Feeding and oviposition rates in the pine weevil *Hylobius abietis* (Coleoptera: Curculionidae)

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Abstract

Feeding and oviposition in the pine weevil *Hylobius abietis* (Linnaeus) were monitored under laboratory conditions in two long-term experiments lasting over an extended breeding season. Data were also collected from weevils under semi-natural conditions outdoors. In addition, the effects of crowding and starvation were studied in separate experiments. During the main peak oviposition period, female *H. abietis* consumed 50% more bark tissue than males. When oviposition ceased, the feeding rate of the females declined to the same level as in the males. The rates and spatial distribution patterns of oviposition and feeding were clearly affected by climatic conditions and the degree of crowding. Females were estimated to lay on average 0.8 eggs per day during the season under outdoor conditions. The realized fecundity of a female weevil during the first season was estimated to be approximately 70 eggs. The estimated average rate of feeding was 23 mm^2 of Scots pine bark per weevil per day. This implies that planted seedlings can only constitute a minor part of the food resources needed to sustain *H. abietis* populations of the size that usually appear on fresh clear-cuttings in northern Europe.

Introduction

Adult pine weevils, *Hylobius abietis* (Linnaeus) (Coleoptera: Curculionidae), cause extensive damage to European forest regeneration by feeding on the bark of newly planted coniferous seedlings (Eidmann, 1974; Day & Leather, 1997; Örlander & Nilsson, 1999). In managed forests, reproduction is concentrated in fresh regeneration areas, where the larvae live under the root-bark of coniferous stumps. High population levels build up and can be maintained because of the large amount of breeding substrate that is constantly available; i.e. new areas are harvested every year and these areas are distributed fairly evenly in the landscape within the weevils' dispersal range (Eidmann, 1977; Nordlander, 1998).

New breeding areas are colonized during spring migration, when *H. abietis* may disperse up to about 100 km (Solbreck, 1980). The flying weevils are attracted to freshly felled areas by conifer volatiles emanating from stumps and logging waste (Solbreck & Gyldberg, 1979). After arrival, immigrant *H. abietis* may feed intensively in the crowns of adjacent coniferous trees for a short period of time, during which most of the females become sexually mature (Örlander *et al.*, 2000). Prior to migration, the flight muscles develop while the weevils feed at the site of emergence (Nordenhem, 1989). Two to three weeks after arrival at the breeding site, the flight muscles degenerate and their ability to fly is lost, although it can be regained the following season (Nordenhem, 1989). Both before and after the flight period, most of the feeding occurs at or below ground level, i.e. mainly on stems and twigs or on roots of conifers (Örlander *et al.*, 2000). Feeding continues throughout the summer until the adult weevils start preparing for hibernation. Trap catch data have indicated that the immigrant *H. abietis* terminate their activity earlier in the

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season (late August in southern Sweden) than pre-reproductive weevils of the following generation (Örlander *et al.*, 1997). These emerge in August the following year and remain active for one or two months before hibernation (Nordenhem & Eidmann, 1991).

Immigrant females of *H. abietis* oviposit throughout the active period, which lasts most of the summer. Eggs appear to be predominantly laid in the soil near stump roots (Nordlander *et al.*, 1997), but they may also be inserted into niches chewed in the inner bark of such roots. Larvae hatching from eggs in the soil find their way to roots suitable for their development in response to host volatiles (Nordenhem & Nordlander, 1994). Factors such as soil moisture and soil texture affect the females' choice of sites to lay their eggs (Munro, 1927; Nordlander *et al.*, 1997). Females may also respond to the presence of other *H. abietis* when choosing oviposition sites. It has been suggested that a reason to avoid oviposition in bark could be that such eggs suffer the risk of being destroyed by *H. abietis* feeding on root bark or to avoid competition with other pine weevil larvae (Nordlander *et al.*, 1997).

Although *H. abietis* is a significant pest species, much of the basic information needed to understand its population dynamics and the relationship between population size and the damage it causes is still lacking (Eidmann & Lindelöw, 1997; Örlander *et al.*, 1997, 2001; Leather *et al.*, 1999). Its preferred temperature, humidity and light conditions ranges for feeding and oviposition have been investigated (Christiansen & Bakke, 1968; Havukkala, 1980) and its rates of feeding have been quantified, but only in short-term experiments (Pohris, 1983). The age-structure, physical ageing, sexual maturation and development of *H. abietis* in clear-cuttings of different age classes have also been described (Nordenhem, 1989; Örlander *et al.,* 2000). However, neither its variation in realized fecundity during the course of a season nor its potential fecundity under optimal conditions have been described. Furthermore, no reliable estimates of per capita feeding rates through the season are available. Data on survival and longevity in *H. abietis* are also incomplete.

The aims of this study were to describe and analyse the rates of feeding and oviposition of *H. abietis* and to determine the species' reproductive capacity. This was done under optimal and semi-natural conditions during periods about equivalent to a breeding season. The study focuses not only on the relationship between feeding and realized fecundity, but also on the correlation between the distribution of eggs and feeding sites and the possible effects of competition and periodic lack of food on these traits.

Materials and methods

Four experiments were performed in order to study different aspects of feeding and oviposition, and their possible interactions. Temporal variations in feeding rates and egg production were studied in two experiments (I and II). In addition, the spatial distribution of eggs was recorded in experiment II. The experimental designs differed between the two experiments, partly because it was not known when the first experiment was set up that a proportion of the *H. abietis* eggs are laid outside the plant tissue (Nordenhem & Nordlander, 1994; Nordlander *et al.*, 1997). In a third experiment the distribution of eggs and feeding rates were studied at different levels of crowding. Effects of temporary

starvation on feeding rates were examined in a fourth experiment.

