados solamente en larvas de estadios tempranos. La supervivencia de larvas desde eclosion del huevo hasta 4 semanas de edad fue afectada significativamente por los compuestos de cobre comparado con la dieta artificial sola, y más mortalidad fue asociada con $CuSO_4$ comparado con $Cu(OH_2)$. Los dos compuestos tuvieron efectos equivalentes en el aumento de peso de larvas de estadios tempranos. El aumento del peso fue correlacionado negativamente con el aumento en la concentración de cobre. No se observó ningún efecto del cobre en las larvas de estadios mayores (>30 d). Para estas larvas, el período larval y pupal, el aumento de peso, y la supervivencia eran similares estadísticamente cuando fueron criadas en todos los tratamientos de dieta incluyendo el control. No se observó ningún efecto sobre aumento larval de peso o la supervivencia cuando las soluciones de cobre fueron aplicadas en los niveles nofitotóxicos a dos variedades de patrones de cítricos. El potencial para manipular el contenido de cobre en árboles de cítricos para controlar este plaga se discute.

Translation provided by author.

Predation of tree roots by larvae of the Diaprepes root weevil, *Diaprepes abbreviatus* (L.), has become a major arthropod constraint to productivity of citrus in Florida. Copper compounds (fixed copper and Bordeaux mixture) have been widely used as fungicides and bacteriocides in citrus to control scab, melanose, blast (*Pseudomonas syringae*), and other pathogens. Copper compounds are inexpensive and broad-spectrum. Approximately one half of the United States citrus crop is treated with copper-based fungicides representing the largest single use of copper as a fungicide (Gianessi & Puffer 1992). Copper ions destroy proteins in plant tissues and the phytotoxicity of copper fungicides has led to replacement with safer chemical fungicides when available. However, copper fungicides are applied to over half of Florida's orange crop acreage, and over 75% of the state's grapefruit to control diseases such as brown rot, melanose, scab, Alternaria brown spot, and greasy spot (Florida Cooperative Extension Service 2000). Copper accumulates in the soil and can become phytotoxic to citrus rootstocks at high concentrations, especially to young trees in acidic soils (Alva et al. 2000). In soils with pH 8.2, copper content up to 400 mg kg^{-1} (ppm) was not phytotoxic to Swingle; in soils with pH 5.7 and 6.2, significant growth reduction occurred when copper levels exceeded 200 mg kg^{-1} (Alva et al. 2000).

Observations in this laboratory led us to hypothesize that copper content in the root tissue of citrus rootstocks might confer resistance to the Diaprepes root weevil through inhibition of its digestive enzymes such as polysaccharide hydrolyzing enzymes known to degrade plant material (Doostdar et al. 1997). The purpose of the research reported here was to document the effect of diet-incorporated copper on survival and growth of *D. abbreviatus* larvae.

Materials and Methods

Diet Incorporation

Diaprepes root weevils were obtained from a laboratory colony maintained by the U.S. Horticultural Research Laboratory, Ft. Pierce, FL. Larvae were reared on artificial diet according to Lapointe and Shapiro (1999). Neonate larvae (<24 h old) were placed in cups (PC100 1-oz. cups and lids, Jet Plastica, Harrisburg, PA) containing either a commercially prepared insect diet (product no. F1675, Bio-Serv, Inc., Frenchtown, NJ) or the same diet with one of three concentrations of cupric sulfate ($CuSO_4$*$5H_2O$, Sigma Chemical Co.) or cupric hydroxide [$Cu(OH)_2$, Sigma Chemical Co.]. The pH of the prepared diets was tested and adjusted to 7.0 while the diets were still liquid. Treatments consisted of control (diet only) and the equivalent of 250, 500, and 1,000 ppm Cu for each of the two copper compounds. Ten neonates were placed in each of thirty cups per treatment for a total of 2,100 larvae. Initial weight of larvae was approximately 0.1 mg (Lapointe 2000). Diet cups infested with larvae were kept in sealed plastic bags in an incubator at 27°C, 24:0 D:L. At 27 d after infestation of the diet cups, surviving larvae were recovered from individual cups, counted, and weighed. Survival of early instars from neonate to 27 d was expressed as percent survival per cup and transformed (arcsine) to normalize variance. Larval survival and weights were analyzed by ANOVA and means compared by Tukey's Honestly Significant Differences (HSD) test (SAS Institute 1999). Nontransformed means of larval survival are presented. For larval weights, "diet cups" was used as the error term because larvae were nested within cups. Thirty larvae were randomly selected from each treatment and placed individually in cups containing fresh diet of the respective treatment. These larvae were replaced in the incubator and allowed to complete development. Survival, larval period, pupal period, and adult weight upon emergence were recorded and analyzed by ANOVA.

