Enhancement of the activity of a nuclear polyhedrosis virus by an optical brightener in the eastern hemlock looper, *Lambdina fiscellaria fiscellaria* (Lepidoptera: Geometridae)

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ABSTRACT

The pathogenicity of a nuclear polyhedrosis virus originally isolated from *Lambdina fiscellaria lugubrosa* (Hulst) was compared between treatments with and without the optical brightener Blankophor P167 against *L. f. fiscellaria* (Guenée) in the laboratory. The brightener significantly enhanced viral activity by 7.5-fold in terms of LD_{50}, and by 22.9-fold in terms of LD_{95}. With the addition of the brightener, the virus killed *L. f. fiscellaria* larvae 1.5- to 1.8-fold faster than without the brightener.

Key words: nuclear polyhedrosis virus, *Lambdina fiscellaria fiscellaria*, optical brightener, virus enhancement

INTRODUCTION

The eastern hemlock looper (EHL), *Lambdina fiscellaria fiscellaria* (Guenée) (Lepidoptera: Geometridae), is one of the most destructive defoliators of balsam fir, *Abies balsamea* (L.), in eastern Canada (Hudak and Raske 1995). Outbreaks of EHL occur periodically, and each outbreak lasts several years before populations collapse abruptly. Viral diseases are suspected to play an important role in the collapse of outbreaks of this insect, although other field studies suggested that fungi are important mortality factors (Otvos *et al.* 1973). Cunningham (1970) isolated a multicapsid nuclear polyhedrosis virus (*Lff*MNPV) from an outbreak population of EHL. Previous laboratory studies revealed that larvae of EHL were susceptible to *Lff*MNPV and to other NPV viruses originally isolated from the western hemlock looper, *L. f. lugubrosa* (Hulst) or from the western oak looper, *L. f. somniaria* (Hulst) (Cunningham 1970). However, no satisfactory results have been obtained in a field spray trial with *Lff*MNPV against EHL (Cunningham and Kaupp 1995).

Optical brighteners act as whiteners, ultraviolet absorbers and protectants, thus they are widely used in detergent, paper, plastics, and organic coatings industries (Villaume 1958). Recently, optical brighteners were shown not only to protect insect viruses from the denaturing effect of UV radiation (Shapiro 1992), but also to enhance biological activity of several insect viruses against their respective hosts (Shapiro and Robertson 1992; Li and Otvos 1999a). Enhancement of viral activity by optical brighteners varied greatly from one virus-host system to another. Whether optical brighteners could enhance NPV activity against EHL was not determined previously. Here we report the results of laboratory

experiments on EHL larvae exposed to various dosages of a nuclear polyhedrosis virus with and without Blankphor P167, an optical brightener that significantly enhanced activity of another NPV against the western spruce budworm (Li and Otvos 1999a). The objectives of this study were to determine 1) effects of the brightener on larval mortality of EHL (enhancement of viral activity), 2) effects of the brightener on the time-to-death of larvae killed by the virus.

MATERIALS AND METHODS

Experimental insects. Larvae were obtained from a laboratory colony of *L. f. fiscellaria*. The colony originated from larvae collected from the field in Québec and Newfoundland, and had been reared in the laboratory for one generation on artificial diet and natural foliage before the experiment (Li and Otvos 1999b). Following a 3 month diapause in darkness at 2.0 ± 0.5^{0}C and 100% R.H., the eggs of EHL were moved to a rearing room under conditions of 20 ± 1^{0}C, 55-60% R.H., and a photoperiod of 16:8 (L:D) h. Eggs were checked twice a day, and newly hatched larvae were transferred into 20-ml creamer cups (five larvae/cup) that contained a modified spruce budworm artificial diet without formalin (Robertson 1979). Larvae in the cups with diet were kept at the above rearing conditions for about 2 weeks before being transferred onto flushing young (1 m-high) grand fir trees, *Abies grandis* (Dougl. ex D. Don) housed in a cage (0.8 x 1.0 x 1.5 m) at a rate of about 100 larvae per tree. The detailed larval rearing techniques were reported by Li and Otvos (1999b). Fourth-instar larvae, < 24-h-old, were removed from the trees in the cages, and placed in 24-well tissue culture plates (one larva/well) without food for 16-20 h before bioassays. Fourth-instar larvae were chosen for the tests because they are large enough to consume a virus-contaminated artificial diet pellet within 24 h (see below), and because their susceptibility to virus is not significantly different from those of younger larvae (Cunningham 1970).

Virus inocula. A multicapsid nuclear polyhedrosis virus originally isolated from *L.f. lugubrosa* (*Lfl*MNPV) was found to infect EHL larvae (Cunningham 1970). *Lfl*MNPV was purified by repeated centrifugation (3,000 - 8,000 rpm for 30 min each time at 15 ^{0}C) and resuspension in sterile distilled water. Stock suspensions of *Lfl*MNPV were quantified by counting polyhedral inclusion bodies (PIB) using Wigley's method (1980) and stored at 2 ^{0}C before use. Inocula were diluted in distilled water or in the final concentration of 1% (wt/wt) of optical brightener Blankophor P167 (Bayer Corp., Pittsburgh, PA) to the desired concentrations in the bioassays. The 1% concentration was tested because it was an optimal concentration for enhancing viral activity (Argauer and Shapiro 1997; Li and Otvos 1999a).

