

Evaluation of copper hydroxide as a repellent and feeding deterrent for Cuban brown snail (Mollusca: Gastropoda: Pleurodontidae)

John L. Capinera*

Abstract

Repellents and feeding deterrents are underutilized tactics for plant pest damage suppression. Here I report on laboratory and screenhouse studies that assessed a copper hydroxide-based fungicide as a chemical barrier to prevent the Cuban brown snail, *Zachrysia provisoria* (Pfeiffer, 1858) (Gastropoda: Pleurodontidae) from accessing plants and from feeding on foliage. This snail species is considered to be one of the most important molluscs affecting plants in Florida. Studies were conducted as a follow-up to previous research with different molluscs that demonstrated the potential usefulness of copper hydroxide formulated in a commercial fungicide as a repellent. Although the residue of copper hydroxide displayed repellency to *Z. provisoria* in laboratory studies, it did not effectively prevent snails from climbing the sides of pots to access plants in screenhouses. Addition of 2 spreader-stickers, Bonide Turbo® and Southern Ag®, to the copper hydroxide to enhance retention of the fungicide did not improve performance. However, when copper hydroxide was applied to pots and foliage, feeding on vegetation was significantly suppressed under screenhouse conditions. I attribute these results to better fungicide residue persistence on foliage than on plastic pots.

Key Words: *Zachrysia provisoria*; fungicide; ornamental plants

Resumen

Los repelentes y disuasorios de alimentación son tácticas pocas utilizadas para la supresión de daño de las plagas en las plantas. Aquí se informa sobre los estudios de laboratorio e invernadero con malla que evaluaron un fungicida a base de hidróxido de cobre como una barrera química para evitar que el caracol marrón cubano, *Zachrysia provisoria* (Pfeiffer, 1858) (Gastropoda: Pleurodontidae) tenga acceso a las plantas e inhibir su alimentación sobre el follaje. Se considera esta especie de caracol como uno de los moluscos más importantes que afecta a las plantas en la Florida. Se realizaron los estudios como seguimiento de investigaciones anteriores con diferentes moluscos que demostraron la utilidad potencial del hidróxido de cobre formulado en un fungicida comercial como repelente. Aunque el residuo de hidróxido de cobre mostró repelencia a *Z. provisoria* en estudios de laboratorio, no evitó eficazmente que los caracoles trepan por los costados de las macetas para acceder a las plantas en los invernaderos con malla. La adición de 2 adhesivos spreader, Bonide Turbo® y Southern Ag®, al hidróxido de cobre para mejorar la retención del fungicida no mejoró el rendimiento. Sin embargo, cuando se aplicó hidróxido de cobre a las macetas y al follaje, se suprimió la alimentación de la vegetación significativamente en las condiciones de invernadero con malla. Se atribuye estos resultados a una mejor persistencia de los residuos de fungicidas en el follaje que en las macetas de plástico.

Palabras clave: *Zachrysia provisoria*; fungicida; plantas ornamentales

Despite the successful and widespread use of compounds such as DEET and picaridin for disrupting biting and feeding by blood-feeding arthropods (Naucke et al. 2007; Carroll et al. 2010; Rodriguez et al. 2015), chemical repellents and feeding deterrents are not usually considered an option for plant protection. However some compounds, especially plant-derived materials such as neem (azadirachtin), can disrupt feeding on plants by insects (Senthil-Nathan 2013) and cinnamamide has been reported to deter feeding by birds and mammals (Gill et al. 1995). Indeed, crude formulations of plant-based material have been used for thousands of years to repel insects (Maia and Moore 2001). Moreover, identification of bioactive repellents remains an active area of research in some parts of the world.

Less effort has been devoted to the problem of plant destruction by terrestrial slugs and snails. Most current management recommendations focus on killing the offending molluscs rather than protecting the plants. Controlling molluscs should eventually reduce herbivory

(assuming a finite pool of herbivores or limited recruitment) but in the short term, plant damage can occur before the pests consume a poison bait and succumb to its toxicants. Oftentimes it is quite difficult to eliminate molluscs from an area because a toxicant may not be palatable, they recover from poisoning, or they are sheltered deep in the soil and lack opportunity to feed on a poison bait. Also, molluscs can be surprisingly vagile, reinfesting areas quickly after they have been eliminated or suppressed. In addition, mollusc toxicants have a history of causing ecological damage (Kozłowski et al. 2010). Fairly recent introduction of iron-based toxicant baits has somewhat reduced these concerns. However, the efficacy of iron-based baits have been questioned compared with older metaldehyde-based formulations (Edwards et al. 2009; Laznik et al. 2010; Ciomperlik et al. 2013; Capinera 2013; Capinera & Guedes Rodrigues 2015).

