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Nuclear polyhedrosis virus as a biological control agent for *Malacosoma americanum* (Lepidoptera: Lasiocampidae)

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Abstract

In addition to damaging trees, the eastern tent caterpillar is implicated in early fetal loss and late-term abortion in horses. In a field study, we evaluated the potential biological control of the caterpillar using eastern tent caterpillar nuclear polyhedrosis virus (ETNPV), a naturally occurring virus that is nearly species-specific. Egg masses were hatched and second instar larvae were fed virus-inoculated foliage to propagate the virus *in vivo*. Then, a viral pesticide was formulated at concentrations of 10^4, 10^6 and 10^8 polyhedral inclusion bodies per ml. The pesticide was applied to foliage on which second, third and fourth instar caterpillars were feeding. When the majority of surviving larvae reached the sixth instar, colonies were collected and the surviving caterpillars counted. Mean numbers of surviving caterpillars per treatment were compared via 95% bootstrap confidence intervals. The data indicate second instar caterpillars were highly susceptible to the virus, but only at the highest concentration tested. Third instar caterpillars were also somewhat susceptible to high virus concentrations, while fourth instar caterpillars were fairly resistant. Our data provide the strongest evidence to date that ETNPV can be propagated, harvested and refined for formulation as a biological control agent for eastern tent caterpillar. Its use on this insect may be merited in circumstances where landowners and managers need to protect trees and horses.

Introduction

Eastern tent caterpillar [*Malacosoma americanum* (F.)] is a defoliator of trees and shrubs. Caterpillar populations cycle over an 8–10 year period (Meyers 1990) and occasionally become so large that trees may be completely defoliated (Stehr and Cook 1964; Kulman 1965). Eastern tent caterpillar is particularly injurious to ornamental and fruit trees of *Prunus* and *Malus* spp. Defoliation can cause reduced growth and loss of branches (Kulman 1965) and even tree death (Stehr and Cook 1964).

In addition to feeding damage, eastern tent caterpillar is implicated in mare reproductive loss syndrome (MRLS). This syndrome causes early fetal losses and late-term abortions in thoroughbred and saddlebred horses (Kane and Kirby 2001; Dwyer 2003; Webb et al. 2004). From 2000 to 2003, an MRLS outbreak cost the Kentucky equine industry an estimated $336 million (Thalheimer and Lawrence 2001), and Sebastian et al. (2003) showed the outbreak was caused by exposure to eastern tent caterpillar.

Recommended insecticides for eastern tent caterpillar control include malathion and tebufenozide (McDowell 2002), and biological agents include Spinosad, *Bacillus thuringiensis* and the entomogenous fungi (Lacey et al. 2001). Other means of

control include cutting and removing tents, parasitic wasps and entomogenous nematodes (Leathers and Gupta 1993). The recent outbreak of MRLS in Kentucky spurred interest in new, non-chemical controls for eastern tent caterpillar (McDowell 2002). *Malacosoma* spp. can be difficult to control because of their congregational behaviour inside a protective tent shielded from most predators and from exposure to insecticides. However, because eastern tent caterpillar has a gregarious behaviour, a contagious virus may be particularly effective in its control. Caterpillars emerge from tents to forage at night, and if the virus is foliarly applied, some caterpillars will likely contract the virus during feeding and then infect other caterpillars upon returning to the tent. The use of nuclear polyhedrosis virus (NPV) as a biological control has already proven effective with hymenopterous and other lepidopterous pests (Bergold 1953; Smith 1976; Granados 1980). Furthermore, susceptibility of *Malacosoma* spp. to NPV has been noted on numerous occasions (Weed 1896; Headlee 1934; Wadley 1938; Clark and Thompson 1954; Clark 1955; Kulman 1962, 1965; Benz 1963; Stairs 1964, 1965a; Kulman and Brooks 1965; Stairs 1966; Tompkins et al. 1981; Yearian and Young 1976; Dethier 1980; Stehr and Cook 1964).

The objective of this study was to determine the feasibility of using nuclear polyhedrosis virus as a biological control of eastern tent caterpillar. We hypothesized that increasing concentrations of the virus would reduce caterpillar survival. We also hypothesized that the effectiveness of the virus would decrease with increasing larval age as has been found in Engelhard and Volkman (1995).

Materials and Methods

During the first year of the study, virus polyhedra were propagated in host caterpillars, purified and freeze-dried. The following year, a field trial was carried out to assess the effect of a range of virus concentrations on survival of second, third and fourth instar caterpillars.

