Influence of Some Plant Extracts and Neem Preparations on the Maturation Feeding of the Large Pine Weevil, *Hylobius abietis* L.

ANNE LUIK
Institute of Plant Protection, Estonian Agricultural University
Kreutzwaldi 64, 51014 Tartu, Estonia
IVAR SIBUL
Department of Silviculture, Estonian Agricultural University
Kreutzwaldi 5, 51014 Tartu, Estonia
KALJO VOOLMA
Forest Research Institute, Estonian Agricultural University
Kreutzwaldi 5, 51 014 Tartu, Estonia

Luik A., Sibul I., Voolma K. 2000. Influence of Some Plant Extracts and Neem Preparations on the Maturation Feeding of the Large Pine Weevil, *Hylobius abietis* L. *Baltic Forestry*, 1: 53–58.

In laboratory choice feeding tests with large pine weevil, *Hylobius abietis* L., it was established that the water emulsion of the NeemAzal-T/S (content of azadirachtin 1%) in concentrations 5%, 10% and NeemAzal-T (content of azadirachtin 5%) emulsions in concentrations 2%, 10% and 20% provoked antifeedant activity for weevils during 24-72 h. It was stronger in higher concentrations (10%, 20%) than in lower ones (2%, 5%) whereas males were more sensitive than females and responded significantly already to a lower concentration. 20% water extracts of raw leaves of *Phaseolus vulgaris* L. and *Tussilago farfara* L. depressed the feeding activity in females only slightly, but significantly in males. The effect might be caused by the glycosides like phasein and tussilagin. In forest conditions the treatment of spruce seedlings with 20% water emulsion of the NeemAzal-T preparation significantly decreased the number of seedlings damaged by weevils during the first vegetation period after planting.

Key words: *Hylobius abietis*, neem preparation, plant extract, maturation feeding

Introduction

The large pine weevil, *Hylobius. abietis* L (*Coleoptera, Curculionidae*), is one of the most destructive insects on conifer reforestation areas in the Palearctic region. The weevils begin to get out from hibernation sites when the mean diurnal temperature is around +9 °C (Eidmann, 1964; Haritonova, 1965). More active spring movement of weevils starts generally in the second half of May when temperatures are around 18 °C (Christiansen & Bakke, 1968; Låångström, 1982). Usually in Northern European conditions the mass flight occurs from the middle of May till the second half of June (Eidmann, 1974; Solbreck & Gyldberg, 1979; Lekander et al., 1985). It could also be earlier or later depending on ecological peculiarities of the conditions where weevils are breeding and hibernating (Luik & Voolma, 1989). During this time weevils guided by olfaction concentrate on fresh clear-cut areas where they are looking for maturation feeding sites on the cambial layers of young conifer tree stems and shoots and for oviposition sites on the roots of the stumps in recently felled conifer trees or in the logging slash mixed with soil and ground litter (Nordlander et al., 1986; Luik & Voolma, 1989). Heavy conifer seedling mortality is common, particularly, where seedlings have been planted on recently clear-cut areas (Bejer-Petersen et al., 1962; Eidmann, 1974; Heritage et al., 1989).

A wide variety of methods has been employed for weevil control, including various mechanical and chemical means to protect the plants against weevil damage (Eidmann, 1974; Annila, 1982; Lindström et al., 1986). From the mechanical methods different types of protective collars are most promising (Lindström et al., 1986).

For the monitoring of the pine weevils the baited pitfall traps with standardized baits (aa-pinene, ethanol 96%, water) have been developed (Tilles et al., 1986; Nordlander, 1987). However, all these methods are not sufficient for large pine weevil control.

