ANTIFEEDANT EFFECT OF NEEMAZAL-T/S ON THE LARGE PINE WEEVIL *HYLOBIUS ABIETIS* L.

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Abstract

The paper presents three laboratory experiments aimed to establish how long NeemAzal-T/S can protect the seedlings against the weevils, and if the effective lifespan of insecticide could be prolonged by mixing the emulsion with the adjuvant Nu-Film 17. Tests with mature as well as with young, immature insects, have established the efficiency of protection against the damages caused by the large pine weevil when the treatment was conducted with 5 %, 10 % and 20 % NeemAzal-T/S (having 1 % azadirachtin A), as well as with a mixture of 20 % NeemAzal and 10 % Nu-Film 17. The insect feeding has been affected by all treatments and the magnitude of effect has increased with the concentration of insecticide, but it has also been influenced by the gender and physiological state of the weevils. In mature, post oviposition insects, females have been more sensitive to insecticide than males, and the opposite was true in young, immature weevils, as well in mature insects during oviposition. The best results have been achieved with 20 % NeemAzal and 10 % Nu-Film 17 against the male young weevils. A good protection (efficacy higher than 75 %) is possible only a few days after treatment, but sufficient protection is achieved after two weeks, because most of the wounds are superficial, without severe impact on the plants. Consequently, we suppose that treating the seedlings with 20% NeemAzal (or higher concentrations) at time intervals shorter than two weeks, during the periods with high rates of feeding, will keep the damages and seedling losses at a normal level. The efficiency could be improved and the period between treatments prolonged by mixing NeemAzal with Nu-Film. Field experiments are necessary to validate our conclusions.

Keywords: antifeedant effect, *Hylobius abietis*, large pine weevil, NeemAzal-T/S, Nu-Film 17.

Rezumat

EFECTUL INSECTICIDULUI NEEMAZAL-T/S DE INHIBARE A HRĂNIRII GÂNDACILOR DE *Hylobius abietis* L.

Lucrarea prezintă trei experimente de laborator menite să stabilească cât timp durează protecţia insecticidul NeemAzal-T/S (care contine 1 % azadiractin A), prin efectul său de inhibare a hrănirii, asupra puieţilor de răşinoase împotriva atacului de *Hylobius abietis* şi dacă durata de viaţă a insecticidului ar putea fi prelungită prin utilizarea adjuvantului Nu-Film 17.

În testele efectuate cu gândaci maturi, precum şi cu gândaci tineri, în care insectele au putut să aleagă între hrana tratată şi cea netratată, s-a stabilit evoluţia eficienţei protecţiei împotriva vătămărilor cauzate de trombar când tratamentele au fost efectuate cu 5%, 10 % şi 20 % NeemAzal, precum şi cu un amestec de 20 % NeemAzal şi 10 % Nu-Film 17. Hrănirea gândacilor a fost afectată de toate tratamentele şi amploarea efectului a crescut o dată cu creşterea concentraţiei insecticidului. Sexul şi starea fiziologică a gândacilor au influenţat de asemenea eficienţa tratamentelor. În cazul gândacilor maturi, aflaţi în perioada de după ovipoziţie, femelele au fost mai sensibile la insecticid decât masculii, dar în cazul gândacilor tineri, precum şi a celor maturi aflaţi în perioada de ovipozitie, masculii au fost mai sensibili decât femelele. Efectul cel mai intens de inhibare a hrănirii s-a obţinut cu amestecul de 20 % NeemAzal şi 10 % Nu-Film 17 în cazul gândacilor tineri. O protecţie bună (eficienţă mai mare de 75 %) este posibilă doar câteva zile după tratament, însă putem aprecia drept suficientă protecţia asigurată pe durata a două săptămâni, întrucât majoritatea roaderilor sunt superficiale, fără un impact grav asupra plantelor. În consecinţă, e de presupus că vătămările şi pierderile de puieţi ar putea fi ţinute la un nivel normal (acceptabil) prin tratarea repetată a puieţilor, în perioadele de hrănire intensă a gândacilor, cu emulsie de NeemAzal în concentraţie de 20 % sau mai mult la interval de maximum două săptămâni. Eficienţa ar putea fi ameliorată şi perioada dintre dintre tratamente prelungită prin utilizarea adjuvantului testat, Nu-Film 17. Pentru validarea acestor concluzii sunt însă necesare experimente în condiţii de teren.

