OVIPOSITION PREFERENCES OF THE LARGE PINE WEEVIL, *HYLOBIUS ABIETIS* (L.), FOR DIFFERENT CONIFEROUS SPECIES

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Abstract

In this paper we present the results of field and laboratory experiments conducted with the purpose to establish which coniferous species are preferred by weevils for the oviposition as well as by the first-instar larvae. The coniferous species used in field and laboratory experiments were Norway spruce (*Picea abies* L. (Karst)), Scots pine (*Pinus sylvestris* L.), European silver fir (*Abies alba* Mill.) and European larch (*Larix deciduas* Mill.). In the field experiments, the highest density of insects (larvae, pupae or young adults) was found in Norway spruce billets and the lowest one in silver fir, the ratio between the highest density and the lowest one varied from 15.3 to 62.4 in different experimental areas.

In laboratory experiments, the weevils equally preferred Norway spruce and Scotch pine, the lowest density of eggs and larvae being also in silver fir, the ratio was 6.7-7.3 to 1.0. When we used eggs or first-instar larvae, the most numerous larvae were recovered from pine twigs and the least from silver fir.

All the results have indicated that the large pine weevil and its larvae prefer Scots pine or Norway spruce, not silver fir. This could explain the low level of weevil populations in the regions with a high proportion of silver fir in the composition of tree stands. Consequently, the silver fir occurrence in mature mixed resinous stands could significantly contribute not only to higher stand stability, but also to the decrease of weevil populations and of their attack risk at the beginning of the next stand generation.

Keywords: *Hylobius abietis*, oviposition preferences, *Picea abies*, *Pinus sylvestris*, *Abies alba*, *Larix decidua*

Rezumat
PREFERINȚELE GÂNDACILOR DE *HYLOBIUS ABIETIS* (L.) PENTRU DIFERITE SPECII DE CONIFERE ÎN CAZUL OVIPOZIȚIEI

În această lucrare, noi prezentăm rezultatele experimentelor de teren şi de laborator efectuate în vederea stabilirii preferinţelor adulţilor şi larvelor de vârsta I de *Hylobius abietis* pentru diferite specii de conifere, ca substrat de ovipoziţie şi respectiv ca sursă de hrană. Speciile de conifere utilizate în aceste experimente au fost: molidul (*Picea abies* L. (Karst)), pinul silvestru (*Pinus sylvestris* L.), bradul (*Abies alba* Mill.) şi laricele european (*Larix decidua* Mill.). În experimentele de teren, cea mai mare densitate de insecte (larve, pupe sau gândaci tineri) s-a găsit în parii-cursă de molid, iar cea mai mică în cei de brad, raportul între densitatea cea mai mare şi cea mai mică variind între 15,3 şi 62,4 în diferite suprafeţe experimentale.

În experimentele de laborator, gândacii au preferat în egală măsură molidul şi pinul, cea mai mică densitate de ouă şi larve fiind tot în cazul bradului, raportul densităţilor fiind 6,7-7,3 la 1. Când în experimente s-au folosit ouă sau larve de vârsta I, cele mai multe larve s-au recuperat din segmentele de ramuri de pin, iar cele mai puţine din segmentele de ramuri de brad. Toate aceste rezultate indică faptul că adulţii de *Hylobius abietis* şi larvele lui preferă pinul sau molidul, dar nu bradul. Aceasta ar putea explica nivelul redus al populaţiilor de trombar în regiunile cu o pondere mare a bradului în compoziţia arboretelor. În consecinţă, prezenţa bradului în arboretele de amestec mature ar putea contribui în mod semnificativ nu doar la o mai mare stabilitate a arboretelor, ci şi la reducerea populaţiilor de trombar şi a riscului de atac din partea lui în perioada în care noua generaţie de arbori ar putea fi vătămată de acesta.

Cuvinte cheie: *Hylobius abietis*, ovipoziţie, preferinţa pentru diferite specii, *Picea abies*, *Pinus sylvestris*, *Abies alba*, *Larix decidua*

1. INTRODUCTION

Hylobius abietis is a very important insect pest of coniferous plantations in many countries of the Europe where the forestry is based on clear-cuttings, and it can cause severe damages during the first 3-4 (5) years after the cutting of the mature stands, according to the development time of a generation (Eidmann 1974; Langström 1982; Örlander et al. 1997). In addition, the weevils could be vectors of pathogenic fungi for coniferous trees (Kadlec et al. 1992; Lévieux et al. 1994).

