Assessment of Barrier Materials to Protect Plants from Florida Leatherleaf Slug (Mollusca: Gastropoda: Veronicellidae)

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Assessment of barrier materials to protect plants from Florida leatherleaf slug (Mollusca: Gastropoda: Veronicellidae)

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Abstract

Potential barrier materials (diatomaceous earth, hydrated lime, sulfur, fumed silica, wood ash) and chemical repellents (2 essential oil-based sprays) were evaluated for their efficacy to prevent herbivory by the Florida leatherleaf slug, *Leidyula floridana* (Leidy, 1851) (Gastropoda: Veronicellidae) by placing a band of material around the plant tissue or by direct application to foliage. Hydrated lime and sulfur effectively prevented foliage damage compared with the other 3 materials when presented as barriers. Dusting or sprinkling these barrier materials on foliage generally did not inhibit feeding by slugs, but sprinkling hydrated lime or wood ash onto foliage significantly reduced herbivory. Two commercially available essential oil formulations were evaluated for their effectiveness at repelling *L. floridana*. Pure 'N' Natural Snail & Slug Away (a cinnamon oil-based product) was quite effective whereas Slug & Snail Defense (containing cedar oil, pine oil, peppermint oil, and white pepper) was ineffective. Contact toxicities of barrier materials and essential oil products were assessed, and compared with a metaldehyde-based bait (Corry's Slug and Snail Pellets), an iron phosphate-based bait (Ecosense Slug and Snail Pellets), and a sulfur-based bait (Ortho Bug-getta Snail and Slug Killer$_2$). Mortality of slugs was high when slugs were exposed to metaldehyde, hydrated lime, and the cinnamon oil-based spray. Iron phosphate bait, sulfur bait, and fumed silica were less effective but provided some reduction in herbivory. Soil moisture adversely affected efficacy of barrier materials, significantly compromising functionality relative to dry soils. Water uptake from soil by barrier materials was positively correlated with foliage consumption but pH was negatively correlated. Because of the negative correlation of pH with consumption, the negative effects of soil moisture on barrier effectiveness, and the rapid physiological response of slugs to some materials, these barrier materials are perhaps better considered to be physiochemical barriers rather than physical barriers.

Key Words: *Leidyula floridana*; repellents; hydrated lime; sulfur; molluscicides

Resumen

Materiales de barrera potencial (tierra de diatomeas, cal hidratada, azufre, sílice pirógena, ceniza de madera) y repelentes químicos (2 aerosoles a base de aceites esenciales) fueron evaluados por su eficacia para prevenir la herbivoría por *Leidyula floridana* (Leidy, 1851) (Gastropoda: Veronicellidae) colocando por medio de bandas de material alrededor del tejido de la planta o por aplicación directa al follaje. La cal hidratada y el azufre previnieron eficazmente el daño del follaje en comparación con los otros 3 materiales presentados como barreras. La aplicación de estos materiales de barrera en forma de polvo o rociando en el follaje generalmente no inhibe la alimentación por las babosas, pero rociando con cal hidratada o ceniza de madera en el follaje reduce significativamente la herbivoría. Se evaluaron dos formulaciones de aceites esenciales comercialmente disponibles para determinar su efectividad para repeler *L. floridana*. "Pure 'N' Natural Snail & Slug Away" (un producto basado en aceite de canela) fue bastante eficaz, mientras que "Slug & Snail Defense" (que contiene aceite de cedro, aceite de pino, aceite de menta y pimienta blanca) no fue eficaz. Se evaluaron las toxicidades de contacto de los materiales de barrera y aceites esenciales, se compararon con cebos basados en metaldehído ("Correy's Slug & Snail Pellets"), un cebo basado en fosfato de hierro ("Ecosense Slug y Snail Pellets") y un cebo a base de azufre "Ortho Bug -getta Snail and Slug Killer$_2$". La mortalidad de las babosas fue alta cuando estas estuvieron expuestas a metaldehído, cal hidratada y aceite de canela en forma para rociar. Cebo de fosfato de hierro, cebo de azufre y sílice pirógena fueron menos efectivos pero proporcionaron cierta reducción en herbivoría. La humedad del suelo afectó negativamente la eficacia de los materiales barrera, comprometiendo significativamente la funcionalidad relativa a suelos secos. La absorción de agua del suelo por materiales barrera se correlacionó positivamente con el consumo de follaje, pero el pH se correlacionó negativamente. Debido a la correlación negativa del pH con el consumo, los efectos negativos de la humedad del suelo sobre la efectividad de la barrera, y la respuesta fisiológica rápida de las babosas a algunos materiales de barrera son mas bien considerados como inhibidores físicoquímicos en lugar de barreras físicas.

Palabras clave: *Leidyula floridana*; repelentes; cal hidratada; azufre; molusquicidas

Terrestrial mollusc plant pests commonly are managed by application of chemical toxicant-containing baits. However, these baits often are not suitably attractive, or molluscs do not eat enough of the toxicant to be killed, resulting in plant damage. Physical barriers are sometimes suggested as alternatives to toxicants. Efficacy data to support use of barriers are limited and sometimes suspect. Probably the best example of this is the reputed benefits of diatomaceous earth, which has been reported to kill insects by absorbing the oily or waxy outer layer of the cuticle, and may possibly cause physical abrasion (Quarles 1992; Korunic 1997, 1998). Toxicity and repellency of diatomaceous earth

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have been suggested not only for insects but also for slugs; claims can be traced back to unsubstantiated reports communicated by DeCrosta (1979) in the organic gardening literature. This author reported that a reduction in plant damage by slugs was achieved by placement of an irritating physical barrier of diatomaceous earth around each plant. However, the epidermis of slugs and snails is nothing like the cuticle of insects, and even if it was, diatomaceous earth works best under dry conditions (e.g., in stored grain and indoors [Korunić et al. 2016]), an environment not favored by molluscs. Molluscs are largely protected from abrasion and desiccation by a layer of slime that they deposit while they move. Nevertheless, the organic gardening literature continues to promote diatomaceous earth as a remedy for slug herbivory.

