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Use of Rapeseed Meal to Control Black Vine Weevil Larvae Infesting Potted Rhododendron[1]

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Abstract

Defatted rapeseed meal (RSM) from *Brassica napus* L. was applied as a mulch to potted rhododendron and strawberry (companion plants) infested with black vine weevil larvae to evaluate its potential as a natural soil fumigant. In the first year of study, three potting media (Sunshine® mix #1, 9:1 bark:sand, and 1:1 bark:peat [by vol]) were tested for their effects on larval survival. In the second year, 0, 50, 100, or 200 g (0, 1.8, 3.5, or 7 oz) of RSM were added to potted plants of rhododendron cv. 'Ignatius Sargent' grown in 1:1 bark:peat media in #1 (3-liter) containers. Meal was added approximately 16 weeks after planting rhododendron and strawberry plants. In the first study potting media type was found to have a significant effect on insecticidal activity of the meal resulting in a higher rate of larvae surviving in pots with Sunshine® mix than those with custom mixes of bark and sand or bark and peat. In the second study the highest rate of RSM, 200 g (7 oz)/1 gal pot, reduced larval survival by 70% compared to the lowest rate, 50 g (1.8 oz) RSM, and the control. Shoot growth of rhododendron was unaffected by all rates of RSM, however strawberry leaf margins became necrotic with RSM rates of 100 (3.5 oz) and 200 g (7 oz). Rapeseed meal is toxic to black vine weevil larvae, but rates necessary for effective control in a nursery application may be too high for practical use.

Index words: *Otiorhynchus sulcatus*, *Brassica napus*, isothiocyanates, *Rhododendron* 'Ignatius Sargent', *Fragaria* x *ananassa* Dushesne, alternative insecticide, soil fumigant.

Significance to the Nursery Industry

The pest status of black vine weevil has increased significantly throughout the United States during the past 20 years. Increased production of weevil host plants in containers has resulted in greater host density and optimal microhabitat conditions. In addition, registration of insecticidal soil drenches has decreased due to their residual activity and risk of contaminating ground water, limiting effective control of this pest. Current management techniques for black vine weevil fail to provide complete control. Incorporation of *Brassica* spp. tissues into the soil shows potential as replacement for conventional soil fumigants. In this study, RSM applied as a mulch strongly affected survival of black vine weevil larvae infesting potted rhododendron and strawberry companion plants. The highest application rate, 200 g (7 oz) RSM per pot, reduced larval numbers by at least 70% compared to the lowest rate, 50 g (1.8 oz) RSM, and the control. Potting media composition was also shown to affect weevil larval survival. Rhododendron shoot growth was unaffected by RSM, but rates of 100 (3.5 oz) and 200 g (7 oz) appeared to be phytotoxic to strawberry. Small scale tests of RSM application to different nursery crops species to assess phytotoxicity would be a prerequisite to large scale tests for larvae control. The increasing production of rapeseed and other *Brassica* oil seed species in the US provides a readily available source of this alternative to conventional insecticides.

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Introduction

Black vine weevil, *Otiorhynchus sulcatus* (Fabricius), is a widespread insect pest of nursery, greenhouse and field-cultivated crops throughout the continental United States. Although injury to plants was reported as early as 1890, the weevil formerly held minor pest status (12, 36). This weevil is now a serious problem in the Pacific Northwest and an increasing problem in other areas of the country. Root weevils are reported to be the most important insect pests of woody ornamentals in Oregon (9, 14) and a growing control problem in California nurseries (30) and vineyards (32).

The increase in pest status of black vine weevil in nurseries and greenhouses is due to the concentration of favored host plants, the suitability of potting mix, and environmental management of nursery stock (moisture and temperature) in ways which also favor larval survival (27, 36, 40). Distribution of this pest is believed to be caused primarily by the movement of infested plant material from propagation sources or growing areas (27, 36, 40). For example, weevils infesting vineyards of south central California coast may have been introduced from potted roses planted at vineyard entrances (32). An additional factor favoring the increase of black vine weevil is the extensive host range of more than 100 susceptible plant species (12, 14, 16, 21, 26, 34, 41). Susceptible plants include azalea, cyclamen, heather, kinnikinnick, manzanita, maples, pieris, primrose, rhododendron, salal, viburnum, yew, and most conifers.

Plant damage is primarily caused by black vine weevil larvae, which inhabit the soil, feeding on roots and boring into underground plant parts (24, 37). Economic damage may result from relatively low larval densities; for example, threshold estimates are 3 larvae per one-year-old potted rhododendron and 2–8 larvae per mature (berry producing) field-grown strawberry plant (19, 31). Adult weevil feeding on foliage is less damaging, although leaf notching reduces the quality and marketability of landscape plants and indicates infestation, which may result in consignment rejection.