Recently the new interest has arisen in natural botanical insecticides. They are environmentally more friendly than chemical pesticides and acting in many insects in different ways – as repellents, growth regulators or as toxicants (Bergen, 1994; Schmutterer, 1990, 1992). Among natural pesticides, the compounds from neem (*Azadirachta indica* A. Juss) have a number of properties useful for insect pest management. These include repellency, feeding and oviposition deterrence, insect growth regulator activity, low mammalian toxicity and low persistence in the environment (Koul, 1992; Schmutterer, 1990). Neem is also less toxic than many conventional insecticides to nonphytophagous insect species, including pests' natural enemies and insects-pollinators (Hoelmer et al., 1990; McCloskey et al., 1993; Nauman et al., 1994). Some experiments are made with neem and other plant compounds also for forest pest management. The neem seed extracts had a systemic influence on the bark beetle *Dendroctonus ponderosae*. Lodgepole pines treated with neem were less attacked by *D. ponderosae* and mortality of larvae was increased on applicated trees (Naumann et al., 1994). In weevils *Hylobius pales* the application of pine logs with neem extract significantly inhibited feeding during 24 h, some other natural compounds as borneol, bornyl acetate, cucurbitacin, limonin, myrcene S(+) and R(-) carvone and verbenone also acted as feeding deterrents during that time (Salom et al., 1994). In feeding choice tests where weevils of *H. abietis* chose between Scots pine twigs treated with plant extracts and untreated ones it was clarified that 20% water extracts of *Asarum europaeum* and *Narcissus poeticus* influenced *H. abietis* as feeding stimulants. The extracts of *Allium sativum, Taxus baccata, Primula veris* and *Heracleum sosnowsky* acted as feeding deterrents for a short term (24-48 h) in laboratory conditions (Luik, 1997; Luik et al., 1998). It would be important to detect natural compounds which would have a high deterring or repellent activity for the weevils of *H. abietis*. The aim of the present study was to establish the influence of water extracts of *Phaseolus vulgaris* and *Tussilago farfara* and neem preparations NeemAzal-T/S (1% content of azadirachtin) and NeemAzal-T (5% content of azadirachtin) on maturation feeding of *H. abietis*. The experiments were carried out in the spring-summer periods of 1997 and 1998.

Material and methods

For laboratory experiments the weevils of *H. abietis* were collected from trapping pits from a fresh clear-cutting area in the forest district of Räpina in South Estonia. Scots pine twigs of 10 cm long and 5 mm in diameter were halved, one half was treated with plant extracts or neem emulsions, the other was an untreated control twig. A treated and untreated twigs were placed in individual moistened paper sleeves (to prevent contact between twigs) within petri dish. One weevil, starved for 12 h was placed in each dish (100x15 mm). The petri dishes were exposed to natural light conditions in temperature +20±1 °C. In every variant of choice tests 35-40 weevils were used and every specimen was tested only one time. An area estimate of weevil feeding on cambial tissue was made with the help of the transparent mm^2 paper after 24 h, 48 h and 72 h for each twig. All treatments were replicated two times. The sex of beetles was established for the estimation of feeding differences between sexes. The mean feeding area of weevil per different periods and their standard deviation were calculated. The significance of differences was controlled by the Student-t test at 0.05 level.

For the extract the raw leaves of *Phaseolus vulgaris* and *Tussilago farfara* were ground and exposed in water for 24 h. The content of plant material was 20%. After filtration the extracts were used for pine twigs dipping for 5 seconds. After dipping the pine twigs were dried for 5 seconds on filter paper and put together with the untreated control half to petri dish. NeemAzal-T/S (1% azadirachtin) and NeemAzal-T (5% azadirachtin) formulations are produced by Trifolio M GmbH in Germany. These two preparations differ by the concentration of active compound azadirachtin, and also NeemAzal-T/S contains some additional plant oils. NeemAzal-T/S 5% and 10% water emulsions were tested in laboratory conditions in 1997. In 1998 the experiments with NeemAzal-T 2%, 10% and 20% water emulsions were carried out in laboratory and forest conditions. In field conditions differently treated (2%, 10%, 20% NeemAzal-T) three-year-old Norway spruce seedlings were planted in randomised blocks (every of 40 blocks contained all differently treated seedlings and an untreated control seedling) on a fresh clear-cut area. Before planting, the root collars of seedlings were treated with different emulsions with painting brushes. The number of trees damaged by weevils was established with weekly intervals during the vegetation period. The mean of trees damaged by weevils per variant and their

standard deviation were calculated in different periods. The significance of differences between variants was controlled by the Student-t test.

Results

In the experiments carried out in 1997, the effect of NeemAzal-T/S and plant extracts on feeding of *H. abietis* weevils depended on the feeding time and the weevils' sex. After 24 h weevils fed significantly less on twigs treated with the extract of *P. vulgaris* and with the 10% emulsion of NeemAzal-T/S, whereas the feeding of males was more inhibited than that of females (Table 1). NeemAzal-T/S acts for males already in a lower concentration significantly whereas it depresses feeding in females only slightly. After 48 h and 72 h cambial tissues on average were eaten significantly less only on twigs treated with NeemAzal-T/S 10% emulsion. Also the untreated control twigs were less damaged in NeemAzal-T/S treatment variants than in other variants. It indicates that NeemAzal-T/S inhibits the total feeding activity of weevils and its rate depends on the concentration. To all tested plant compounds the males are more sensitive than females (Table 1). Even after 48 h and 72 h the twigs treated by extract of *P. vulgaris* were significantly ($p<0.05$) less damaged by males than by females. The extract of *T. farfara* depressed feeding considerably ($p<0.05$) only in males but not in females (Table 1).