Some data suggest that plant feeding by terrestrial molluscs can be disrupted without using a toxicant. Cinnamamide, a synthetic material that is based on the plant-produced cinnamic acid, was shown to be useful as a seed repellent against the slug *Deroceras reticulatum* (O.F. Müller, 1774) (Gastropoda: Agrolimacidae) (Watkins et al. 1996). Copper-based products, urea-formaldehyde, and garlic concentrate were reported by Schuder et al. (2003) to adversely affect the movement of the slug *Deroceras panormitanum* (Lessona and Pollonera, 1882) (Gastropoda: Agrolimacidae) and the snail *Oxyloma pfeirreri* (Rossmassler, 1835) (Gastropoda: Succineidae). Thompson et al. (2005) found that copper hydroxide affected feeding by *Deroceras laeve* (Müller, 1774) (Gastropoda: Agrolimacidae) and *Ambiogolimax valentianus* (*Lehmannia valentiana*) (Ferussac, 1822) (Gastropoda: Limacidae) in choice tests; similarly, Capinera & Dickens (2016) reported that 2 species of snails (*Ventridens demissus* [A. Binney, 1843] [Gastropoda: Zonitidae] and *Lissachatina fulica* [Férussac, 1821] [Gastropoda: Achtinidae]) and 2 slugs (*Deroceras laeve* and *Leidyula floridana* (Leidy, 1851] [Gastropoda: Veronicellidae]) were deterred from feeding by copper hydroxide in choice- and no-choice tests. Laznik and Trdan (2016) reported that hydrated lime and wood ash could be used to form a barrier and provide some protection of leaf tissue from herbivory by *Arion* slugs (Gastropoda: Arionidae). Lindqvist et al. (2010) found that birch tar oil would repel the snail *Arianta arbustorium* (Linnaeus, 1758) (Gastropoda: Helicidae) and the slug *Arion lusitanicus* Mabille, 1868 (Gastropoda: Arionidae). Kheirodin et al. (2012) reported that mineral oil could prevent *Caucasotachea lencoranea* (Musson, 1863) (Helicidae) snails from climbing citrus trees. Kozlowski et al. (2010) compared several toxicants and antifeedants for foliar protection from *A. lusitanicus*, with some antifeedants (e.g., cinnamamide, scopoletin, copper compounds) providing a measure of foliage protection.

Clearly, there are materials that have the potential to limit or reduce the damage potential of terrestrial molluscs, but data on effectiveness are lacking. Here I report on the feeding deterrent and toxic effects of potential barrier materials (diatomaceous earth, hydrated lime, sulfur, fumed silica, wood ash) and 2 commercially available essential oil-based products reputed to possess repellent properties. For comparison purposes, 3 conventional bait products, (a metaldehyde-based bait, an iron phosphate-based bait, and a new sulfur-based bait) were included in the toxicity portion of the study.

Methods

MOLLUSC CULTURE

Leidyula floridana collected in Florida, USA, were cultured in the laboratory for 6 to 8 generations before use in these studies. Slugs were fed romaine lettuce (*Lactuca sativa* L. var. *longifolia*) ad libitum, maintained at 24 °C and a photoperiod of 14:10 h (L:D). They were reared in 30 × 22 × 10 cm (L × W × H) plastic boxes (TriStatePlastics, Dixon, Kentucky, USA) 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, and 92.4% sand. The organic matter content was 11.5%.

MATERIALS TESTED

Several materials were evaluated for feeding deterrent and toxic properties, though the methodology varied depending on their physical properties. The diatomaceous earth was Safer Brand diatomaceous earth (Woodstream Corporation, Litilz, Pennsylvania, USA), and is sold for pest control. Amorphous fumed silica was obtained from Cab-o-sil, Cabot Corp., Tuscola, Illinois, USA. Hydrated lime (Mrs. Wage's Pickling Lime, Kent Precision Food Group Inc., St. Louis, Missouri, USA) used in these bioassays was a commercial product formulated for human consumption. This form of lime is more consistent and contains fewer contaminants than agricultural lime, so is more desirable for research purposes. The sulfur used in these studies was Snake Eyes Brand, Hi-Yield Dusting & Wettable Sulfur (Voluntary Purchasing Group, Bonham, Texas, USA) and also is sold for pest control. Two new products based on essential oils and claiming effectiveness as snail and slug repellents recently became available and also were evaluated. One product is Slug & Snail Defense (Pinelake Industries, Carollton, Texas, USA), and is marketed as a repellent for slugs and snails, but does not claim to be a toxicant. Its active ingredients are 0.05% cedar oil, 0.0025% pine oil, 0.0025% peppermint oil, and 0.00125% white pepper. It is pre-formulated and is ready to apply from a spray bottle. The other is the similar-sounding Pure 'N' Natural Snail & Slug Away (Gro-Power, Inc., Chino, California, USA), which contains 0.6% cinnamon oil as the active ingredient. As with the aforementioned product, it is pre-formulated and ready to apply. However, Snail & Slug Away is claimed to provide repellent and toxic properties. It also is available as a granular formulation but this was not evaluated. For comparative toxicity purposes, 3 conventional bait products also were evaluated on *L. floridana*: a metaldehyde-based bait (Corry's Slug and Snail Pellets, Matson LLC, North Bend, Washington, USA), an iron phosphate-based bait (Ecosense Slug and Snail Killer, Ortho, Marysville, Ohio, USA), and a new sulfur-based bait (Ortho Bug-getta Snail & Slug Killer$_2$, The Ortho Group, Marysville, Ohio, USA) also were included.

INHIBITION OF FEEDING

Barrier Studies

Laboratory studies were conducted to evaluate the effectiveness of various materials to prevent slug feeding. In some of these assays, slugs were required to cross through or over a physical barrier to access food. The bioassay chamber (Fig. 1) consisted of a 22 L × 30 W × 10 H cm plastic box containing about 4 cm of moist garden soil. Slugs (2–5 g) were introduced to region Fig. 1a. The center of the box contained a 14-cm diam plastic Petri dish (Fig.1b) containing soil. A smaller (8 cm diam) Petri dish was completely filled with moist soil (about 25% moisture), and a 4.8-cm diameter disc of lettuce was placed on the soil surface to serve as an attractant (food source) for the slug. The smaller dish (Fig. 1c) was recessed in soil contained within the larger dish (Fig. 1b). Soil of the larger dish was either dry (< 1% moisture) or moist (about 25% moisture) depending on treatment. The larger dish (Fig. 1b) was recessed in moist soil (about 25% moisture) contained in the bottom of the box (Fig. 1a) and the level of soil and Petri dishes adjusted to a uniform depth in the box. Treatment material was heaped to a depth of about 2 cm onto the narrow (3 cm) ring of soil (Fig. 1b) surrounding the small dish and food. Thus, to access food the slug would pass

