LARVAL SUSCEPTIBILITY OF BALSAM FIR SAWFLY (HYMENOPTERA: DIPRIONIDAE) TO NEEM

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Susceptibility of different larval stages of balsam fir sawfly, *Neodiprion abietis* (Harris) to Neemix 4.5 EC, a commercial neem preparation, was determined by using foliage dip bioassays. Larval responses to neem were concentration dependent. The LC_{50} values on 12 days after the treatment for second, third and fourth instars were 1.73 (0.99–2.71, 95% CL), 5.03 (3.29–7.16) and 13.37 (9.31–18.71) ppm azadirachtin, respectively, suggesting that younger larvae were more susceptible to neem than older instars. The acute toxicity of Neemix 4.5 to *N. abietis* larvae was comparable to that of the organophosphate insecticide Dylox 420 EC.

Introduction

The balsam fir sawfly, *Neodiprion abietis* (Harris) (Hymenoptera: Diprionidae), feeds primarily on balsam fir, *Abies balsamea* (L.) Mill., and sometimes on several species of spruce, *Picea* spp. and hemlock, *Tsuga* spp. in North America (Wallace and Cunningham 1995). Usually the outbreak of this sawfly lasts for 2–4 years and then populations suddenly collapse. Since 1991 in western Newfoundland, epidemic populations of this sawfly on balsam fir stands have persisted and the infestation is still increasing. Severe defoliation reduces tree growth and may cause tree mortality, resulting in significant economic losses. The current infestation of over 30,000 hectares of balsam fir in western Newfoundland poses a great threat to spaced stands that represent a significant silvicultural investment and are crucial to future timber supplies. At present in Newfoundland, no environmentally acceptable control strategies for this sawfly are available although some promising results with a nuclear polyhedrosis virus against this insect have been obtained. To operationally manage this pest, effective control agents are urgently needed.

Insecticides derived from the neem tree, *Azadirachta indica* A. Juss (Meliaceae), are considered less harmful to the environment and relatively safe to beneficial insects because of their requirement for oral ingestion, their low mammalian toxicity and limited persistence in the environment (Schmutterer 1990). Results from previous studies indicated that neem extracts have great potential for the control of forestry insects (Helson 1992; Naumann et al. 1994; Wanner et al. 1997; Helson et al. 1998; Lyons et al. 1998), and that several species of sawflies are particularly susceptible to neem (Schmutterer 1985; Larew et al. 1987; Lyons et al. 1998; Helson et al. 1999). Several neem-based products have been tested against *N. abietis* in the field and the results are encouraging (West et al. unpublished data).

The relationship between life stages of insects and their susceptibility to neem is not clear. Prabhaker et al. (1989) reported that early instars of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) are more susceptible to neem than older larvae, while Banken and Stark (1997) demonstrated that the fourth instars of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) are more susceptible to azadirachtin than the first instars. Susceptibility of different life stages of a given insect is important and crucial to its control programs, because such information is highly

relevant to spray targets in the field. Although there is more than one strain or form in *N. abietis* complex elsewhere, Carroll (1962) reported that there is only one form of balsam fir sawfly in Newfoundland. There are five larval stages for males and six for females of *N. abietis*, and the last instars of both sexes do not feed (Carroll 1962). The larval feeding occurs between July and August in Newfoundland.

The objective of this study was to determine the toxicity of Neemix 4.5 EC, a commercial neem preparation, to different larval stages of *N. abietis* in the laboratory.

Materials and Methods

Experimental Insects. Larvae were collected from heavily infested balsam fir trees near Corner Brook, Newfoundland, in July 1999. The stands from which larvae were collected had not been previously treated with pesticides. Larvae were obtained by cutting branches from the lower crown of the infested trees that were 5–7 m in height. Larvae were transferred to the laboratory immediately following collection. In the laboratory, less than one-day-old second, third and fourth instars were selected for the following bioassays. Larval stages were determined according to characteristics of the size and color of their body and head capsule (Carroll 1962).