Cuvinte cheie: efect de inhibare a hrănirii, *Hylobius abietis*, NeemAzal-T/S, Nu-Film 17, trombarul puieţilor de molid

1. INTRODUCTION

In Romania, like in many other countries, *Hylobius abietis* is the main pest of coniferous plantations installed shortly after wood harvesting. Without control measures it could damage 50-80 % of the seedlings and sometimes the cultures have been totally compromised. In order to avoid the damages, we have elaborated a methodology for the quantification of attack risk and recommended the differentiation of protective measures according to the risk degree (Olenici & Olenici, 2003). In the most cases with a high risk of attack, a postponement of planting for 2 years after clear cutting is accepted, but during the 3rd or the 4th season (according to the length of generation development) the risk is still high enough to require other protection measures. One of them is to catch the weevils using traps baited with alpha-pinene and ethanol (Olenici & Olenici, 2004), a procedure that is already largely used in Poland (Stocki, 2000), but the young weevils emerging in July-September are not attracted to this kind of stimuli (Nordenhem & Eidmann, 1991; Malphettes et al., 1994; Zumr et al., 1995; Olenici & Olenici, 2002a). For such situations, spraying of seedlings with synthetic pyretroids like deltamethrin, cypermethrin etc. is usually the only possible protective measure. The pyretroids are also used to treat the seedlings before planting. Due to the short persistence of this kind of insecticide, we have tried to improve the efficiency of the treatment by combining the insecticides with the adjuvant Nu-Film 17 (Olenici & Olenici, 2002b), but in a few years such insecticides will no longer be allowed for use in Europe. Therefore, starting from the results published by Luik (2000) and Thacker & Bryan (2003a), in 2004 we began the laboratory experiments aimed to study the possibilities of NeemAzal-T/S employment in the protection of coniferous seedlings against the large pine weevil. This product proved to be efficient against many insect species (Anonymous, 2004) and is accepted in European Union for organic farming (Anonymous 2006).

Using 5 % and 10 % water emulsions, Luik (2000) pointed out a significant antifeedant effect, especially in mature males, during 72 hours after treatment. However, the published data denote a clear tendency of efficiency decreasing, although the observation period was very short compared to the feeding period of the insects in the field. On the other hand, Thacker & Bryan (2003) used undiluted neem extract and obtained very good results for the three-week period of observation, but the temperature was quite low (10-15oC), influencing negatively the weevil feeding and favourably the lifespan of insecticide. In addition, the use of undiluted extract could be too expensive and possibly toxic for plants, especially if the treatment is applied by dipping the seedlings into insecticide or by spraying. Therefore, the aims of our first experiments were to establish how long lasts the antifeedant effect of NeemAzal-T/S on the large pine weevil, and if the effective lifespan of insecticide could be prolonged by mixing the emulsion with the adjuvant Nu-Film 17.

2. MATERIALS AND METHODS

We have conducted three laboratory experiments with NeemAzal-T/S (1 % azadirachtin A) from Trifolio-M Gmbh (Table 1). In the first experiment we have used basal segments (8 cm long, without needles) of 4 year-old Norway spruce seedlings, but in the other two experiments twigs (6 cm long) of Scots pine from only one tree have been used. All the pieces have been selected to have no wounds, no exfoliation of the outer layer and about the same diameter (Table 2), in order to avoid any unwanted influence from them to weevil feeding. Cutting of segments took place shortly after the harvesting of twigs and their ends have been dipped into melted wax to prevent the dessication as well as to avoid a very high release rate of terpenes from the bark and wood sections. After that, the twig segments have been treated with water emulsion of NeemAzal-T/S or with water (control segments), by dipping them in liquid for de 10 seconds. The twigs remained 4-5 hours in vertical position to dry before placing them in glass jars with weevils.

During the observation period, the twigs remained also in vertical position, on a moistened layer of peat and sand in glass jars of 300-400 ml capacity. A treated segment (respectively two treated segments, in experiment III) and an untreated segment were placed in each jar, about 4 cm apart. In the experiments I and II there were two control series (one for each treatment), but in the experiment III only one.

One weevil was confined in each jar. In experiments I and II, we used mature weevils that had been collected from the field in June and supplied with fresh Scots pine twigs until the beginning of the experiments. For the 3rd experiment, we used young, imature weevils, obtained from Norway spruce trap billets. Each weevil was used in only one experiment.