The adults are polyphagous insects, but prefer to feed on thin bark of conifers while the larvae develop only in conifer bark, usually on stumps and their roots (Eidmann 1974). The feeding preferences of the large pine weevil have been extensively studied (Langström 1982; Leather et al. 1994; Manlove et al. 1997; M?nsson & Schlyter 2004), but the oviposition preferences took less attention although exist some information that larval mortality, development time, weight of adults and their biological performances differ among conifer hosts (Butovitsch & Heqvist 1961; Bejer-Petersen et al. 1962; Thorpe & Day, 2002), a fact which could have significant importance on insect population management.

On the other hand, recently Nordlander et al. (1997) discovered that the weevil females lay the eggs in the soil near the roots and not directly in the host bark. Therefore, the objectives of this study were to establish the oviposition preferences of weevil adults for the following conifer species: Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), European larch (*Larix decidua*), silver fir (*Abies alba*), and to prove if the first-instar larvae are able to choose between different host species and which of them are preferred.

2. MATERIALS AND METHODS

2.1. The field experiment

In the field we have used the method of breeding billets described by Bejer-Petersen et al. (1962). Breeding billets of Scots pine, Norway spruce, European larch and European silver fir, measuring 1 m, were cut from freshly cut trees and buried in the soil (Fig. 1), in three fresh clear-cut areas (Table 1). In each area, a ditch of 30 cm in depth along one side and sloped evenly upward to the other side was dug. The billets of different species were placed alternating at 5-10 cm intervals, with the thick end upwards, and thereafter covered with the soil which had been excavated from the ditch. After colonization by insects, they were dug up at different time intervals and inspected in laboratory, to find the insects or pupal cells of *Hylobius abietis* (Fig. 2).

2.2. Laboratory experiments

In the first laboratory experiment (A) we tried to imitate the field experiment, but controlling the most important factors. Therefore, in June 2004, we have collected mature *Hylobius abietis* weevils, that were fed on fresh Scots pine twigs until the experiment was set up. As substrate for oviposition we used twig segments of 30-40 mm diameter and 60 mm length (Fig. 3), which have been treated at their ends with melted wax in order to prevent the dehydration, as well as the penetration of fungi in the phloem through the section surface. All the twig segments of a host species were cut from the same tree to avoid the possible variation of attractiveness between the trees. In order to assure living conditions similar to those in the field, an 8 cm layer of moist peat mixed with sand was put in plastic

Fig. 1. Ditch with breeding billets of Norway spruce and silver fir alternating
Şanţ cu pari-cursă de molid şi brad dispuşi alternativ

Table 1. Details concerning the experiment set up, harvesting and analysing of breeding billets used in the field to establish the weevil oviposition preferences
Detalii privind experimentul de teren efectuat în vederea stabilirii preferințelor gândacilor pentru diverse specii de rășinoase ca substrat de ovipoziție

Experi-mental area	Alti-tude (m)	Aspect	Date of billets burying	Date of billets digging out	Host tree	No. of analysed billets	Analysis period
Tomnatic, I, 10B	810	N	9-10.05 2001	1.02. 2002	S+F	5+5	1-6.02.2002
				25.06.2002	S+F	5+5	8-11.07.2002
				2.08.2002	S+F	3+3	3.08.2002
				14.05.2004	S+F	15+15	14-18.05.2004
Tomnatic, I, 68C	862	NW	12.05 2001	21.02.2002	S+F	5+5	21-28.02.2002
				15.06.2002	S+F	5+5	20-30.06.2002
				16.07.2002	S+F	2+2	18.07.2002
				2.08.2002	S+F	3+3	5-6.08.2002
				23.08.2002	S+F	4+4	26.8-7.09.2002
				4.04.2003	S	5	12.4-10.5.2003
				24.05.2004	S+F	7+8	26-27.05.2004
Tomnatic, I, 94B	830	SW	2.07. 2002	11.07.2003	S+P+L+F	5+4+6+5	11-26.07.2003
				05.12.2004	S+P+L+F	5+5+5+5	22.1-6.2.2004
				28.05.2004	S+P+L+F	7+6+7+7	28.5-4.7.2004

Notes: S - Norway spruce; F - Silver fir; P - Scots pine; L - European larch

Fig. 2. *Hylobius abietis* developmental stages from breeding billets: larva under the bark (upper-left), larva in pupal cell (upper-right), pupa (bottom-left) and young weevil (bottom-right)
Stadii de dezvoltare ale insectei *Hylobius abietis* din parii-cursă: larvă sub scoarță (stânga sus), larvă în leagănul de împupare (dreapta sus), pupă (stânga jos) și gândac tânăr (dreapta jos)

jars (Fig. 3). Thus we kept the freshness of the twig segments and provided a shelter for weevils which during the day retire in the -litter or in the soil. A twig segment of each host species (Scots pine, Norway spruce, European larch and silver fir) was buried in horizontal position at 2 cm depth in each of the 15 plastic jars. Thin (5-20 mm diameter) Scots pine twigs, as food source for weevils, were put on the peat layer in each jar. On the 29th of June, in each of the 15 jars, two pairs of weevils were confined. After three weeks, on the 19th of July, the experiment was ceased and the twig segments analysed to find the eggs or larvae in the bark (Fig. 4).

