

# Techniques in Black Vine Weevil Research: Part II, Adulticide Studies

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The effectiveness of black vine weevil control with an adulticide strategy can be measured by two principal approaches: field plots and laboratory assays. Field experiments are most easily attempted where there are preexisting high population densities of root weevils. Crops where I've successfully conducted such trials include Colorado blue spruce grown as Christmas trees, field-grown yew, and strawberries. The most important factor in being able to obtain data from these tests is isolation of treatment units. To prevent movement of weevils from one plot to another, I surround plots with exclusion barriers. These consist of 15-cm (6-inch)-wide aluminum flashing inserted into a slit in the soil to a depth of 5 cm. Grease is applied to the top 5 cm and foliage overhanging the barrier is clipped off. Intersecting corners are fitted together by slitting the aluminum halfway on both pieces so that they may interlock (like cardboard inserts in multicompartment cartons).

Following a spray, populations of weevils are assessed with 5-minute timed counts of live and dead weevils, or with a combination of counts, pitfall catches, and counts of larvae from fixed-volume soil and root samples taken in October. After doing a 5-minute timed count, any remaining dead weevils are removed from the plots to prevent including their mortality during the next assessment. From these studies, I've found populations of black vine weevil for which labeled dosages of azinphos-methyl and deltamethrin are ineffective (no difference between the treated group and the untreated check). A well conducted test should demonstrate that the activity of adult weevils (measured with pitfall traps) is inversely proportional to the number of dead weevils, and is correlated with the resulting number of larvae (Cowles 1996).

There are many designs of pitfall traps. My current method is to use two 530-ml (18 fl oz) plastic cups with four holes (2 mm diameter) drilled into the bottom of each for drainage. The bottom cup serves to hold the soil in place while emptying out the top cup. The top cup is then coated along the inside top 5 cm with Rain-X (Blue Coral-Slick 50, Ltd., Cleveland, OH) to make it unclimbable. This Teflon-like coating lasts through a full field season, and unlike grease, will not become coated with soil. Both cups have to be buried into the soil so that the topmost edge is level with the soil

surface. A No. 2 plastic pot is then modified to make a rain shelter. Cut three supporting legs into the rim and invert it over the pitfall, then tape over any drainage holes in the center of the pot. Insert two metal stakes through side drainage holes to prevent the rain shelter from being disturbed by the wind. The most effective use of pitfall traps is to place them at the corners of the enclosed plots. My field trapping in nurseries has shown that placing the traps at the corners increases the catches about 5- to 7-fold over having the pitfall trap next to the side of a barrier or placed in the middle of the plot. The adult weevils exhibit wall-following behavior; when they encounter a corner of the plot, their turning probably increases the chance that they'll fall into the pitfall trap.

Laboratory studies permit detailed evaluation of insecticide characteristics. If sufficient weevils can be collected from a problem field, various insecticides can be sprayed at the normal field rate onto foliage and caged with those weevils to directly evaluate mortality. In this manner, I've found populations that were not killed by acephate, carbaryl, chlorpyrifos, endosulfan, and fenpropathrin. Additional studies I've found useful include tests of field residual activity, field half-life, and feeding bioassays to determine dose response, insect growth regulator effects, and synergism; examples of these studies are given below.

## Field Half-life Determination

Analytical chemistry can be used to directly determine how rapidly insecticide residues degrade under field exposure to sun, wind, and rain. However, some insecticides, including fipronil and imidacloprid, may break down into metabolites that are also insecticidal. Calculating the toxicity of the resulting mixtures could be extremely difficult, so direct evaluation through bioassay can be the easiest method to determine the "effective" half-life of the insecticide/metabolite mixture. By "effective" half-life, I mean the time that is required for the toxicity to be reduced to material sprayed at half the concentration. To conduct one of these tests, apply an insecticide in a doubling series of rates, and then measure mortality at several dates after spraying for each dosage.

The effective field half-life for fipronil was investigated by spraying yew foliage in the field (200 gal/acre) with dosages of 0, 10, 20, 40, 60, 80, 160 and

320 g ai/ha. There were three replicates in a randomized complete block design. Adult black vine weevils (20 per experimental unit) were caged with shoots clipped at 0, 3, and 7 days after spraying; a total of 1,440 weevils were used in this experiment. Mortality was recorded at 3, 6 or 7, and 10 days after caging adults on foliage. The mortality response at 6–7 days of weevil exposure to treated foliage shows very clearly the decline in residual activity due to degradation of residues in the field (Fig. 1). The lines connecting the points for each dosage are generated by the “spline” feature from the graphical package (SigmaPlot, SPSS, Chicago, IL).