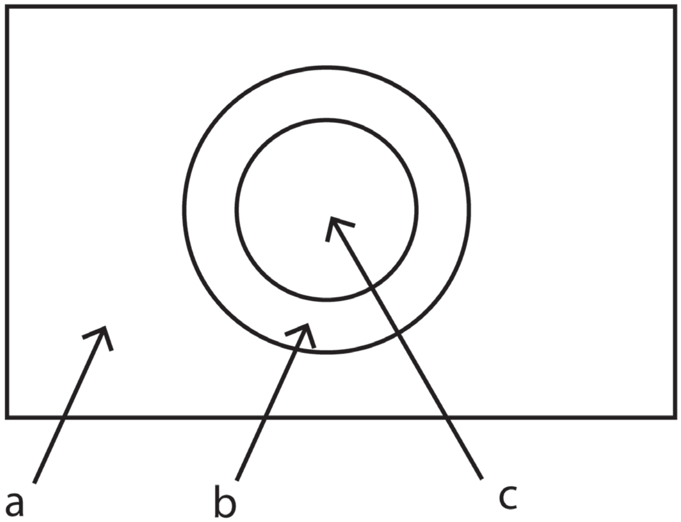

Fig. 1. Diagram of barrier and soil moisture evaluation arena. Slugs were inoculated onto moist soil in region "a," and could access a leaf disc on moist soil in region "c" after traversing region "b" that contained wet or dry soil that was either with or without physical 'barrier' material. The circles represent a small plastic Petri dish within a larger Petri dish; the walls of dishes preserved the integrity of the soil moisture treatments.

through or over the 2 × 3 cm band of treatment material. Consumption of the lettuce in the center-most dish was used as evidence that the slug entered and displayed feeding behavior. The level of foliage consumption was determined using a LI-COR 3000 leaf area meter (LI-COR Corporation, Lincoln, Nebraska, USA). Bioassays were conducted through 1 dark cycle during a 14:10 h (L:D) photoperiod, and at 24 °C. Slugs were tested only once for their response to each material, then held for 2 wk to assure that they were not adversely affected by the treatment before being used for other evaluations.

Each potential barrier material was evaluated by adding it atop a narrow (3 cm wide, 2 cm height) ring of soil surrounding the center-most dish (Fig. 1b) containing food. Moisture treatments consisted of: (1) dry soil atop moist soil; (2) barrier material atop moist soil; (3) dry soil atop dry soil; (4) barrier material atop dry soil. Dry soil was air dried and contained < 1% moisture when assessed by oven drying to a constant mass. Moist soil was adjusted to about 25% moisture by adding water to dry soil. Because the dry soil was contained within a closed box containing moist soil, a small amount of moisture was acquired from the atmosphere. Also, when dry soil or barrier material were placed atop moist soil they soon became moist.

For the purpose of these studies, 4 containers, each with 1 of the 4 moisture treatments, constituted a replicate and were assessed simultaneously. Fifteen replicates (60 slugs in total) were assessed, each on a different day. The numbers of slugs that consumed leaf material in each treatment were analyzed (GraphPad Prism, Graphpad Software, San Diego, California, USA) with Chi-square, and then (as needed) with Fisher's exact test to determine the statistical significance of specific contrasts. Leaf consumption data (cm^2) were assessed with the D'Agostino and Pearson omnibus normality test and transformed (log [X + 1]) before analysis with a 1-way ANOVA (GraphPad Prism). Slugs varied in mass from about 2 to 5 g each, but within each replicate the sizes of the slugs were equivalent (± 0.5 g). Treatment differences in mean numbers of slugs feeding and mean levels of consumption were considered significant at $P < 0.05$. The following potential barrier materials, each applied under the 4 moisture conditions, were used in bioassays: diatomaceous earth, hydrated lime, sulfur, amorphous fumed silica, and wood ash.

Snail & Slug Away also was assessed, but using a modification of the laboratory physical barrier tests. As with the physical barrier tests, a single slug was released into a moist, soil-filled container and had to traverse a 'barrier' on the surface of the soil to access a disc of foliage. However, the barrier was simply the outer 3 cm (rim) of a 14 cm diam plastic plate from which the middle 8 cm diam had been removed. The plastic rim was sprayed with Snail & Slug Away, and air-dried prior to introducing the slug; thus, in this study the slugs were confronted with a chemical barrier rather than a physical barrier. I compared the number of slugs feeding and the level of consumption, as in the barrier studies, for Snail & Slug Away-treated plate rims and tap water-treated plate rims (control). A total of 20 slugs were tested with the Snail & Slug Away treatment and their corresponding water controls. The numbers of slugs that consumed leaf material in each treatment was analyzed with Fisher's exact test. The leaf consumption data (cm^2) were assessed with the D'Agostino and Pearson omnibus normality test, and transformed (log [X + 1]) before analysis with a *t*-test (GraphPad Prism).

Feeding Deterrent Properties

The effectiveness of the materials used in barrier assays to deter leaf consumption by slugs also was assessed by choice tests. Each material was separately applied to the adaxial leaf surface of 4.8-cm diam discs of lettuce as either a dust, or sprinkled as small clumps, in choice tests (treated versus untreated leaf discs). Following is the basic protocol used for diatomaceous earth, followed by any modifications for other treatments.

Diatomaceous earth in dust form was applied with a DeVilbis model 15 atomizer (DeVilbis Healthcare, Port Washington, New York, USA); about 5 puffs were applied to 4.8-cm diam discs of lettuce, resulting in a very light but visually discernable covering of dust over the entire surface of the leaf. The amount of dust applied was too small (< 0.0001 g) to be detected with an analytical balance (Mettler Toledo AL104, Greifensee, Switzerland). Diatomaceous earth also was sprinkled in clumped form by shaking onto separate leaves with a modified pepper shaker (holes enlarged to 2 mm diam). The application resulted in 2 to 5 mg of material per leaf disc, but in an intermittent pattern, with clumps separated by small areas of untreated leaf surface. For both application methods, a treated and an untreated (control) leaf disc of the same size were placed on a moist paper towel in an 18 cm diam, 8 cm H plastic container (TriStatePlastics, Dixon, Kentucky, USA), and a slug added. Total consumption of treated and untreated leaf discs was assessed using a LI-COR 3000 leaf area meter (LI-COR Corporation, Lincoln, Nebraska, USA). Assays were conducted through 1 dark cycle during a 14:10 h (L:D) photoperiod, at 24 °C. I evaluated 40 slugs in paired-choice feeding tests for dusting, and 30 slugs for sprinkling, with each slug in a separate container. Slugs were selected to be similar in size, and varied in mass from about 3 to 5 g each. After assessment with the D'Agostino and Pearson omnibus normality test, the leaf consumption data were transformed (log [X + 1]) and analyzed with a paired *t*-test (GraphPad Prism). Replicates were not included in the analysis when there was no consumption of a leaf disc. Treatment differences in choice tests were considered significant at $P < 0.05$.