In all experiments tender bark on freshly cut young stems or branches of Scots pine, *Pinus sylvestris* (L.) (Pinaceae), was used as a feeding or oviposition substrate. The pine substrate was on each occasion cut from one tree and distributed equally between the treatments. Pine weevils used in the study were collected at different locations in the vicinity of Uppsala, Sweden. *Hylobius abietis* vary considerably in size but but the sexes are on average of similar size (width of pronotum not significantly different between samples of 3245 females and 3628 males, $P = 0.63$, ANOVA, unpublished data). Extremely small or large individuals were not included in the experimental populations.

Feeding and oviposition: experiment I

In order to test if female *H. abietis* have higher feeding rates than males, and to investigate the relationship between feeding rates and oviposition, these traits were recorded in the laboratory over an 18-week period.

Weevils in their migratory phase were collected at a sawmill, north of Uppsala, in late May and stored in darkness at +10°C until the experiment was started five months later. These storage conditions interrupt reproductive development, so oviposition did not start until the weevils were transferred to the experimental conditions (temperature, +20°C; light regime, L18: D6).

At the start of the experiment, 110 male and 110 female *H. abietis* were placed in a large cage (width × length × height: $800 \times 800 \times 400$ mm) filled with a 50 mm deep layer of sand. Ten pieces of Scots pine stem (length 500 mm, diameter 40 mm) with smooth bark surfaces were half buried in the sand to serve as breeding and feeding substrates. Males and females were differently marked using Tipp-Ex® to facilitate subsequent re-collection and sorting by sex.

Feeding intensity was measured over 24 h every fourth day when groups of ten weevils of the same sex were transferred from the cage to large glass Petri dishes (diameter 190 mm), each containing a piece of Scots pine branch (length 100 mm, diameter 15 mm) with thin bark serving as a feeding substrate. A wet filter paper was placed in each dish to keep the humidity high. The feeding experiment included ten replicates for each sex. The individual weevils in each replicate were randomly picked from the cohort each time. To measure bark consumption, the bark surface was covered with transparent acetate film and the bark areas where weevils had been feeding were outlined using a felt-tip pen (Leather *et al.*, 1994). The entire feeding areas were outlined, irrespective of depth, but feeding marks generally extended down to the xylem on the feeding substrate used. The acetate film was attached to paper and photocopied, providing an image of the mapped feeding areas. The amount of bark area removed by weevils feeding and the number of feeding marks were then estimated by analysing the feeding area images, using a photographic image analysing system (Quantimet 720, Imanco Ltd, Denmark).

After measuring feeding, all weevils were transferred back to the large cage containing humid sand and a fresh piece of pine, for another three-day period. The piece of Scots pine constituting the combined breeding and feeding

substrate was collected and replaced after each period. The numbers of eggs and larvae found in the bark layer of the breeding substrate were recorded. The sand was not replaced in the large container between each three-day period. It was assumed that larvae that emerged from eggs laid in the sand would eventually find their way to the breeding substrate. Thus, an estimate of the total numbers of eggs laid per three-day period in the large container could be calculated. This was done by adding the number of larvae found in the breeding substrate to the number of eggs found in the breeding substrate from the preceding eight-day period. That period corresponds roughly to the time it takes for the eggs to hatch at room temperature (+20°C) and for the larvae to find the breeding substrate (Eidmann, 1974; Nordenhem & Nordlander, 1994). The experiment was finished after 133 days, by which time 33 records had been collected for both one-day consumption and the rate of oviposition over three-day periods.

Feeding and oviposition: experiment II

In this experiment, feeding and oviposition rates of *H. abietis* were measured over time and the spatial distributions of feeding and egg-laying were recorded in order to investigate the effects of (i) microclimate and (ii) the presence of other *H. abietis*. Adult pine weevils were collected from a fresh clear-cutting at the end of May with the aid of covered pitfall traps (Nordlander, 1987) baited with newly-cut pieces of Scots pine. The weevils were stored for one day at +12°C before the experiment started.

Egg laying and feeding patterns of *H. abietis* were recorded both indoors and outdoors. The indoor part of the experiment was continued for 29 weeks at room temperature (+20°C) with a light regime of L18: D6. The outdoor part, which had a similar design, was started simultaneously, but was interrupted after six weeks due to the weather being warmer than normal. It was set up in a field laboratory, at the university campus (Ultuna, Uppsala, Sweden), under a roof and thus shaded for most of the day, but was otherwise exposed to ambient light and temperature conditions. Air temperature data were obtained from the Ultuna meteorological station 200 m from the field laboratory. In both parts of the experiment, two treatments were included, one with a solitary female and the other with one female and one male. Each treatment had 12 replicates indoors and 12 outdoors, placed in separate plastic containers (width × length × height; 350 × 550 × 280 mm) ventilated through a hole in the lid covered by a fine metal mesh. Each container was supplied with a 120 mm thick layer of sand on the bottom and a piece of fresh Scots pine stem (length 100 mm, diameter 30 mm) with thin, smooth bark that was half buried in the sand. This piece of pine served as both a breeding (oviposition) and a feeding substrate. The weevils were moved to new containers with fresh pine pieces every seventh day. The boundary line between the upper exposed surface and the buried lower surface of the pine piece was marked before it was removed from the container. These pine pieces were stored at room temperature for approximately ten days to enable the eggs to hatch and to verify that they were viable and then moved to a freezer until they were examined.