The jars were held in laboratory, at room temperature (about 18-22oC), in natural light regime. At the top, they have been covered with plastic fiber mesh, allowing a free air movement. The area of bark removed from the twigs was measured periodically. Each time the areas of outer and inner bark (phloem) removed were quantified separately.

The mean area of removed bark was calculated for each observation time and the significance of differences was controlled by Student-t test or by Bonferroni test, depending on the distribution normality that was checked by Shapiro-Wilk test.

The insecticide effect on insect feeding was expresed not only through the treatment efficiency (E), as usualy, but also through the antifeedant index (AFI), used in similar experiments by Klebzig and Schlyter (1999) and by other authors. We used the following relations: E (%) = 100 (C-T)/C and AFI = (C-T)/(C+T), where: C - area of removed bark on control twigs and T - area of removed bark on treated twigs. According to Klebzig and Schlyter (1999), AFI = - 1 is indicative of the best possible feeding stimulant, AFI = 0 means no effect and AFI = + 1 is indicative of the best possible antifeedant

Table 1. Detailes concerning the laboratory experiments with NeemAzal - T/S

Detalii de la experimetele de laborator cu NeemAzal - T/S

Experiment	Period of observation	Weevils	Vegetal material	Emulsion concentrations and number of replications
I	14-26.07 2004	mature males	stem segments of spruce seedlings	5 % and 10 % NeemAzal; 15 replications
II	3.08-7.09 2004	mature males and females	twig segments of Scots pine	10 % and 20 % NeemAzal; 15 replications
III	16-25.09 2004	imature males and females	twig segments of Scots pine	20 % NeemAzal and 20% NeemAzal + 10% Nu-Film; 20 replications

Table 2. Mean diameter of twig segments used in the experiments

Diamentrul ramurilor folosite la experimente

Experiment	Treatment	Mean ± standard deviation (mm) in ...			
		male weevils		female weevils	
		treated twigs	untreated twigs	treated twigs	untreated twigs
I	NeemAzal 5 %	6.7 ± 1.6[a]	6.7 ± 1.6[a]	-	-
	NeemAzal 10 %	6.5 ± 1.4[a]	6.5 ± 1.5[a]	-	-
II	NeemAzal 10 %	7.8 ± 1.2[a]	8.0 ± 1.1[a]	7.6 ± 1.2[a]	7.3 ± 1.1[a]
	NeemAzal 20 %	7.8 ± 1.1[a]	7.7 ± 0.7[a]	7.9 ± 1.4[a]	7.8 ± 1.0[a]
III	NeemAzal 20 %	9.1 ± 0.8[a]	8.8 ± 1.1[a]	9.9 ± 0.8[a]	9.3 ± 1.2[a]
	NeemAzal 20 % + Nu-Film 10 %	8.6 ± 1.1[a]	8.8 ± 1.1[a]	9.6 ± 0.8[a]	9.3 ± 1.2[a]

Notation: For each treatment, apart for male and female weevils respectively, means bearing the same superscript within each line are not significantly different at P = 0.05.

3. RESULTS

In the experiment I, 5 days after treatment application, the total area of bark removed by the weevils on stem segments treated with 5 % NeemAzal was 41,5 % smaller than that eaten on the control segments, whereas on those treated with 10 % NeemAzal the area was 31,2 % smaller than that consumed on the control pairs (Table 3). However, the reduction of the deep wounds (with bark and phloem removed) area was by 69 % and 28.2 %, in the case of the treatment with 10 % and 5 % NeemAzal, respectively.

Seven days later, the differences between the mean area of bark removed from the treated segments and their pair control decreased to 8,6 % for 5% NeemAzal, and 15 % for 10% NeemAzal. Apparently, it means that weevils caused about the same damages irrespective of the treatment, but - after 12 days from the beginning of the experiment - the average area of deep wounds was 38,5 % and 46,7 % respectively smaller than that on control segments. The differences between the means were not statistically significant, because of very high variability of the data. The values of antifeedant index were small and decreased very much between the two observation moments, suggesting that the insecticide had no or very litile effect after the first 5 days.

Data from the second experiment (Table 4) denote that the weevils gnawed less bark and phloem on twigs treated with 20% NeemAzal and their control pairs, than on twigs with 10 % NeemAzal and corresponding controls, regardless of the weevil's gender and the observation time. It means that the higher concentration (20 %) was more efficient than the lower one (10 %), but the differences between the treatments vanished gradually in one month.

