Influence of Neem Oil on the Large Pine Weevil, *Hylobius abietis* L. (*Coleoptera, Curculionidae*)

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Abstract

The large pine weevil, *Hylobius abietis* (L.) (Coleoptera, Curculionidae), is the most important pest affecting boreal and temperate conifer forest regenerations in Europe and Asia. Weevils feed on the bark of newly planted coniferous trees causing seedling mortality, stem deformation, and reduced growth. The loss of seedlings without synthetic pesticide treatment may be very high during the first 2–3 years after planting. Having regarded to environmental protection the natural insecticides like neem formulations could replace the chemical control. The effects of natural cold-pressed neem (*Azadirachta indica* A.Juss) oil (biopreparation NEEM EC (10 000 ppm azadirachtin) produced by the Indian Neem Tree Company) on the maturation feeding of *H. abietis* was tested in laboratory and field experiments. NEEM EC was tested at concentrations of 2% and 10%. Feeding on food sources treated with neem oil was significantly depressed in laboratory choice tests. A strong antifeedant effect (antifeedant index (AFI) = 0.35–0.62) was revealed for both sexes depending on the concentration of neem oil. In field test, during the whole season weevils damaged the treated 4-year-old Norway spruce seedlings significantly less (< 50%) than untreated control plants (100%).

Key words: antifeedant, biopreparation, Estonia, *Hylobius abietis,* NEEM EC, neem oil

Introduction

The pine weevils (*Hylobius* spp.) are common insects in coniferous forests. There are three species of the genus *Hylobius* (Coleoptera, Curculionidae) in Estonia, two of them endanger reforestation at clear-cut areas (Sibul 2000). The most widespread, economically important and abundant species is the large pine weevil (*H. abietis*) whose habitat ranges from Western Europe to Eastern Siberia and Japan (Eidmann 1974, Gourov 2000). It is one of the most dangerous pests of conifers in the areas where felling has been done in the form of clear-cutting, and subsequent rapid reforestation of clear-cut areas with coniferous seedlings has taken place (Wilson and Day 1994; Voolma et al. 2003, Långström and Day 2004). Pine weevils use the roots of the fresh conifer stumps as their main breeding sites (Nordlander et al. 1986, Voolma 1994, Hannerz et al. 2002). The economic damage is caused by adults that feed on the phloem tissues of coniferous seedlings where they bite at the bark in patches (Leather et al. 1999). The plants damaged by the pine weevils become weak, extensively damaged plants are killed. In large numbers, the pine weevils are capable of completely destroying the reforestation seedlings planted at clear cut area, causing considerable economic losses (Örlander and Nilsson 1999, Wainhouse et al. 2001, 2004, Voolma and Sibul 2002).

Over time, various chemical insecticides ranging from ultratoxic chlorine- (DDT, hexaclorane) and phosphorus organic (chlorophos, bensophosphate, etc.) insecticides to synthetic pyrethroids (permethrin, deltamethrin) have been used against the pine weevil (Žiogas and Valenta 1977, Tuomainen et al. 2003). The ever-increasing pollution of the environment and partial or full restrictions imposed in many countries of the European Union on using chemical insecticides in the forests (Anon. 2000, Långström and Day 2004) encourage finding new means and methods for pest control (Thacker and Bryan 2003). One option to keep the pine weevil under control and decrease the damages caused by it, is to treat seedlings with natural bioactive compounds, e.g. with insecticidal plant extracts and plant oils, or other botanical biopesticides which disguise the scent of the feeding plant from the pest insects, thus affecting their behaviour.

The advantages of biopreparations over chemical pesticides are their rapid decomposition in nature, selective effect on insect pests, relatively small or non-existent risk of environmental pollution and no safety hazard for human health (Johnson 1998, Sibul et al. 2004). As the composition and concentration of the bioactive ingredients vary, the affected pests do not develop resistance towards biopreparations as rapidly as they do towards ordinary insecticides. Likewise, the botanical toxic substances only have effect on specific insect species populations and their natural biotic systems of interactive self-regulation (Isman 1997, Regnault-Roger 2005).

During recent years, one of the leading topics in the pine weevil research has been the use of bioactive compounds and natural insecticides derived from host or non-host plants for pine weevils repellents or antifeedants for controlling their population (Månsson and Schlyter 2004, Olenici and Olenici 2006, Sibul et al. 2001, 2002, 2004, 2005, 2006, Schlyter et al. 2004, Thacker and Bryan 2003, Thacker et al. 2002, 2003, Thompson 2004, Bohman et al. 2008, Eriksson et al. 2008). During the last decade, a series of studies on the effect of plant extracts on feeding behaviour of the pine weevil have also been carried out in Estonia (Luik et al. 1995, 1998, 2000, Sibul et al. 2001, 2002, 2004, 2005, 2006).