Assessment of hydrated lime and fumed silica were conducted in the same manner as the diatomaceous earth study, except as follows: the paired-choice feeding deterrent assessments using the dusting and sprinkling methods were conducted with 20 slugs in individual containers in each case.

Assessment of sulfur was conducted the same manner as the diatomaceous feeding deterrent studies, except as follows: because sulfur can be applied as a wettable powder, in addition to application via dusting and sprinkling, this material was applied as a wettable powder by misting onto leaf discs with a sprayer according to the manufacturer recommendations (1.6 g sulfur per 100 mL water) and allowed to dry for 2 h before being presented to slugs in a choice test with water-treated (control) foliage. Also, the number of replicate slugs in the paired-choice tests was either 20 or 25.

Assessment of wood ash was conducted in the same manner as the diatomaceous earth study, except as follows: the paired-choice feeding deterrent assessments using the dusting and sprinkling methods were conducted with 25 slugs in individual containers in each case.

Two products containing essential oils (Slug & Snail Defense and Pure 'N' Natural Snail & Slug Away) were included in paired-choice foliage consumption studies in the same manner as the diatomaceous earth feeding deterrent (choice) studies, except that they were sprayed onto foliage discs directly from the spray bottle. Untreated leaf discs were sprayed with tap water as a control. For Slug and Snail Defense, 40 slugs were evaluated while 20 slugs were evaluated in Snail & Slug Away tests.

TOXICITY

Toxicities of all materials used in barrier assays on *L. floridana* were evaluated and compared with conventional bait-based treatments. Most products were applied by rolling 2 to 5 g slugs in the test materials; however, the essential oils (Slug & Snail Defense, Snail & Slug Away) were applied by spraying until the slugs were thoroughly wetted. Also, the 3 baits were fed to the slugs. Baits were evaluated by scattering 1 g of bait pellets plus an individual slug in the bottom of a 500 mL plastic container with moist filter paper in the bottom and lettuce as food for a 24 h period.

After exposure to each treatment material, 4 slugs were confined to each of 5 replicate cylindrical plastic cages (15 cm diam, 6 cm H) containing a single layer of moist paper towel, plus lettuce, in the bottom of the cage. Barrier test material initially adhered and often was retained, at least in part, on the dorsal surface of their bodies for 18 h. Slugs were monitored for 7 d, with the towel and lettuce replaced, and the cage cleaned daily. Untreated controls consisting of 5 cages, each with 4 slugs not exposed to treatment materials, were similarly confined to cages with moist paper towel and monitored in the same manner. I used 220 slugs in this study (10 treatments plus an untreated control, each with 5 replicate cages containing 4 slugs per cage). After 5 d, each cage was provided with a premeasured 8 × 8 cm piece of lettuce for 24 h, then leaf consumption assessed by measuring remaining leaf tissue with the leaf area meter. The proportions of dead slugs per container after 7 d were arcsine square root transformed before analysis with a 1-way ANOVA. Leaf consumption data (cm^2) on d 5 were log (x + 1) transformed and also subjected to a 1-way ANOVA. Significantly different treatment means for mortality and consumption were determined with Bonferroni's multiple comparison test ($P < 0.05$).

CHARACTERISTICS OF DRY BARRIER MATERIAL

It was immediately apparent that there were changes in the physical character of the dry materials when they were applied to wet soil. The dry material absorbed a considerable amount of liquid, which could affect the structure, and potentially the efficacy. To assess this potential issue, diatomaceous earth, hydrated lime, sulfur, fumed silica, and wood ash were spread over moist or dry soil to determine moisture uptake (increase in mass) by the material. All soil, and plastic containers, used in these studies were the same as specified under "mollusc culture." For preparation of moist soil, tap water was added to a large batch of dry soil to bring the soil to about 25% moisture (wt/wt). The moist soil was partitioned into 3 containers to a depth of 5 cm. Then, 50 mL of each barrier material was spread in a small area of each container to a depth of about 2 cm, and the container lid affixed. After 20 h, the lid was temporarily removed and a Hobo data logger (Onset Computer Corporation, Bourne, Massachusetts, USA) inserted to monitor humidity of the headspace above the soil. After another 4 h, with the lid in place, the humidity of the air was determined, and soil and barrier material samples (10–15 g each) were taken from each box. After determining wet mass, each sample was dried to a constant mass and the difference used to calculate moisture uptake. The same process was used to assess dry soil and moisture uptake potential from dry soil. To assess the capability of the different materials to absorb moisture from the soil, moisture levels (%) were transformed to arcsine square root prior to analysis with 1-way ANOVA.

The relationship of barrier material moisture-holding capacity on foliage consumption was assessed with Pearson correlation analysis (r) by correlating maximum water uptake on wet soil with actual leaf consumption by slugs when the barrier was applied to wet or dry soil. Similarly, barrier material pH was correlated with actual leaf consumption by slugs when the barrier was applied to wet or dry soil.

To assess the effects of relative humidity in the air above the moist soil on moisture content of the barrier material, independent of contact with soil, 15 g samples of each material were placed into 8 cm diam Petri dishes without lids, then inserted into 3 replicate boxes containing wet or dry soil. Conditions were the same as mentioned previously for barrier material contact with soil. Samples were weighed after 24 h and the difference in mass was used to calculate moisture uptake from the atmosphere. To assess the ability of the different materials to absorb moisture from the air, moisture levels (%) were transformed to arcsine square root prior to analysis with 1-way ANOVA. Differences within each statistical analysis for the measured physical parameters were considered significant at $P < 0.05$.

The pH of the physical barrier materials was assessed with an ExStick model 100 pH meter (Extech Instruments, FKIR Systems, Inc., Nashua, New Hampshire, USA) with resolution of 0.01 pH. Ten g of each barrier material was stirred with 90 mL of distilled water for 15 s prior to acquiring the readings, following the method of Korunić (1997) for diatomaceous earth. All physical characteristic measurements were conducted in the laboratory at 24 °C.

In addition, the 2 forms of silica (diatomaceous earth and fumed silica) evaluated in these tests were compared microscopically. Samples were examined, and photographs taken, with Auto-Montage Pro software (Version 5.02, Syncroscopy, Cambridge, United Kingdom) linked to a Leica DMLB compound microscope.