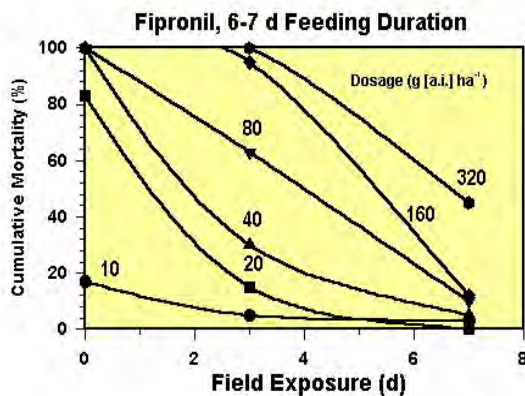
The next step in determining the field half-life is to draw a horizontal line across the graph at selected mortality levels. For example, the horizontal line representing 63 percent mortality intersects the curve for the 80-g dosage at 3 days field exposure, and also intersects the 20-, 40-, 160-, and 320-g dosages at various days of exposure. The difference in days between each of these successive curves represents independent assessments of the effective half-life of fipronil. The difference in days for two successive curves represents the time required for fipronil to decay to 25 percent of the higher dosage, and the difference in days between three curves (doses of 320 g and 40 g, or 160 g and 20 g) represents the time to decay to 12.5 percent of the higher dose. When these data are graphed as the difference in days of field exposure versus the log of the percent of the dosage remaining (50, 25, 12.5, or 6.25 percent), the resulting regression gives the slope for the exponential decay equation:  $C_t = C_0 e^{rt}$ , where  $C$  is the concentration at time 0 and  $t$ ,  $t$  is the number of days, and  $r$  is the decay constant (Fig. 2). By defining the concentrations of interest to be  $C_0 = 2$  and  $C_t = 1$ , we can calculate the half-life by solving the equation for  $t$ . In the case of these fipronil data, this estimated effective half-life is 1.63 days.

### Dose-response Feeding Bioassay and Synergism

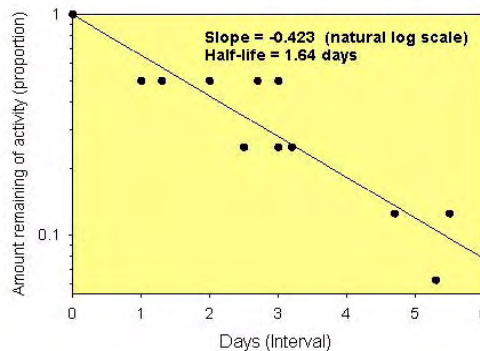
Laboratory dose-response bioassays typically consist of a logarithmic concentration series of insecticides applied as a small droplet (in an acetone solution) to the insect, by allowing adults to walk over a coating on the inside of a glass petri dish, or by feeding on sprayed foliage or dipped leaves. These studies almost always use probit analysis (a form of non-linear regression), which permits calculation of the median lethal concentration ( $LC_{50}$ ), other LCs (e.g.,  $LC_{90}$ ), and the slope of the dose-response relationship (Robertson and Preisler 1992).

The toxicity of very slow-acting ingested toxicants must be evaluated with a different approach. Even when presented with different concentrations on leaf disks, adults will simply continue to feed until they

have ingested a lethal dose (LD) (which makes the usual method for analysis impossible). However, if the actual LD for each individual can be calculated, based on quantitatively coating the leaf disks and measuring the area consumed, then the dose-response can be visualized by graphing these ranked LDs vs. the cumulative proportion of the population each individual represents. The calculated dosages actually give the LD relationship directly, as the dosage required to kill the 30<sup>th</sup> individual out of a population of 60 individuals is by definition the median LD.



**Figure 1.** Mortality response of black vine weevils after 6–7 days of exposure to different concentrations of fipronil.



**Figure 2.** Days of field exposure in relation to the remaining dosage.

To quantitatively dose leaves with fipronil, sugar maple leaf disks (20 for each concentration) were cut with a 19.7 mm-diameter cork borer, and then dipped into 7, 10, 14, and 20 ppm insecticide solutions to which 0.02 percent Silwet L-77 organosilicone surfactant had been added. The surfactant allowed sheeting of liquid over the leaf surface. The dipped leaf

disk was held vertically for a few seconds with the edge of the disk just touching the dipping solution to allow the excess to drain. The insecticide solution on the leaf disks was then allowed to dry on a sheet of aluminum foil. To investigate the synergistic interaction of fipronil with acetamiprid, a formulation containing 4 percent fipronil and 16 percent acetamiprid was diluted to generate concentrations of 7, 10, 14, and 20 ppm of fipronil, 0.02 percent Silwet L-77 was added, and the leaves dipped as before. The loading of insecticide solution onto the leaf was determined by weighing a set of leaf disks before and immediately after dipping. The increase in weight was converted to the volume of liquid adhering to the disk (16 mg, equivalent to 16  $\mu$ l). With the concentration of the insecticide solution and the leaf area known, the average quantity of insecticide per unit of leaf area could then be calculated.