Table 3: Bark area removed by the weevils in the experiment I (mean ± standard deviation, mm^2) and the mean antifeedant index

Suprafețe de scoarță consumată de gândaci în experimentul I (media +/− abaterea standard, mm^2) şi indicele inhibitor mediu

Treat-ment	19.07.2004				26.07.2004			
	Type of wound			Mean AFI	Type of wound			Mean AFI
	shallow	deep	total		shallow	deep	total	
5 %	7.7 ± 14.3[a]	8.9 ± 22.8[a]	16.5 ± 34.1[a]	0.26	50.8 ± 78.4[a]	7.2 ± 16.5[a]	57.5 ± 89.0[a]	0.04
Control for 5 %	15.8 ± 19,5[a]	12.4 ± 22.7[a]	28.2 ± 23.9[a]		51.3 ± 65,1[a]	11.7 ± 15.2[a]	62.9 ± 66.9[a]	
10 %	12.1 ± 20.2[a]	2.7 ± 4.4[a]	14.8 ± 19.7[a]	0.18	43.7 ± 45.4[a]	5.6 ± 7.5[a]	49.3 ± 44.3[a]	0.08
Control for 10 %	12.9 ± 12.7[a]	8.7 ± 18.8[a]	21.5 ± 26.2[a]		47.4 ± 40.3[a]	10.5 ± 21.5[a]	58.0 ± 45.5[a]	

Notation: Apart for each choice combinatation, means bearing the same superscript within each column are not significantly different at P = 0.05.

On the other hand, one can see that when considering the total area of removed bark (Table 5), the treatment was more effective against the female weevils, except the first 5 days. The males gnawed on both kinds of twigs, either treated or untreated, at least the same quantity of bark as females had knawed, or more. In addition, during the first 5 days, they caused preponderantly deep wounds on treated twings, and shallow wounds on control ones. At the end of the experiment, the shallow wounds represented more than 50 % of removed bark area on both treated and untreated twigs. By contrast, the females caused mainly deep wounds on twigs treated with 10 % NeemAzal and on their control pairs, but shallow wounds on the counterparts with 20 % NeemAzal.

Table 4. Bark area removed by the weevils in the experiment II (mean ± standard deviation, mm^2)

Suprafețe de scoarță consumată de gândaci în experimentul II (media +/− abaterea standard, mm^2) şi indicele inhibitor mediu

Datum	Weevils	Type of wound	Treatment			
			10 % NeemAzal	Control for 10% NeemAzal	20% NeemAzal	Control for 20% NeemAzal
5.08	males	shallow	6.6 ± 9.3^{a}	31.0 ± 37.4^{b}	5.3 ± 11.6^{a}	23.0 ± 30.2^{a}
		deep	15.4 ± 18.7^{a}	13.3 ± 13.7^{a}	10.0 ± 13.3^{a}	15.2 ± 12.9^{a}
		total	**22.0 ± 17.2^{aA}**	**43.6 ± 37.3^{bA}**	**15.3 ± 21.1^{aA}**	**38.2 ± 31.6^{bA}**
	females	shallow	6.2 ± 19.0^{a}	15.4 ± 19.1^{a}	7.7 ± 11.7^{a}	7.2 ± 7.9^{a}
		deep	24.2 ± 27.5^{a}	25.9 ± 19.4^{a}	5.0 ± 6.9^{a}	13.6 ± 15.6^{a}
		total	**30.3 ± 31.4^{aA}**	**41.2 ± 25.4^{aA}**	**12.7 ± 14.2^{aA}**	**20.8 ± 15.4^{aA}**
8.08	males	shallow	16.6 ± 14.3^{a}	52.0 ± 54.9^{b}	12.5 ± 18.0^{a}	43.6 ± 62.9^{a}
		deep	28.0 ± 31.0^{a}	21.4 ± 21.2^{a}	20.0 ± 19.2^{a}	35.7 ± 35.1^{a}
		total	**44.5 ± 31.4^{aA}**	**73.4 ± 54.0^{aA}**	**32.6 ± 31.4^{aA}**	**79.3 ± 94.4^{aA}**
	females	shallow	12.5 ± 38.1^{a}	23.7 ± 25.2^{a}	15.0 ± 20.9^{a}	17.8 ± 16.6^{a}
		deep	34.9 ± 42.5^{a}	31.2 ± 20.2^{a}	10.7 ± 9.7^{a}	18.4 ± 19.6^{a}
		total	**47.4 ± 54.2^{aA}**	**54.9 ± 33.5^{aA}**	**25.7 ± 22.8^{aA}**	**36.2 ± 30.1^{aA}**
7.09	males	shallow	110.1 ± 56.1^{a}	218.3 ± 145.7^{b}	102.1 ± 112.3^{a}	159.6 ± 103.3^{a}
		deep	93.1 ± 66.3^{a}	136.1 ± 74.7^{a}	96.1 ± 58.3^{a}	111.1 ± 79.9^{a}
		total	**203.2 ± 48.1^{aA}**	**354.4 ± 162.1^{bA}**	**198.2 ± 124.4^{aA}**	**270.6 ± 159.5^{aA}**
	females	shallow	69.3 ± 94.6^{a}	109.8 ± 85.8^{a}	62.3 ± 60.8^{a}	117.1 ± 142.2^{a}
		deep	74.8 ± 51.4^{a}	133.3 ± 136.3^{a}	41.6 ± 30.5^{a}	60.9 ± 33.0^{a}
		total	**144.1 ± 113.4^{aA}**	**243.1 ± 137.6^{bA}**	**104.0 ± 63.1^{aB}**	**178.0 ± 141.2^{aA}**