Results

The total number of slugs feeding on foliage was not significantly affected ($X^2 = 5.77$; df = 3; $P = 0.123$) by the diatomaceous earth barrier or soil moisture (Table 1). Similarly, mean foliage consumption was not affected by soil moisture conditions or the presence or absence of diatomaceous earth on the soil ($F = 1.475$; df = 3,56; $P = 0.231$) (Table 2). In paired-choice tests, dusting or sprinkling plant discs with diatomaceous earth did not have a statistically significant effect (Table 3).

Hydrated lime was considerably more active than diatomaceous earth. In the barrier and soil moisture test, a highly significant reduction in the number of slugs feeding occurred ($X^2 = 32.85$; df = 3; $P < 0.001$) (Table 1). Foliage consumption was significantly ($F = 13.05$; df = 3,56; $P < 0.001$) affected by lime and soil moisture in the barrier study (Table 2); foliage consumption was high in containers with wet soil without lime. Dusting foliage with lime did not significantly affect leaf consumption in the paired-choice tests. Slugs consumed about

Table 1. Total number of *Leidyula floridana* slugs consuming or not consuming foliage in relation to soil moisture level and barrier material.

		Wet soil		Dry soil	
Barrier material	Consumption status	Soil only	Soil + barrier	Soil only	Soil + barrier
Diatomaceous earth	Yes	15	14	11	12
	No	0	1	4	3
Hydrated lime	Yes	15	8	11	0
	No	0	7	4	15
Sulfur	Yes	15	8	12	2
	No	0	7	3	13
Fumed silica	Yes	15	14	9	12
	No	0	1	6	3
Wood ash	Yes	15	14	13	12
	No	0	1	2	3

the same amount of foliage when lime dust was absent (controls) as when present (Table 3). However, when lime was sprinkled onto foliage, resulting in greater quantity per leaf, consumption of foliage was considerably reduced compared with untreated foliage. Efforts by slugs to avoid the sprinkled lime were evident as they fed in small patches forming a serrated edge on the leaf disc.

Sulfur affected the total numbers of slugs feeding in the barrier and soil moisture tests (X^2 = 26.72; df = 3; P < 0.001) (Table 1). The amount of foliage consumption also was significantly (F = 11.32; df = 3,56; P < 0.001) affected, especially on dry soil (Table 2). In paired-choice tests, dusting with sulfur did not significantly affect leaf consumption (Table 3). Similarly, sprinkling sulfur on leaf discs did not significantly affect foliage consumption. Likewise, a dried aqueous sulfur suspension, previously applied as a spray onto foliage, did not statistically reduce leaf consumption.

A statistically significant difference in the number of slugs feeding (X^2 = 10.08; df = 3; P = 0.018) occurred in the fumed silica barrier and soil moisture tests (Table 1). However, this was a relatively weak response and when the effects were partitioned using Fisher's exact test, the silica did not exert a significant effect (P = 0.08). Consumption of foliage was significantly (F = 5.489; df = 3,56; P = 0.002) affected by the silica barrier and soil moisture treatments. However, consumption of foliage on wet soil did not differ from consumption on wet soil plus silica. Likewise, consumption of dry soil did not differ from dry soil plus silica. This indicates that the slugs were responding to moisture levels more than the presence of silica. In paired-choice tests, dusting or sprinkling silica on leaves did not have a statistically significant effect on leaf consumption (Table 3).

The number of slugs feeding on foliage disks was not significantly (X^2 = 3.074; df = 3; P = 0.295) affected in the wood ash barrier and soil moisture test (Table 1). Likewise, foliage consumption (Table 2) was not significantly affected by the wood ash regardless of substrate moisture (F = 0.909; df = 3,56; P = 0.443). In choice tests, wood ash dusted on foliage did not significantly reduce feeding. In contrast, when a larger amount of wood ash was applied by sprinkling on foliage, it significantly reduced herbivory (Table 3).

Response of slugs to the essential oil-based products varied. The Slug & Snail Defense product had no effect on feeding by slugs in paired-choice tests. In contrast, the Pure 'N' Natural Snail & Slug Away product significantly reduced leaf consumption by slugs (Table 3).

In the treated plastic plate chemical 'barrier' no-choice test, Pure 'N' Natural Snail & Slug Away significantly affected the number of slugs feeding (P < 0.001). Foliage consumption also was significantly affected (t = 4.344; df = 38; P < 0.001). An 82% reduction in foliage consumption was attributable to Snail & Slug Away.

TOXICITY

Not surprisingly, slug toxicity varied significantly by material and mode of exposure (F = 21.73; df = 10,44; P < 0.001; Table 4) 7 d after treatment. Hydrated lime, Snail & Slug Away, and Corry's metaldehyde-based bait caused rapid death of slugs and prevented leaf consumption. Within minutes of being rolled in hydrated lime or sprayed with the Snail & Slug Away, slugs were exuding copious amounts of slime and lost their mobility. Within 1 h of treatment, all individuals in these treatments were swollen and dead. The metaldehyde product was slightly slower acting, but nearly all slugs were dead by the following d. Some of the barrier materials displayed no toxicity, or at least did not display a statistically significant level. Two products that are sold as toxic baits (iron phosphate bait and sulfur bait), and also fumed silica, induced moderate levels of toxicity.

Some toxicants, or potential toxicants, significantly reduced leaf consumption when it was assessed 5 d after treatment (F = 42.13; df = 10,44; P < 0.001; Table 4). Not surprisingly, the materials that induced

Table 2. Mean (± SE) leaf consumption (cm^2) by *Leidyula floridana* slugs of foliage (lettuce) surrounded by a ring of wet or dry soil, with or without a 'barrier' material.

	Wet soil		Dry soil	
Barrier material	Soil only	Soil + barrier	Soil only	Soil + barrier
Diatomaceous earth	9.4 ± 7.5 a	9.1 ± 1.3 a	5.5 ± 1.4 a	8.8 ± 1.7 a
Hydrated lime	9.9 ± 1.4 a	3.5 ± 1.1 bc	6.4 ± 1.5 ab	0 c
Sulfur	10.7 ± 1.2 a	6.3 ± 1.4 ab	9.1 ± 1.7 a	1.0 ± 0.7 b
Fumed silica	12.7 ± 1.7 a	9.3 ± 1.5 ab	4.7 ± 1.7 b	5.5 ± 1.7 b
Wood ash	10.3 ± 1.4 a	6.6 ± 1.0 a	8.3 ± 1.9 a	6.7 ± 1.6 a

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

Table 3. Consumption by *Leidyula floridana* slugs of treated and untreated foliage discs in choice tests when potential feeding deterrents were applied by various means.