Bark consumption was measured with a similar technique to that used in experiment I. Debarked areas were outlined using a felt-tip pen on transparent acetate film. The parts representing the upper (exposed) and lower (buried) bark surfaces were marked and the film was attached to a piece of paper. The feeding mark pattern was scanned separately for each piece of pine by using a flatbed scanner (HP Scan Jet 4C). The amount of bark area removed, the numbers of feeding marks and their perimeters were measured from the scanned bitmap images.

Eggs in the breeding substrate were located by peeling off the bark layer of each piece of pine with a scalpel. The numbers and position (upper or lower surface) of all eggs and larvae found were recorded. To estimate the number of eggs deposited in the sand, a new piece of Scots pine was buried in the containers after the adult weevils had been removed. This piece was entirely covered by sand and left for two weeks to attract the emerging larvae (Nordenhem & Nordlander, 1994; Nordlander *et al.*, 1997). The number of larvae in each piece was subsequently recorded as described above.

Effects of crowding on feeding and distribution of eggs

A laboratory experiment was conducted to test if the distribution of eggs was affected by female responses to crowding by conspecifics. One reproductively active, mated female *H. abietis* was placed alone or together with one, two or five juvenile (pre-reproductive) females. There were 12 replicates of each of the four treatments. Reproductive females were collected from a fresh clear-cutting in May and stored at +10°C to slow down life processes and development. The juvenile females were collected from a one-year-old clear-cutting in September. At this time of year, a new generation of weevils is emerging from the pupal chambers in the roots. These pre-reproductive weevils feed for a period before hibernating in the ground. The two groups of weevils were stored separately at +10°C until the experiment was started on November 16. Some juvenile females were dissected and it was established that their gonads had not developed, i.e. they were still pre-reproductive. The experiment was continued for a week and the same procedure as described for experiment II was used to obtain estimates of area consumed and realized fecundity.

Compensatory feeding after a period of starvation

A field experiment was carried out to test the effects on feeding rates of continuous and discontinuous access to food and exposure vs. shading. The experiment was conducted outdoors in June at a clear-cutting 40 km north of Uppsala, Sweden. Prior to the experiment, weevils were collected at a sawmill during swarming. Groups of five *H. abietis* of the same sex were placed in well-drained, plastic containers (width × length × height: 200 × 200 × 120 mm) with metal mesh lids that had been half-filled with sifted peat. Five replicates of groups of each sex were included in each treatment. A total of 20 groups of each sex was used. Half of the groups had continuous access to food that was renewed every week. The other groups had access to food every second week, starting with one week of starvation. Five groups of female and five groups of male *H. abietis* were provided with either discontinuous or continuous access to food and were placed in containers in the clear-cutting in a position that was exposed to the sun. In addition, 20 containers with similar treatments were placed in the shade of a neighbouring forest stand. Thus, the experiment had a

split plot design. The feeding substrate consisted of Scots pine branches (length 100 mm, diameter 30 mm). On each feeding occasion the branches were taken from a single tree. All containers were buried so that the lid was level with the ground. The area of bark consumed on the feeding substrate was estimated using transparent graph paper. The pieces of pine were searched for eggs and the same procedure as described for experiment II was used to estimate the number of eggs laid in the peat substrate.

Statistical analyses

Temporal patterns in realized fecundity and feeding were analysed using repeated measure analyses (GLM procedure, SAS) when several measurements were made on the same individuals or groups (experiment II). The design of experiment I did not fulfil the requirements for repeated measures analysis, because the individuals were randomized between each recording of feeding. These data were analysed by univariate variance analyses with time and sex as fixed factors using the GLM procedure in SAS, and in a multivariate analysis to complement the univariate analyses. When multiple correlations or regressions were analysed a standard Bonferroni correction of the *P*-values was applied table-wise.

Results

Feeding and oviposition: experiment I

A total of 84 out of 110 (76%) females and 92 out of 110 (84%) male *H. abietis* survived for the entire period of 128 days (18.3 weeks) that the experiment lasted.

Females consumed more bark than males (fig. 1, table 1). The mean bark area consumed per individual per day was 62 mm^2 (SE = 3) for females and 40 mm^2 (SE = 1) for males. In females, the feeding rate varied over time, reaching a peak about 40 days after the experiment started. Males showed a more constant rate of feeding throughout the experiment, with only minor fluctuations.

The size and density of feeding marks differed between the sexes (table 1). Male *H. abietis* made significantly larger, but fewer, feeding marks than females. The average number of feeding marks made per weevil per day was 2.3 for males and 4.5 for females. The mean area of each feeding mark made was 18 mm^2 (SE = 0.3) for males and 15 mm^2 (SE = 0.3) for females. The size of each feeding mark did not vary over time between sexes, i.e. no time* sex interaction was found (table 1).

The average egg production during the entire experiment (18 weeks) was 0.58 eggs (SE = 0.06, *n* = 30) per female per day (fig. 2). During the first week, very few eggs were laid. A peak in oviposition followed during the second and third weeks, when a maximum of 1.5 eggs were laid per female per day (fig. 2). During the first six weeks of the experiment, the mean egg production for all females was 0.81 (SE = 0.14) eggs per female per day. The daily oviposition rate was fairly stable at approximately 0.7 eggs per female for the following four weeks, and then declined to about 0.2–0.3 eggs per female per day for eight weeks. No data are available for the fraction of eggs laid in the sand after this time. The oviposition rates were measured over the three-day periods that the weevils were kept in the large cage. Some eggs were probably also laid during the intervening one-day periods when the weevils were kept in the Petri dishes, but these were not recorded. Hence, the egg-laying
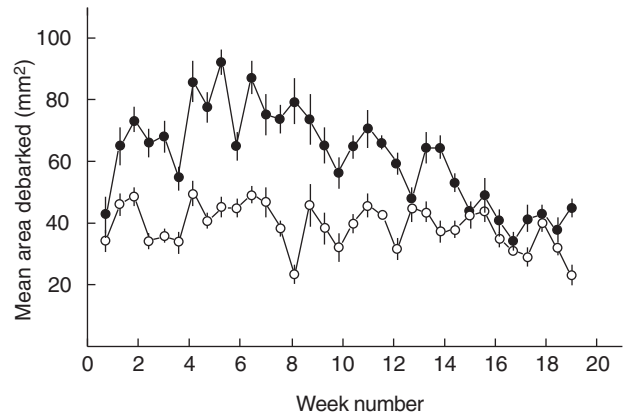