The past research works indicated the efficacy of the neem preparations (NeemAzal-T, NeemAzal-T/S) on *H. abietis*. The neem-based biopesticides contain many biologically active compounds extracted from neem: triterpenoids, phenolic compounds, carotinoids, steroids and ketons. Tetranortriterpenoid azadirachtin ($C_{36}H_{44}O_{16}$) and other limonoids (salinnin, nimbin) are the main bioactive ingredients in neem seeds. The content of azadiractin in neem kernels depends on geographical location, methods of extraction etc. (Ermel et al. 1987; Schmutterer 1995; Sanguanpong 2006). In this paper, the effect of cold-pressed neem oil (NEEM EC) on the maturation feeding of *H. abietis* was tested in laboratory and field conditions. The influence of neem oil is not studied on *H. abietis* before.

Materials and methods

Insect

The *H. abietis* adults were collected in May–June 2007 from trapping pitfalls in a freshly cut area in the forest district of Räpina, Southern Estonia, where beetles are very abundant. After collection, the weevils were kept in groups of ten in glass jars with moistened moss in a refrigerator at 2–3°C. Water and fresh Scots pine (*Pinus sylvestris* L.) twigs were added to the jars as needed. Prior to the experiments weevils were transferred to ambient laboratory conditions (22°C, 75% r.h. and a photoperiod of L14:D10) for 12 h without food but with access to water. Only healthy, medium-sized individuals were accepted as experimental insects. The body mass of the weevils ranged in males from 136–192 mg (mean 173±21 mg) and females 200–230 mg (mean 198±32 mg). The sex of the weevils was determined and the females and the males were tested separately.

Biopesticide

The natural cold-pressed neem oil biopreparation NEEM EC (10 000 ppm Azadirachtin; produced by the Indian Neem Tree Company) was used. NEEM EC (emulsifiable concentrate) was tested at concentrations of 2% and 10% (rate of application 20ml/l and 100ml/l, respectively).

NEEM EC is the general purpose botanical pesticide of choice for organic agriculture. NEEM EC is widely used in several countries around the world today either singly in Integrated Pest Management or in combination with synthetic pesticides (for review see Mordue (Luntz) and Nisbet 2000). NEEM EC belongs to the category of medium to broad spectrum pesticides. NEEM EC works by intervening at several stages of the life of an insect. It does not kill the pests instantaneously but incapacitates it in several other ways (Anon. 2005).

Laboratory test

The laboratory tests were carried out in the laboratories of the Estonian University of Life Sciences. Effects of the NEEM EC on the large pine weevil' feeding was tested in 9.5 cm Petri dishes with Scots pine twigs with a length of 8 cm and a diameter of 5–7 mm. The ends of the pine twigs were dipped in melted wax and the twigs were then dipped in a solution of NEEM EC. The treated and the untreated twigs were placed in individual moistened paper sleeves (to prevent contact between the twigs) within a Petri dish. One weevil was supplied with two twigs that were either both untreated (control), or with one twig untreated and the other one treated (choice). In all choice test and control variants 24 weevils (12 females + 12 males) were used and every specimen was tested only one time. Twelve replicate Petri dishes were used per treatment variants and control. The laboratory assays were conducted at room temperature (22±1°C), in natural light regime (L14:D10). The sex of the beetles was determined for the estimation of the feeding differences between the females and the males. The feeding area of bark (outer cortex and phloem collectively) removed from the twigs was measured periodically after 24 h, 48 h and 72 h for each twig. The amount of bark consumed on the treated, untreated and the control twigs were measured using a transparent scale-grid (mm^2) held over a twig.