Treatment material	Method of application	Consumption of treatment (cm^2 ± SE)	Consumption of control (cm^2 ± SE)	Statistics (*t*; df; *P*)
Diatomaceous earth	dust	6.0 ± 1.4	7.1 ± 1.6	1.730; 35; 0.092
Diatomaceous earth	sprinkle	4.2 ± 1.0	5.7 ± 0.5	0.737; 23; 0.468
Hydrated lime	dust	5.8 ± 0.8	7.4 ± 1.1	0.934; 18; 0.363
Hydrated lime	sprinkle	3.8 ± 0.7	7.3 ± 1.0	2.375; 17; 0.029
Fumed silica	dust	4.9 ± 1.2	3.2 ± 1.0	1.398; 15; 0.183
Fumed silica	sprinkle	5.0 ± 0.7	6.5 ± 1.2	0.161; 19; 0.874
Sulfur	dust	4.6 ± 1.0	4.7 ± 0.9	0.734; 21; 0.471
Sulfur	sprinkle	8.4 ± 1.3	7.9 ± 0.9	0.260; 22; 0.797
Sulfur	spray	7.1 ± 1.5	8.7 ± 1.4	1.174; 19; 0.255
Wood ash	Dust	3.7 ± 0.8	4.1 ± 0.9	1.173; 22; 0.253
Wood ash	sprinkle	3.8 ± 1.1	8.5 ± 1.3	4.2; 18; <0.001
Slug & Snail Defense	spray	6.0 ± 1.4	7.1 ± 1.6	0.199; 38; 0.843
Snail & Slug Away	spray	1.9 ± 0.9	10.8 ± 1.3	5.656; 20; <0.001

the highest mortality (metaldehyde, hydrated lime, Snail & Slug Away) caused the greatest suppression in consumption, though iron phosphate was also quite efficacious in this regard. The sulfur bait, which caused a moderate level of mortality by d 7, was not very effective at suppressing consumption when evaluated on d 5.

PHYSICAL CHARACTERISTICS OF BARRIER MATERIAL

The barrier materials, when applied to wet soil, absorbed a considerable amount of liquid almost immediately. Dry barrier materials were light in color, but within 10 min of application they darkened as water and minute organic matter were absorbed.

Water content of the 'wet' soil in this study was determined to be 25.4 ± 0.5%, and 'dry' soil contained 0.4 ± 0.2%. The moisture content of the headspace of the box containing wet soil was 97% RH, and of the container with dry soil it was 48% (close to the ambient humidity of the laboratory: 50%). After 24 h, all materials in contact with wet soil displayed a considerable increase in moisture, though the moisture uptake by barrier materials varied significantly ($F = 372$; df = 4,10; $P < 0.001$) (Table 5). In contrast, water uptake of materials in contact with dry soil was less than wet soils, though some significant differences were observed among materials ($F = 7.04$; df = 4,10; $P < 0.006$) (Table 5).

Table 4. Effects of potential toxins on mean (± SE) mortality (7 d after treatment) and mean (± SE) leaf consumption by *Leidyula floridana* slugs (5 d after treatment). Slugs were fed commercial metaldehyde-, sulfur-, or iron phosphate-based baits; sprayed with essential oil-based mollusc repellent; or rolled in various materials evaluated as barriers to herbivory.

Treatment	Mortality (%)	Leaf consumption (cm^2)
Untreated control	5.0 ± 5.0 c	7.9 ± 2.0 a
Slug & Snail Defense	5.0 ± 5.0 c	6.3 ± 1.8 b
Wood ash	10.0 ± 6.1 c	7.6 ± 2.9 a
Diatomaceous earth	20.0 ± 9.3 c	10.4 ± 2.3 a
Sulfur dust	25.0 ± 13.7 c	6.3 ± 2.5 b
Fumed silica	40.0 ± 10.1 bc	5.6 ± 1.6 bc
Sulfur bait	40.0 ± 12.7 bc	6.8 ± 4.2 ab
Iron phosphate	50.0 ± 7.9 b	2.8 ± 2.6 c
Metaldehyde	95.0 ± 5.0 a	0 d
Hydrated lime	100.0 a	0 d
Snail & Slug Away	100.0 a	0 d

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

The mass of barrier materials also increased in the boxes where they were exposed to different levels of humidity but not in direct contact with soil, with the mass following a similar pattern as with those exposed to soil, but to a lesser degree (Table 4). There were statistically significant differences ($F = 372$; df = 4,10; $P < 0.001$) in moisture uptake when barrier materials were exposed to high humidity, or to low humidity ($F = 66.6$; df = 4,10; $P < 0.001$). Very little moisture was acquired from the air in boxes with dry soil. The only exception was fumed silica, which exhibited an unusually large mass increase following exposure to high humidity.

Although the physical characteristics of barrier materials were about the same (powdery) when dry, they differed considerably when wet, or dried after being wetted. The physical characteristics most noticeably different when these materials were wetted were the occurrence of cracks and the degree of adhesiveness. The frequency of cracking by these materials after exposure to moisture can be described (from most to least) as diatomaceous earth > fumed silica > hydrated lime > sulfur = wood ash. The pattern of adhesiveness by these materials, following exposure to moisture, can be described (from most to least) as diatomaceous earth > fumed silica = hydrated lime > sulfur = wood ash. Thus, the pattern of cracking and adhesiveness were quite similar, as greater stickiness led to clumping of materials and formation of cracks. At the extremes, diatomaceous earth was slimy and sticky, and formed wide, deep cracks when wetted, whereas wood ash performed in an opposite manner.

When dried after being wetted, all materials solidified. Hydrated lime became very hard after drying, diatomaceous earth hardened but to a lesser degree, sulfur hardened somewhat but regained a powdery surface after drying, silica also hardened slightly though it crumbled easily when touched, and ash solidified least and crumbled easily.

The maximum potential level of water uptake by barrier materials (Table 5, first column of data) was positively correlated with the amount of slug feeding on wet soil ($r = 0.359$; $P < 0.002$) as well as dry soil ($r = 0.4211$; $P < 0.001$) in the barrier tests. Thus, water-uptake capacity appeared to affect foliage consumption, with wetter barrier material conducive to feeding. Also, barrier material water-uptake capacity seemed to increase consumption more under dry soil conditions than wet soil conditions.

