Final report for the OAN/ODA, 2005(combined report for 2004-2005)

Influence of soil mixes on the efficacy of chemical pesticides for preventative, rescue, and curative treatments for black vine weevils

Amount funded: $5000

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The black vine weevil, *Otiorhynchus sulcatus,* and other related root feeding weevils are a bane to nursery growers in the Pacific Northwest. Regular control by any means or any combination of means has proven at best to be relatively erratic. Some growers have learned to just live with this pest. Other growers may have stopped growing certain varieties because of these pests while others may have developed systems of constant vigilance and spraying in attempts to ameliorate these pests.

Screening of chemical and biological control agents for larvae of these weevils usually is conducted either topically (direct contact with technical grade product) or by finding plants with problems and then treating the soil and/or plants with compound or by digging field or pot grown larvae and seeding the larvae (of unknown age) into a pot that is then treated with compound. All of these methods have sufficed as there has not really been an alternative method or bioassay that can quickly screen multiple dilutions of product. Antidotal reports continue to accumulate about how some control product works well at field X but not at field Y or how it did better last year, etc. Why is this? I would hypothesize that part of the problem is soil types and maybe watering. Over 30 years ago it was realized that "soil type has a major influence on the effectiveness of insecticides in soil" (Harris, 1972). He and later Getzin (1985) stated that soil moisture and soil organic matter content had varying influences on pesticide activity in the soil. Alternatively it may be that we (researchers) cannot control the system enough to have accurate and conclusive results from which we can develop recommendations.

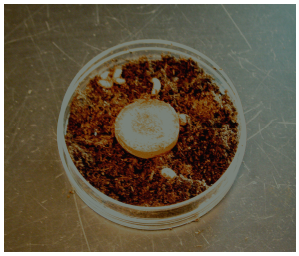
With soil insects that damage major row crops (corn, wheat, etc.) soil bioassays have been developed that use many larvae in Petri dishes with different soil or other factors that may receive dilutions of a chemical, dilutions of a biological or while in one dilution of a product, receive different moisture and temperature regimes to measure efficacy of the product. This has been easily affordable as there are rearing programs for the major row crop soil pests and they are abundantly (many millions) available for testing. Unfortunately, until now, black vine weevil, although a challenge for growers, has only been sporadically abundant for testing. We (USDA, ARS, HCRL, Corvallis, OR) (Fisher and Bruck, 2004) have developed a rearing system for black vine weevils that is capable of producing more that 1000 larvae of a certain stage per week, year – round. Given this abundance, we have been able to proceed, for the first time, with development of a bioassay procedure that can test, quickly and unequivocally, the efficacy of control products placed in soil and the effect of control products, in situ (in plant pots with plants), and the effects of moisture and temperature on efficacy while in those situations and, particularly, for the nursery industry, the effects of additives, i.e. charcoal, humus, water holding granules, bark types etc. on efficacy. Similarly, we can develop methods to look at efficacy of product over a series of dilutions in these situations and establish actual baseline effectiveness of product against any larval stage.

Over the past two years and with funding OAN/ODA, I have been developing a quick soil bioassay and a pot bioassay for determining efficacy of chemical or biological products that are or may

be possible candidates for use to control of black vine weevil larvae and other root weevil feeders. For the pot bioassay we have been using nursery liners (*Picia abies* 'Nidiformis') for host plants since the larval damage the roots quickly and damage is also measurable.

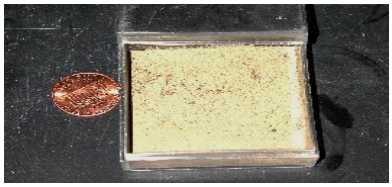
Part 1 (funding for 2004). Soil bioassay

Methods: The commercially formulated insecticides, bifenthrin granules (0.2% a.i.), bifenthrin liquid (7.9% a.i.), and chlorpyrifos (50% a.i., i.e. Dursban® W) were used. The soil was a potting mix, OBC # 1. I chose this for the soil because it is a controlled mix that is commercially available and thus, able to use repeatedly for establishing base-line information and it is available for others to compare their results with my results. For bifenthrin F the dilution series was 0, 5, 10, 15, 20, and 30 ppm FP, v/v replicated 4x (trials)(25 ppm is the recommended high rate). For Dursban the dilution series was 0, 0.5, 1, 1.5 and 2.5 PPM FP, v/v/ replicated 4x. There were 5 replications of each treatment per each time a trial was run. Basically, a single treatment consisted of a standard Petri dish with 20, 6 week old black vine weevil larvae [~ 3-4th instar (Tom and Fisher, 2006)] placed in a designated dilution of product mixed with potting mix and with a slice of carrot (a common lab food for the pests) in the dish.