Notations: 1) Separate for each concentration, the means bearing the same small superscript within each row are not significantly different P = 0.05. 2) Separate for each datum, the means bearing the same capital superscript within each column are not significantly different at P = 0.05.

Table 5. The efficiency (%) of treatments in the second experiment (expressed as reduction of total damage area and of deep wounds area, respectively) and the mean antifeedant index

Eficiența (%) tratamentelor din al doilea experiment (exprimat ca reducere a suprafeței totale vătămate, respectiv a adâncimii) şi indicele inhibitor mediu

Treatment	Datum	Reduction of ...				Average antifeedant index	
		total removed bark area		deep wounds area			
		Males	Females	Males	Females	Males	Females
10 % NeemAzal	5.08	49.5	26.5	-15.8	6.6	0.33	0.15
	8.08	39.4	13.7	-30.8	-11.9	0.25	0.07
	7.09	42.7	40.7	31.6	43.9	0.27	0.26
20 % NeemAzal	5.08	59.9	38.9	34.2	63.2	0.43	0.24
	8.08	58.9	29.0	44.0	41.8	0.42	0.17
	7.09	26.8	41.6	13.5	31.7	0.15	0.26

The efficiency of the treatments and antifeedant index were quite low even 2-5 days after the application of insecticide (Table 5) and - in most cases - the differences between the average values were not statistically significant because of very high variability of the data.

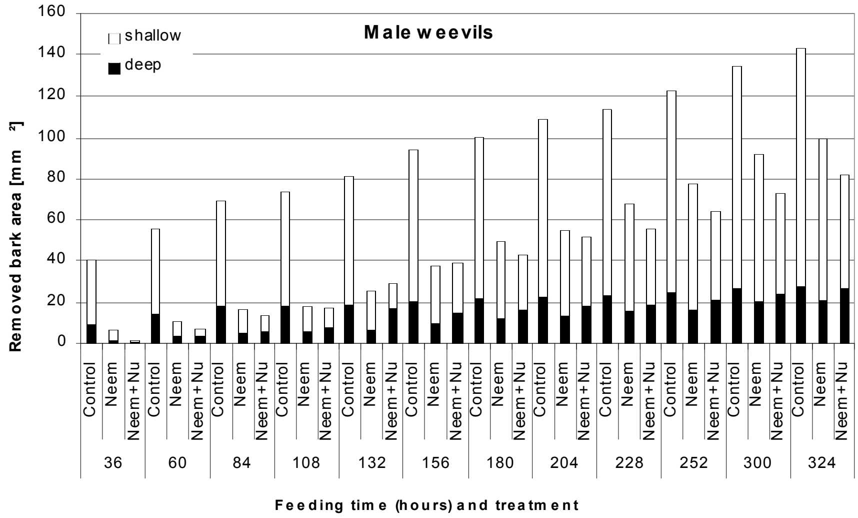
Data from the 3rd experiment (Table 6 and fig. 1-2), when we used young weevils and a concentration of 20 % NeemAzal, revealed that treatment efficiency was dependent on the weevil's sex, the males being more affected than females. A quite high efficiency (more than 75 %) was achieved only during the first 3 days for females, and 5 days for males. After that, the weevils consumed about the same quantity of bark and phloem on both treated and untreated twigs, so that two weeks after the treatment the female weevils damaged equally the treated and untreated twings. The adjuvant Nu-Film 17 increased the efficiency of treatment and this was more obvious with increasing the time elapsed from the beginning of treatment. Like in the other experiments, the shallow wounds were prevailing, especially in control twigs.