In the experiments carried out in 1998, it became also evident that males were more sensitive to NeemAzal-T water emulsions of lower concentration (2%, 10%) (Table 1). Higher concentration (20%) of the emulsion (1% of active compound azadirachtin) completely depressed the feeding of specimens of both sexes during 72 hours on treated twigs. Observations showed that beetles moved also to treated twigs and palpated them, only after that they left the twigs and started feeding on untreated control twigs. Their feeding rate on control twigs was higher than in case of NeemAzal-T/S experiments where the concentrations of active compound azadirachtin was lower (0.05% and 0.1%) and the depression of feeding was not so high but the feeding rate on untreated control twigs was lower. This could be caused by the additional plant oil content in NeemAzal-T/S preparation.

NeemAzal-T 20% water emulsion effected significantly ($p=0.00019$) the feeding behaviour of pine weevils in forest conditions (Figure 1). During the first two weeks weevils did not eat the bark of seedlings treated

Table 1. Mean feeding area (mm^2 ± standard deviation) of pine weevil females (f) and males (m) depending on treatment of pine twigs and feeding time

Treatment	Sex	24 h		48 h		72 h	
		Treated	Control	Treated	Control	Treated	Control
NeemAzal-	f	7.8±3.6	10.4±3.0	15.8±4.3	16.1±4.1	16.8±4.7	19.7±4.5
T/S 5%	m	3.3±1.0*	8.5±3.5"	4.4±1.2*	16.4±5.4"	8.2±2.4*	19.0±6.1"
NeemAzal-	f	0.8±0.3	9.0±2.5"	3.8 ±1.2	13.8±3.3"	14.1±2.2	18.7 ±5,7
T/S 10%	m	0.3±0.1*	8.1±1.6"	1.5±0.6*	13.2±2.7"	2,0±0.9*	17.7±4.6"
NeemAzal-T	f	17.4±3.1	35.6±5.3"	31.1±6.2	53.5±8.6"	44.0±5.9	73.3±7.2"
2%	m	9.2±3.7*	26.3±4.4"	18.4±3.5*	49.7±7.5"	28.5±3.9*	64.3±6.6"
NeemAzal-T	f	0.0±0.0	30.3±4.6"	1.2±0.1	47.5±8.4"	6.5±1.1	57.2±8.8"
10%	m	0.5±0.0	35.9±5.3"	1.2±0.1	60.9±9.6"	5.1±1.4	70.9±9.8"
NeemAzal-T	f	0.0±0.0	27.3±3.5"	0.0±0.0	44.1±3.7"	0.1±0.0	71.5±9.3"
20%	m	0.0±0.0	22.8±6.4"	0.0±0.0	51.0±8.2"	0.0±0.0	75.7±8.7"
P. vulgaris	f	6.1±3.2	13.8±3.3"	31.1±11.1	35.4±6.8	49.1±9.0	53.6±9.5
	m	2.5±0.7*	13.5±3.5"	10.0±3.7*	30.8±5.9"	17.0±5.8*	51.0±7.9"
T. farfara	f	6.9±1.7	12.5± 4.9	22.1±5.7	35.6±9.6	29.0±5.9	52.5±8.9
	m	4.5±1.5	11.1±2.7"	12.0±4.6	32.1±8.7"	18.3±8.4	48.6±10.2"

* *Significant difference between males and females by Student t-test, $p<0.05$*
" *Significant difference between treated and untreated control twigs by Student t-test, $p<0.05$* (n = 35-40)