Fig. 1. Mean daily bark area consumed (mm^2) per female (●) or male (○) *Hylobius abietis,* measured every fourth day. Mean values are based on initially 11 groups of ten females and 11 groups of ten males feeding on Scots pine bark. Vertical bars denote standard error of the mean (SE).

Table 1. *P*-values of F-statistics for univariate analyses of variance (Proc GLM, SAS) of consumption and feeding patterns of *Hylobius abietis* under laboratory conditions.

Source	df	*P* area	*P* no. of marks	*P* area/ feeding mark	MANOVA Pillai's Trace
Time	32	0.0001	0.0001	0.0001	0.0001
Sex	1	0.0001	0.0001	0.0001	0.0001
Time*sex	32	0.0001	0.0001	0.075	0.0001
Error	544				

Response variables tested were mean area consumed per individual, number of feeding marks and mean area per feeding mark in relation to the fixed factors time, sex and the interaction between time and sex. Instead of Bonferroni corrections, figures in the last column represent Pillai's Trace *P*-values of a multivariate analysis including all the response variables used in the three tests performed.
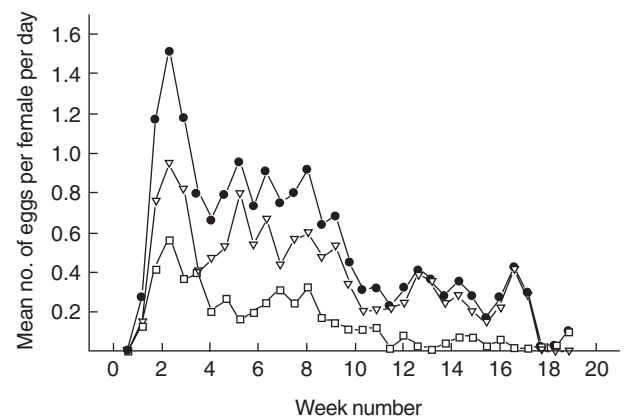

Fig. 2. Mean daily number of eggs laid per female *Hylobius abietis* during three-day periods in the pine substrate (□) or in the sand (▽) and their total (●). The number of eggs deposited in the sand is represented by the number of larvae found in the pine substrate. These data are shifted eight days back to coincide with the timing of egg laying.

figures should be adjusted by adding up to approximately 25% to the estimated daily rates, i.e. 0.73 eggs per female per day female over 14 weeks.

Most of the eggs (71%) were deposited in the sand (fig. 2). This value was calculated from the number of larvae found in the pine substrate and the total recorded numbers of eggs and larvae. The percentage of eggs placed in the sand increased with time from 50–60% initially to over 90% during the last weeks of measurements.

The bark area consumed by female *H. abietis* and the numbers of eggs laid were positively correlated. The association was significant in all but one case, in which the factors were displaced by one or two weeks with respect to each other (table 2).

Feeding and oviposition: experiment II

Under indoor conditions, 67% (16/24) of females survived for the 14 weeks that the experiment lasted, and six females (25%) were still alive at the point, after 29 weeks (203 days), when observations ended. The average longevity of females under indoor conditions was 20 weeks (SE = 1.6, n = 24, range 7–29). During the first six weeks of the outdoor part of the experiment, weekly mean air temperature increased from +7.7°C to +13.0°C (minimum and maximum temperatures during the period were −2.5°C and +26.0°C, respectively). The outdoor part of the experiment was disrupted after six weeks due to unusually warm weather that killed several weevils.

Adults of the pine weevils in the outdoor experiment removed half as much bark as individuals in the corresponding treatments indoors (fig. 3). The average consumption per female per day was 37 (SE = 6) mm^2 in the outdoor experiment and 80 (SE = 4) mm^2 indoors during the first six weeks. Pairs of one male and one female *H. abietis* consumed 50% more than the single females indoors. The difference (40 mm^2) is assumed to be equivalent to the area consumed by the males, if effects of competition are neglected (see below). In the outdoor experiment, a somewhat larger difference (59%) was found, and the estimated area consumed by males was 22 mm^2 (fig. 3).

The mean consumption per weevil was fairly constant during the first six weeks of the experiment for all groups, but the consumption by groups feeding outdoors tended to slightly increase during this period (fig. 3). The different trends in consumption are reflected in the results of the repeated measures MANOVA-analysis (table 3), since the within-subject effects were significant for the factor time. The effect of the interaction time*experiment indicated that feeding rates indoors and outdoors varied through time in different ways (table 3). The feeding rate declined after the initial peak indoors, while it was more constant or slightly

Table 2. Pearson correlation coefficients for testing the strength of association between feeding and oviposition by female *Hylobius abietis* under laboratory conditions.