Bioassays. The response of second, third and fourth instars of *N. abietis* to Neemix 4.5 EC (Thermo Trilogy Corp., Columbia, MD, 4.5% azadirachtin) was determined by using a foliage dip technique. Neemix 4.5 was diluted in distilled water (pH = 6.8) into a series of six concentrations. Distilled water treatment served as controls. Based on preliminary tests, concentrations ranged from 2.8 to 90 ppm azadirachtin for all instar tests, following a 2-fold dilution. Healthy balsam fir branches with all age classes except for the current year needles were cut into 4–5 cm long twigs. These twigs were immersed in each dilution of Neemix 4.5 or distilled water for 10 seconds. Dipped twigs were allowed to air dry at room temperature and then placed in groups of 6–7 in 340 ml clear plastic cups (Solo Cup Co., Urbana, IL). Ten larvae of appropriate stage (second, third or fourth) were transferred into the cups with a camel-hair brush. Each concentration was replicated three times, and 20 larvae were tested per replicate. Thus, 60 larvae were tested per concentration or control. Larvae in the cups were placed in a growth chamber at 20± 1°C, 55–60% RH, and 16:8 (L:D) h, and allowed to feed on the treated foliage for four days before the foliage was removed and replaced with fresh, untreated twigs. Thereafter, the twigs were changed each time larval mortality was checked.

Similar bioassays were also conducted simultaneously with the organophosphate insecticide Dylox 420 (Bayer, Etobicoke, ON, a.i. trichlorfon 420 g/l), being temporarily registered for the control of *N. abietis* in Newfoundland in 1999, for comparison with Neemix 4.5.

Data Analysis. Larval mortality was checked and recorded at 4, 7, and 12 days after treatment. The cumulative mortality 12 days following treatment was corrected for control mortality using Abbott's formula (Abbott 1925). The corrected mortality data were subjected to probit analysis using POLO-PC (LeOra Software 1994) to estimate the LC_{50}, and the slope of the regression line for each instar. The chi-square goodness-of-fit was used to test whether each instar data set fit the probit model. Regression lines for different instars were subjected to the likelihood ratio tests for the equality and parallelism of slopes (Robertson and Preisler 1992). Differences in LC_{50} between any two instars were compared for significance ($P < 0.05$) using the lethal-dose ratio test (Robertson and Preisler 1992).

TABLE I. Larval susceptibility of *Neodiprion abietis* to Neemix 4.5 EC and to Dylox 420 EC.

Instar	*n*	Slope ± SE	χ^2	df	LC_{50}* (95% CL) (ppm)
Neemix 4.5 EC					
Second	420	2.14 ± 0.32	2.56	4	1.73 (0.99–2.71)
Third	420	1.22 ± 0.18	1.79	4	5.03 (3.29–7.16)
Fourth	420	1.06 ± 0.18	2.50	4	13.37 (9.31–18.71)
Dylox 420 EC					
Second	420	1.76 ± 0.32	0.69	4	1.06 (0.40–2.15)
Third	420	1.30 ± 0.20	3.23	4	2.87 (1.47–4.77)
Fourth	420	1.09 ± 0.17	4.12	4	5.73 (3.37–9.01)

* The values of LC_{50} were estimated based on the cumulative larval mortality by 12 days after the treatment.