Bioassays. Five viral concentrations from 39 to 5,000 PIB/µl and six from 39 to 10,000 PIB/µl were used in the treatments with and without brightener, respectively. In addition, one control (distilled water alone) was made for the treatment without brightener, and 1% Blankophor P167 without virus was used as a control for the treatment with brightener. One µl of each viral dilution or control was applied onto a small pellet [4.4 mg ± 0.1 (SE), n = 20] of artificial diet inside each well of a 24-well tissue culture plate. The diet pellets were large enough to fully absorb 1 µl of liquid and allowed larvae to ingest known amount of virus. Immediately after the virus was added to the pellets, one fourth-instar larva, fasted for 16-20 h, was placed into each of the wells. Larvae were confined in the wells by covering the tissue culture plates with lids and were allowed to feed on the treated diet plug for 24 h under conditions of darkness, 20 ± 1^{0}C, and 55-60% R.H. Preliminary tests indicated that higher proportion of larvae consumed the entire diet plug within 24 h in the dark than in the light. Twenty-four larvae were tested for each replicate,

and three replicates were made for each dilution or control. Those larvae that consumed the entire pellet of diet were transferred to untreated fresh one-year-old foliage of grand fir in a 170-ml fluted food cup (Sweetheart Cup Co. Inc., Chicago, IL) (five per cup) and placed at 25 ± 1°C, 55-60% RH, and 16:8 (L:D) h. Larvae that did not consume the entire pellet of diet were discarded.

Data analysis. Mortality was checked twice per week, and foliage changed. Tests were terminated 31 d after treatment, by which time larvae had either died or pupated. The cumulative mortality by 31 d was analyzed using probit analysis (LeOra Software 1994) to estimate the lethal doses of LD_{50} and LD_{95}. Differences in LD_{50} or LD_{95} between treatments with and without Blankophor P167 were compared for significance ($P < 0.05$) using the lethal-dose ratio test (Robertson and Preisler 1992).

To determine the effect of the optical brightener on the time to death (LT_{50} or LT_{95}), the data on larval mortality over time was analyzed with a complementary log-log model (Preisler and Robertson 1989; Robertson and Preisler 1992). The LT_{50} and LT_{95} for both treatments were estimated at the concentration of 5,000 PIB/µl of *Lfl*MNPV. The lethal-dose ratio test (Robertson and Preisler 1992) was used to determine significant differences ($P < 0.05$) in LT_{50} or LT_{95} between the two treatments.

RESULTS AND DISCUSSION

Effects of the optical brightener on larval mortality of EHL. Larval mortality was low in both controls [4.2% ($n = 72$) for distilled water alone, and 4.3% ($n = 70$) for 1% optical brightener alone], indicating that the larvae tested were healthy and that optical brightener was not toxic to EHL larvae. The LD_{50} and LD_{95} for the treatment with *Lfl*MNPV plus 1% Blankophor P167 were significantly ($P < 0.05$) lower than those for the treatment with *Lfl*MNPV alone (Table 1), indicating that the brightener enhanced *Lfl*MNPV activity against *L. f. fiscellaria*. About 174 PIB per larva were required to kill 50% of the test larvae when virus was used alone, while only 23 PIB per larva were needed to kill the same percentage of larvae when 1% brightener was added to the *Lfl*MNPV suspension. To increase larval mortality from 50 to 95%, 42.8 times as much virus was required for the treatment using *Lfl*MNPV alone (i.e., an increase from 173.9 to 7443.3 PIB per larva). In contrast, only 14.1 times as much virus was needed for the treatment with *Lfl*MNPV plus 1% brightener to increase larval mortality from 50 to 95% (i.e., an increase from 23.1 to 326.6 PIB per larva). In terms of LD_{50}, the addition of the brightener enhanced viral activity by 7.5 times. In terms of LD_{95}, the brightener enhanced *Lfl*MNPV activity by 22.9 times (Table 1). The 7.5- to 22.9-fold enhancement of viral activity in this study was much lower than the 90- to 1,500-fold increases previously reported when stilbene brighteners were added to *Ld*MNPV against *Lymantria dispar* (L.) (Shapiro and Robertson 1992; Argauer and Shapiro 1997), but was comparable to the 1.8- to 13-fold increase in the virus-host system of *Cf*MNPV and *Choristoneura occidentalis* Freeman (Li and Otvos 1999a).

Several bioassays have been developed to study insect viruses in the laboratory. In this study, we used a bioassay in which the same known amount of virus was consumed by each test larva. This technique may have some advantages over surface-contamination or foliage dipping bioassays where larvae ingested unknown amount of virus. With the addition of optical brighteners to the virus, feeding behavior of the larvae may have changed and they may not have consumed the same amount of virus. Thus, the bioassay used in this study may give more reliable results on the effects of optical brighteners on viral activity.