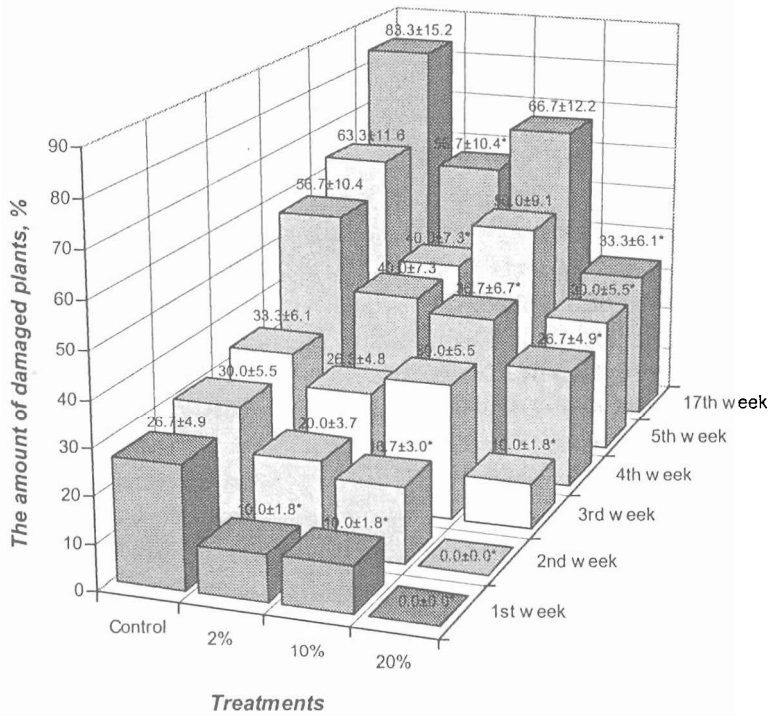

Figure 1. The percentage (%% ± standard deviation) of damaged 3-year-old spruce seedlings treated with water emulsion of NeemAzal-T in concentration 2%, 10% and 20% and untreated seedlings by *Hylobius abietis* L. on fresh clear-cut area after 1. . .5 and 17 weeks after planting.
* *Significant differences between control by Student t-test, $p<0.05$*

with 20% emulsion. During the whole season weevils damaged the treated seedlings significantly less (33.3%) than untreated control plants (83.3%). Only some slight phytotoxic influence of the 20 % NeemAzal-T emulsion on the spruce seedlings was noticed.

Discussion and conclusions

A phytophagous insect finds a host-plant by certain chemical stimuli, whereas secondary metabolites give the species specific chemistry which is of paramount importance in the selection of hosts by phytophagous insects (Bernays & Chapman, 1994). On the other hand, secondary compounds of plants are a part of the plants' defence against plant-feeding insects and other herbivores (Rosenthal & Janzen, 1979) and, therefore, they can actively influence insects. By treating host-plant with non-host compounds its smell and taste can become unacceptable for a phytophagous insect. From the point of view of ecological pest control the most promising are the plant-produced compounds which are acting as repellents, inhibitors, or antifeedants as they only disorientate insects but do not directly kill them. What kind of compounds acts as repellents or deterrents depends also on insect species (Bernays & Chapman, 1994).

P. vulgaris contains secondary compounds like glycoside phasein and linol-, palmitin-, stearin acids (Vogel, 1996). Glycosides are water soluble and probably the feeding inhibiting effect of the water extract of *P. vulgaris* on *H. abietis* was caused by phasein. The water extracts are not stable and the concentrations of influencing ingredients can change more easily. Apparently, the antifeedant effect diminished rapidly. After a longer time (48-72 h) only the males' feeding was significantly depressed (Table 1). Evidently lower concentrations can act more on males than on females.

The leaves *T. farfara* should contain secondary metabolites like glycoside-tussilagin, saponines and some organic acids (Tammeorg et al., 1972). The feeding depression expressed in males of *H. abietis* could be caused by tussilagin and saponins but apparently their concentration was too low for the significant influence of females.

It is possible to conclude that both tested plant extracts also contain feeding inhibiting ingredients for the pine weevils of *H. abietis* but probably their concentration was too low for a stronger effect and, therefore, only more sensitive sex – males – reacted on them significantly. For practical use the further study is needed for the stabilization of the extract composition.

NeemAzal-T/S and NeemAzal-T contain azadirachtin – triterpenoid, the secondary metabolite of the neem tree. In tests with weevils of *H. pales* azadirachtin had an antifeedant activity and was stronger with a higher concentration (Salom et al., 1994). In the case of *H.*

abietis the situation is the same and neem preparations seem to have an influence mainly – *via* taste as weevils first were checking also treated twigs by touching them with *palpi* and after that the untreated control twigs were preferred for feeding. Some vapours of the NeemAzal-T/S could also have influenced the behaviour of weevils as the feeding rate of specimens on control twigs was lower, in comparison with plant extracts variants. 0.05% and 0.1% azadirachtin (5% and 10% NeemAzal-T/S water emulsions, respectively) considerably depressed feeding of males even during 72 hours but was not so effective against females (Table 1). Males were also more sensitive to alteration of light conditions (Merivee et al.,1998). Specimens of both sexes were significantly affected only by 1% azadirachtin (20% of NeemAzal-T emulsion) and this antifeedant activity was maintained also in forest conditions during summer. It is possible to conclude that the NeemAzal-T in 20% of water emulsion was longer active against pine weevils than any other tested plant compounds.