There are instances where feeding deterrents or repellents might be more desirable than toxicants or as a supplement to toxicants. In

University of Florida, Entomology & Nematology Department, Gainesville, Florida 32611, USA; E-mail: capinera@ufl.edu

*Corresponding author; E-mail: capinera@ufl.edu

Florida, terrestrial molluscs are occasional pests of plants grown as ornamentals as well as vegetables grown in home gardens and organic production systems. Plants grown in greenhouses or shadehouses, as well as outdoors, are susceptible to damage. Although a variety of procedures and products are reputed to deter herbivory by molluscs, there is a dearth of supporting data.

Previously, we reported that copper hydroxide fungicide significantly suppressed foliage consumption by 4 mollusc species in choice and no-choice laboratory tests (Capinera & Dickens 2016). Copper hydroxide also has been reported to function as a repellent in laboratory bioassays against *Leidyula floridana* (Binney, 1851) (Gastropoda: Veronicellidae). Here I report on laboratory and screenhouse investigations to assess the potential of using copper hydroxide to protect flowering plants from herbivory by a different mollusc, the Cuban brown snail *Zachrysia provisoria* (Pfeiffer, 1858) (Gastropoda: Pleurodontidae). The Cuban brown snail is probably the most important mollusc pest in Florida, as well as some islands in the Caribbean region (Capinera 2013).

Methods

MOLLUSC CULTURE

Cuban brown snails were collected from various areas in Florida, USA, and cultured in the laboratory for 3 to 4 generations before use in these studies. Snails were reared on romaine lettuce (*Lactuca sativa* L. var. *longifolia* [Asteraceae]) fed ad libitum, and maintained at 24 °C and a photoperiod of 14:10 h (L:D). Garden lime (20% by weight) incorporated in gelled agar was provided and is considered essential for shell growth. This mineral sometimes can become limiting under laboratory conditions. Snails were maintained in 30 × 22 × 10 cm (L × W × H) plastic boxes containing 5 to 6 cm of moist garden soil (Robin Hood garden soil, Hood Landscaping, Adel, Georgia, USA) with a mineral content of 3.6% clay, 4.0% silt, 92.4% sand, and 11.5% organic matter.

LABORATORY REPELLENCY TESTS

This bioassay takes advantage of the natural tendency of snails to move to the top of a covered container. CuPro 5000[®] fungicide/bactericide (61.3% copper hydroxide [40% metallic copper equivalent]; SePRO Corporation, Carmel, Indiana, USA) was applied at the recommended rate, 0.5 g per 100 mL tap water, by misting it onto the inside wall of 18 × 8 cm (D × H) plastic cylindrical containers. After allowing time for the fungicide droplets to dry, moist paper toweling and lettuce leaves were placed in the center of each of 5 containers. Four snails, each of which was 2 to 5 g in mass, were added to each of the 5 containers (20 snails in total). The same numbers of containers and snails were used as controls but with the sides of the containers treated with tap water only. Location of individuals in containers was tabulated at 24 h intervals for 7 d and numbers of snails resting on lids recorded. Snails then were returned to the bottom of the container and fed. Mean Cuban brown snail distribution data were transformed ($\log [x + 1]$) to meet the assumptions of normality and analyzed with a 2-way repeated measure ANOVA (treatment, days) with GraphPad Prism (GraphPad Software, San Diego, California, USA). Also, the means were separated using the Bonferroni multiple comparison test ($P < 0.05$).

SCREENHOUSE BARRIER TESTS

Two screenhouse (10 × 5 × 3 m; L × W × H) evaluation trials of copper hydroxide were conducted using the aforementioned product at the same rate of application. These studies provided outdoor exposure

of Cuban brown snails, treated pots, and plants to natural levels of heat, humidity, and rainfall.

Trial 1 was conducted using zinnia, *Chrysogonum peruvianum* L. (Asteraceae), plants in Jun 2016 over an 8-d period. For this study, commercially produced zinnia plants (Lowe's Home Center, Gainesville, Florida, USA) growing in 10 cm diam plastic pots were sprayed to runoff then allowed to dry and consisted of: (1) untreated pot and plant, placed on a copper hydroxide-treated substrate (plastic cafeteria tray); (2) sides of pot sprayed with copper hydroxide, but plant foliage and substrate not treated; (3) plant foliage and pot treated with copper hydroxide and placed on untreated substrate; (4) untreated plant and pot (water spray only) placed on untreated substrate (untreated control). A total of 32 (8 replicates of the 4 treatments) plants were used in each trial. Pots were grouped into clusters (1 plant and pot from each of the 4 treatments) but clusters were randomly distributed in the screenhouse. The floor of the screenhouse was sprayed with water each morning and evening, and 24 small (10 cm diam) pots were distributed regularly around the inside perimeter of the screenhouse as shelters for snails. At the initiation of the study, 125 snails, each of which was 2 to 5 g in mass, were released into the center of the screenhouse at about 3:00 PM. Thereafter, each morning at about 9:00, each pot containing a plant was examined for the presence of snails. The cumulative number of snails observed on each plant and pot after 8 d of observation was totaled. At this time, the total number of zinnia leaves damaged by the snails was determined. Mean snail abundance and leaf damage data were subjected to the D'Agostino and Pearson omnibus normality test. These data then were transformed using ($\log [x + 1]$) then analyzed with 1-way ANOVA. The Bonferroni multiple comparison test (GraphPad Prism) was used to determine differences in the datasets ($P < 0.05$).

