Techniques in Black Vine Weevil Research: Part I, Pot Studies and Rearing

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Over the past 10 years, my efforts in conducting container studies with black vine weevil have progressed from failing to achieve larval-infested pots to consistently obtaining meaningful results. The following procedures are successful methods I have developed or adapted from Carl Shank's lab at Washington State University.

I collect adult weevils from the field, either at night with a sweep net in strawberry fields or by beating during the day or evening in infested field-grown yew nurseries. Live adults are also recovered from pitfall traps in my field studies. Adults are held in 5-gal (20-liter) plastic buckets with ample yew foliage. The bucket is greased along the inside top 2 inches (5 cm), and is covered with a wooden-framed aluminum screen. Foliage is not allowed to touch the screen. Adults tolerate crowded conditions; we've kept ~10,000 weevils in one oviposition bucket. Despite disagreements in the literature regarding the optimal diet for vine weevil adults (Nielsen and Dunlap 1981, Hanula 1988), I have had great success with a pure yew foliage diet, resulting in 99+ percent viable eggs. I also refrigerate first-year adults for 1–2 weeks just as they are starting to lay eggs, then return them to the warmer insectary (see conditions given below). This interval of refrigeration appears to allow them to bypass the interval during which they normally would lay a high proportion of inviable eggs. The increased oviposition rate and egg viability following refrigeration are currently unexplained phenomena. When there are sufficient eggs for setting up experiments, adults can be refrigerated with yew foliage in a plastic container. One must limit the ventilation to prevent drying, and check weekly to ensure that adults do not have dry food. It is important to remove them from the refrigerator every 6 months for a couple of days of intensive feeding.

Conditions for egg laying are 75°F (24°C) or cooler, greater than 85 percent relative humidity (Shanks and Finnigan 1973), and light conditions of 16:8 L:D (Nielsen and Dunlap 1981). It is extremely important not to allow foliage to dry or to become depleted. If the rearing room is too dry, then cover the top of the screen with plastic to conserve moisture. High humidity is required for eggs to properly develop, but cool temperatures then become necessary to prevent fungal epizootics among the adults (John Buxton, ADAS, Worcester, UK, personal comm.).

While fresh foliage may need to be added every 2–3 days, eggs should be harvested on a weekly basis. More frequent egg collection results in many eggs being immature, white, and sticky (which makes cleaning them difficult). Collection frequency greater than 11 days may result in some of the eggs hatching in the oviposition bucket (Smith 1932). Beating the yew branches in an ungreased 5-gal bucket shakes weevil adults, eggs, and frass from the foliage. The material at the bottom of the oviposition cage is also collected to separate out the eggs; it may have to dry to allow sieving. The material containing eggs from both buckets is then screened through standard soil sieves: No. 10 (2000-µm opening), No. 20 (850 µm), and No. 40 (425 µm). Adults and leaf fragments accumulate in the top sieve, frass in the middle, and fine frass mixed with eggs in the bottom. Do not use a No. 25 mesh, as the eggs fit into and stick in the center of the openings. Eggs are rolled on a sheet of paper to separate them from the remaining frass. The eggs are next measured volumetrically to determine their number (there are 4,620 eggs per ml), then disinfected with a 2-minute soak in 1:10 bleach solution. Surface disinfection is essential, as otherwise the hatched larvae turn a distinctive magenta color and succumb to *Serratia marcescans* infection. Following the bleach treatment, eggs are rinsed with water in a Büchner funnel, and the moist filter paper supporting the eggs placed in a Parafilm-sealed petri dish. Eggs can be refrigerated for 4 weeks before using them in experiments, but we generally use them immediately to artificially infest pots.

The number of eggs needed to infest pots is determined by the quality of the root systems. I have had success with 100 eggs per No. 1 pot (2.4 liters), with average larval recoveries in untreated checks of 25–50 percent with those excellent hosts listed above. Larval recovery is reliable enough to permit six replicates to statistically separate treatment effects. To measure the eggs easily, allow them to dry just enough to be able to pour or roll them. A convenient method for "counting" the eggs needed for infesting a pot is to manufacture a measuring scoop that has the volume for the required number of eggs (100 eggs equals 21.6 µl). Place the eggs 3–5 cm deep in a slit or hole adjacent to plant roots, and then cover them.

For larvae to develop successfully, hosts must have extensive, highly nutritious root systems. Poor choices are mint (Cowles 2001) or rhododendron (Hanula 1988). Excellent hosts include arborvitae, *Astilbe*, *Heuchera*, *Primula, Sedum*, strawberry, or yew (Blackshaw 1987, Cowles 2001). The most readily available, fastest-growing host may be strawberry. Crowns with some roots can be obtained from nurseries; these should be disinfected for 20 minutes in 1:10 bleach solution and then rinsed thoroughly before planting. This disinfection procedure is intended to kill any insect pathogenic nematodes that may adhere with soil to the roots. The best plants are those grown for 2–3 months before infesting with weevil eggs.

There are variations in survival of larvae related to potting mix characteristics. The optimal media have adequate pore space and allow excellent drainage, which will improve root growth and prevent anaerobic conditions if the pots are overwatered. Overwatering often becomes an issue because in pots with greater numbers of larvae, plants may be stripped of their roots, and without effective evapotranspiration, these pots become increasingly wet. The ideal media do not contain perlite, vermiculite, styrofoam pieces, or white gravel, as these make recovery of larvae difficult later. Composted pine bark mixed with peat and sand is a medium that has worked very well.

An absolutely critical consideration is to maintain soil temperatures for larvae below 27°C (81°F) (Stenseth 1979). During the summer, this requires burying pots into the soil. This method may increase the risk of inadvertent entry of insect pathogenic nematodes from the splashing of surrounding soil. For these reasons, I prefer running bioassays during the winter in a moderately heated greenhouse (16°C, 60°F).

Whenever possible, I allow experiments to run for 100 days. Sometimes plants die too soon, in which case I may end an experiment earlier, or insert disinfected carrots or yew shoots into the pots to provide supplemental food for the larvae. To evaluate larval populations, plants and media are removed from pots and the larvae are picked from media. If the roots allow internal feeding, they may have to be dissected to recover all the larvae.

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