Table 6: Bark area removed by the weevils in the experiment III (mean ± standard deviation, mm^2)

Suprafața de scoarță consumată de gândaci în experimentul III (media ± abaterea standard, mm^2)

Feeding time (hours)	Weevils	Treatment		
		20% NeemAzal	20% NeemAzal + 10% Nu-Film	Control
36	males	6.7^{a} ± 11.7^{aA}	1.8 ± 5.7^{aA}	36.2 ± 31.7^{bA}
	females	11.4 ± 20.3^{aA}	8.4 ± 18.9^{aA}	44.3 ± 34.2^{bA}
60	males	11.4 ± 17.9^{aA}	9.2 ± 11.6^{aA}	54.5 ± 42.6^{bA}
	females	15.5 ± 25.1^{aA}	11.1 ± 21.9^{aA}	64.7 ± 40.7^{bA}
84	males	16.6± 18.5^{aA}	13.6 ± 15.7^{aA}	69.0 ± 50.8^{bA}
	females	23.3 ± 33.4^{aA}	17.1 ± 26.6^{aA}	72.5 ± 44.7^{bA}
108	males	18.3 ± 19.5^{aA}	17.5 ± 18.9^{aA}	73.4± 52.3^{bA}
	females	32.3 ± 44.8^{aA}	23.7 ± 30.6^{aA}	82.0± 49.1^{bA}
132	males	25.6 ± 25.2^{aA}	28.9 ± 32.4^{aA}	81.2 ± 58.7^{bA}
	females	43.3 ± 64.9^{aA}	34.3 ± 39.3^{aA}	92.9± 58.7^{bA}
156	males	37.4 ± 29.2^{aA}	38.6 ± 40.1^{aA}	93.8 ± 62.1^{bA}
	females	55.7 ± 71.6^{aA}	42.1 ± 45.8^{aA}	100.7 ± 63.5^{bA}
180	males	49.5 ± 32.5^{aA}	42.6 ± 40.6^{aA}	100.5± 66.6^{bA}
	females	66.1 ± 82.6^{aA}	56.9 ± 53.8^{aA}	108.3 ± 64.9^{bA}
204	males	55.7 ± 34.6^{aA}	51.2 ± 40.5^{aA}	108.7 ± 69.9^{bA}
	females	85.1 ± 93.1^{aA}	65.7 ± 59.6^{aA}	112.7 ± 66.9^{aA}
228	males	67.6 ± 70.5^{aA}	56.1 ± 44.9^{aA}	113.9± 70.5^{bA}
	females	98.0 ± 95.2^{aA}	77.1 ± 58.6^{aA}	117.7 ± 70.4^{aA}
252	males	77.5 ± 48.1^{aA}	64.1 ± 46.1^{aA}	123.0 ± 76.2^{bA}
	females	105.9 ± 98.8^{aA}	89.3 ± 61.0^{aA}	123.5 ± 70.6^{aA}
300	males	91.4 ± 56.0^{aA}	72.9 ± 51.0^{abA}	134.3 ± 85.7^{bA}
	females	125.7 ± 102.1^{aA}	115.6 ± 71.9^{aA}	132.7 ± 73.3^{aA}
324	males	99.1 ± 59.0^{aA}	81.4 ± 53.5^{aA}	142.5 ± 89.2^{bA}
	females	144.8 ± 110.9^{aA}	122.4 ± 77.1^{aA}	143.4 ± 69.1^{aA}

Notation: 1) Means bearing the same small superscript within each row are not significantly different at P = 0.05. 2) Separate for each feeding time, the means bearing the same capital superscript within each column are not significantly different at P= 0.05.