Table 1

Effect of Blankophor P167 on activity of *Lambdina fiscellaria lugubrosa* multicapsid nuclear polyhedrosis virus (*Lfl*MNPV) against *L. f. fiscellaria* larvae.

Treatment	n^a	Slope ± SE	χ^2	df	LD_{50} (PIB per larva)[c]		LD_{95} (PIB per larva)	
					Value (95% CL)	Ratio (95% CL)	Value (95% CL)	Ratio (95% CL)
*Lfl*MNPV alone	399	1.01 ± 0.11	7.18ns[b]	4	173.9 (49.4-401.1)	7.5 (3.5-16.3)	7443.3 (2494.9-73365.0)	22.9 (2.3-230.5)
*Lfl*MNPV + 1% Blankophor P167	322	1.43 ± 0.26	2.67ns	3	23.1 (9.7-37.1)		326.6 (203.0-783.8)	

[a] number of larvae that consumed the entire diet pellet, not including control larvae.

[b] ns, not significant, ($P > 0.05$); implying the probit model is appropriate.

[c] Ratio was calculated by dividing the LD_{50} or LD_{95} for the treatment with the virus alone by the LD_{50} or LD_{95} for the treatment with the virus plus 1% Blankophor P167. Lethal dose values (LD_{50} and LD_{95}) are considered different if the 95% confidence limits of the ratio do not include 1 (Robertson and Preisler 1992).

Table 2

Effect of Blankophor P167 on time to mortality of *Lambdina fiscellaria fiscellaria* larvae by *L. f. lugubrosa* multicapsid nuclear polyhedrosis virus (*Lfl*MNPV) at 5000 PIB/µl.

Treatment	n^a	Slope ± SE	χ^2	df	LT_{50} (days)[c]		LT_{95} (days)	
					Value (95% CL)	Ratio (95% CL)	Value (95% CL)	Ratio (95% CL)
*Lfl*MNPV alone	63	4.49 ± 0.38	3.78ns[b]	5	13.6 (12.5-14.6)	1.5 (1.3-1.7)	31.5 (27.9-37.1)	1.8 (1.5-2.2)
*Lfl*MNPV + 1% Blankophor P167	56	5.87 ± 0.57	0.95ns	4	9.1 (8.3-9.8)		17.3 (15.6-19.8)	

[a] number of larvae that consumed the entire diet pellet, not including control larvae.

[b] ns, not significant, ($P > 0.05$).

[c] Ratio was calculated by dividing the LT_{50} or LT_{95} for the treatment with the virus alone by the LT_{50} or LT_{95} for the treatment with the virus plus 1% Blankophor P167. Lethal time values (LT_{50} and LT_{95}) are considered different if the 95% confidence limits of the ratio do not include 1 (Robertson and Preisler 1992).

Effects of the optical brightener on time to death of EHL larvae killed by *Lfl*MNPV. The times to death (LT_{50} or LT_{95}) for the treatment with *Lfl*MNPV plus 1% Blankophor P167 were significantly ($P < 0.05$) shorter than those for the treatment with *Lfl*MNPV alone (Table 2), indicating that larvae died faster when the brightener was added to *Lfl*MNPV suspensions. At the concentration of 5,000 PIB per larva of *Lfl*MNPV, 13.6 days were required to kill 50% of the larvae when virus was used alone, while only 9.1 days were needed to kill the same percentage of larvae when 1% brightener was added to *Lfl*MNPV suspensions. To increase the lethal time from LT_{50} to LT_{95}, 2.3-fold as long was required for the treatment using *Lfl*MNPV alone (i.e., an increase from 13.6 to 31.8 days). In contrast, only 1.9-fold as long was needed for the treatment with *Lfl*MNPV plus 1% brightener to increase the lethal time from LT_{50} to LT_{95} (i.e., an increase from 9.1 to 17.3 days). In terms of LT_{50}, larvae in the treatment with virus plus brightener died 1.5 times faster than those in the treatment with virus alone. In terms of LT_{95}, the brightener reduced the time to death by 1.8 times (Table 2).

The addition of 1% Blankophor P167 not only increased *Lfl*MNPV activity against *L. f. fiscellaria*, but also hastened larval death in the laboratory. Although several insect viruses have been operationally used in pest management programs (Cunningham and Kaupp 1995), one of the drawbacks is the slow killing of pests by viruses. In most cases, foliage protection is the main aim of pest management strategies. Thus, the slow-action of insect viruses may be a serious disadvantage in pest control programs because severe damage may occur before pests are killed. Hastening larval death by the addition of optical brighteners to insect viruses may be of significance in crop protection, because viruses may kill pests before serious damage occurs. Hastening larval death may also lead to earlier and greater horizontal transmission of the virus, which enhances the development of secondary infection and possibly terminates the outbreak of the pest (Otvos *et al.* 1989). More research is needed to test the efficacy of virus with the addition of optical brighteners in the field under natural conditions.

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