

EFFICACY OF CONIFER SEEDLING PROTECTION AGAINST PINE WEEVIL DAMAGE USING NEONICOTINOIDS AND METAFLUMIZONE INSECTICIDES

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Abstract: *In a laboratory experiment, we studied how effective three neonicotinoids (acetamiprid, imidacloprid, thiacloprid) and metaflumizone are in preventing the pine weevil (*Hylobius abietis*) damage and what the effects of insecticides on insects. None of the tested insecticides could prevent the damage and 30-86.7% of twigs suffered deep wounds before all weevils died in about three weeks. However, the mean area of deep wounds was greatly reduced compared with that in the control treatment, representing only 0.4% - in thiacloprid, 0.6% - in acetamiprid, 2.4% - in imidacloprid and 6.9% - in metaflumizone treatments. The tested neonicotinoids might be sufficiently effective in the field conditions at a lower population density.*

Key words: *Hylobius abietis, chemical seedling protection, nicotinoids, metaflumizone, insect mortality.*

1. Introduction

Hylobius abietis (Linnaeus 1758) is the most important pest of conifer plantations in Europe, causing major damage especially where forest regeneration is mainly based on clear cuttings immediately followed by planting [9]. Therefore, over the time many methods and ways to protect the seedlings against the damage caused by adults of this species have been tried [4]. Considerable progress has been made with the use of physical barriers to protect seedlings [16], [12], applying various silvicultural measures such as soil scarification or other methods of soil

preparation [13], [14], [15], [18], [10] or biological control [1], [2], [3], [6], [19]. However, the treatment of seedlings with insecticides in the nursery, before planting, or even in the forest land is still the main strategy to prevent the attack in most European countries [9], but many pyrethroid insecticides are banned or severely restricted by European Union regulations or by forests' certification. Consequently new insecticides are sought for seedling protection against injury caused by the weevils.

The research presented in this paper had two objectives: to establish the efficiency of seedling protection against *Hylobius*

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abietis damage by dipping into emulsion of insecticides, and to learn the effects these insecticides have on insects.

2. Material and Methods

Weevil collection. During the period 17th-22nd July 2013, using 20 pitfall traps baited with alpha-pinene, ethanol and fresh bark of Norway spruce, we collected 803 living weevils from a clear cutting area exploited in April 2012 in the forest district Pojorâta, in Suceava county (47° 25'20"N, 25°23'31"E). For two days they were fed with fresh Scots pine twigs and fresh spruce bark. On the third day, on July 24, they were randomly allocated into experimental treatments. Those who had to be subjected to starvation, were set aside only on the fourth day, on July 25.

Twig treatment. Instead of coniferous seedlings or saplings, we used in this experiment segments of freshly picked Scots pine twigs. They were chosen to have similar dimensional characteristics (mean length \pm standard error: from 13.8 \pm 0.2 mm to 14.3 \pm 0.2 mm, $df = 4$, Chi-square = 3.231, $p = 0.520$, Kruskal-Wallis

test; mean diameter \pm standard error: from 9.0 \pm 0.3 mm to 9.4 \pm 0.3 mm, $df = 4$, $F = 0.178$, $p = 0.949$ one-way ANOVA test), to avoid any influence on the results. In order to maintain the freshness of the twig segments throughout the experiment, their ends were treated with paraffin immediately after cutting.

The insecticides were applied by completely submerging the twig segments into insecticide emulsion. Thereafter, they were removed and allowed to be dried before "planting" in the peat bed within the jars.

Experiment design. A laboratory experiment was conducted to test four different insecticide active ingredients (Table 1), three of them belonging to the chemical group of the neonicotinoids (acetamiprid, imidacloprid, thiacloprid) and the fourth was metaflumizone.

Apart from the four mentioned treatments, a control treatment (untreated food) was used to serve as a reference for the assessment of the effectiveness of insecticides we tested. Consequently, it was an experiment with 5 treatments, each repeated 5 times.

Brief characterization of the four insecticides tested in laboratory

Table 1

Treatment	Trade name	Active ingredient	Toxicity class	Action by	Tested concentration*
T1	Mospilan 20 SP	acetamiprid 200 g/kg	III	contact and ingestion	0.2% a.i.
T2	Nuprid 200 SC	imidacloprid 200 g/l	IV	contact and ingestion	0.2% a.i.
T3	Alverde 240 SC	metaflumizone 240 g/l	IV	mainly ingestion	0.24% a.i.
T4	Calypso 480 SC	thiacloprid 480 g/l	III	contact and ingestion	0.48% a.i.
T5 - control	-	-	-	-	-

Note: *) weight/volume - in V1 and the volume/volume - in V2-V4; a.i. – active ingredient

Each replication was constituted of 6 segments of pine twigs placed in a plastic jar of 2 liters capacity.