Glucosinolates are sulfur-containing organic anions that are found exclusively in dicotyledonous plants and commonly

in the order Capparales. High concentrations are found in a few families, including the economically important Brassicaceae (17). Incorporation of these plant tissues into soil shows promise for reducing soil-borne plant pathogens and other pests (8, 23). Glucosinolates have limited biological activity, but enzymatic degradation by myrosinase (thioglucoside glucohydrolase, E.C. 3.2.3.1) results in the formation of a number allelochemicals, including organic isothiocyanates, which are assumed to be responsible for much of the observed pest inhibition associated with these compounds (3, 11, 38). For example, methyl isothiocyanate, is commercially available and used directly as a soil fumigant. More than 20 different aliphatic and aromatic isothiocyanates result from degradation of glucosinolates associated with commercially produced oilseed rape and mustards, *B. napus*, *B. campestris, B. juncea*, *B. hirta (= Sinapis alba)*, and *B. nigra* (13, 39). Isothiocyanates have been produced in soil amended with intact rapeseed meal (7, 8).

Our objective in the first phase of our study was to assess the influence of potting media type on insecticidal activity of defatted rapeseed meal *(B. napus)* applied as a mulch and targeted at larvae of black vine weevil. We used results of our first trial in designing trials testing higher rates of rapeseed meal on black vine weevil larvae infesting rhododendrons. Our overall objective was to determine the suitability of rapeseed meal as an amendment to control black vine weevil larvae in potted nursery stock.

Materials and Methods

Adult black vine weevils were collected from a hop (*Humulus lupulus* L. 'Chinook') yard in Greenleaf, Idaho, in June 1994 and 1995. Weevils were maintained in a laboratory colony at 21C (70F) with a 16:8 hour (L:D) daily photoperiod, using a modified rearing procedure described by Doss and Shanks (15). Weevils were fed 'Shuksan' strawberry foliage, *Fragaria* x *ananassa* Duchesne, renewed as needed every 3–4 days. Adults began oviposition during the last week of June in 1994 and 1995. Eggs were collected at 2- to 3-day intervals and held for 10 days at about 76% RH and 21C (70F) (35).

Experiment 1. 1994. Bare root plants of 'Shuksan' strawberry were individually planted in 3-liter (#1) pots in one of three potting media in April. Media used included Sunshine® mix #1 (peat and perlite), a custom mix of shredded Douglas fir bark and sand (9:1 by vol) obtained from Monrovia Nursery Company, Dayton, Oregon, or a custom mix of shredded Douglas fir bark and peat (1:1 by vol) obtained from Klupenger's Nursery, Aurora, Oregon. Custom mixes were free from pesticides. Plants were placed in an outside bed and watered regularly to maintain moisture. A shade cloth was constructed over the bed in July. Each pot was infested with 100 black vine weevil eggs, placed on soil surface at the crown of the strawberry plant. All eggs were inspected for viability before placement (1). Infestation proceeded over time, and was completed within one week in mid-July.

Defatted rapeseed seed meal, a by-product from seed pressed for industrial quality oil, was obtained by extracting the oil from machine-harvested mature seed of *B. napus* ('Dwarf Essex') (5, 8). Treatments consisted of two rates of rapeseed seed meal, two rates of detoxified rapeseed seed meal, and a control. High, 33.5 g (1.2 oz), and low, 12.5 g (0.4 oz), rates of rapeseed seed meal and of detoxified rapeseed seed meal were incorporated into the top 2.5 cm (1 in) of potting media around the strawberry plants. Control treatment consisted of light cultivation of the top 2.5 cm (1 in) of media without RSM applied. Rates were determined from previous laboratory bioassays of black vine weevil larvae (2). Meal was detoxified by saturating intact rapeseed meal with water for 48 h and allowing it to air dry. The addition of water initiates enzymatic degradation of glucosinolates, producing volatile isothiocyanates which are removed in the vapor phase during the saturation period (5). Treatments were blocked over time, August 22–30. Experimental design consisted of a 3 × 5 factorial (3 potting mixes and 5 rapeseed treatments) in a randomized complete block design with 10 replications. Surviving larvae were recovered by sifting through the potting media by hand in late fall. Analysis of variance was used to test for RSM treatment effects on surviving number of larvae by using the GLM procedure, and significant differences between treatment means were determined by a protected LSD (33).