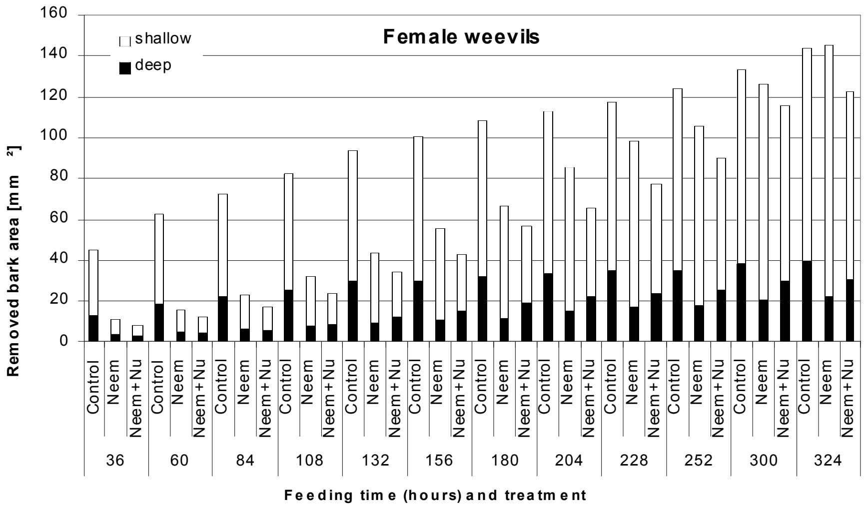


Fig. 1. Weevil feeding dynamics in the 3rd experiment, showing the differences between males (top) and females (bottom) as well as the extent of deep and shallow unds.

Dinamica hrănirii gândacilor în cel de-al treilea experiment, prezentând diferențele dintre masculi (graficul de sus) şi femele (graficul de jos), precum şi amplasarea roaderilor pro funde şi superficiale

4. DISCUSSION

The laboratory experiments usually hardly simplify the condition existing in the field. Therefore, good results from laboratory could not assure similar results in the field. This is the reason why we have tried to simulate in laboratory experiments the field conditions. However, there lacked the rain and the strong solar radiation from direct light, factors that could have influence on the insecticide decomposition and on the treatment efficacy.

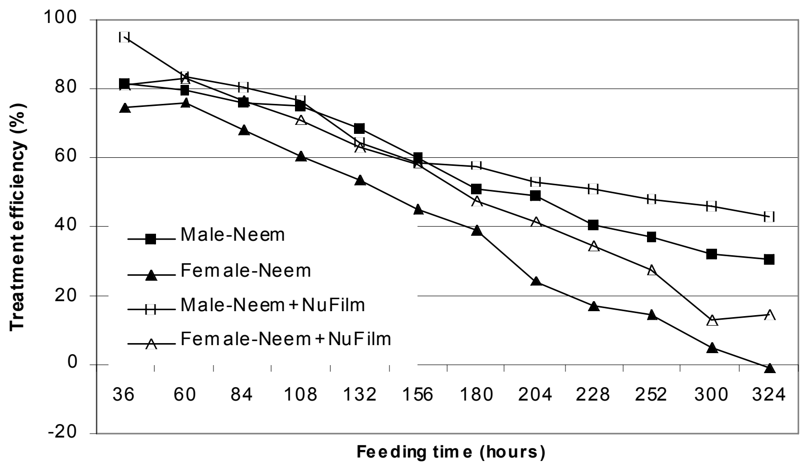


Fig.2 The evolution of treatment efficiency in young beetles when used 20 % NeemAzal

Evoluția eficienței tratamentului în cazul gândacilor tineri şi al utilizării a 20% NeemAzal

Under described conditions, that could be considered as optimum for feeding (Christiansen & Bakke, 1968; Havukkala & Selander, 1976), we tested the protection efficiency against the weevil damages starting with low concentrations of NeemAzal (5 % and 10 %, respectively), but also with male weevils, that proved to be more sensitive than females (Luik, 2000). Both treatments decreased the attack and the effect was stronger in higher concentration, confirming the results of Luik (2000), but the efficacy was quite low and short lasting, comparing with the very long feeding period of Hylobius abietis. In addition, the data were highly variable. This observation is valid for all of our experiments and could be a result of using unstarved insects and moistened substratum, the combination of starvation and thirst contributing to reduction of relative variance regarding the feeding (Schlyter et al., 2004). The variability from the 1st experiment could be also due to variable attraction of seedlings for weevils, but such a thing is normal in field condition.