In another laboratory experiment (B) we placed, on moist paper in 12 Petri dishes, eggs or first-instar larvae of *Hylobius abietis* in parallel rows between twig segments of host conifers. In each dish there have been 28 eggs or 18 larvae. After two weeks the segments have been inspected searching for larvae in or under the bark. A few eggs didn't hatch, because of the mould that had developed on them, and some larvae could not be found at all, but we were not able to identify the cause of their disappearing, and supposed that they had escaped from the dishes.

Fig. 3. Twig segments (left) and plastic jars with peat and sand (right) used in laboratory experiment A
Segmente de ramuri (stânga) şi borcane de plastic cu turbă şi nisip (dreapta) ce s-au folosit în experimentul de laborator A

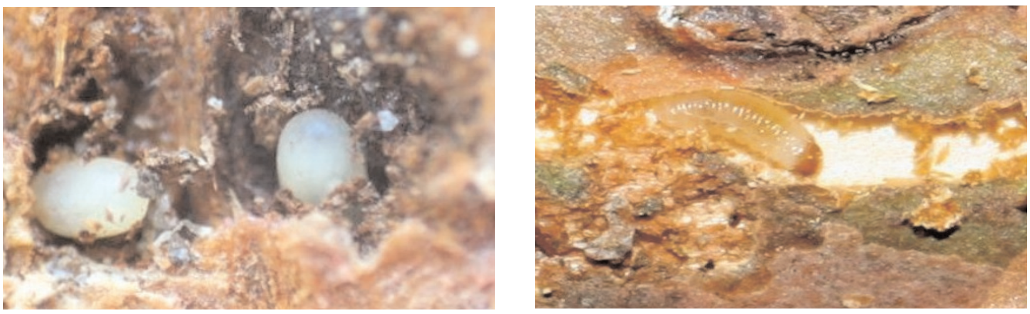

Fig. 4. Eggs (left) and larva (right) of *Hylobius abietis* from twig segments used as oviposition substrate in the laboratory experiment A
Ouă (stânga) and larvă de *Hylobius abietis* (dreapta) în segmentele de ramuri utilizate ca substrat în experimental de laborator A

2.3. Statistical analysis

In order to establish the preference of adults or larvae for any host species, we compared the proportion of infested billets or twig segments, as well as the mean number of insects that colonized each substrate unit (breeding billet or twig segment). The significance of differences between proportions was established using Chi-square test, and for differences between means we used 1-way ANOVA and Tukey or Kruskal-Wallis test when the distributions were normal, respectively when the distributions did not fulfil the assumption of normality.

3. RESULTS

In the field experiment, all the breeding billets of Norway spruce, Scots pine and European larch have been colonized by *Hylobius abietis*, whereas 46.4-59.3 % of silver fir billets remained un-colonized (Table 2). At the same time, silver fir billets had the lowest average density of infestation, and statistically significant differences have been found between this tree species and the other ones in all experimental areas. One can also see that - in the management unit 94B - the infestation density of Norway spruce billets exceeds about three times that found in Scots pine and European larch.

The results from laboratory experiment A (table 3) show that weevils succeeded in colonizing only 40-80 percent of the twig segments during the three weeks of experimentation, most of them being of pine and larch. Average infestation density was highest in spruce and pine, and lowest in silver fir. Because of high variability of data, there were no statistically significant differences. However, the ranking of species according to weevil preference is quite obvious.