The second screenhouse trial was conducted in the same manner during Sep 2016, but with several differences. Madagascar periwinkle plants, *Cantharathus roseus* (L.) (Apocynaceae) were used in evaluations because zinnia plants were not available commercially at the time of the study; there were 5 treatments, necessitating use of 40 plants (5 treatments, 8 replicates); additional snails (a total of 150) (2–5 g in mass) were released; and the treatments were modified. Treatments consisted of: (1) copper hydroxide applied to the pot only, not on the substrate and plant foliage; (2) copper hydroxide plus Bonide Turbo[®] spreader-sticker (Bonide Products, Oriskany, New York, USA) applied only to the pot, no treatment of substrate and plant foliage; (3) copper hydroxide plus Southern Ag[®] spreader-sticker (Southern Agricultural Insecticides, Palmetto, Florida, USA) applied only to the pot, no treatment of substrate and plant foliage; (4) plant and pot treated with copper hydroxide but no substrate treatment; (5) untreated plant and pot (water spray only), no substrate treatment (untreated control). Both spreader-stickers were applied at the recommended rate, 0.4 mL of spreader sticker per 100 mL copper hydroxide suspension.

Results

LABORATORY TESTS

The copper hydroxide-based fungicide was repellent, significantly reducing the number of *Z. provisoria* snails crawling over the treated (chemical 'barrier') area of the container ($F = 57.14$; $df = 1,56$; $P < 0.001$) (Fig. 1). Snail response varied significantly through time ($F = 4.29$; $df = 6,56$; $P = 0.001$) but the interaction was not significant ($F = 0.430$; $df = 6,56$; $P > 0.853$). Over the course of 7 d there was a tendency for an increasing number of snails to ascend the sides and attain the top of the container but the effect of the copper hydroxide was clearly evident.

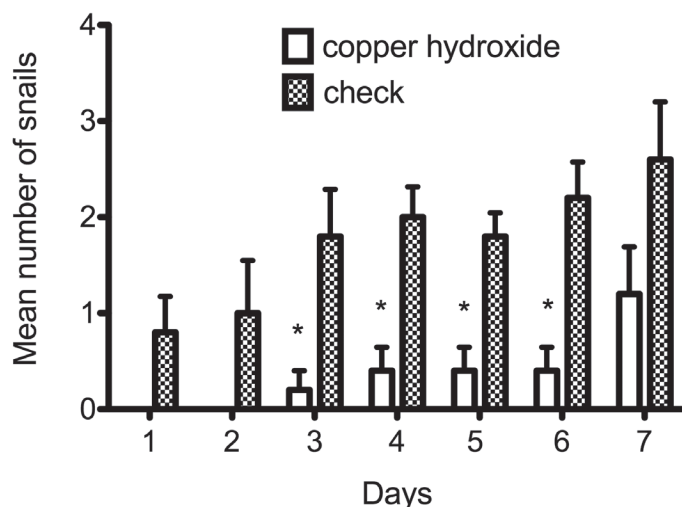


Fig. 1. Mean number (\pm SE) of *Zachrysia provisoria* snails accessing the lid of their container over a 7-d period when the interior walls of the container were coated with copper sulfate residue, or treated only with tap water (control). Days with statistically significant ($P < 0.05$) differences in snail behavior are marked with an asterisk (*).

SCREENHOUSE BARRIER TESTS

In the first test, the number of snails observed on zinnia plants did not differ significantly ($F = 0.724$; $df = 3,28$; $P = 0.546$) among treatments (Table 1). However, there were significantly more damaged leaves in the untreated control ($F = 4.064$; $df = 3,28$; $P = 0.016$) than in the treatment where the pot and plant were treated. Intermediate leaf damage occurred to plants in the pot-only treatment and the substrate treatment, but did not differ significantly compared with control or the pot plus plant treatments.