Greenhouse Trial

The effect of soil copper concentration on survival and growth of *D. abbreviatus* larvae feeding on the roots of two citrus varieties was evaluated in greenhouse trails by using a factorial design. The citrus plants used in the experiment were one-year-old seedlings of Swingle citrumelo [*Citrus paradisi* Macf. × *Poncirus trifoliate* (L.) Raf.] and Sun Chu Sha mandarin (*C. reticulata* Blanco) potted individually in 946-cm^3 containers with 180 g of potting soil (Metromix 500, Scotts, Marysville, OH). A range of soil copper concentrations (0, 50, 150, or 300 ppm) was established by amending the soil (180 g) in each pot with 0, 9, 27, or 54 mg copper applied as soluble copper chloride in 100 ml water to each container. Treatments were applied as two partial applications ten days apart. Each treatment was replicated 15 times. Total number of plants was 120 (15 replications × 2 citrus species × 4 copper concentrations). Each container was infested 30 d after the final copper application with 5 neonatal larvae (≤24-h-old). The experiment was maintained in a greenhouse and plants were watered (100 ml per pot) twice weekly. The number and mean fresh weight of surviving larvae in each container, dry weights of plant roots, and height of plant shoots were recorded after eight weeks. Data were analyzed by the General Linear Models Procedure (SAS Institute 1999) to determine if soil treatments or citrus varieties affected the outcome of the experiment.

Results

Diet Incorporation

The dose of copper had no effect on survival of early instars ($F = 0.57$; df = 2, 174; $P = 0.57$). However, there was an effect of Cu source ($F = 9.71$; df = 1, 174; $P < 0.01$). Both Cu sources reduced larval survival of early instars compared with the control. Survival was reduced by 20% with $Cu(OH)_2$ and by 33% with $CuSO_4$ compared with the control (Table 1). There was a significant effect of Cu on the weight gain of larvae that survived to 27 d ($F = 66.3$; df = 6, 29; $P < 0.01$). There was no interaction between rate and Cu source ($P = 0.30$), so data for the two sources were combined and the rates were compared with the control. The mean weight of larvae reared on diet containing the low rate (250 ppm) of copper was not different from that of larvae reared on the control diet (12 ppm); the medium rate (500 ppm) of copper reduced larval weight by 27% and the high rate (1,000 ppm) by 82% compared with the control (Fig. 1) (α = 0.05, Tukey's HSD).

There was no effect of gender ($F = 0.53$; df = 1, 121; $P = 0.47$) or of treatment ($F = 0.66$; df = 6, 121; $P = 0.68$) on larval period (124.8 ± 2.9 d, n =

TABLE 1. MEAN (± SEM) SURVIVAL (n = 30) AND WEIGHT OF EARLY INSTARS OF THE DIAPREPES ROOT WEEVIL AFTER FEEDING FOR 27 D ON ARTIFICIAL DIET CONTAINING BACKGROUND (12 PPM), CUPRIC SULFATE OR CUPRIC HYDROXIDE INCORPORATED AT THREE RATES.

Copper source	Rate (ppm)	Survival (%)	Weight (mg)	n
$CuSO_4$	250	48.2 ± 3.2 a	44.2 ± 2.5 ab	137
$CuSO_4$	500	58.0 ± 4.6 a	36.6 ± 2.2 bc	159
$CuSO_4$	1000	49.1 ± 3.5 a	7.8 ± 0.5 d	139
$Cu(OH)_2$	250	61.5 ± 3.4 ab	41.4 ± 2.1 ab	170
$Cu(OH)_2$	500	60.4 ± 3.7 ab	32.8 ± 2.0 c	172
$Cu(OH)_2$	1000	64.6 ± 5.5 ab	9.5 ± 0.7 d	167
Diet only	12	77.2 ± 5.0 b	47.7 ± 2.5 a	201
$CuSO_4$	all	51.8 ± 2.2 a		435
$Cu(OH)_2$	all	62.1 ± 2.5 b		509
Diet only	12	77.2 ± 5.0 c		201

Means within columns and sections followed by the same letter are not significantly different (α = 0.05, Tukey's HSD after a significant ANOVA).