The twigs were “planted” in a peat layer 4 cm thick, which served as a shelter for weevils during the day, too. The peat was periodically moistened to ensure optimum living conditions for *Hylobius abietis* adults.

In each of the 25 jars (5 treatments x 5 replications), 20 beetles were placed, so as to simulate the conditions of a very high infestations (a plantation being considered heavily infested - under normal site conditions - at a pest density higher than 0.5 weevils/seedling). Consequently, there were 100 weevils/treatment, enough to obtain adequate data regarding the dynamics of weevil mortality as a result of their coming into contact with insecticides and their ingestion, as well.

To compare the dynamics of weevil mortality caused by insecticides with that of mortality from starvation, other 70 beetles were kept in two jars with damp peat, but without food.

Data collection. After the start of the experiment, periodically (at most 2-3 days interval) we checked how many weevils were still alive, as well as the bites they made on the pine twigs. At each inspection, the size of bites was measured and deep bites, which reached the wood, were recorded separately from shallow bites, which did not yet reach the cambium. The bite inspections were stopped on August 17, when only three weevils in insecticide treatments were alive, and those unable to feed.

The survival of starved weevils was monitored until August 23, when the last beetle died. A weevil was considered dead when it gave no signs of life to poking of its antennae and ventral side of abdomen. During this operation, the weevils were carefully observed by stereomicroscope.

Temperature and relative humidity recording. Because the feeding of pine weevil depends on the temperature and the air relative moisture, these parameters were recorded into laboratory every 15 minutes during the experiment using a HOBO® Pro v2 data logger. Recorded data showed that daily mean temperature varied between 22.5°C and 25.9°C and air relative humidity varied between 54.2% and 62.6%. It can be said that it was an optimum temperature for beetles’ feeding, but a suboptimal air relative humidity.

Data analysis. For assessment of insecticide efficacy we took into account both the incidence of attack (percentage of twigs with bites caused by the weevils) and its intensity (mean size of the deep bites). All data were analyzed by using the XLSTAT program (Addinosoft), which works under Microsoft (TM) Excel. The normality of distributions was tested using the Shapiro-Wilks test and the homogeneity of variance with Levene's test. When the assumptions of normality and homogeneity of variance were met (diameter of twig segments), the ANOVA test followed by the Tukey (HSD) test was used to compare the five independent samples. When the assumptions were not met (length of twig segments and size of bites), the non-parametric test Kruskal-Wallis was used, followed by the Steel-Dwass test. To compare the proportions of attacked twig segments in the five treatments, we used the Chi-square test followed by the Marascuilo procedure that enables to simultaneously test the differences of all pairs of proportions.

3. Results and Discussions

3.1. Treatment efficacy

Significant differences between treatments, both in terms of frequency (Figure 1 and Table 2) and the intensity of the

attack (Table 3) have been noted just two days after the start of the experiment. Given the very high experimental population density, in the first two days over 60% of twig segments suffered injuries, most of which, however, were superficial, especially in the treatments T1 and T4.

This rapid differentiation of treatments is due to the specific mode of action the tested insecticides have (Table 1). Those acting by contact were more effective in keeping the weevils away in the first two days, while Alverde 240 SC, mainly acting by ingestion, had only a very low effect in diminishing the attack frequency, but reduced the size of deep bites by 50%, in comparison with the control treatment.

Because the tested insecticides do not kill insects immediately, the frequency of attack increased for another 3-11 days or longer, depending on the insecticide.

Consequently, after 24 days, when the bite inspections were stopped, the frequency of twig segments with deep galls exceeded 30% in all tested treatments, reaching 83-86% in T2 and T3, which do not differ significantly - from this point of view - from the control treatment. If the

treatment efficiency is evaluated by the reduction of the attack frequency, the most effective was the T4 treatment (20% for all bites and 70% for deep bites) and the least effective was T3 (3.3% and 13.3%, respectively).

Regarding the intensity of the attack, expressed by the mean size of the deep bites, it can be noted that after two days since the experiment initiation there was a significant increase only in the control treatment (T5) and in T2. Between July 26 and August 17 the average bite size in T5 and T2 increased 7.4 times and 2.8 times, respectively. In T2 this happened simultaneously with an important increase of attack frequency (from 80% to 96.7% for all bites and from 40% to 83.3% for deep bites) suggesting that insects did not completely paralyze after first contact with the insecticide and were able to feed.