The pH values of the barrier materials were: diatomaceous earth, 7.8; hydrated lime, 12.9; sulfur, 7.7; fumed silica, 4.35; wood ash, 10.98. These values were significantly negatively correlated ($r = -346$;

Table 5. Mean % (± SE) moisture content of material applied as barrier when in contact with wet or dry soil, and high or low humidity, for 24 h. Soil moisture levels averaged 25 and 0.4% at the high and low soil moisture levels, respectively. Relative humidity levels in the headspace of the containers averaged 97 and 48% at the high and low soil moisture levels, respectively.

	Soil moisture level (%)		Air humidity level (%)	
Barrier material	High	Low	High	Low
Diatomaceous earth	60.6 ± 0.3 c	0.7 ± 0.4 b	11.6 ± 0.4 b	0.0 ± 0.1 b
Hydrated lime	46.6 ± 0.5 d	4.5 ± 0.2 a	4.1 ± 0.7 c	2.1 ± 0.1 a
Sulfur	24.0 ± 0.1 e	0.3 ± 0.1 b	3.2 ± 0.3 c	0.4 ± 0.2 b
Fumed silica	76.7 ± 0.9 a	1.7 ± 0.5 ab	41.7 ± 4.6 a	2.1 ± 0.3 a
Wood ash	66.7 ± 2.1 b	0.2 ± 0.1 b	7.7 ± 0.7 bc	0.0 ± 0.1 b

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

$P = 0.002$) with foliage consumption on wet soil. However, pH was not significantly correlated ($r = -0.172$; $P = 0.149$) with foliage consumption on dry soil. Overall, there was a trend for high pH barrier materials to inhibit feeding.

Microscopic examination of the barrier products indicated that the diatomaceous earth particles were comprised of a mixture of intact and broken, but small, diatom skeletons (Fig. 2a). The fumed silica consisted of formless particles of varying sizes (Fig. 2b) but considerably larger than diatomaceous earth. These 2 siliceous materials are quite different structurally.

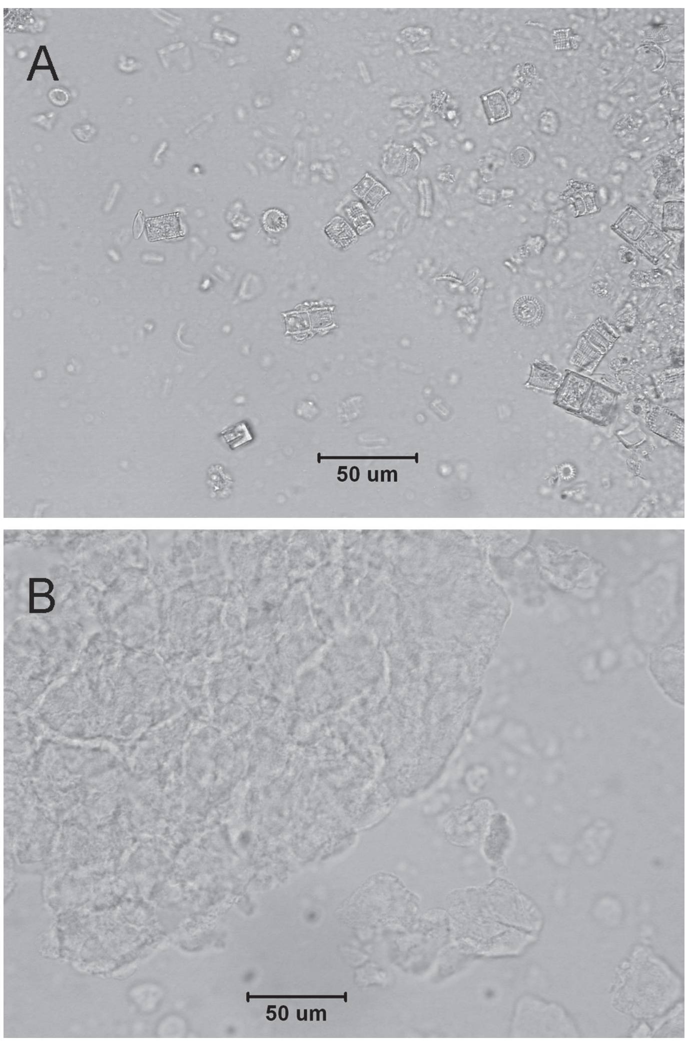

Fig. 2. Microscopic views (40×) of (A) diatomaceous earth, and (B) fumed silica.

Discussion

The materials evaluated in this study on *L. floridana* slugs varied considerably in their effectiveness at preventing foliage consumption and inducing mortality. Diatomaceous earth applied as a barrier, dust, or by sprinkling foliage did not reduce the number of slugs feeding on foliage nor the amount of foliage consumed. The recommendation to use this material to kill molluscs, or to deter their feeding on foliage, lacks consistent scientific support. Recently, the lack of diatomaceous earth efficacy also was noted by Laznik and Trdan (2016), although Ciomperlik et al. (2013) observed some bioactivity depending on the species of snails evaluated. Even with insects, diatomaceous earth is not always an effective insecticide (Martindale & Newlands 1981). Generally, similar results were obtained with wood ash, but when sprinkled on foliage it caused a significant reduction in consumption compared with no treatment. I found no evidence that fumed silica applied as a barrier reduced feeding. Dusting or sprinkling of fumed silica on foliage did not influence slug feeding in choice tests.

In contrast to the poor performance of diatomaceous earth, wood ash, and fumed silica on *L. floridana* slugs, exposure to sulfur or hydrated lime applied as barriers significantly reduced feeding regardless of soil moisture. However, both products were notably more efficacious under dry soil conditions. A similar pattern was evident in the amount of foliage consumed, wherein consumption was reduced (to a greater degree) under dry soil conditions compared with wet. In choice tests, foliage consumption was unaffected by dusting or spraying an aqueous suspension of sulfur, and by sprinkling dry sulfur. Hydrated lime had no effect when dusted on foliage, but feeding was reduced when sprinkled onto foliage. If a barrier is desired to keep slugs from accessing plants, sulfur or hydrated lime appear to be suitable choices. Laznik and Trdan (2016) observed that hydrated lime was an effective barrier treatment, but did not evaluate sulfur. Due to the ability of lime and sulfur to affect soil pH, it probably would be advisable to use lime where the soil is acidic, and sulfur where it is alkaline. I also found that fumed silica displayed some slug repellency because leaf consumption was slightly reduced in barrier tests, but from a practical perspective it was not effective.