Field test

Field trials were established in a fresh clear-cutting area in the forest district of Räpina, Southern Estonia (58°09' N, 27°08' E). Four-year-old Norway spruce (*Picea abies* (L.) H.Karst.) seedlings were planted in grids with 2 m spacing, using a randomised block design. The seedlings were planted in early summer (15.06.2007). The experiment was set up according to randomised blocks where every of 40 blocks contained all differently treated seedlings (n=240) and an untreated control seedling (n=120). After planting, different solutions were applied around the root collar of the seedling trees using a paintbrush. The trees were treated with 5 ml of neem oil solutions 0–10 cm above the root collar. The control seedlings were left untreated. Feeding damage to the planted seedlings was assessed weekly (during 4 months period) after treatment using a visual percentage scoring system. The debarked area (consumed outer and inner bark in percentage) of the stem was recorded on a four-step scale (personal method): 0 = no feeding, 1 = 1–25%, 2 = 26–50%, 3 = 51–75% and 4 = 76–100% loss of bark. Damage was assessed 0–10 cm above the root collar. The mean of the trees damaged by weevils per variant and their standard deviations were calculated for different periods.

Data analysis

The mean feeding area of a weevil for different periods and its standard deviation were calculated. The significance of the differences was compared by ANOVA Student's t-test at p<0.05. Also, antifeedant index (AFI) (Blaney et al. 1984; Klepzig and Schlyter 1999; Schlyter et al. 2004) was calculated for each treatment. AFI = (C–T) / (C+T), where C = feeding area on the untreated twig; T = feeding area on the treated twig. AFI = 0 is indicative of a zero effect, the negative values (< 0) indicate a feeding stimulant and the positive values (> 0) indicate feeding inhibition. AFI = +1 is indicative of the best possible antifeedant and AFI = –1 is indicative of the best possible feeding stimulant.

Results

Laboratory experiment

The mean amount of bark removed on the twigs (mm^2) is shown in Table 1. Comparison with the control neem oil reduced significantly the feeding activity of the weevils (P<0.05).

During the choice trial period was a clear effect of preference against treatment concentrations. The differences between the average values were not statistically significant because of very high variability of the data within first day (24 h) except between the mean feeding areas of male weevils on NEEM EC 10% treated and untreated twigs. After 24 hours the amount of bark consumed varied between 0 mm^2 (80% of tested weevils) and 22±8.2 mm^2 (20% of tested weevils) on treated twigs in both treatments. At the same time in control variant all twigs were consumed.

After 48 hours in choice tests significant differences were found between the mean feeding areas of treated and untreated twigs. At the end of the experiment (after 72 hours) the damaged bark area was larger on the twigs with lower concentration (Table 1). The mean weevil male feeding rate was also higher on untreated twigs in 2% NEEM EC choice trial variant (P<0.05). The sex of the weevils had significant influence on the feeding activity after 2 days (P = 0.033). 2% and 10% NEEM EC solutions considerably depressed feeding of specimens of both sexes during 72

Table 1. Bark area (mean ± SD, mm^2) removed from treated, untreated and control twigs by the weevils in choice experiments through time with respect to neem oil concentration. Antifeedant index (AFI) indicates the antifeedant effect. AFI < 0 – feeding stimulant, AFI = 0 – no effect, AFI > 0 – antifeedant

Feeding time, h	Weevil`s sex	Treatment						Control
		NEEM EC 2%	Untreated	AFI	NEEM EC 10%	Untreated	AFI	
24	♀♀	7.1±12.2^	9.9±4.4	0.16*"	2.0±5.4^	5.0±6.4	0.43*"	21.5±7.0^*
	♂♂	4.7±5.5^	11.5±10.7	0.42*"	1.4±4.2^	21.1±3.7**	0.88*"	32.9±11.7^*
48	♀♀	16.8±5.3^	36.0±7.5**	0.36	8.7±5.5^	28.5±4.0**	0.53	57.3±8.1^*
	♂♂	13.1±5.5^	26.6±5.9**	0.34	6.9±3.1^	40.2±14.2**	0.71	79.8±26.3^*
72	♀♀	29.7±7.0^*	58.1±9.0**	0.32	13.2±6.9^	46.8±9.4**	0.56	88.8±10.0^*
	♂♂	22.0±8.0^*	32.7±6.8	0.20	14.4±3.2^	48.1±14.3**	0.54	123.9±41.0^*

* significant difference between the males and the females by the Student t-test, p<0.05
** significant difference between the treated and the untreated twigs by the Student t-test, p<0.05
*" significant difference between antifeedant indexes(AFI) by the Student t-test, p<0.05
^ significant difference between the treated and control twigs by the Student t-test, p<0.05

hours, but at the end (72 hours after beginning) of the experiment males were statistically more susceptible to the neem oil.