135). The experimental design did not allow for statistical comparison of larval survival of late instars. However, survival ranged from 53 to 73% and was not associated with the copper compounds or concentration (Table 2). There was no effect of treatment on pupal period (F = 1.6; df = 6, 121; P = 0.15). Mean (± SEM) pupal period was 18.9 ± 0.4 d (n = 128). Upon emergence, female adults were heavier than males (F = 43.1; df = 1, 120; P < 0.01), but there was no effect of incorporation of copper compounds on adult weight (F = 0.9; df = 6, 120; P = 0.50). Mean (± SEM) male weight was 280.5 ± 5.1 mg (n = 62); mean female weight was 349.6 ± 8.4 mg (n = 66).

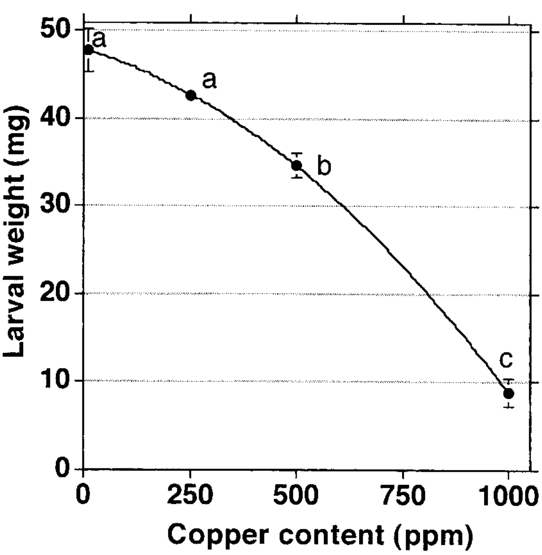

Fig. 1. Effect of rate of incorporation of copper in artificial diet on live weight of early instars of Diaprepes root weevil after 27 d at 26°C. Background copper concentration in artificial diet was 12 ppm. Bars are SEM (n > 200). Means with the same letter do not differ significantly at α = 0.05 by Tukey's HSD after a significant ANOVA.

Greenhouse Trial

Soil amendments with copper, applied as copper chloride, did not affect survival (F = 0.15; df = 3, 112; P = 0.93) or weight gain (F = 0.11; df = 3, 105; P = 0.96) of *D. abbreviatus* larvae over the range of concentrations evaluated in this experiment. After eight weeks, the mean (± SEM) number of larvae surviving per container was 2.0 ± 0.1 (n = 120 containers). The grand mean (± SEM) of weight of surviving larvae after eight weeks across all treatments was 196.4 ± 6.6 mg (n = 113 containers with surviving larvae). There was no effect of copper treatments on the dry weights of citrus roots (F = 0.01; df = 3, 112; P = 0.99) or height of the plant shoots (F = 1.03; df = 3, 112; P = 0.38). The overall means for dry weight of roots and height of shoots were 4.4 ± 0.3 g (n = 120) and 50.4 ± 1.4 cm (n = 120), respectively.

The numbers (F = 1.15; df = 1, 114; P = 0.29) and weights (F = 0.10; df = 1, 114; P = 0.76) of surviving larvae were similar whether the insects fed on the roots of Swingle citrumelo or Sun Chu Sha mandarin. However, the dry weight of roots (F = 50.66; df = 1, 114; P ≤ 0.01) and height of plant shoots (F = 95.35; df = 1, 114; P ≤ 0.01) differed by citrus variety. Both the mean root weight and shoot height for Swingle citrumelo (5.9 ± 0.4 g and 60.0 ± 1.5 cm, n = 60) were greater than those for Sun Chu Sha mandarin (2.8 ± 0.3 g and 40.7 ± 1.4 cm, n = 60) although the plants were similar in age and size when the experiment was initiated. The differences in the values for plant measurements were due to inherent differences between the two citrus varieties. No interactions between copper treatment and citrus variety were detected for any of the measured variables in the experiment.