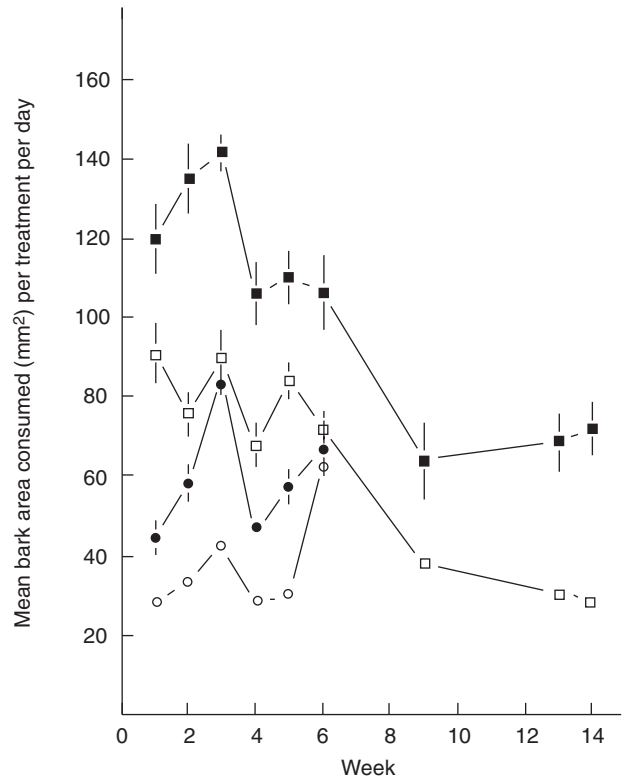| Time lag	n	r	P	Bonferroni confidence limit
No time lag	33	0.640	0.0001	0.013
Area + 1 week	31	0.496	0.005	0.025
Eggs + 1 week	31	0.521	0.003	0.017
Area + 2 weeks	29	0.349	0.060	0.05 ns
Eggs +2 weeks	29	0.701	0.0001	0.01

Fig. 3. Mean bark area (mm^2) consumed by *Hylobius abietis* per treatment per day in parallel indoor and outdoor experiments. Treatments are paired weevils (one female, one male) feeding together, or a solitary female (n = 12). Vertical bars denote standard error (SE). ■, Female + male indoors; ●, female + male outdoors; □, female indoors; ○, female outdoors.

Table 3. Repeated measures multivariate analysis (Proc GLM, SAS) of variance for bark area consumed by male and female pairs or solitary female *Hylobius abietis* per week under indoor or outdoor conditions.

Source, within-subject effects	df	Wilks' lambda	F	P
Time	5	0.331	16.2	0.0001
Time*experiment	5	0.534	6.97	0.0001
Time*treatment	5	0.517	7.49	0.0001
Time*experiment* treatment	5	0.860	1.30	0.284

Source, between-subject effects	df	Type III SS	F	P
Experiment	1	947.6	79.7	0.0001
Treatment	1	328.56	27.6	0.0001
Experiment* treatment	1	30.19	2.54	0.118
Error	44	523.38	11.9	–

Data from weeks 1–6 included. Experiment factor is indoor or outdoor experiment, treatment factor includes two treatments, solitary female or paired female and male.

increased outdoors (fig. 3). The factors experiment (indoors/outdoors) and treatment (female and male/solitary female) had a significant between-subject effect on area consumed (table 3) while the interaction (experiment*treatment) was not significant.

The total egg yield of *H. abietis* for the first six weeks was higher in the indoor than in the outdoor experiment (fig. 4). Females indoors laid on average 3.21 (SE = 0.14) eggs per day while females outdoors produced 1.27 (SE = 0.09) eggs per day over six weeks. After an initial peak in the first or second week, the mean number of eggs produced per female per day declined throughout this period in both experiments (fig. 4). During the remaining time in the indoor experiment, egg production decreased further and finally ceased. Females that were confined with a male showed a tendency to lay more eggs on average compared to females that were kept separate. Indoors, females from the paired male and female treatment laid on average 142.3 (SE = 11.9, *n* = 12) eggs over six weeks compared to 127.3 (SE = 12.8) eggs by isolated females. Outdoors, the corresponding figures were 57.2 (SE = 5.8) and 49.8 (SE = 6.1), respectively. However, no statistically significant difference in realized fecundity was found between the treatments, neither indoors nor outdoors (two-sample t-tests; indoors: t = 0.857, *P* = 0.60, *n* = 12, outdoors: t = 0.875, *P* = 0.59, *n* = 12). The average sum of eggs registered during a female's lifetime (range 7 to >29 weeks) was 172 (range 84–242, SE = 15.3) in the paired female and male treatment, whereas solitary females laid, on average, 156 eggs (range 56–265, SE = 18.5). The six females that survived for 29 weeks laid on average 204 (SE = 18.9) eggs per individual (range 56–418). Note that egg production samples were not registered in weeks 7–8 and 11, and that only eggs laid in the sand were counted after week 14. Hence these values are below maximum values.
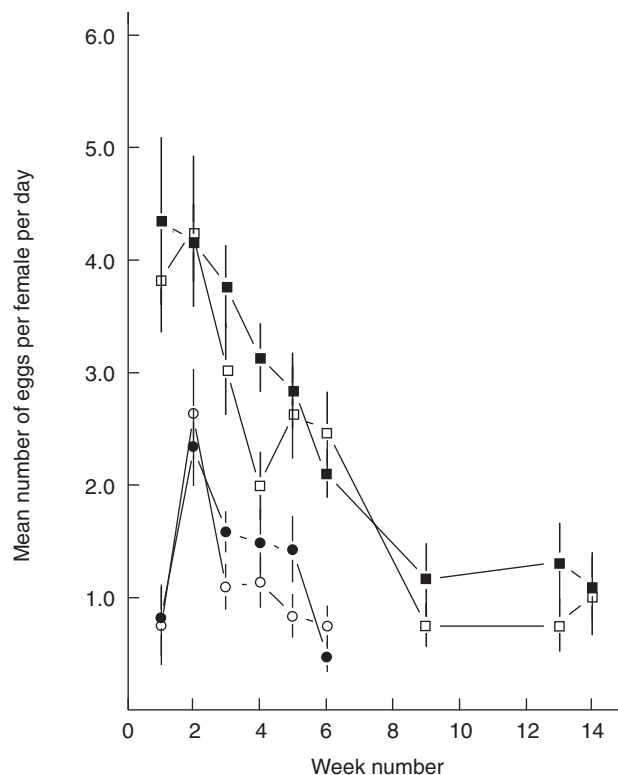
During the initial six weeks, solitary females indoors fed equally on the upper and lower surfaces of the pine pieces (fig. 5). In contrast, pairs of male and female *H. abietis* mainly fed on the upper surface. In the outdoor experiment, the weevils fed mostly on the upper surface of the feeding and breeding substrate. Most of the eggs were deposited in the bark of the pine pieces, both indoors and outdoors (fig. 6). About two thirds of the eggs laid in the breeding substrate were found in the upper bark surface in the indoor experiment (61%), while in the outdoor experiment the majority of eggs in the pine pieces were placed in the lower bark surface (91%) (fig. 6). Single females deposited a larger percentage of their eggs in the breeding substrate than the females paired with male weevils (indoors = 79% vs. 69% outdoors = 84% vs. 64%; Log –likelihood G-test, *P* = 0.0001). Further, solitary female weevils indoors distributed their eggs more evenly between the upper (51%) and lower bark surfaces compared to the females paired with males, which laid most of their eggs in the upper surface (71%).