Acknowledgements

They belong to the Estonian Science Foundation which has supported the present study (grants 2620 and 2684), to firm Trifolio M GmbH for sending neem preparations, to Mrs Valli Viidalepp for laboratory and field assistance and to Mrs Mall Tamm for English language help.

References

Annila E. 1982. Lindaanin käyttö männun paperkennotaimien suojaamiseksi tukkimichentäin tuhoilta. (The effect of Lindaan on control of the large pine weevil on pine paper-pot seedlings). Folia For. 512: 1-14. (in Finnish).

Bejer-Petersen B., Juutinen P., Kangas E., Bakke A., Butovitsch V., Eidmann H., Hedqvist K. J., Lekander B. 1962. Studies on *Hylobius abietis* L. development and life cycle in the Nordic countries. Acta Ent. Fenn. 18: 1-107.

Berger A. 1994. Using natural pesticides: current and future perspectives. Swedish University of Agricultural Sciences, Alnarp, 67 pp.

Bernays E. A., Chapman R. F. 1994. Host-plant selection by phytophagous insects. Chapman and Hall, New York, 141 pp.

Christiansen E., Bakke A. 1968. Temperature preference in adults of *Hylobius abietis* L. (Coleoptera: Curculionidae) during feeding and oviposition. Z. ang. Ent. 62: 83-89.

Eidmann H. H. 1964. Studien über die Entwicklung von *Hylobius abietis* L. im Freiland und in Laboratoriumszuchten. Z. ang. Ent. 54: 140-149.

Eidmann H. H. 1974. *Hylobius* Schönh. In: W. Schwenke (ed.), Die Forstschädlinge Europas. 2. Bd. Käfer. Verlag Paul Parey, Hamburg, Berlin, pp. 272-293.

Haritonova N. Z. (Харитонова Н. З.) 1965. Большой сосновый долгоносик и борьба с ним. [The pine weevil, *Hylobius abietis*, and its control]. Moscow. 88 pp. (in Russian).

Heritage S. G., Collins S., Evans H. F. 1989. A survey of damage by *Hylobius abietis* and *Hylastes* spp. in Britain. In: R. I. Alfaro and S. G. Glover (Editors), Insects affecting reforestation: biology and damage. Victoria, BC, Canada, pp. 28-33.

Hoelmer K. A., Osborne L. S., Yokomi R. K. 1990. Effects of neem extracts on beneficial insects in greenhouse culture. In: J. C. Lovke and R. H Lawson (Editors). Neem potential in pest management programs. U. S. Dep. Agric. Res. Serv. 86: 100-105.

Koul O. 1992. Neem allelochemicals and insect control. In: R. S. J. H. Rizvi and V. J. Rizvi (Editors). Allelopathy: basic and applied aspects. Chapman and Hall, London, pp. 389-412.

Långström B. 1982. Abundance and seasonal activity of adult *Hylobius*-weevils in reforestation areas during first years following felling. ,Comm. Inst. For. Fenn. 106: 1-3.

Lekander B., Eidmann H. H., Bejer B., Kangas E. 1985. Time of oviposition and its influence on the development of *Hylobius abietis* (L.) (Col., Curculionidae). Z. ang. Ent. 100: 417-421.

Lindström A., Hellqvist C., Gyldberg B., Langström B., Mattson A. 1986. Field performance of a protective collar against damage of *Hylobius abietis*. Scand. J. For. Res. 1: 3-15.

Luik A. 1997. Taimed putukate mõjutajaina. (Influence of plants on insects). Tartu, 87 pp. (in Estonian with English summary).

Luik A., Sibul I., Voolma K. 1998. Taimetõmmised männikärsaka küpsussööma mõjutajatena. (Influence of plant extracts on the maturation feeding of the large pine weevil, *Hylobius abietis* L.). Metsanduslikud Uurimused XXIX. Tartu. pp. 146-154. (in Estonian with English summary).

Luik A., Voolma K. 1989. Some aspects of the occurrence, biology and coldhardiness of *Hylobius* weevils. In: R.I. Alfaro and S.G. Glover (Editors), Insects affecting reforestation: biology and damage. Victoria, BC, Canada, pp. 28-33.