The insects in T1 and T4 also continued to feed after the first two days, but their deep bites were dwindling, so that mean size decreased between July 26 and August 17, while the weevils in T3 represent a special situation. In the first two days, they consumed about 10 times more food than

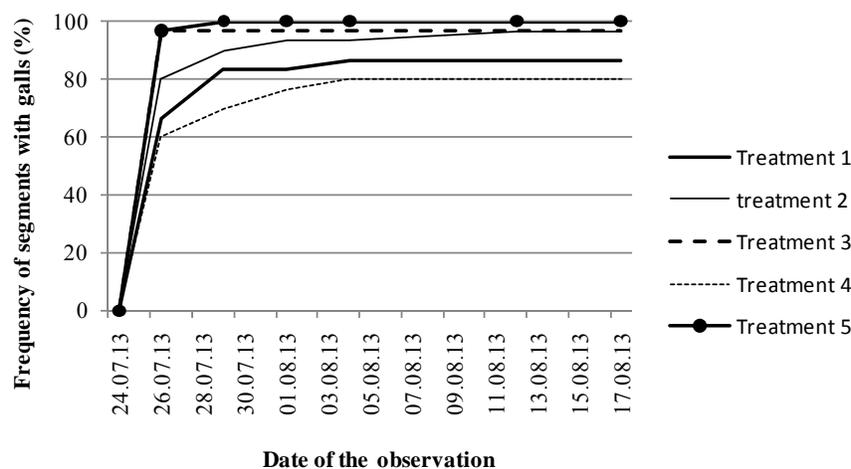


Fig.1. *Hylobius abietis* attack evolution in the laboratory experiment, expressed through frequency dynamics of twigs with galls of both types (deep and shallow)

Proportion of twig segments with deep galls

Table 2

Treatment	Date of the observation					
	26.07.13	29.07.13	01.08.13	04.08.13	12.08.13	17.08.13
T1	26.7 ^a	46.7 ^{ad}	46.7 ^{ad}	50.0 ^{ac}	50.0 ^{ac}	50.0 ^{ac}
T2	40.0 ^{ac}	56.7 ^{ac}	63.3 ^{ac}	76.7 ^{ab}	83.3 ^{ab}	83.3 ^{ab}
T3	70.0 ^{bc}	86.7 ^{bc}	86.7 ^{bc}	86.7 ^b	86.7 ^b	86.7 ^b
T4	10.0 ^a	16.7 ^d	16.7 ^d	30.0 ^c	30.0 ^c	30.0 ^c
T5 - control	86.7 ^b	100.0 ^b	100.0 ^b	100.0 ^b	100.0 ^b	100.0 ^b

Note: Proportions in the same column followed by the same letter do not differ significantly at $p = 0.05$ (Chi-square test).

Table 3

The intensity of the attack expressed by size of deep galls cumulated from the start of the experiment (mean \pm standard error, mm²)

Treatment	Date of the observation					
	26.07.13	29.07.13	01.08.13	04.08.13	12.08.13	17.08.13
T1	4.3 \pm 0.9 ^a	4.1 \pm 0.6 ^a	4.1 \pm 0.6 ^a	4.1 \pm 0.6 ^a	4.1 \pm 0.6 ^a	4.1 \pm 0.6 ^a
T2	5.8 \pm 1.2 ^a	5.8 \pm 1.2 ^a	9.3 \pm 1.6 ^a	11.1 \pm 2.2 ^a	15.7 \pm 2.4 ^b	16.0 \pm 2.5 ^b
T3	45.6 \pm 9.0 ^b	45.6 \pm 9.0 ^b	45.8 \pm 9.0 ^b	45.9 \pm 9.0 ^b	46.0 \pm 9.0 ^c	46.0 \pm 9.0 ^c
T4	4.7 \pm 0.7 ^a	3.6 \pm 0.7 ^a	3.6 \pm 0.7 ^a	2.7 \pm 0.6 ^a	2.7 \pm 0.6 ^a	2.7 \pm 0.6 ^a
T5 - control	89.7 \pm 14.3 ^b	195.7 \pm 22.1 ^c	268.2 \pm 25.9 ^c	361.7 \pm 30.8 ^c	551.3 \pm 35.6 ^d	666.7 \pm 33.2 ^d

Note: Mean values in the same column followed by the same letter do not differ significantly at $p = 0.05$ (testul Kruskal-Wallis urmat de testul Steel-Dwass)

insects in T1, T2 and T4, but thereafter fed extremely little. This means that the insecticide was effective only after being ingested in a sufficient amount.

At the end of the experiment, the average size of deep bites on treated twigs was only a small percentage (0.4% - in T4, 0.6% - in T1, 2.4% - in T2, 6.9% - in T3) of the size of bites on untreated twigs. Consequently, one can state that the most effective active ingredient was thiacloprid, followed by acetamiprid, imidacloprid and the least effective was metaflumizone.

Mospilan 20 SP containing acetamiprid is currently used in Romania for sapling protection against *Hylobius abietis* and it offers sufficient protection for a season

where the population density is much lower than in our experiment.