The repeated measures analysis performed on average egg production per week for the first six weeks of the experiment detected effects of time and the two-way interactions time*experiment and time*treatment (table 4). The significance of these interaction terms indicated that the differences in the average number of eggs laid between the indoor and outdoor experiments, and between solitary and paired females, changed through time. Although the total number of eggs laid was somewhat higher in the groups with a male and female in both the indoor and outdoor experiments, there was no between-subject effect of treatment in the repeated measures analysis (table 4). Neither did the interaction (experiment*treatment) help

Fig. 4. Mean number of eggs laid per female *Hylobius abietis* per day over six weeks in parallel indoor and outdoor experiments, (*n* = 12). Vertical bars denote the standard error of the mean. ■, Female + male indoors; ●, female + male outdoors; □, female indoors; ○, female outdoors.
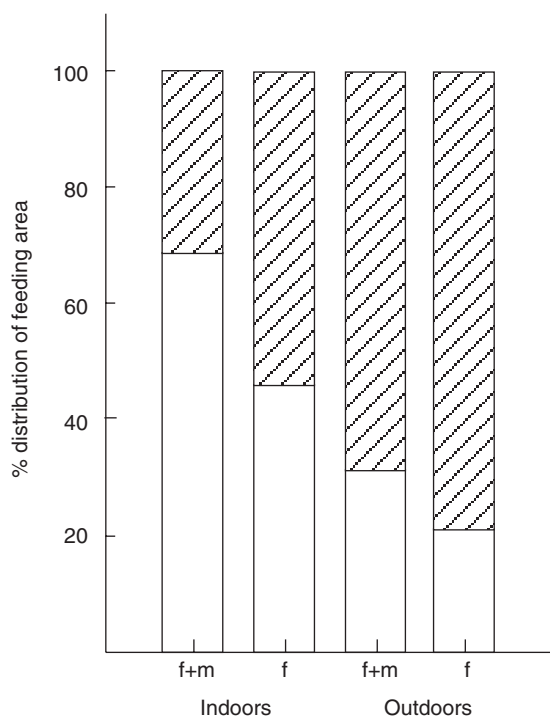

Fig. 5 Percent distribution of feeding sites of *Hylobius abietis* per treatment over six weeks under indoor and outdoor conditions on exposed (▨) and buried (▢) bark surfaces of pieces of Scots pine.
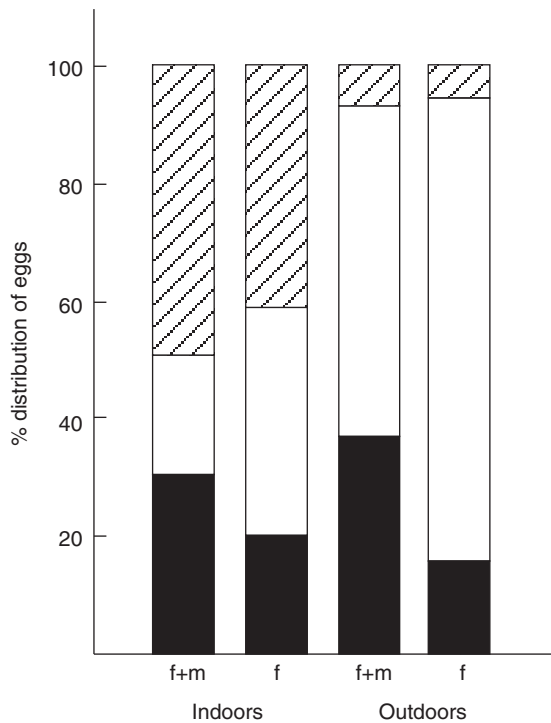
Fig. 6. Percent distribution of eggs by *Hylobius abietis* per treatment over six weeks under indoor and outdoor conditions. Eggs were recorded on the exposed (▨) and buried (□) bark surfaces of the Scots pine pieces and in the sand (■).