Results from the current study showed that some materials used as barriers against slugs can absorb a considerable amount of moisture when in direct contact with moist soil, resulting in reduced effectiveness. Therefore, it may be necessary to ring a plant with a collar constructed from water-resistant material, then place the barrier material on top of the collar. Additionally, ambient precipitation and above-ground irrigation can be problematic, perhaps necessitating frequent replacement of the material. Plant collars are recommended occasionally for suppression of oviposition by root feeding insects such as cabbage maggot, *Delia brassicae* (Wiedemann) (Diptera: Anthomyidae) (Boiteau & Vernon 2001), and are available commercially for such uses, though I am unaware of research on use of collars in conjunction with physical or chemical barriers or repellents for slugs.

Although significant reduction in leaf consumption by slugs was noted for some of the dry foliar treatments, it would be difficult to maintain an effective residue on leaves, especially at heavier application rates. Moreover, the level of leaf damage incurred by some of the foliar treatments would make them impractical for use.

Application of the essential oil-based Slug & Snail Defense product provided no detectable benefit in paired-choice tests or by spraying the slugs. The purported active ingredients in this product have little scientific literature to support their use as repellents for molluscs, although some essential oils certainly display pesticidal properties, as well as repellency (Isman 2000). Mc Donnell et al. (2016) reported that peppermint oil (an active ingredient in Slug & Snail Defense) was toxic to juvenile *Cornu aspersum* (O.F. Müller, 1774) (Gastropoda: Helicidae), though at a much higher concentration than the commercial product. Indeed, the concentrations of essential oils in Slug & Snail Defense appear low because commercial repellents and insecticides based on these compounds often contain concentrations of 0.25 to 3.0%, or greater (Pavella 2016). The manufacturer of Slug & Snail Defense states that the product is not toxic, so it is not surprising that survival of *L. floridana* was not affected. Presumably, there are research data to support the marketing claims of Slug & Snail Defense as a repellent, but this is not presently available in the scientific literature. Isman (2000) noted that bioactivity of essential oils can vary among target species, but I saw no evidence of feeding deterrence by *L. floridana* in my study, nor with the snail *Zachrysia provisoria* (Pfeiffer, 1858) (Gastropoda: Pleurodontidae) (JLC, unpublished).

The cinnamon-oil based Snail & Slug Away did not prevent leaf feeding by *L. floridana* slugs but greatly reduced feeding in paired-choice tests. Importantly, this product also was a fast-acting and effective toxicant when applied directly to the mollusc. Nevertheless, Snail & Slug Away did not completely prevent access to vegetation by *L. floridana* slugs in a no-choice test. However, this product reduced the frequency of slug access when applied as a chemical repellent barrier. Snail & Slug Away might prove useful to alleviate herbivory by molluscs in home gardens, although the product's interactions with environmental factors, such as rainfall, are yet to be determined.

Categorizing and interpreting the mode of action of toxicant bait products used against molluscs is difficult because some materials may be toxic upon contact, whereas others require ingestion. The efficacy of mollusicides that act as feeding deterrents (in order to reduce or prevent foliage consumption) are dependent upon the amount of active ingredient ingested. The results of iron phosphate- and metaldehyde-based bait tests on *L. floridana* reported previously by Capinera and Guedes Rodrigues (2015) were similar to those reported here, wherein metaldehyde killed slugs quickly (with nearly complete mortality) while feeding was eliminated quickly. Iron phosphate killed *L. floridana* slugs more slowly, killing only about half of the slugs within 5 d, but eliminated feeding almost immediately (d 1). Both bait formulations were tested previously on *Z. provisoria* snails and produced substantially the same effects on mortality and feeding (Capinera 2013).

Diatomaceous earth and fumed silica are amorphous silica products, but quite different structurally. Diatomaceous earth consists predominately of the remains of fossil diatoms, with the balance consisting of inorganic oxides and salts (Quarles 1992). Fumed silica usually is made from silica sand that has been modified by heat, initially aggregating into large, 3-dimensional particles, and then aggregating further to produce fluffy material with a very large surface area. As noted previously, neither product seemed to consist of particulate material that would damage a slime-producing organism. However, Korunić (1997) noted that the toxicity of diatomaceous earth to insects can vary greatly depending on such factors as pH, shape and size of particles, size distribution, SiO_2 content, adsorption, and whether their origin was freshwater or marine diatoms. Laznik and Trdan (2016) were similarly unable to demonstrate significant toxic effects on molluscs when treated with diatomaceous earth. Silica also occurs naturally in some plants where it can have a major role in defense against insect herbivory (Reynolds et al. 2009). Selvi et al. (2015) reported that silica synthesized from rice husk ash was a desiccant that would inactivate and then kill snails and slugs. It should be noted that in the studies reported herein, a moderate level of mortality (equivalent to the mortality induced by the commercial iron phosphate bait and sulfur bait) was associated with slugs coated in fumed silica, although diatomaceous earth was not toxic. Also, the diatomaceous earth tested was about mid-range in size for diatomaceous earths (Petrović et al. 2011). Clearly, diatomaceous earth, if it affects slugs, does not do so consistently.

In summary, the responses of slugs to the materials evaluated in this study were quite variable. The materials were: neither repellent nor toxic (essential oils of Slug & Snail Defense, wood ash, diatomaceous earth), repellent but not toxic (sulfur dust), moderately toxic but not very repellent (fumed silica, sulfur bait, iron phosphate), not very repellent but quite toxic following either contact or ingestion (metaldehyde bait), or repellent and toxic (hydrated lime, cinnamon oil-based Snail & Slug Away). The use of physical barriers to protect plants from molluscs may be feasible but the practicality of barriers is questionable except perhaps in a home garden environment, because they likely would require frequent reapplication. Because the materials tested in this study absorbed moisture from the soil very quickly, application as barriers would likely necessitate application of a waterproof layer beneath them to prevent water uptake. Indeed, a method of repelling moisture from above (e.g., rainfall, irrigation) also might be required to eliminate the need for frequent re-application of material. Although only a few potential barrier materials were evaluated here, it is noteworthy that the water uptake potential by these materials was positively correlated with foliar damage potential by *L. floridana*. Also, pH of the powdered barrier materials was negatively correlated with foliage consumption. Thus, these materials should be considered as physiochemical barriers, not just physical barriers. These findings may provide clues to identification of other, more effective barrier materials.

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