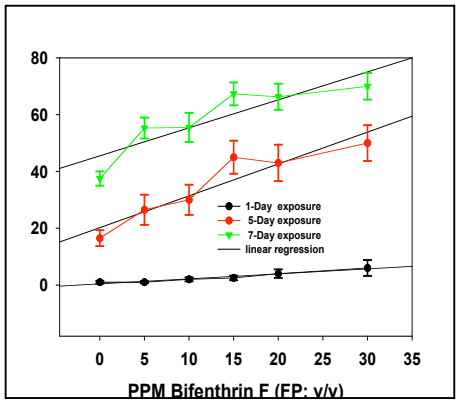
Any single dilution series for testing was set-up and run on the same day. The experiment then was placed in an incubator at 21ºC for 7 days. Because the assay system was observable and larvae can be removed and placed back on the substrate, if not dead, we took observations of mortality at 1, 3, 5, and 7 days.

Initial trials with bifenthrin G found no mortality with 4th instars. But, from numerous antedotal and pot studies, we know that some sort of protection is afforded when added to potting mixes before or at planting. Thus an assay was devised to test for mortality of hatchings. The test arena was a small 5 cm square box with some fine (120 mesh) field soil and a few granules of bifenthrin G. The soil and granules were moistened and 20 eggs that were ready to hatch (mandibles can be seen inside of the eggs) were placed on top of the soil. Each day they were checked for hatch and mortality.

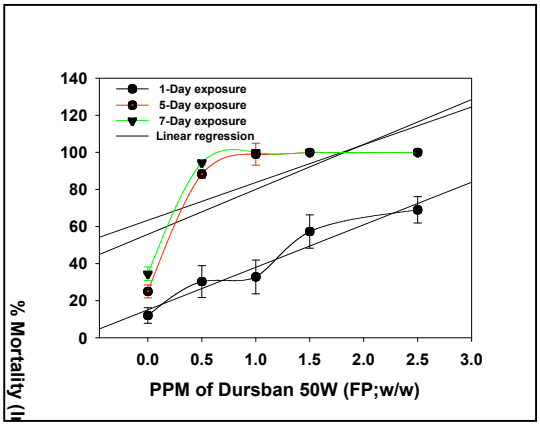


Results Part 1:

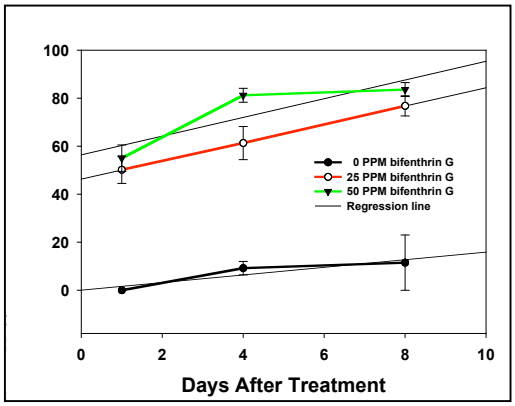
With both the Dursban and bifenthrin F trials there were significant differences among treatments for days after treatment and for increasing PPM. It should be noted that mortality with bifenthrin F did not approach 100% even after 7 days exposure. When compared with Dursban 50 W that had 100% mortality at 1 PPM in 5 days and >60% mortality in 24 hrs at 2.5 PPM, bifenthrin did not appear to have as great a 'kill' as the previous 'chemical of choice', chlorpyrifos (Dursban). I would interpret these findings to mean that increasing amounts of bifenthrin F (even beyond the recommended rates) as a drench will take care of most of the black vine weevil present. However, we (Denny Bruck and I) are finding that some biological pesticides may do



just as good a job if not better than bifenthrin. It would be great to have a product like Dursban were less PPM will be as effective as more. The future for that kind of chemical does not look promising. I think we will have to struggle with the likes of bifenthrin and other products. We will need to find other compatible products, possibly biological agents and/or cultural techniques that will, in combination, deliver some reasonable level of management.



For the hatchling assay, I present the data on 1, 4, and 8 days after treatment. The treatments were 0 PPM, 25 PPM (high recommended rate) and 50 PPM. All treatments were significantly different from each other for each day. However, it appears the trial would have to run longer than 8 days to get 100% mortality. 50 PPM paralleled the effect of 25 ppm but not at double the effect. In fact, although significantly different, the effect was only half again that of 25 PPM. The test implies that using lesser quantities than the maximum recommended rate may give some protection, but, it may not be as desirable as 25 PPM. Certainly using more than 25 PPM is not necessary to maintain efficacy.