Results and Discussion

Control mortality for second, third and fourth instars treated with distilled water were 11.8 ± 3.2% (mean ± SE), 15.5 ± 2.5%, and 8.9 ± 2.7%, respectively. Response of *N. abietis* larvae to Neemix 4.5 was concentration dependent, and satisfactorily described by the probit model (see χ^2 values in Table I). The likelihood ratio tests indicated that regression lines for second, third, and fourth instars were significantly different ($\chi^2 = 95.8$, df = 4, $P < 0.05$), and that the slope of the line for second instars was not parallel with that for either third ($\chi^2 = 7.2$, df = 1, $P < 0.05$) or fourth instars ($\chi^2 = 10.2$, df = 1, $P < 0.05$). However, slopes of the lines for third and fourth instars did not deviate significantly from being parallel ($\chi^2 = 0.4$, df = 1, $P > 0.05$). Regression slopes estimate the change in biological activity per unit change in concentration of the stimulant. Parallel lines may signify that the test organisms have qualitatively identical but quantitatively different levels of detoxification enzymes (Robertson and Preisler 1992). Thus, our results suggest that the second instars may differ both qualitatively and quantitatively in detoxification enzymes from third or fourth instars. This may be related to larval feeding behavior of this species.

Lethal concentration (LC_{50}) of Neemix 4.5 increased with larval instars (Table I), indicating that younger larvae were affected more by foliage surface applications of Neemix 4.5 than were older instars. All differences in LC_{50} between any two instars were significant (Table II). Scraping the surface of the treated foliage by younger sawfly larvae may result in ingesting more toxin per unit of consumed food than older larvae, yielding a higher larval mortality. Although the exact relationship between larval instars of *N. abietis* and their susceptibility to Neemix 4.5 cannot be concluded from this study because the actual amount ingested by individuals of each instar was not known, the results show that younger larvae were more easily killed by Neemix 4.5 than were older instars. This information is highly relevant to operational spray programs. If possible, operational sprays with neem-based products for the control of *N. abietis* should target younger larvae. This strategy would maximize control efficacy and minimize foliage damage by older larvae. Targeting young larvae would also minimize direct adverse effects of neem, if any, on parasitoids of *N. abietis* because most parasitoids do not become active until late in the season (Carroll 1962).

TABLE II. Comparisons of larval susceptibility of *Neodiprion abietis* to Neemix 4.5 EC.

Comparison	LC_{50} ratio (95% CL)*
3rd versus 2nd	2.91 (1.57–3.92)
4th versus 2nd	7.73 (4.52–9.75)
4th versus 3rd	2.66 (1.34–5.33)

* The ratio of lethal concentrations was calculated by dividing the lethal concentration (LC_{50}) for older instar by the lethal concentration (LC_{50}) for young instar. Lethal concentrations are considered different if the 95% confidence limits (CL) of the ratio do not bracket 1 (the lethal dose ratio test; Robertson and Preisler 1992)

Besides direct toxicity, we noticed that adult emergence from pupae in Neemix 4.5 treatments was lower than that in the control (approximately 30% versus 90%). Furthermore, emerged adults from the Neemix 4.5 treatments had about 50% individuals with malformed wings, compared with about 7% in control groups. Fecundity of these surviving adults may be reduced as reported by others with *Ceratitis capitata* (Diptera: Tephritidae) (Di Ilio et al. 1999).

Although acute toxicity of Neemix 4.5 was lower than that of Dylox 420, it was comparable (Table I). In terms of LC_{50}, Dylox 420 was approximately 1.6-, 1.8- and 2.3-fold more toxic than Neemix 4.5 to second, third and fourth instars, respectively. During the experiments, we observed that Neemix 4.5 killed larvae more slowly than did Dylox 420. From foliage protection viewpoint, the slow action may present a limitation in using neem-based products against defoliators. This limitation, however, may be overcome by the antifeedant and repellent properties of neem. Following neem applications, larvae may not die immediately, but may not continue to feed. Thus, foliage protection may be possible. Furthermore, non-feeding larvae may provide significant food sources for predators or hosts for parasites that could augment control of *N. abietis*.

In summary, younger larvae of *N. abietis* were more susceptible to Neemix 4.5 than older instars. Acute toxicity of Neemix 4.5 to the sawfly larvae was comparable with that of Dylox 420. Because of less harmful effects of neem on the environment than synthetic chemicals, Neemix 4.5 is a promising and strong candidate of alternatives for the control of *N. abietis*.

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