The NEEM EC acted as antifeedant (AFI > 0) during experiment, while the 10% solution inhibited weevils feeding more (AFI = 0.43 – 0.88) than that of 2% solution (AFI = 0.16 – 0.42). There were differences between males and females antifeedant indexes after 24 hours of insecticide application whereas male feeding more depressed (AFI = 0.42 – 0.88) than female (AFI = 0.16 – 0.43).

Neem oil had no toxic effect on *H. abietis*. All weevils were alive after the experiment.

Field experiment

Neem oil significantly influenced the feeding behaviour of pine weevils in forest conditions (Figure 1). During the first four weeks after planting the number of damaged seedlings was similar in both neem oil treatments. Since the midsummer pine weevils' damage did not rise to the end of test period (October). Throughout the experimental period, treated plants (2% NEEM EC 53% ± 8.7, 10% NEEM EC 40% ± 9.3) were significantly ($P<0.05$) less damaged compared with the control (100% ± 0.0). At the end of first week 93% of the control plants were damaged, whereas more than 50% of bark at the seedlings root collar was consumed. A week after planting in injured control transplants all damage rates existed and in following weeks the damage increased. The damage rate of treated transplants was low or medium (up to 75% from bark area in root collar) and it escalated a bit during summer (Figure 2). Transplants inspection in the spring of 2008 showed

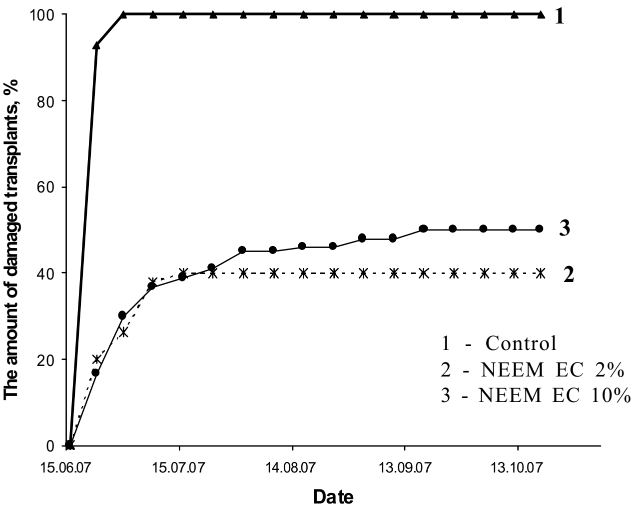

Figure 1. The effect of NEEM EC treatments on the 4-year-old Norway spruce transplants by *Hylobius abietis* in a fresh clear-cut area 4, 8, 12 and 16 weeks after planting

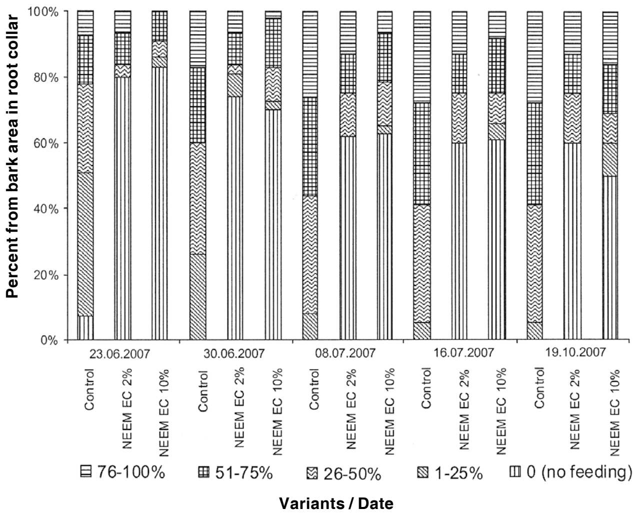

Figure 2. Dynamics of damage rate (%) by *Hylobius abietis* in root-collar bark area in 4-year-old Norway spruce transplants in a fresh clear-cut area depending on level of NEEM EC treatments

that 88% of treated plants were alive, but only 15% of the control plants were survived. There were no differences between NEEM EC 2% and 10% treatments.

Discussion

The presented data indicate that assayed neem oil acted as significant (AFI = 0.88) antifeedant by the *H. abietis*. Previous choice experiments with neem preparations and some other biopesticides have shown similar results to the large pine weevil (Luik et al. 1998, 2000, Sibul et al. 2001, 2002, 2006, Thacker et al. 2002, 2003, Thacker and Bryan 2003, Olenici and Olenici 2006). The repellent and antifeedant effect of neem-based preparations have been reported for a wide range of insect pests (Schmutterer 1990, Schmutterer and Singh 1995, Koul 2004), in addition for mites and nematodes (Musabyimana and Saxena 1999, Akhtar 2000). Due to antifeedant effect of neem, the insects on neem-treated plants probably die because of starvation (Zabel et al. 2002). It is recognized that neem affects pests also in many other ways such as oviposition deterrence, growth inhibition, mating disruption, chemosterilization, etc. (Schmutterer 1995, 2002).