Part 2. (funding for 2005) Pot bioassay

In this study I wanted to determine the efficacy of available chemical products in different soil mixes when used as a preventative (before eggs) or curative treatment (4-5 instar).

Methods: For all of the parts of these experiments I used an extruded, long, 4 inch pot for our container and a liner of birds nest spruce (*Picea abies* 'Nidiformis') as our plant. Standard watering and fertilization procedures were used. Six soil mixes were used: SB-40 and AWDE, from Sunshine® Mixes and OBC#1, and OBC#2 from Oregon Bag Co. and 9B, and 85 from Pro-Gro Mixes. The BVW egg infestation rate was 50 eggs per pot (a total of 161,000 eggs was used).

***Preventative Treatments*:** Pesticides were applied to the amount of soil needed for each pot in the treatment and then thoroughly mixed. The bifenthrin granular treatment was at 50ppm FP, W/W (2X recommended rate); the bifenthrin flowable treatment was at 25 ppm FP, W/W(recommended high rate); and the thiamethoxam treatment was at 40 ppm FP, W/W (recommended high rate); the control pots for each time did not receive any insecticide. The plants were then planted in the pots. There were 15 pots / treatment / egg - infestation date / soil and 15 pots / infestation date / soil without pesticide treatment as the control. Eggs were be added on day 0 (planting day), day 60 and day 90. After each egg treatment, the pots were allowed to set for 70 days to allow larvae to grow to a

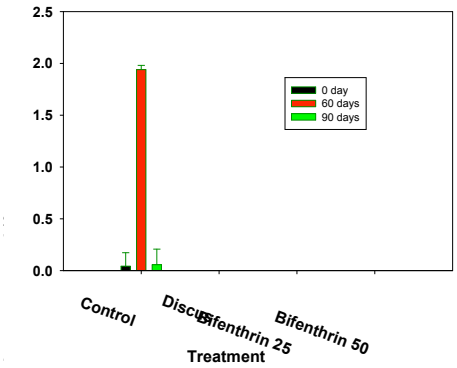
searchable size. At that time pots were emptied and larvae counted and graded for size (instar) and weight. All roots were kept for dry weight.

Curative Treatments: Plants were planted as above, but without pesticide, and allowed to grow for about 2 months. Then, ten 4-5 instar black vine weevils were added and allowed to acclimate for about 6 days. Pesticides were applied to the top layer of soil for all pots in the treatment. There was a control of 20 plants per treatment / soil without pesticide. There were 2 treatments / pesticide / soil and a no pesticide control treatment. There were 20 pots per treatment. The bifenthrin granular treatments were 25 ppm (recommended rate) and 50ppm FP, W/W (2X recommended); the bifenthrin flowable treatments were 10 ppm and 25 ppm FP, W/W(low and high recommended rate); and the thiamethoxam treatments were 30 and 40 ppm FP, W/W (recommended rate range). Fourteen days after the pesticide treatment, the treatments were evaluated by searching for and counting and weighing the larvae. Dry weight of roots was also recorded.

Results Part 2.

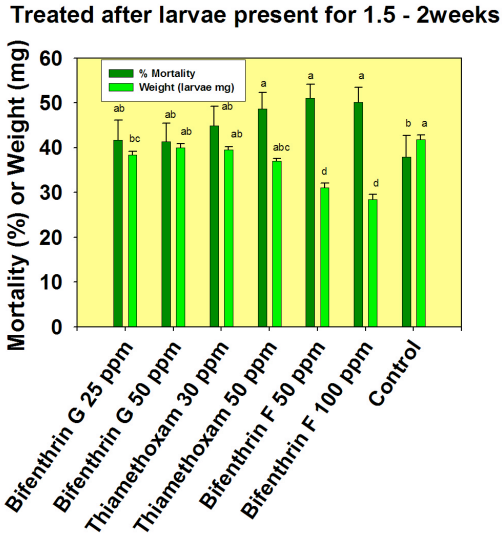
Preventative treatments:

All eggs that we took as samples to estimate the hatch of the eggs we used averaged >80% hatch. At each of the egg application times, all chemical treatments prevented hatchlings from establishing. I say this as I do not believe the treatments acted as an ovicide (kills the eggs). If we look at the previous information from the egg and hatchling bioassay, you will notice that the treatments did not prevent hatching, but it tended to kill the small hatchling larvae after several days exposure. Apparently the hatchlings are extremely vulnerable. Additionally, you may notice that the percent recovered as larvae in the control treatments was very low. This is not unusual. It is commonplace among all insects, particularly with those that have soil stages to have this kind of survival rate. However, at no time did we find even one larva in the treated pots. Thus, I must conclude that, indeed, the treatments were really statistically different than the control and that the chemicals prevented establishment



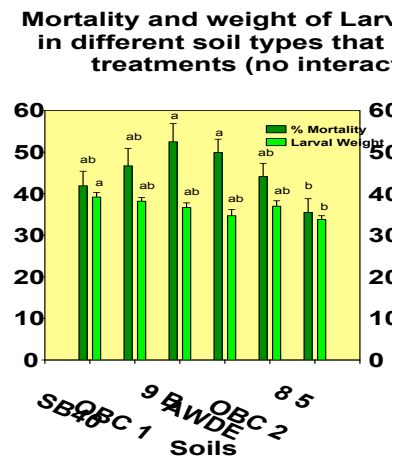
Curative treatments:

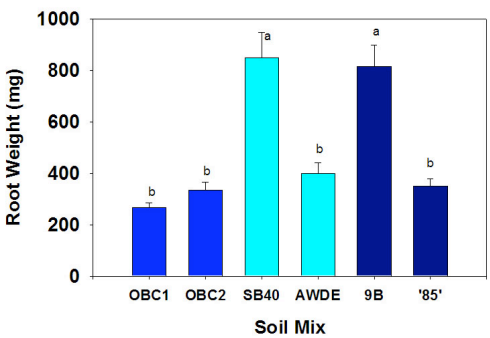
All of the chemical treatments produced a greater mortality among the 5th instars than the non-treated control. The treatments thiamethoxam (50 ppm) and the two liquid bifenthrin treatments produced the greatest mortality. However, the mortality after about two week's exposure to the treatment was less than 60%. Even the controls had close to 40% mortality from natural causes. We took the measurement of larval weight because in past experiments we had noticed that larvae did not appear to grow when placed in pesticide treated soil when compared with the untreated soil. In this study the larvae recovered from the 2 liquid bifenthrin treatments had



significantly lower weight than the other pesticide treatments and the control. I think this indicates that the larvae are 'sick' from the pesticide and may not make it to the adult stage. But, when we analyzed our data for differences in root weight, we found that the dry root weights of the pesticide treated plants did not differ from the non-treated control.

Another objective of this study was to see if soil type affected the efficacy of the pesticides. In our statistical analysis of our measured parameters, we found no interactions among the measurements for mortality, larval weight or dry root weights in regards to the various chemical treatments and soil mix. Thus, we concluded that the effects soil mix and pesticide treatment acted independently on the measured parameters. In the accompanying illustration you can see that the soil '85' had the least mortality and that 9B and AWDE had the greatest mortality. Larval weights did not show a lot of differences but those on SB40 were the heaviest and those on '85' were the lightest.





The soils SB40 and 9B had significantly greater dry root weight than the dry root weights from the other soils (all others were statistically the same). There was no correlation between root weights and larval weights or larval mortality. Of interest, the soil '85' had a low root weight, low larval weight but, contrarily, had low mortality.

Note: *In all figures, bars followed by the same letter are not significantly different from each other according to the Tukey-Kramer test for significance among means of fixed data when a parameter is found significant at the probability level not to exceed the 5 % level of confidence (have a 95+% chance of being different) with the analysis of variance procedure. Lines on the bars represent the Standard Error of the mean of the indicated effect.*

What does this all mean?

1. We now have a useful soil bioassay for determining efficacy of products and a useful pot bioassay to quickly (~3 weeks) determine the efficacy of formulated products when placed in the presence of a plant and a definitive latter larval stage.
2. Soils may affect efficacy, but they most certainly have an effect on pest mortality.
3. Commercially available potting mixes exhibited variable but definitive influences on plant growth. I would suggest that more research be funded to look at the effects of mixes from off of the "shelf" and custom mixes on plant growth and development.

4. Bifenthrin and possibly other new chemistries may be effective in the mitigation of BVW but do not meet the muster of previous chemistries such as chlorpyrifos.
5. Bifenthrin granular is useful only when used to prevent establishment of these pests. It is not efficacious against medium to large larvae.
6. Do not expect rapid kill with Bifenthrin.
7. The future of managing these pests lies with a mix of techniques, such as pesticide, biologicals and cultural practices.

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