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Some effects of copper-based fungicides on plant-feeding terrestrial molluscs: A role for repellents in mollusc management

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ABSTRACT

The effects of three copper-based fungicides on feeding by four terrestrial molluscs (two slugs and two snails) were assessed in laboratory choice and no-choice tests. These products functioned effectively as short-term feeding deterrents. However, a copper hydroxide-based fungicide was more persistent than copper octanate- and copper diammonia diacetate-based fungicides under field conditions. Tests with foliage treated in the field and fed to *Leidyula floridana* and *Lissachatina fulica* showed that significant feeding suppression persisted for several days. We also determined that copper hydroxide was repellent, affecting movement of *L. floridana* slugs independent of food. Further, we assessed copper hydroxide effects with and without iron phosphate molluscicide bait. Copper hydroxide alone significantly suppressed foliage consumption, but did not affect slug survival. Iron phosphate bait alone caused slug mortality, and significantly suppressed feeding, but only after 24 h. Significantly improved foliage protection, relative to iron phosphate bait application alone, was attained when copper hydroxide repellent was used simultaneously with iron phosphate bait. Where terrestrial molluscs are a plant protection issue, it appears that ancillary benefit can be derived from using copper hydroxide fungicides. These data provide evidence for the potential application of the "push–pull" or "stimulo-deterrent" concept relative to mollusc management; plant protection may be enhanced when attractant and repellent treatments are applied simultaneously.

1. Introduction

Copper is an essential element for growth of animals, and for humans, there seems to be no evidence of systemic toxicity associated with dietary exposure to copper; indeed, there may be a greater risk from deficiency of copper than from excess uptake (US EPA, 2008). Under normal conditions, especially where soil pH is controlled, copper toxicity to plants is rare (Rusjan, 2012). However, copper can inhibit fungi, bacteria, algae, and some forms of animal life, especially animals living in aquatic environments. Fish and aquatic invertebrates are quite susceptible to copper-related mortality (US EPA, 2008). On the other hand, due to their "natural" origin, several copper-containing materials are considered suitable for use in organic farming (Edwards-Jones and Howells, 2001).

Metallic copper foil has long been known to be an impediment to slug and snail movement. Slugs produce large quantities of mucus and withdraw their tentacles when they come into contact with copper foil, indicating that it is an irritant (Schüder et al., 2003). Thus, copper foil is sometimes used to limit access of molluscs to trees, greenhouse planting benches, and pots. This is not practical in outdoor planting beds due to the large amount of material needed to surround outdoor plantings, and to the ability of molluscs to dig beneath such barriers. Copper-impregnated matting and copper fungicides have also been reported to have antifeedant, repellent, irritant, growth inhibition, or lethal effects (Marigomez et al., 1986; Schüder et al., 2004). Copper toxicity often seems to be related to copper concentration level. Copper fungicides are particularly interesting as a potential mollusc management tool because many are registered for use on ornamental and food crops.

The use of copper-based fungicides for mollusc management is not a well-researched area and the results are not entirely consistent (Prystupa et al., 1987; Davis et al., 1996; Hollingsworth and Armstrong, 2003; Schüder et al., 2004; Thompson et al., 2005; Koslowski et al., 2010). Here we report studies of three copper fungicide formulations with four terrestrial molluscs of economic

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importance. The fungicides were selected to represent currently available and popular commercial formulations. We did not include copper sulfate because it is declining in popularity due to concerns about toxicity and persistence, and because of the availability of alternative products. We were particularly interested in the effects of different products on mollusc feeding behavior, and persistence under field conditions, but also examined repellency and mortality. Finally, we assessed the potential interaction of copper fungicide with an iron phosphate-based molluscicide.

2. Material and methods

2.1. Mollusc culture

The molluscs studied were two slugs: *Leidyula floridana* (Leidy, 1851) (known as Florida leatherleaf slug), and *Deroceras laeve* (O.F. Müller, 1774) (known as marsh slug), and two snails, *Ventridens demissus* (A. Binney, 1843) (known as perforate dome snail), and *Lissachatina* (*Achatina*) *fulica* (Férussac, 1821) (known as giant African snail). These molluscs damage principally ornamental plant crops. All were collected in Florida, USA, and cultured in the laboratory for 1–3 generations, where they were fed romaine lettuce (*Lactuca sativa* L. var. *longifolia*) *ad libitum*, and maintained at 24 °C and a photoperiod of 14:10 h (L:D). The snails were also supplied with garden lime mixed with gelled agar because lime is essential for shell growth, and can become limiting under laboratory conditions.