Table 2. Frequency[1] of breeding billets with *Hylobius abietis* and infestation density[1] (mean ± standard error) depending on the tree species
Frecvenţa parilor-cursă cu *Hylobius abietis* şi densitatea infestării (media ± eroarea mediei) în funcţie de specia de arbore

Clear-cutting area	Tree species	No. of analysed billets	Mean[2] billet diameter (cm)	Mean[2] bark thickness (mm)	Infestation frequency (%)	Average[2] density (insects/billet)
10B	Spruce	28	$8.8^{a} \pm 0.3$	$1.9^{a} \pm 0.08$	100^{a}	$19.9^{a} \pm 2.4$
	Fir	28	$9.0^{a} \pm 0.3$	$2.1^{a} \pm 0.11$	53.6^{b}	$1.3^{b} \pm 0.3$
68C	Spruce	31	$8.9^{a} \pm 0.3$	$1.8^{a} \pm 0.10$	100^{a}	$39.3^{a} \pm 4.8$
	Fir	27	$9.6^{a} \pm 0.4$	$2.5^{b} \pm 0.18$	40.7^{b}	$1.9^{b} \pm 0.3$
94B	Spruce	17	$10.6^{a} \pm 0.3$	$1.9^{a} \pm 0.14$	100^{a}	$99.8^{a} \pm 6.0$
	Pine	15	$11.2^{ab} \pm 0.4$	$2.3^{a} \pm 0.41$	100^{a}	$31.9^{b} \pm 6.0$
	Larch	18	$11.8^{b} \pm 0.3$	$5.1^{b} \pm 0.42$	100^{a}	$35.7^{b} \pm 5.5$
	Fir	17	$12.3^{b} \pm 0.4$	$3.6^{c} \pm 0.18$	52.9^{b}	$1.6^{c} \pm 0.4$

[1]) There have been taken into account all the developmental stages of *Hylobius abietis* (larvae, pupae, adults), alive or dead, as well as the pupal chambers. [2]) For each clear-cutting area, means bearing the same superscript within each column are not significantly different at the 95 % level of probability.

In the second laboratory experiment (B), 86.9 % of eggs hatched, but only 125 larvae from a total of 146 have been found. When we started the experiment using first-instar larvae, only about half of them (53 %) were found after two weeks. In both cases, the most larvae have been recovered from pine twigs (Table 4).

4. DISCUSSION

In the field experiment, we have found many more insects of *Hylobius abietis* in Norway spruce breeding billets than in those of other species. Scots pine billets and those of European larch had about 33-36 % and silver fir about 1.6 % as much insects as Norway spruce. Similar results have been reported by Kuziemska-Grzeczka (1984), who studied the oviposition preferences of *Hylobius abietis* for many coniferous species, including *Pinus sylvestris*, *Picea abies*, *Larix decidua* and *Abies alba*.

Table 3. Frequency of twig pieces with *Hylobius abietis* and infestation density (mean ± standard error) depending on the tree species in laboratory experiment A
Frecvenţa segmentelor de ramuri cu *Hylobius abietis* şi densitatea infestării (media ± eroarea mediei) în funcţie de specia de arbore în experimentul de laborator A

Tree species	No. of analysed twig segments[1]	Mean[2] diameter of twig pieces (mm)	Mean[2] thickness of alive bark (mm)	Frequency (%)	Average density (mean[2;3] ± standard error)
Spruce	10	$32.4^{a} \pm 1.7$	$1.9^{a} \pm 0.1$	50.0^{a}	$7.3^{a} \pm 3.4$
Pine	10	$32.8^{ab} \pm 2.3$	$1.1^{b} \pm 0.2$	80.0^{a}	$6.7^{a} \pm 1.5$
Larch	10	$34.9^{b} \pm 1.9$	$1.5^{c} \pm 0.2$	70.0^{a}	$3.1^{a} \pm 1.2$
Fir	10	$33.9^{ab} \pm 2.9$	$1.9^{a} \pm 0.2$	40.0^{a}	$1.0^{a} \pm 0.4$

[1]) There have been discarded 5 replications in which one weevil female died before finishing the experiment. [2]) Means bearing the same superscript within each column are not significantly different at the 95 % level of probability. [3]) There have been taken into account only the eggs and larvae of *Hylobius abietis* found in twig pieces from the peat layer, although 30 eggs and larvae have been found in the bark of twig segments provided for feeding, which were of Scots pine.

Table 4. Frequency of twig pieces with *Hylobius abietis* and infestation density (mean ± standard error) depending on the tree species in laboratory experiment B
Frecvenţa segmentelor de ramuri cu *Hylobius abietis* şi densitatea infestării (media ± eroarea mediei) în funcţie de specia de arbore în experimentul de laborator B

Tree species	No. of analysed twig segments	Frequency (%) in the experiment with....		Average density (mean[1] ± standard error) in the experiment with....	
		eggs	larvae	eggs	larvae
Spruce	6	100	100	$6.5^{a} \pm 1.1$	$3.3^{a} \pm 0.8$
Pine	6	100	100	$11.5^{b} \pm 0.3$	$5.0^{a} \pm 0.6$
Fir	6	100	83.3	$2.8^{c} \pm 0.7$	$1.2^{b} \pm 0.3$

[1]) Means bearing the same superscript within each column are not significantly different at the 95 % level of probability.