In the second test, results were largely the same, with numbers of snails not significantly affected ($F = 0.724$; $df = 4,35$; $P = 0.582$) among the treatments, but leaf damage was significantly affected ($F = 3.125$; $df = 4,35$; $P = 0.27$) (Table 2). As in the first trial, significantly more leaf damage occurred in the untreated control than in the pot plus plant treatment. Thus, addition of spreader-stickers did not significantly enhance repellent performance of copper hydroxide.

Discussion

Copper products have been shown repeatedly to have bioactivity with respect to slugs and snails (Marigomez et al. 1986; Davis et al. 1996; Moran et al. 2004; Schüder et al. 2002, 2003, 2004, 2005; Synman et al. 2005; Thompson et al. 2005; El-Gendy et al. 2009), although the nature of the bioactivity appears to take many forms. Antifeed-

Table 1. Occurrence of *Zachrysia provisoria* snails and their feeding damage to zinnia in relation to pot and plant treatments with copper hydroxide fungicide, Jun 2016.

Fungicide treatment	Cumulative mean (\pm SE) no. snails observed on plant or pot	Mean (\pm SE) no. leaves damaged
Pot only	2.1 \pm 0.8 a	3.5 \pm 0.9 ab
Substrate only	2.1 \pm 0.6 a	3.4 \pm 0.7 ab
Pot plus plant	3.1 \pm 1.1 a	1.4 \pm 0.5 b
Untreated control	3.2 \pm 0.6 a	6.4 \pm 1.4 a

Means within a column followed by the same letter are not significantly different ($P > 0.05$; Bonferroni multiple comparison test).

Table 2. Occurrence of *Zachrysia provisoria* snails and their feeding damage to Madagascar periwinkle plants in relation to pot and plant treatments with copper hydroxide fungicide, Sep 2016.

Fungicide treatment	Mean (\pm SE) no. snails observed on plant or pot	Mean (\pm SE) no. leaves damaged
Pot only	1.2 \pm 0.5 a	1.3 \pm 0.5 ab
Pot only + Bonide sticker	1.5 \pm 0.5 a	2.0 \pm 0.8 ab
Pot only + Southern Ag sticker	2.2 \pm 0.9 a	2.2 \pm 0.6 ab
Pot + plant	1.6 \pm 0.6 a	0.7 \pm 1.0 b
Untreated control	3.1 \pm 0.9 a	4.1 \pm 0.9 a

Means within a column followed by the same letter are not significantly different ($P > 0.05$; Bonferroni multiple comparison test).

ant, repellent, irritant, growth inhibition, and lethal effects have been reported, but they are not always reproducible, possibly due to the concentration of the copper molecule or the method of contact with the mollusc. Previously, we demonstrated that the copper hydroxide fungicide functioned as a repellent and feeding deterrent for 4 mollusc species (Capinera & Dickens 2016). Results of the current study reported herein provided the same responses for the snail *Z. provisoria* under laboratory conditions.

However, in screenhouse tests simulating production of zinnia and Madagascar periwinkle flowers under more environmentally adverse conditions, copper hydroxide applied as a barrier to substrate and pots did not significantly deter snails from accessing and climbing the pots. Although snails accessed plants, leaf damage was significantly reduced relative to controls when zinnia plants (and pots) were sprayed with copper hydroxide fungicide. In a follow-up test, the addition of stickers (to enhance retention of the fungicide) to the spray mixture on Madagascar periwinkle plants did not significantly reduce snail numbers in the pot or on the plants. Indeed, copper hydroxide residues did not appear to adhere well to the plastic pot, as judged by visible deposition. However, consistent with the previous test, treatment of plants with copper hydroxide significantly reduced plant damage by *Z. provisoria*. Fungicide residue seemed to be more persistent on foliage than on the plastic pots.

In summary, a clear pattern is emerging, wherein there is a consistent benefit associated with use of copper hydroxide-based fungicide for foliage protection. A clear advantage of this commercially available product is that it is registered for use on many crops as a fungicide treatment. Although fungicide residues may be objectionable in ornamental production systems, they may be acceptable in the ornamental landscape (especially if only the most susceptible plants were treated). Unfortunately, copper hydroxide residues on commercially produced ornamentals might affect acceptability by consumers and deter plant producers from using the fungicide (especially if close to the date of sale). Ideally, protection of ornamental plant foliage would occur without spraying the plants directly. Unfortunately, I did not find evidence that pot or substrate treatment was very effective. Some benefit accrued, but intermediate levels of foliage damage still occurred. There are other alternatives for mollusc management for plant producers, such as copper foil and screening, which can be used to deter mollusc movement onto greenhouse benches. Nevertheless, copper hydroxide could provide an ancillary benefit beyond the fungicidal and bactericidal properties normally associated with this pesticide.

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