To run the bioassay, individual black vine weevil adults were caged with a leaf disk in a petri dish. To prevent drying (and shrinking) of the leaf disk, filter paper dampened with 1 ml water lined the bottom of the dish, and petri dishes were held in plastic bags. Leaf disks were removed for measurement as soon as the weevil became incapacitated. The leaf area consumed was calculated by measuring the remaining portion of each leaf disk with a flatbed scanner and SigmaScan Pro software (SPSS, Chicago, IL), and then compared with the average area of intact leaf disks.

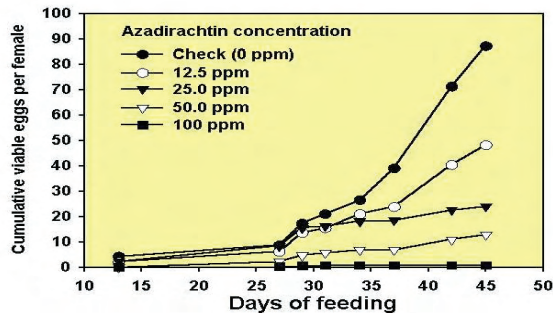


Figure 3. Effect of azadirachtin on black vine weevil viability.

The dose response of the black vine weevils was somewhat biased by the concentration of the solution into which the leaf disks were dipped. At the higher concentrations, the weevils were able to consume a larger dose before they became intoxicated and died (Fig. 3). The “true” median lethal dose, therefore, is best estimated by the lowest concentration tested, and is approximately 18 ng per weevil. However, the composite graph from the four dosages demonstrates

how the LD<sub>50</sub> can be directly estimated (Fig. 4). Fipronil was synergized when combined with acetamiprid. The dose-response has the same slope (through the mid-portion of the curve) but the LD<sub>50</sub> is shifted to 9 ng (fipronil) per weevil. When synergized by acetamiprid, there were no indications of an upward bias in the estimation of median lethal dose with increasing concentration.

### Effect of Azadirachtin on Black Vine Weevil Reproduction

Previous authors have discovered through adult feeding studies that some insect growth regulators (apholate and dimilin) interfere with black vine weevil oviposition (Cram 1967, Zepp et al. 1979). Growth regulator effects are too subtle to be easily observed in field studies, and so they have to be evaluated with laboratory studies. I investigated the effects of

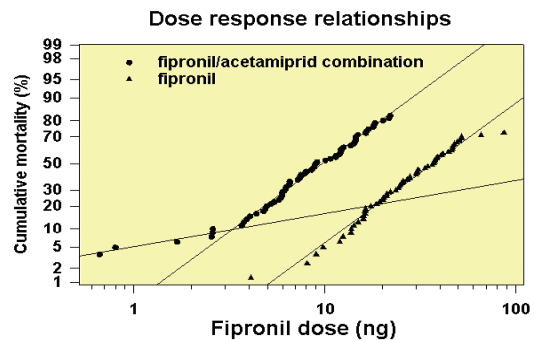


Figure 4. Dose response relationships.

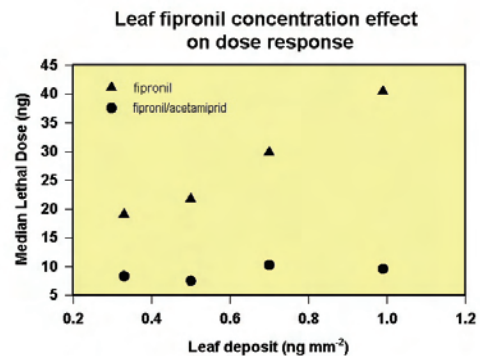


Figure 5. Leaf fipronil concentration effect on dose response.

azadirachtin (SureFire Japanese Beetle Repellent) on black vine weevil reproduction by caging weevils with yew foliage dipped in 0, 12.5, 25, 50 and 100 ppm of azadirachtin. Each adult was individually caged in a petri dish with moist filter paper and treated foliage. Eggs were counted and removed, then held in petri

dishes to evaluate whether they would mature to the brown stage, indicating viability (Smith 1932). From this test, it is evident that black vine weevil reproductive function can be interrupted with azadirachtin in a dosage-dependent manner (Fig. 5, page 25). Unlike dosage-mortality relationships, the response of the weevils in this bioassay is not quantal (dead or alive, an all or nothing response), but was a

graded response. Individual weevils laid fewer eggs as the dosage of azadirachtin increased, and of these eggs, the proportion of viable eggs also decreased. At 100 ppm, there were no viable eggs laid. Future studies will determine whether these effects are reversible, either by removing the weevils from the azadirachtin-containing diet, or by dosing the weevils with an oviposition-enhancing growth regulator (fenoxycarb).

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