McCloskey C., Arnason J., Donskov N., Chenier R., Kaminski J., Philogene B. J. R. 1993. Third troffic level effects of azadirachtin. Can. Entomol. 125: 163-165.

Merivee E., Sibul I., Luik A. 1998. Diel dynamics of feeding and locomotor activities in the large pine weevil, *Hylobius abietis*. Baltic Forestry, 4(2): 59-62.

Nauman K., Currie R. W., Isman M. B. 1994. Evaluation of the repellent effect of a neem insecticide on foraging honey bees and other pollinators. Can. Entomol. 126: 225-230.

Naumann K., Rankin L. J., Isman M. B. 1994. Systemic action of neem seed extract on mountain pine beetle (Coleoptera: Scolytidae) in lodgepole pine. J. Econ. Entomol. 87(6): 1580-1585.

Nordlander G. 1987. A method for trapping *Hylobius abietis* (L.) with a standardized bait and its potential for forecasting seedling damage. Scand. J. For. Res. 2: 199-202.

Nordlander G., Eidmann H. H., Jacobsson U., Nordenhem H., Sjödin K. 1986. Orientation of the pine weevil *Hylobius abietis* to underground sources of host volatiles. Entomol. exp. appl. 41(1): 91-100.

Rosenthal G. A., Janzen D. H. 1979. Herbivores their interaction with secondary plant metabolites. Academic Press, New York, 234 pp.

Salom S. M., Carlson J. A., Ang B. N., Grosman D. M., Day E. R. 1994. Laboratory evaluation of biologically based compounds as antifeedants for the pales weevil, *Hylobius pales* (Herbst.) (Coleoptera: Curculionidae). J. Entomol. Sci. 29(3): 407-417.

Schmutterer H. 1990. Properties and potential of natural pesticides from neem tree, *Azadirachta indica*. Ann. Rev. Entomol. 35: 271-297.

Schmutterer H. 1992. Higher plants as sources of novel pesticides. In: Insecticides: mechanism of action and resistance. Intercept Ltd., Andover, pp. 3-15.

Solbreck C., Gyldberg B. 1979. Temporal flight pattern of the large pine weevil, *Hylobius abietis* L. (Coleoptera, Curculionidae), with special reference to the influence of weather. Z. ang. Ent. 88: 532-536.

Tammeorg J., Kook O., Vilbaste G. 1972. Eesti NSV ravimtaimed. (Medical herbs of the Estonian SSR). Valgus, Tallinn, 287 pp. (in Estonian).

Tilles D. A., Nordlander G., Nordenhem H., Eidmann H. H., Wassgren A. B., Bengström G. 1986. Increased release of host volatiles from feeding scars: a major cause of field aggregation in the pine weevil *Hylobius abietis* L. (Coleoptera; Curculionidae). Environ. Ent. 15(5): 1050-1054.

Vogel G. 1996. Handbuch des speziellen Gemüsebaues. Ulmer-Verlag, Stuttgart, 324 pp.

Received 11 October 1999

ВЛИЯНИЕ НЕКОТОРЫХ РАСТИТЕЛЬНЫХ ВЫТЯЖЕК И НЕЕМ ПРЕПАРАТОВ НА ДОПОЛНИТЕЛЬНОЕ ПИТАНИЕ БОЛЬШОГО СОСНОВОГО ДОЛГОНОСИКА, *HYLOBIUS ABIETIS* L.

А. Луйк, И. Сибул, К. Воолма

Резюме

В лабораторных экспериментах по питанию большого соснового долгоносика было установлено, что водная эмульсия NeemAzal-T/S (содержание действующего вещества азадирахтина 1%) при концентрациях 5%, 10% и NeemAzal-T (содержание действующего вещества азадирахтина 5%) при концентрациях 2%, 10% и 20% оказывают антифидантное действие в течение 24-72 часов. Высшие концентрации имели более сильное действие чем низкие, причем самцы оказались более чувствительными чем самки, прореагировав даже на самые низкие концентрации. При использовании 20%-ных водных экстрактов *Phaseolus vulgaris* L. и *Tussilago farfara* L. питательная активность у самцов подавлялась также гораздо сильнее чем у самок.

Обработка еловых сеянцев 20%-ной эмульсией NeemAzal-T в лесных условиях значительно сократила повреждения, причиняемые долгоносиком, в течение первого вегетационного периода после посадки.

Ключевые слова: *Hylobius abietis*, неем препарат, растительные вытяжки, дополнительное питание.