Very low damage areas were recorded in imidacloprid treated twigs also in England [17] in a short time (7 days) laboratory experiment. The same active ingredient was quite effective when applied as a granular product in the soil, also [7]. In Sweden the seedlings treated with the cypermethrin or imidacloprid insecticides suffered the same mortality level after two seasons since planting [12].

3.2. Insecticide effects on insects

All weevils in insecticide treatments and those kept without any food died gradually less than 30 days since the beginning of the

experiment. At the same time, 17% and 27% of weevils died in the control treatment until the 17th and 23rd of August, respectively. However, the dynamics of mortality were quite different from one treatment to another (Figure 2).

If we consider the time when fifty percent of the insects in the treatment died (lethal time 50% - LT50), it is noteworthy that only insects in T1 and T4 died earlier than starved weevils, while those in treatments T2 and T3 died later. LT50 was 8 days in T1, 10 days in T4, 12 days for starved weevils, and 14 days in T2 and T3. These facts could mean that the weevils in treatments T2 and T3 died due to starvation rather than due to insecticide poisoning, while those in T1 and T4 died because of combined effect of poisoning and starvation.

In a similar experiment [17] the insects fed on imidacloprid treated twigs died much earlier than those kept without food and there being concluded that there may be a different underlying cause of death between starved weevils and those fed on treated twigs. On the other hand, in their experiment, LT50 was of 12 days for

imidacloprid, although the emulsion concentration was only 0.1%.

In another experiment, 100% mortality was recorded after only 4 days of weevil exposure to 0.1% and 0.05% acetamiprid treated twigs [11].

These differences could have several causes: different physiological state of insects used in the experiments due to different periods from the season when insects were collected, different environmental conditions during experimentation, starvation or feeding of insects before experiment etc.

Insects fed on treated twigs behaved differently depending on the insecticide used. Metaflumizone acts through blocking the sodium channel of the nervous system and causes “relaxed” paralysis of the insect [8], while neonicotinoids bind to nicotinic acetylcholine receptors of cells of the central nervous systems and trigger a response by that cell. By overstimulation the receptors are blocked and paralysis occurs, which leads to death [5].

The weevils fed on metaflumizone treated twigs could not feed anymore after the first two days.

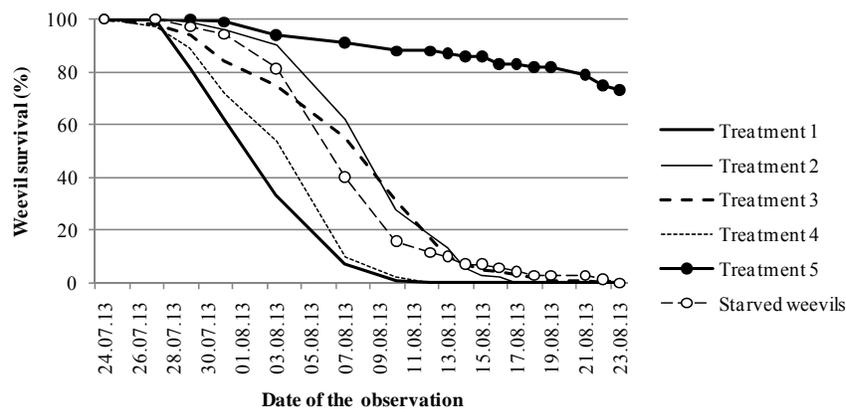


Fig. 2. Percentage of original *Hylobius abietis* population remaining alive after different treatments

They were almost completely inert and only moved their tarsi. Some of them opened their elytra and extended their hind wings. In contrast, the beetles fed on twigs treated with the other insecticides were affected by paralysis, but not continuously and from time to time, they had the possibility to move. Yet they lost their balance, sooner or later and fell back, staying in this position for a while until they came back to their senses and tried to walk again. In the field, these weevils could probably find untreated food and survive. Even so, the seedlings treated with neonicotinoids are much better protected than those treated with metaflumizone, because neonicotinoids act by contact and the weevils recognizing the treated seedlings from untreated ones will search for untreated food [17], while metaflumizone acts by ingestion that means seedling damage. The insects fed on them will die, but the seedlings will suffer severe damage from successive feeding of different beetles.

4. Conclusions

Based on the results regarding the size of deep bites (which can lead to the death of seedlings) it can be concluded that an adequate level of protection of seedlings could be achieved using one of the insecticides that have as active ingredient thiacloprid, acetamiprid or imidacloprid.

Insects feeding on treated twigs died in about three weeks, during which they could intermittently move (those that consumed neonicotinoid treated food) or remained almost completely inert (those fed on metaflumizone treated food).

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