Table 4. Repeated measures multivariate analysis of variance (Proc GLM SAS) for number of eggs laid per female *Hylobius abietis* per week under different experimental conditions.

Source, within-subject effects	df	Wilks' lambda	F	P
Time	5	0.215	28.4	0.0001
Time*experiment	5	0.686	3.58	0.0093
Time*treatment	5	0.62	4.78	0.0017
Time*experiment* treatment	5	0.860	1.27	0.2959

Source, between-subject effects	df	Type III SS	F	P
Experiment	1	12634.4	66.1	0.0001
Treatment	1	283.4	1.48	0.230
Experiment* treatment.	1	17.16	0.09	0.766
Error	43	8221.8	191	–

Data from week 1–6 included. Experiment factor is indoor or outdoor experiment, treatment factor includes two treatments, solitary female or paired female and male.

explain the variance in average number of eggs laid. Thus, egg-laying showed similar responses in the two experiments over time, independently of treatment.

The area of bark consumed was positively correlated with the total number of eggs deposited (in sand or bark; Pearson correlation, r = 0.360, n = 72, P = 0.002), i.e. the more bark removed, the more eggs were laid during the same week. Feeding and oviposition were positively associated for the lower side of the pine piece for both treatments and for both indoor and outdoor experiments (table 5). The correlation was not significant for egg laying and feeding on the upper side. However, the association was fairly strong (giving high r-values) for the lower side of the substrate, indicating that some of these 'feeding-marks' may have been created in connection with oviposition. The total number of eggs laid in the piece of pine and the amount of feeding were associated for the treatments with paired male and female weevils (table 5). Further, the total number of eggs produced and the amount of feeding were clearly associated in all treatments (r = 0.24–0.44, P = 0.000–0.04, n = 71–72).

As the outdoor experiment was disrupted after six weeks, the seasonal feeding and oviposition rates of *H. abietis* were estimated from data collected in indoor treatments. The estimated feeding rate for 14 weeks outdoors was calculated by extrapolating from data for weeks 9, 10 and 12–14 in the indoor treatment. Fourteen weeks is roughly equivalent to the length of a breeding season. The average amount of feeding was estimated to amount to 23 mm^2 per capita per day, and females laid on average 0.8 eggs per day during the 14-week season.

Crowding

The additional presence of one or several juvenile females reduced the per capita consumption (mean bark area consumed) significantly (Kruskal-Wallis Test, df = 3, P = 0.036). One juvenile female feeding together with a reproductive female reduced the per capita consumption by about 10% compared to a reproductively active female feeding alone. Consumption, relative to solitary females, decreased by 46 and 53% in the presence of two and five juvenile females, respectively (table 6).

The percentage of eggs deposited in the breeding substrate declined with the number of juvenile (pre-reproductive) females placed together with the single reproductive female (table 6). However, the mean number of eggs produced per reproductive female was not affected by the density of juvenile females in the same container (Kruskal-Wallis test df = 3, P = 0.80).

Compensatory feeding after starvation

Weevils that had been starved for a week consumed more when they were fed the following week than did weevils with continuous access to food (table 7). The largest difference between feeding treatments was recorded after the second period of starvation for females in the exposed groups (40% higher consumption). No significant difference was found between groups exposed or in the shade. The lack of effect of the exposure treatment was probably due to cold and rainy weather during the experiment (table 8).

Discussion

The general temporal patterns of feeding and oviposition rates were similar in the two long-term experiments (experiments I and II). Females of *H. abietis* ate 40–50% more bark tissue than males during the first 6–10 weeks, which coincided with the main oviposition period. This difference later decreased when females reduced their rates of both feeding and egg-laying. Males fed at a fairly constant rate throughout the experiment. Thus, higher feeding rates in female *H. abietis* coincided with the main egg-laying period, indicating that energy costs were associated with egg

Table 5. Pearson correlation coefficients testing strength of associations between bark area consumed and number of eggs laid on upper (r_{upper}), lower (r_{lower}), and entire bark surfaces (r_{pine}) by *Hylobius abietis* under indoor or outdoor conditions.

Experiment	Treatment	n	Upper		Lower		Pine	
			r	P	r	P	r	P
Indoor	F+M	72	0.222	0.058 ns	0.678	0.0001	0.318	0.006
	F	72	0.168	0.156 ns	0.499	0.0001	0.191	0.104 ns
Outdoor	F+M	71	0.261	0.026 ns	0.591	0.0001	0.388	0.001
	F	72	0.147	0.216 ns	0.388	0.001	0.247	0.034 ns

Significance levels of *P*-values adjusted according to sequential Bonferroni test, ns indicates not significant *P*-values. F, female; M, male.

Table 6. The effect of crowding on the relative proportion of eggs deposited in sand and mean number of eggs laid per female *Hylobius abietis* during one week.

Treatment	n	Relative proportion of eggs laid in sand %	Mean number of eggs (SE)	Mean area (cm^{-2}) consumed per capita (SE)
0	10	42.1	15.9 (2.84)	2.71 (0.45)
1	9	52.9	14.0 (2.89)	2.50 (0.44)
2	10	56.5	13.8 (2.78)	1.45 (0.18)
5	10	68.5	12.7 (2.84)	1.28 (0.08)

Treatments consisted of one reproductive female and 0, 1, 2 or 5 juvenile pre-reproductive females.

Table 7. Mean consumption of bark (mm^2 of Scots pine bark area) per *Hylobius abietis* per week with continuous or discontinuous access to food ($n = 5$).