We began our study by collecting 15 eastern tent caterpillar egg masses from black cherry (*Prunus serotina* Ehrh.) and apple (*Malus* spp.) in the winter. These egg masses, which served as hosts for propagating ETNPV, were layered between cotton gauze and stored at 4°C in a cardboard box. Similarly, an additional 100 egg masses were collected and stored, and these egg masses later served as experimental units for testing the biological control agent. In early March, the egg masses used for viral propagation were removed from cold storage, surface sterilized by immersion in 5.25% sodium hypochlorite solution for 1 min and rinsed for 5 min under cold running tap water (Ignoffo and Dutky 1963; Grisdale 1969). Three egg masses were placed into each of five sterile Petri dishes (14.5 cm dia.) with moist filter paper to maintain humidity and were maintained at 25°C under a 12 h light–dark cycle (Addy 1968). Egg masses began to hatch mid-March. Caterpillars were fed apple leaves collected from greenhouse-reared branches. By late March, nearly all caterpillars were in the second instar and were placed onto bouquets of *Malus* spp. with stems immersed in water inside wood-frame cages with cellophane sides.

Eastern tent caterpillar nuclear polyhedrosis virus stabilate was obtained from the USDA Forest Service Northeastern Forest Experiment Station at Hamden, Connecticut. The stabilate was prepared by microblending 10 mg of ETNPV in 100 ml of distilled water, followed by serial dilution to give a concentration of 1.0×10^6 polyhedral inclusion bodies (PIB) per ml as assayed with an AO Bright-line Hemocytometer® (American Optical Company, Buffalo, NY, USA).

The second instar caterpillars were subjected to bouquets of *Malus* spp. that were sprayed to the dripping point with the blended inoculum using a hand-held atomizer. During the following 3 weeks, infected, dead caterpillars were removed and stored at 4°C. The effort yielded 3.75 l of caterpillar carcasses. The carcasses were suspended in distilled water and blended in a Waring blender for 15 min. Sodium deoxycholate and Triton X-100 were each added to a final concentration of 1%, and the suspension was incubated overnight at 37°C with stirring to promote protein denaturation (Arif and Brown 1975). The solution was filtered through three layers of cheese cloth to remove large debris. The filtrate was centrifuged at 1500 ***g*** for 30 min and the pellet resuspended in distilled water. This suspension was homogenized in a ground glass homogenizer to break-up clumps. Centrifugation and homogenization were repeated six times. This procedure allows the recovery of more than 95 percent of the PIB in the pellet while leaving most of the contaminating materials in the supernatant fluid (Arif and Brown 1975). Two sucrose solutions were prepared, one with a specific density of 1.20 (43.9% w/w) and the other with a specific density of 1.30 (61.7% w/w) (Van der Geest 1968). The occlusion body suspension was layered on top of a 4 cm thick layer of sucrose of specific density 1.30 and centrifuged at 8000 ***g*** for 30 min. The polyhedra (specific

density 1.268) accumulated at the separation of the sucrose and lighter aqueous phase while the heavier particles passed through the sucrose solution to the bottom of the centrifuge tube. The polyhedra were removed from the tube, re-suspended in distilled water, homogenized and placed over the sucrose solution with a specific density of 1.20. After centrifugation at 8000 ***g*** for 30 min, the polyhedra precipitated to the bottom of the tube leaving bacteria and lighter tissue fragments above (Van der Geest 1968). This procedure was repeated three times to isolate purified virus. The polyhedra were then suspended in distilled water and centrifuged at 1500 ***g*** for 30 min to remove remaining sucrose. The virus was serially diluted to make a concentration that could be assayed with an AO Bright-Line Hemocytometer®. The polyhedra were freeze-dried and stored at 0°C.

Just prior to application in our field trial the following spring, a viral solution was prepared consisting of 72% distilled water, 25% molasses, 1% folic acid (Shapiro 1985), 2% Rhoplex B-60 A® (Rohm and Haas, Philadelphia, PA, USA) as a spreader-sticker, and a measured quantity of viral polyhedral. The final viral concentrations of the formulations were 10^4, 10^6 and 10^8 PIB/ml.

The field trial occurred at the West Virginia University Forest located in Preston County 32 km east of Morgantown (longitude 79°48′W, latitude 39°41′N). The site was dominated by 40–60-year-old trees, with the predominant species being black cherry (*Prunus serotina* Ehrh.), red maple (*Acer rubrum* L.), red oak (*Quercus rubra* L.), white oak (*Quercus alba* L.) and black birch (*Betula lenta* L.).

During the first week of May, 100 egg masses were individually attached with masking tape near the ends of branches (<3 cm dia. and within 3 m of the ground) at the canopy perimeter of 100 black cherry trees. Main branches below egg masses were coated with petroleum jelly to prevent caterpillars from leaving their respective colonies.

Ten randomly chosen individual egg masses were assigned to each concentration by instar treatment combination, providing nine treatments of 10 replicates. Ten additional egg masses received no virus and served as untreated controls. Treatments were applied to all foliage on each colonized branch to the drip-point with a hand-held pump sprayer. The three virus concentrations were applied to 2nd, 3rd and 4th instar colonies on May 8, 15 and 22 respectively. On 2 June, all tents were brought to the laboratory where the surviving caterpillars from each treatment were counted. The presence of virus in dead caterpillars was confirmed via phase contrast microscopic examination.