2.2. Pesticides

The effects of three commonly available fungicides were assessed for their effects on mollusc feeding. The fungicides were: (1) Bonide Liquid Copper Fungicide, produced by W. Nudorff (Germany) with 0.08% copper octanate (also called "copper soap"), which contained about 0.017% metallic copper equivalent; (2) Liquid Copper Fungicide, produced by Southern Agricultural Insecticides (Palmetto, Florida) with 27.1% copper diammonia diacetate complex, which contained about 8% metallic copper equivalent; and (3) CuPro 5000 fungicide/bactericide, produced by SePRO Corporation (Carmel, Indiana) with 61.3% copper hydroxide, which contained 40% metallic copper equivalent. The fungicides were applied at recommended rates for ornamental plants. The copper octanate product was premixed, and did not require dilution. The copper diammonia diacetate was applied at 1.04 ml formulation per 200 ml water. The copper hydroxide was applied at 0.96 g per 200 ml of water. All were misted onto the adaxial surface of foliage with a hand-held all-purpose spray bottle to runoff, and allowed to dry before being fed to test slugs or snails.

The molluscicide used was Bayer Natria Snail and Slug Killer Bait produced by W. Neudorff, Germany, containing 1% iron phosphate. The bait pellets were applied to the moist surface of soil (Robin Hood garden soil, Hood Landscaping, Adel, Georgia) at the rate of 3 pellets per slug: 21 pellets per container.

2.3. Choice tests

Choice tests were used to determine if the fungicide residue affected consumption of a preferred host, romaine lettuce. Choice tests were conducted by providing test molluscs with a choice between treated and untreated foliage for a 15 h (night-time) period, then recording the proportion of the leaf foliage consumed after visual estimation (using 5% increments). Circular leaf discs were cut from store-purchased lettuce prior to being sprayed with test materials, then allowed to dry before fungicide (treatment) and water (check)-treated discs were offered simultaneously to a mollusc. Each pair (fungicide- or water-treated) of leaf discs was placed on wet paper towels within a 1 L (15 × 7 cm; diam, ht) cylindrical plastic container with a tight-fitting lid to provide a humid environment for both the foliage and a single test animal. The molluscs were selected to be of uniform size for each experiment and evaluated in the laboratory at 24 °C and a photoperiod of 14:10 h (L:D). Weights of molluscs in these tests were 0.2–0.5 g for *D. laeve*, 0.1–0.3 g for *V. demissus*, 1–3 g for *L. floridana*, and 4–8 g for *L. fulica*. We tested 20 molluscs for each combination of fungicide and mollusc species, though in some cases the animal ate nothing, in which case it was not included in the analysis. The leaf disc size was adjusted to the appetite (size) of the animal, with the goal being enough consumption to distinguish preference and not so much that the animal was forced by hunger to eat less preferred host material. Thus, in most cases the molluscs ate at least 20% of a leaf disc, but less than half of the foliage available, or at least less than 100% of any treatment. The foliage disc size was 1.5 cm diam for *D. laeve* and *V. demissus*, 2.0 cm diam for *L. floridana*, and 6.3 cm diam for *L. fulica*.

The proportional consumption data were tested for normality using the D'Agostino and Pearson omnibus normality test. Prior to analysis, percent values were transformed to decimal arcsin square root plus 0.5 values to normalize the data. Data were analyzed using a paired t-test. Data from paired-choice assays are not independent, so paired t-tests are appropriate when both choices are available simultaneously (Horton, 1995). GraphPad Prism (GraphPad Software, San Diego) was used for these and all other analyses.

2.4. No-choice tests

The no-choice tests were conducted in the same manner as choice tests, except that each mollusc was offered only a treated or an untreated foliage disc, and 20 molluscs of each species were individually tested with treated foliage, and another 20 with untreated foliage. Also, the no-choice testing was limited to the copper hydroxide fungicide because after preliminary testing it seemed to be most promising. After testing for normality, data were transformed as in the choice test, and evaluated with an unpaired t-test.

2.5. Fungicide persistence

A preliminary test of outdoor persistence was conducted using all three fungicides or water, but with only *L. floridana* as the assay organism, and field-grown *Erechtites hieracifolia* (American burnweed; Asteraceae) as the food source. The fungicides were applied outdoors to individual plants growing at the edge of a flower garden, at the rates described above. Water was applied to check plants (controls). Plants were separated by a distance of 2 m, and marked after application so they could be re-sampled over time. Leaves were harvested after the foliage dried, returned to the laboratory after various time intervals, and discs were cut from both fungicide- and water-treated foliage. One disc from each fungicide treatment and the control was fed to each slug in a paired arrangement in a 1 L plastic cylinder, as described above for choice tests, using 20 slugs per treatment. Foliage samples were taken daily for up to 3 d after applications. The proportional consumption data were tested for normality using the D'Agostino and Pearson omnibus normality test, then transformed as above and subjected to a two-way ANOVA with fungicide treatment and days post-treatment as the principal variables. Treatment comparisons were made with the Bonferroni multiple comparison test.

More protracted assessments of fungicide persistence were conducted using copper hydroxide as the fungicide and *L. floridana* and *L. fulica* as the assay organisms. The foliage was field-grown *E.*