TABLE 2. SURVIVAL OF LATE INSTARS, LARVAL AND PUPAL PERIODS, AND MEAN WEIGHT OF ADULTS AT EMERGENCE OF DIAPREPES ROOT WEEVIL REARED ON ARTIFICIAL DIET CONTAINING BACKGROUND COPPER (12 PPM) OR COPPER SULFATE OR COPPER HYDROXIDE INCORPORATED AT THREE RATES.

Copper source	Rate (ppm)	Survival (%)	Larval period (d)	Pupal period (d)	Adult weight (mg)			
					Males	n	Females	n
$CuSO_4$	250	70	121.5 ± 6.5	18.1 ± 0.8	279.2 ± 12.6	10	377.0 ± 37.1	11
$CuSO_4$	500	57	130.1 ± 9.7	19.4 ± 1.1	270.5 ± 12.4	5	350.5 ± 16.4	12
$CuSO_4$	1000	73	128.4 ± 5.7	17.7 ± 1.2	259.7 ± 9.8	13	335.0 ± 16.2	9
$Cu(OH)_2$	250	57	120.4 ± 7.5	20.3 ± 0.9	289.9 ± 23.8	8	369.0 ± 12.9	9
$Cu(OH)_2$	500	57	121.8 ± 9.7	18.4 ± 0.6	294.9 ± 7.3	10	334.9 ± 12.6	7
$Cu(OH)_2$	1000	53	133.6 ± 8.9	21.1 ± 1.2	281.7 ± 12.2	6	354.0 ± 21.8	10
Diet only	12	60	117.8 ± 5.7	18.5 ± 0.7	291.3 ± 13.1	10	312.8 ± 14.1	8

Means within columns are not significantly different ($\alpha = 0.05$, ANOVA).

DISCUSSION

Heavy metals are known to have adverse biological effects on insect development (Rayms-Keller et al. 1998; Bischof 1995). For example, 33 ppm copper resulted in 50% mortality of larval *Aedes aegypti* (Rayms-Keller et al. 1998), and retarded development was observed in copper-contaminated larvae of *Lymantria dispar* (Bischof 1995). The toxicity of heavy metals to insects can be synergized by several factors including hypoxia (van der Geest et al. 2002) and the presence of other heavy metal ions (Fargasova 2001). However, the strategic use of a heavy metal ion for controlling an insect pest is restricted by issues of phytotoxicity and long-term contamination of soil. To circumvent these problems, citrus seedlings might be treated in nurseries to raise the concentration of copper in roots before transplanting, thereby conferring a degree of protection to soil insects such as *D. abbreviatus*. However, in this study, treatment of seedlings with nonphytotoxic levels (≤300 ppm) of copper in solution did not confer resistance to larval *D. abbreviatus*.

The toxicity of copper to plant tissue and bioavailability of copper in the environment are known to increase with increasing soil acidity (Alva et al. 2000). The pH of ingested plant tissue by a root weevil would be approximately neutral, the same as that of the artificial diet used in our tests. According to Alva et al. (2000), critical Cu concentration for phytotoxicity in the roots of Swingle citrumelo seedlings ranged from 62 mg kg^{-1} in Myakka fine sand (pH 5.7) to 270 mg kg^{-1} in Candler fine sand (pH = 6.5).

Diet-incorporation assays have been successfully employed to identify compounds with negative effects on the developmental biology of *D. abbreviatus* (Shapiro et al. 1997, 2000; Weathersbee & Tang 2002). In our tests, significant mortality compared with the control diet was associated with $CuSO_4$ but not $Cu(OH)_2$, although the magnitude of the effect was not large and, curiously, did not vary with the concentration of copper. Mean weight gain of larvae reared on either source of copper cation at a concentration of 250 ppm was equivalent to that of larvae reared on the control. Significant inhibition of growth of early instars only occurred at higher concentrations (500 and 1,000 ppm). There was no discernible effect of copper amendments on survival, larval and pupal development, or weight gain on larvae >30 d old. Similarly, larvae of *L. dispar* exposed from the 4th instar were less susceptible to heavy metals than larvae exposed from hatching (Gintenreiter et al. 1993). From these data, we conclude that the concentration of copper required in root tissue to significantly inhibit development and survival of *D. abbreviatus* is too high to avoid phytotoxic effects.