Both NEEM EC concentrations exerted an antifeedant effect on the pine weevil. These results presented also by antifeedant index, and it is highest at the highest concentration of neem oil. In tests with pales weevil (*H. pales*) Azadirachtin had antifeedant activity and it was stronger with higher concentration (Salom *et al.* 1994). In the choice-feeding test, the general smell environment of neem oil, irrespective of the concentration (2% and 10%), inhibited feeding in treated twig (contact influence), whereby general feeding activity (in the control twig) was affected by the volatilising bioactive ingredients. Previous assays with NeemAzal-T/S support results presented in the current experiment (Luik et al. 2000, Sibul et al. 2001, 2006). Antifeedants affect the olfactory and gustatory chemoreceptors on the antennae, the mouthparts (Bland 1983) and the legs (Rees 1969) of the insects and can be both volatile and non-volatile.

The male feeding was more depressed (especially in the end of the experiment) than female. Presumably the feeding rate was different because females need more energy during maturation feeding. According to Bylund et al. (2004) females feeding rate is high during oviposition period and after it ceases, the feeding rate declines to the same level as in the males. Similar male and female weevils' sensitivities to the insecticide during maturation feeding found also by Eidmann and Novák (1970) when DDT was tested on *H. abietis*. They supposed that the higher sensitivity of males reflects the differences between the sexes concerning insect size and weight, males being usually smaller and lighter.

Neem oil solutions showed deterrent and repellent effect on the large pine weevil in forest conditions. NEEM EC can effectively replace conventional chemical insecticides in forestry. Consequently, the neem oil efficiency studies should continually focus on the behavioural and physiological studies of *H. abietis* in laboratory tests. Further research is needed to test the influence of neem oil' higher concentrations and determine its possible phytotoxic effect on conifer seedlings or transplants in field conditions.

Conclusions

Neem oil emulsifiable concentrate (EC) had a repellent and antifeedant effect on the large pine weevil. A strong antifeedant effect (mean antifeedant index (AFI) = 0.35–0.62) was revealed for both sexes depending on the concentration of neem oil, whereas the higher concentration had stronger inhibition. Males were more susceptible to neem oil than the females. Neem oil had significant long-term effect in forest conditions, a year after planting most of treated plants were alive. Neem oil could serve as a perspective preparation for reducing the damage caused by pine weevils in reforestation areas.

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ВОЗДЕЙСТВИЕ НИМОВОГО МАСЛА НА ЖУКОВ БОЛЬШОГО СОСНОВОГО ДОЛГОНОСИКА, *HYLOBIUS ABIETIS* (*COLEOPTERA, CURCULIONIDAE*)

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Резюме

Большой сосновый долгоносик, *Hylobius abietis* (L.) (Coleoptera, Curculionidae), является основным вредителем хвойных культур, заложенных на свежих вырубках. Без защитных мероприятий потери в плантациях в течение первых 2-3 лет после посадки могут быть весьма значительными. По экологическим причинам химические меры борьбы с вредителями необходимо заменить менее опасными. В этом плане большое внимание уделяется препаратам растительного происхождения. Биопрепарат NEEM EC (10 000 ppm azadirachtin, Indian Neem Tree Company), разработанный на базе нимового масла, получаемого из семян тропического дерева ним (neem, *Azadirachta indica* A.Juss), был испытан против большого соснового долгоносика в лабораторных и полевых условиях. NEEM EC использовали в концентрациях 2% и 10%. В лабораторных экспериментах препарат значительно подавлял питание жуков на обработанных с препаратом сосновых ветках. Сильное антифидантное воздействие (антифидантный индекс (AFI) = 0.35-0.62, в зависимости от концентрации препарата) было показано для обоих полов долгоносика. В полевых испытаниях обработка 4-летних саженцев ели обыкновенной (*Picea abies*) также значительно уменьшила поврежденность деревцев большим сосновым долгоносиком.

Ключевые слова: антифиданты, биопрепараты, NEEM EC, нимовое масло, сосновый долгоносик, Эстония