Her results show that in the field experiment the most insects were found in Norway spruce billets, and the Scots pine and European larch were equal infested, but the silver fir was totally avoided. Butovitsch & Heqvist (1961) have also found a lower number of *Hylobius abietis* larvae in Scots pine billets than in Norway spruce billets, while Bakke & Lekander (1965) found about the same number in pine and spruce.

Analysing all these results, we could infer that *Hylobius abietis* preferred to lay the most eggs in Norway spruce billets, and not in those of other species, although in several field trials with trap billets it had been noted that the adults of *Hylobius abiestis* are more attracted by the Scots pine than by the Norway spruce (Christiansen, 1971; Langström, 1982). However, the results do not necessarily reflect the oviposition preferences, but the brood production when the site condition are similar and the oviposition substrate is different, because we do not really know how many eggs were laid by weevils in/on the breeding billets and how many larvae survived. One can suppose that the level of larval mortality differed according to the tree species, as it was already reported by Thorpe & Day (2002) from a laboratory experiment using other tree species.

At the same time, we do not know if all the larvae found in the breeding billets of one species are from eggs laid on those billets, or some larvae have migrated between billets of different species, as the weevil's larvae are able to leave the original substrate and search for another food source (Nordenhem & Nordlander, 1994).

These were the reasons why we simulated the experiment in laboratory conditions and analysed the oviposition substrate shortly after egg laying. In such conditions (experiment A), the Norway spruce twigs were colonized almost as much as those of Scots pine, so we can conclude that both species were equally preferred by female weevils, but much more than European larch and especially silver fir.

Because the large pine weevil can lay eggs in the soil (Nordlander et al., 1997) we have also tested (in laboratory experiment B) the preference of larvae for the development substrates. The first-instar larvae preferred Scots pine and then Norway spruce, avoiding the silver fir. However, some of them colonised the silver fir twigs, but we noted that they were much smaller than those feeding on Scots pine or Norway spruce, although the developmental period was very short. This fact suggests that larvae feeding on silver fir phloem were suffering comparing to those consuming Norway spruce or especially Scots pine phloem. Based on these observations, we suppose that factors depending on feeding substrate caused a higher larval mortality in silver fir billets than in other species and, as a result, we noted higher differences concerning the infestation density between species in the field experiment than in the laboratory experiments.

Kuziemska-Grzeczka (1984), in her study mentioned above, concluded that *Hylobius abietis* cannot develop on silver fir, but we have obtained adult weevils. That means that a few individuals managed to complete their development consuming phloem of silver fir. The difference between these results is, most probably, due to genetic differences of silver fir populations in the natural area of this species, which induce biochemical differences that affected the larval development. In an experiment with Sitka spruce, Thorpe & Day (2002) have reported negative influence of lignin on larval development, but the content of lignin shows high variations between provenances of Sitka spruce (Wainhouse & Ashburner, 1996).

Other influences of host species on larval development time, weight of adults and their fecundity have been already reported in several studies (Butovitsch & Heqvist, 1961; Bejer-Petersen et al., 1962; Guslits, 1970 mentioned by LÍngström, 1982; Thorpe & Day 2002), but no one included the silver fir. Therefore, our research should be continued in this direction, but we could expect that - under the same site conditions and the same adult population level - non-preferred coniferous species will yield a much lower offspring number than preferred species, and probably those insects will have a lower biological potential. In other field studies we have already observed a very low level of weevil populations in the regions with a high proportion of silver fir in the composition of tree stands. Consequently, the silver fir occurrence in mature mixed resinous stands could significantly contribute not only to higher stand stability, but also to the decrease of weevil populations and of their attack risk at the beginning of the next stand generation.

5. CONCLUSIONS

The results o this study demonstrate that *Hylobius abietis* adults prefer Scots pine and Norway spruce as substrate for oviposition. The first-instar larvae are able to choose between different host species and they also preferred the Scots pine and Norway spruce as food. The silver fir can be infested, but it is much less colonised by *Hylobius abietis* when this species is together with Norway spruce or Scots pine, probably because the silver fir phloem is less favourable to larval development. Consequently, the silver fir occurrence in mature mixed resinous stands could significantly contribute not only to higher stand stability, but also to the decrease of weevil populations and of their attack risk at the beginning of the next stand generation.

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