ACKNOWLEDGMENTS

We thank Hunter Smith for technical assistance with bioassays. Research was financed in part by Cooperative Research and Development Agreement 58-3K95-9-657 between ARS and Morse Enterprises Limited, Inc., Miami, FL. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

REFERENCES CITED

ALVA, A. K., B. HUANG, AND S. PARAMASIVAM. 2000. Soil pH affects copper fractionation and phytotoxicity. Soil Science Society of America Journal 64: 955-962.

BISCHOF, C. 1995. Heavy metal concentrations of the endoparasitoid *Glyptapanteles liparidis* Bouche (Hymenoptera) in contaminated *Lymantria dispar* larvae (Lepidoptera). Bull. Environ. Contam. Toxicol. 55: 533-539.

DOOSTDAR, H., T. G. MCCOLLUM, AND R. T. MAYER. 1997. Purification and characterization of an endo-polygalacturonase from the gut of West Indies sugarcane rootstalk borer weevil (*Diaprepes abbreviatus* L.) larvae. Comp. Biochem. Physiol. 118B: 861-867.

FARGASOVA, A. 2001. Winter third- to fourth-instar larvae of *Chironomus plumosus* as bioassay tools for assessment of acute toxicity of metals and their binary combinations. Ecotoxicol. Environ. Saf. 48: 1-5.

FLORIDA COOPERATIVE EXTENSION SERVICE. 2000. Florida Crop/Pest Management Profiles: Citrus (Oranges/Grapefruit). Document CIR 1241, Pesticide Information Office, Food Science and Human Nutrition Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu.

GIANESSI, L. P., AND C. A. PUFFER. 1992. Fungicide use in U.S. crop production: Washington, D.C., Resources for the Future, Quality of the Environment Division (variously paged).

GINTENREITER, S., J. ORTEL, AND H. J. NOPP. 1993. Effects of different dietary levels of cadmium, lead, copper, and zinc on the vitality of the forest pest insect *Lymantria dispar* L. (Lymantriidae, Lepid). Arch. Environ. Contam. Toxicol. 25: 62-66.

LAPOINTE, S. L. 2000. Thermal requirements for development of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). Environmental Entomol. 29: 150-156.

LAPOINTE, S. L., AND J. P. SHAPIRO. 1999. Effect of soil moisture on development of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). Florida Entomol. 82: 291-299.

RAYMS-KELLER, A., K. E. OLSON, M. MCGAW, C. ORAY, J. O. CARLSON, AND B. J. BEATY. 1998. Effect of heavy metals on *Aedes aegypti* (Diptera: Culicidae) larvae. Ecotoxicol. Environ. Saf. 39: 41-47.

SAS INSTITUTE, INC. 1999. StatView Reference. Cary, NC.

SHAPIRO, J. P., K. D. BOWMAN, AND H. S. SMITH. 1997. Resistance of citrus rootstocks and *Glycosmis pentaphylla* against larval *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in live root or diet-incorporation assays. Florida Entomologist 80: 471-477.

SHAPIRO, J. P., K. D. BOWMAN, AND S. L. LAPOINTE. 2000. Dehydrothalebanin, a source of resistance from *Glycosmis pentaphylla* against the citrus root weevil *Diaprepes abbreviatus*. J. Agric. Food Chem. 48: 4404-4409.

VAN DER GEEST, H. G., W. J. SOPPE, G. D. GREVE, A. KROON, AND M. H. S. KRAAK. 2002. Combined effects of lowered oxygen and toxicants (copper and diazinon) on the mayfly *Ephoron virgo*. Environ. Toxicol. Chem. 21: 431-436.

WEATHERSBEE, A. A. III, AND Y. Q. TANG. 2002. Effect of neem seed extract on feeding, growth, survival, and reproduction of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). J. Econ. Entomol. 95: 661-667.