The caterpillar count data were not well-approximated by normal distributions because there were many low counts, including many zeros. Therefore, we used non-parametric statistics (i.e. bootstrap confidence intervals) to estimate effects of the virus on caterpillar survival (Efron and Tibshirani 1993; Hjorth 1994). We acquired 10 000 bootstrap samples for each combination of instar and viral concentration by repeatedly calculating the mean of data points sampled randomly with replacement. The percentile method was used to construct 95% confidence intervals from the bootstrap samples (Dixon 2001).

Results

Our efforts to produce ETNPV yielded 0.64 g of the purified virus, and our data indicate the highest rate of the virus drastically reduced eastern tent caterpillar survival (fig. 1). In fact, no 2nd instar larvae survived exposure to the highest rate. Lack of confidence interval overlap strongly suggests the high rate also reduced survival of 3rd instar larvae below the control (fig. 1). Caterpillars of all instars were not strongly affected by the lowest virus concentration, and fourth instar caterpillars appeared to be resistant to even the highest concentration.

Discussion

Field application of ETNPV resulted in a drastic reduction in caterpillar survival when applied to second instar larvae (fig. 1). Our data only partially

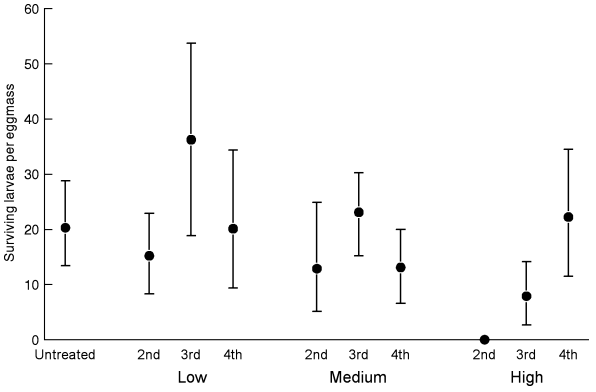

Fig. 1 Ninety-five percent bootstrap means (dots) and confidence intervals (bars) describing mean eastern tent caterpillar densities in untreated control colonies and colonies receiving low ($L = 10^4$ PIB/ml), medium ($M = 10^6$ PIB/ml) and high ($H = 10^8$ PIB/ml) concentrations of nuclear polyhedrosis virus.

agree with the findings of Yearian and Young (1976) who applied ETNPV to fourth and fifth instar larvae. After 14 days, the authors found 3.6, 18.8, 61.4, 94.9 and 99.6 percent mortality at viral concentrations of 10^4, 10^5, 10^6, 10^7, and 10^8 PIB/ml respectively. By contrast, we found fourth instar larva to be highly resistant to the virus at similar concentrations. This supports the findings of Stairs (1965b, 1972), Tanada (1956) and Tanada and Reiner (1962) who found that increasing larval age decreases susceptibility to viral infection in Lepidoptera. One caveat, however, in the experiments conducted by Yearian and Young (1976), is the larvae were treated in the field, collected four days after treatment and subsequently reared in the laboratory. In our study, larvae were collected much later; i.e. 10 days after the final virus application when the presence of sixth instar caterpillars indicated that migration from the tent colony was imminent. Differences in larval survival between the two studies may be attributed to rearing larvae under crowded laboratory conditions. Crowding large numbers of insects into a confined space is considered a key stress factor in inducing viral disease (Steinhaus 1958; Smith 1967; Dwyer 1991; Rothman 1997). Compared to earlier research, our study was more indicative of conditions of natural levels of environmental stress under which biocontrol would be used.

Meyers (1990) cites several authors that note the natural presence of NPV in declining populations of tent caterpillars. Other authors (Meyers 1988; Meyers and Kuken 1995) state that NPV is related to lower fecundity and resistance to the virus is favored in smaller egg masses found at endemic population levels. Sub lethal viral infection may reduce pupal weight and, therefore, cause smaller egg masses in females (Meyers et al. 2000). Meyers (1988) states that the environmental persistence of the virus may also delay the recovery of host populations following outbreaks. Annual or semiannual treatment with ETNPV may suppress population peaks and prevent outbreaks. Also, applying viral pesticides to increase background concentrations of the virus adjacent to pastures and fields occupied by pregnant mares may reduce tent caterpillar populations below hazardous levels.

Our data indicate ETNPV can be propagated, harvested and used as a biological pesticide. Commonly, eradication of indigenous species is undesirable because nearly all insects likely have some role in maintaining ecosystem stability and function. However, population outbreaks can have deleterious impacts on social and economic values. Use of ETNPV as a biological control of the eastern tent caterpillar may be merited in circumstances where land owners and managers want to deter outbreaks or maintain low populations, for example, where breeding mares are pastured, or in forested areas where valuable timber may incur substantial damage or where sensitive habitat may be impacted by defoliation. The specificity (Granados and Federici 1986) of the virus insures that non-target organisms would be unaffected. As the eastern tent caterpillar readily consumes foliage of apple trees, the use of ETNPV as a control in the production of organic fruit (grown in the absence of conventional chemical pesticides) would have considerable merit. Ornamental trees located in urban areas where treatment with chemical pesticides may be undesirable could also be safely treated with a viral pesticide.

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