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TECHNICAL NOTE

Forest Pest Management Institute

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PRODUCTION OF SAWFLY VIRUSES IN PLANTATIONS

Introduction

Two viral insecticides for control of sawflies are produced at the Forest Pest Management Institute for distribution to clients. These are Lecontvirus for control of redheaded pine sawfly, *Neodiprion lecontei*, which was registered under the Pest Control Products Act (Canada) in 1983 and Sertifervirus for European pine sawfly, *N. sertifer*. A registration petition for Sertifervirus is currently being evaluated.

There are no artificial diets available for sawflies. In the laboratory, these insects must be reared on fresh foliage. This is time consuming and virtually precludes handling large numbers of larvae. Nuclear polyhedrosis viruses (NPVs) that infect sawflies have been propagated since the early 1950's in heavily-infested plantations. Sawfly larvae are gregarious and feed in colonies, greatly simplifying harvesting of diseased and dead larvae. Methods have been refined over the years and current techniques are outlined below. Procedures are identical for production of both Lecontvirus and Sertifervirus.

Selecting sites

The aim of a virus production program is to harvest diseased and dead larvae when they are fully grown and yield the greatest amount of virus. Under these circumstances, defoliation and damage are anticipated on infested trees, which may be unacceptable. Ideal sites are plantations that have a history of severe damage and are virtually "write-offs", abandoned Christmas tree farms or plantations with trees large enough to withstand moderate defoliation. Areas naturally infested with sawflies are the first choice as propagation sites, but if inconveniently located, colonies of sawflies can be transferred to a more suitable location by clipping twigs with colonies of larvae, transporting them in paper bags and then tying the twigs with larvae on to other trees using "twist ties" (Fig. 1). As the cut foliage dries out, larvae migrate on to the new host tree.

Timing of virus infection and dosage

Larvae in the fourth-instar are sprayed with virus. An aqueous suspension containing 10^6 polyhedral inclusion bodies (PIB)/mL is applied. This is a higher dosage than is used for control operations when, ideally, first- and second-instar larvae are treated. When infested plantations are used as propagation sites, a mistblower is used, and every third or fourth row is sprayed (Fig. 2). Volume applied is about 20 L/ha.

When individual colonies are transferred to new host trees, they are marked with flagging tape for fast and easy detection. Here, the individual colonies are sprayed with an atomizer spray bottle (Fig. 3). Both the actual colony and surrounding foliage are sprayed: each colony receives about 5 mL of suspension.

Harvesting

The length of time between spraying and death of larvae depends on the ambient temperature and the first check is made at 8 days post-spray. When a plantation is sprayed with a mistblower, mortality occurs over a prolonged period, usually between 8 and 20 days post-spray. When a hand-sprayer is used to treat individual colonies mortality is more uniform, occurring between 8 and 12 days post-spray. Twigs with colonies containing dead and diseased larvae are clipped from the trees and placed in paper bags. Healthy larvae rear up when disturbed and colonies with active larvae are left so that the infection process will proceed. Colonies of larvae are harvested daily, otherwise dead larvae will be removed by predacious and scavenging insects or washed off the foliage by rain.

Bags containing diseased and dead colonies on foliage are stapled shut and kept cool until larvae can be picked off the foliage with forceps and placed in plastic petri dishes (Fig. 4). Larvae that are alive and active are placed in plastic boxes on fresh foliage and reared at room temperature until they either die or pupate. Petri dishes containing dead larvae are frozen and stored at -20°C until processed.

Processing

Frozen, NPV-infected and NPV-killed larvae are freeze-dried and ground to powder for 30 seconds in a Waring blender. To obtain a finer powder, which will pass through a 20 mesh sieve when suspended in water or oil, the ground larvae are mixed with an equal amount of crushed dry-ice and re-ground for 30 seconds in a blender. The powdered material is then stored in tightly sealed containers at 4°C until required for bio-control operations.

The potency of each batch is determined by estimating the number of polyhedral inclusion bodies per gram using the dry counting technique described by Wigley (1980). If larvae are virus-killed or heavily diseased when harvested, the powder contains about 2 x 10^{10} PIB/g. The dead larvae contain some bacteria which are of no concern. However, a quality control check is run to determine if the bacteria are at an acceptable level and that no human pathogens are present. Quality control procedures are described by Podgwaite and Bruen (1978).

Currently, material is shipped to clients as an emulsifiable oil concentrate. The oil used is Abbott Laboratories gelled oil vehicle which is the carrier for *Bacillus thuringiensis* in their product called Dipel 88®. The shelf-life of the NPV in this oil is less than one year and a search is being made for an alternative vehicle. We only formulate sufficient virus to use for one season and recommend that surplus material be discarded.

Conclusions

Production costs of Sertifervirus and Lecontvirus are very low; the main factors are salaries, travelling time to production sites, lodging and related expenses. It takes about 50 virus-infected larvae to produce sufficient material to treat 1 ha. If produced close to the laboratory with no overnight accommodation involved, costs range as low as $.50/ha. Production at a distant site could raise this price to $2.50/ha.

Far greater quantities of material can be produced in naturally infested plantations than by moving colonies of larvae to other sites. In 1984, 6 red and jack pine plantations with a combined area of 9.5 ha yielded 1.0 kg of Lecontvirus which is sufficient to treat 2,000

Figs. 1-4. 1) Colony of European pine sawfly is tied onto a Scots pine with a "twist tie". 2) A red pine plantation infested with redheaded pine sawfly is sprayed with virus using mistblowers. 3) Colonies of redheaded pine sawfly transferred to a convenient location are individually sprayed with an atomizer spray bottle. 4) NPV-killed European pine sawfly larvae are removed from foliage and placed in a petri dish.

ha. In 1985, 200 colonies of European pine sawfly, transferred to suitably located host trees, yielded 50 g of Sertifervirus which is sufficient to treat 100 ha.

We have only produced NPVs for control of *N. sertifer* and *N. lecontei*, but these methods could be adopted for other colonial species of Diprionid sawflies known to be susceptible to NPVs. These include Swaine's jack pine sawfly, *N. swainei*, red pine sawfly, *N. nanulus nanulus*, balsam fir sawfly, *N. abietis* and the jack pine sawflies *N. pratti banksianae* and *N. pratti paradoxicus*.

References

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