Experiment 2. 1995. In April, rhododendrons 'Ignatius Sargent' in 5-cm (2-in) pots were transplanted into 3-liter (#1) pots filled with a custom rhododendron medium of bark and peat (1:1 by vol) obtained from Klupenger's Nursery. A bare root 'Shuksan' strawberry plant, was also planted in each pot to provide early root mass for larval establishment. Drainage holes in pots were covered with nylon screening (18 threads/2.45 cm [1 in] mesh) to minimize larval escape and prevent entrance of other arthropods. Potted plants were placed in an outdoor gravel-lined bed. An automatic watering system with individual spray emitters for each pot was used to maintain adequate moisture to the plants. Tanglefoot was applied to boards lining the perimeter of the bed to exclude local black vine weevil adults. A 5-cm (2-in) gauge wire fence was erected around the bed to exclude rabbits.

Each pot was infested with 100 black vine weevil eggs and 100 first instar larvae. Eggs and larvae were transferred to the base of plant stems with a camel hair brush and covered with 1-cm (0.4-in) of potting medium. Infestations were applied over time, starting mid-July and completed within two weeks.

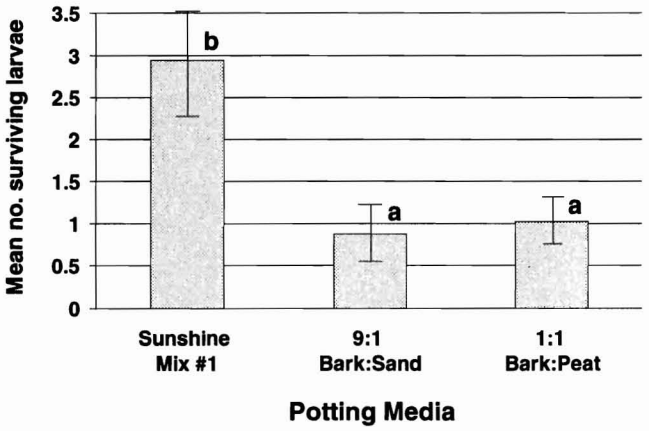

Fig. 1. Mean number of surviving black vine weevil larvae per pot recovered from strawberry plants grown in three separate media and treated with rapeseed seed meal applied as a mulch. Bars with the same letter are similar (Protected LSD, $P = 0.05$, n = 150). Media consisted of Sunshine® mix #1 (peat and perlite), a custom mix of shredded Douglas fir bark and sand (9:1), or a custom mix of shredded Douglas fir bark and peat (1:1).

Treatments consisted of three rates of RSM (50, 100, or 200 g [1.8, 3.5, or 7 oz]), one rate (200 g [7 oz]) of detoxified meal and a control. Meal was cultivated into the top 2.5-cm (1 in) of medium and watered as needed to moisten. Control treatment included mechanical disturbance of the top 2.5 cm (1 in) of potting medium. Each pot was covered with 3 mil plastic sheeting to concentrate volatiles for 48 hr. Treatments were applied over time, beginning mid-August and completed within two weeks. Experimental design consisted of a randomized complete block, with 10 blocks and 20 replications. Surviving larvae were recovered by sifting through potting medium by hand in late fall. Above ground rhododendron biomass was weighed to assess treatment phytotoxicity effects on plants. Analysis of variance was used to test for RSM treatment effects on surviving number of larvae and on rhododendron shoot biomass weight using GLM and ANOVA procedures, respectively, and significant differences between treatment means were determined by a Protected LSD (33).

Results and Discussion

Experiment 1. 1994. Larval survival was unaffected by RSM treatments. However, potting media affected weevil larvae (Protected LSD, $P = 0.0038$ [33]), with a substantially higher rate of larvae surviving in pots with Sunshine® mix than those with custom mixes of bark and sand or bark and peat (Fig. 1). Altered larval survival may result from direct effects related to media moisture and organic matter content or indirect effects of the media on efficacy of soil fumigants released from rapeseed meal.

Moisture can greatly affect the survival and behavior of immature and adult black vine weevil. Relative humidity below 65% at the soil surface substantially reduces egg hatch and survival of neonate larvae (35). Owen et al. (29) found significantly less adult feeding damage in non-irrigated as compared to irrigated landscape beds. Type of potting media affects moisture content as was shown by Nielsen and Roth (28) who found moisture significantly higher in peat than in pine or hardwood bark media. Nielsen and Roth (28) also found larval mortality consistently higher in potting media with low moisture in conventional insecticide bioassays than in media with high moisture content. Our results were consistent with these studies in that more larvae survived in Sunshine® mix #1, which of the three media used has the highest percentage of peat. Although larval survival was similar in the remaining media, the highest mortality was found in the 9:1, bark:sand mix, which lacked peat. In addition to affecting the moisture content, organic matter impacts degradation of glucosinolates and the rate of isothiocyanate breakdown. In studies of glucosinolate transformations in soil, allyl isothiocyanates disappeared most quickly in dry soils having a high concentration of organic carbon (4). The concentration of fumigants in soil is thus reduced by sorption. In testing fumigants with contrasting soil types, Matthiessen et al. (20) found peat to be more absorptive than sand or loam and found methyl isothiocyanate to be rapidly absorbed by soil leaving only ≈1% of the original concentration remaining 2 hr after injection.