The results from the second experiment confirmed that the increase of insecticide concentration from 10 % to 20 % leads to increase in antifeedant effect and treatment efficacy, but they still remained quite low, even in the first days after treatment. However, the effect was detectable after one month. It seems that the males were more sensitive than females, but only during the first 5 days and only if we take into consideration the total area of bark removed by weevils. If we consider the deep wounds, it is obvious that females were more strongly affected than males. In addition, they gnawed less bark and phloem than males. The fact is contrasting with the results already published (Luik, 2000), as well as with our results from the 3rd experiment, and suggests that the reaction of weevils to azadirachtin depends not only on the gender, but also on the physiological state of weevils. The second experiment took place in August, with beetles collected from the field in June and maintained in environmental condition. Because the egg production decreases in August (Lekander et al., 1985), it means that - at the beginning of the experiment - the females were towards the end of oviposition period, and during the most part of the experiment were in post-oviposition period, and this could explain the low feeding intensity, because after the ceasing of oviposition, the feeding rate of the females decline to the same level as in the males (Bylund et al., 2004). On the other hand, the weevils used in the 3rd experiment were immature, and we can infer that also during the maturation feeding, before the oviposition, the females consume more food than males.

A similar difference between male and female weevils, concerning their sensitivity to insecticide during the maturation feeding, was reported by Eidmann and Novák (1970) when tested the sensitivity of Hylobius abietis to DDT. They supposed that the higher sensitivity of males reflects the differences between the sexes concerning insect size and weight, males beeing smaller and lighter.

The average rate of feeding was 8,9-10 mm2 Norway spruce bark per weevil per day in the first experiment (only mature males), 8,3-16,4 mm2 Scots pine bark in the second one (mature males and females), and 23,9-30,4 mm2 Scots pine bark in the 3rd experiment. These results show that young weevils have a higher rate of feeding and consequently the damages caused by this category of insects could be very important in a shorter time than in cases when population is represented predominantly by mature post oviposition insects, if the insecticide protective effect disappeared.

The addition of Nu-Film in insecticide emulsion increased the efficiency of the treatment, but it is difficult to say that the effect is due to a longer lifespan of insecticide or due to physical characteristics and high concentration of the adjuvant. Other tests, including lower concentration of Nu-Film, should be conducted to answer this question.

In our experiments, the shallow wounds had a very high proportion, especially on control twigs. On treated twigs, shallow wounds became prevalent only gradually. It seems that insects avoided to gnaw superficially on treated twigs so long as their surface had high quantity of azadirachtin. It is something opposite to the results reported by MÍnsson & Schlyter (2004), who found large superficial damages in several species that probably possess antifeedant substances but in the inner bark.

Because we had no no-choice situations, it is impossible to say how much the feeding behavior of weevils was altered by the presence of insecticide in nearness of untreated twigs, but it can be seen that, at the end of the observation periods, the average area of deep wounding was quite small on both treated and untreated twigs, and such damages are normally not dangerous for 3-4 year old seedlings. It could mean that treating the seedlings with 20% NeemAzal (or higher concentrations), possibly mixed with Nu-Film 17, every two weeks during the periods with high rates of feeding (depending on the age of clear cutting area), the damages and seedling losses could be kept at a normal level. However, in the field conditions we expect a more rapid degradation of insecticide due to the light and moisture, factors which accelerate azadirachtin decomposition (Kleeberg, 2001; Rao, 2001).

Consequently, economical studies should be conducted to establish if the repeated treatments with or without Nu-Film 17 are acceptable or too expensive, because the number of treatments could rise to 8-10 in areas with very high populations, especially during rainy seasons. So far, good results have been obtained in field tests only with high concentrations or undiluted neem-based products that had much higher content of azadirachtin than NeemAzal-T/S. Thus Metspalu et al. (2003) used 20 % NeemAzal T (with 5 % azadirachtin) and Thaker et al. (2003b) tested undiluted neem oil with 30 % azadirachtin.

5. CONCLUSIONS

Water emulsion of NeemAzal-T/S in concentration of 5-20 % affected the feeding activity of the large pine weevil. The magnitude of the antifeedant effect was dependent on concentration, as well as on the gender and physiological state of the weevils. High efficiency was achieved only during a few days after treatment, but after 2-4 weeks the deep wounds were still small. It seems that the efficacy could be increased mixing the insecticide with Nu-Film. However, in order to achieve an adequate protection of the seedlings, water emulsion of 20 % or more NeemAzal-T/S should be used at intervals no longer than two weeks during the periods with high rates of weevil feeding.

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