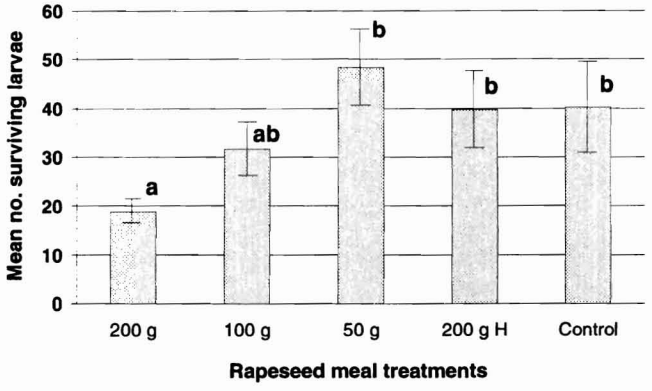

Fig. 2. Mean number of surviving black vine weevil larvae per pot recovered from potted rhododendron plants treated with rapeseed seed meal applied as a mulch. Media with same letter are similar (Protected LSD, $P = 0$, n = 50). Treatments consisted of three rates of rapeseed seed meal (50, 100, or 200 g [1.8, 3.3, or 7 oz]), one rate (200 g [7 oz] H) of detoxified (hydrolyzed) meal and control.

Experiment 2. 1995. Since RSM treatments failed to affect larval survival in the 1994 results, we substantially increased the application rates of rapeseed meal in our trials of 1995. We found that RSM treatments at the higher rates strongly affected weevil larval survival (Protected LSD, $P = 0.0074$ [33]). The highest application rate, 200 g (7 oz) RSM, reduced larvae by at least 70% compared to the lowest rate, 50 g (1.8 oz) RSM, and the control (Fig. 2). Potting medium should have had little, if any, influence on larval survival since all plants were grown in the same custom mix. Rapeseed meal has been shown to be toxic to black vine weevil larvae and LC_{50s} have been estimated in laboratory bioassays (2). These and previous findings have shown that the toxicity is due to organic isothiocyanates, which are degradation products of glucosinolates present in *Brassica* spp. tissues (4, 11, 22). Detoxified rapeseed meal, from which isothiocyanates have been removed, applied at a rate equal to the highest rate of intact RSM (200 g [7 oz]), in our study resulted in larval survival similar to our control treatment.

Visual observation of the potted plants during this study indicated little if any effect of RSM treatments on rhododendron. Mean dry weights of above ground rhododendron biomass were similar among treatments ($F = 0.43$; df = 4, 86; $P = 0.79$, n = 100). However, strawberry plants appeared somewhat desiccated and leaves showed marginal necrosis when RSM rates of 100 or 200 g (3.5 or 7 oz) were used. Strawberry shoots became extremely brittle preventing complete collection of all biomass and accurate weight for analysis at the termination of the experiment.

Current black vine weevil management recommendations emphasize repeated foliar applications targeted at adult weevils (18). Soil drenches to control immature weevils are effective against small larvae, but not against larger (fourth through sixth instar) larvae. In addition, drenches may result in ground water contamination (10, 25, 27). RSM as a biofumigant is a promising alternative to conventional pesticides, which are becoming less available with the reduction in number of registered materials. Plant-generated toxicants, acting as biofumigants, may prove to fill a plant protection need and reduce the risks of pest control for workers and the environment.

Although we found RSM to significantly reduce black vine weevil larval numbers in our study, a method of application which is less labor intensive would be essential in a production setting in order to be economically feasible. In addition, rates of rapeseed meal necessary for effective control in a

nursery application may be too high for practical use, because of the relatively low glucosinolate content in currently available commercially grown rapeseed cultivars. *Brassica* spp. bred to contain higher levels of isothiocyanate-generating glucosinolates would have greater insecticidal potential. Additional rapeseed plant parts might be considered since the release rate of isothiocyanates varies with the tissue. Much of the isothiocyanate produced interacts with protein in the meal. Other tissues such and leaves and stems contain lower glucosinolates concentrations, but release a larger percentage as isothiocyanate because less protein is present (6). Further studies must also include determination of the influence of media and rhizosphere factors on product formation and biological impact of RSM amendments in order to predict efficacy.

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