Week	Exposed to sunlight				Shade			
	Continuous		Discontinuous		Continuous		Discontinuous	
	F	M	F	M	F	M	F	M
1	88.4	163	–	–	130	118	–	–
2	94.8	68.8	140	112	69.6	73.6	94	109
3	103	167	–	–	72.4	51.2	–	–
4	68.0	112	153	187	134	140	197	150
Σ mean	88.5	128	147	149	101	95.7	145	129

F, females; M, males.

Table 8. Results of factorial analysis of variance testing the effect of periodic starvation and sun exposure on the mean consumption (mm^2 bark area) per female or male *Hylobius abietis* per week.

Source	df	Type III SS	f Value	P
Sex	1	22090	0.53	0.472
Exposure	1	158760	3.81	0.06
Continuous/discontinuous	1	835210	20.0	0.0001
Sex*exposure	1	222010	5.32	0.03
Sex*continuous/discontinuous	1	64000	1.53	0.2245
Exposure*continuous/discontinuous	1	118810	2.85	0.1012
Sex*exposure*continuous/discontinuous	1	29160	0.70	0.4093
Error	32	278480	–	–

All factors are fixed factors. Sex (female or male); exposure (sun/shaded); continuous/discontinuous (access to food).

production and that egg-laying was affected by both maternal age and depletion of resources, as previously shown in other insects (e.g. Yanagi & Miyatake, 2002).

The estimated feeding rates of 62 mm^2 per day for females and 40 mm^2 per day for males on Scots pine twigs at 20°C, appear to be in general accordance with results of other studies (Christiansen & Bakke, 1968; Pohris, 1983; Leather *et al.*, 1994). The cited studies measured feeding rates over a shorter time span than in the present study, and they did not compare feeding rates between females and males. In addition to higher consumption in females, the feeding behaviour of *H. abietis* differed between the sexes. Feeding marks made by males were larger, but fewer, than those made by females. Hence, females seem to have a more changeable feeding behaviour, probably due to activities related to oviposition and the placement of eggs. A study of locomotor and feeding activity has shown that female *H. abietis* spend more time feeding than their male counterparts (Merivee *et al.*, 1998).

The most prominent factor affecting feeding and oviposition of *H. abietis* in the present study was the local climate, as evidenced by the 50% lower feeding rate and 60%

lower oviposition rate found in the more variable and, on average, colder outdoor conditions compared to indoor conditions. In a choice experiment, *H. abietis* have previously been shown to prefer relatively high temperatures (range 19–28°C) for oviposition and feeding (Christiansen & Bakke, 1968). However, under natural conditions weevils avoid feeding on plants that are exposed to direct sunlight, and during hot periods they may adopt nocturnal feeding habits at exposed sites (Christiansen & Bakke, 1971). Furthermore, in a field study Sibul *et al.* (1999) found that locomotor activity was lowest around midday, when temperatures were high, and highest just before dusk when relative humidity was high and temperatures had fallen to 17–21°C.

Not only were the rates of feeding and oviposition affected by climatic conditions, the spatial distribution of feeding and oviposition of *H. abietis* also varied in relation to local climate. Outdoors, less than 10% of the eggs were laid on the exposed upper side of the piece of pine provided as a feeding substrate, while 40–50% were deposited on the exposed surface indoors. In contrast, in the outdoor treatments the weevils fed mainly (68–79%) on the exposed surfaces of the pine pieces. Indoors, the weevils fed considerably less (31–54%) on the exposed side (figs 3 and 4). The females apparently choose to oviposit at sites where the risk of desiccation of eggs and larvae was relatively low. This confirms that micro-climatic factors, such as temperature and humidity, have important influences on feeding and the sites chosen by ovipositing female weevils to place their eggs (cf. Christiansen & Bakke, 1968; Havukkala, 1979; Nordlander *et al.*, 1997).

While feeding rates were similar in the two long-term experiments, they differed in terms of numbers of eggs produced. Females in experiment I produced fewer eggs (0.7 eggs per day) than those in experiment II (1.9 eggs per day) over 3.5 months (14 weeks). This discrepancy may be due to the likelihood that some of the eggs laid in the sand and the Petri dishes were missed in experiment I. However, even though egg numbers were lower in experiment I, the temporal trends were similar in the two experiments. Similar temporal patterns in egg-laying, with an initial peak at the beginning of the season (May/June), have been shown in other laboratory studies of *H. abietis* (Novak, 1965; Guslits, 1969). During the peak egg-laying period, female weevils in these studies laid approximately two eggs per day. The realized fecundity and rate of oviposition were affected by the species of plant used for feeding and oviposition in a previous study of maternal effects (Wainhouse *et al.,* 2001). The cited authors found that weevils feeding on Norway spruce and Scots pine over 36 days laid 2.0 and 1.7 eggs per day, respectively, whereas the oviposition rate on Sitka spruce *Picea sitchensis* (Bong.) (Pinaceae) was 0.6 eggs per day. Furthermore, Salisbury (1998) found that *H. abietis* feeding on *Pinus nigra* (Arnold) (Pinaceae) laid on average 0.45 eggs per female per day over 49 days. The realized fecundity in our indoor treatment in experiment II was slightly higher than expected from results of other studies. Novak (1965) reported that females laid up to 150 eggs in 110 days (16 weeks) under laboratory conditions. In our study, females laid on average 196 eggs during a similar time span.

The crowding intensity appeared to be negatively correlated with the per capita consumption rate of bark. However, the reduced rate of feeding per capita in crowded conditions was most likely due to lower feeding rates in the pre-reproductive weevils present (cf. Sahota *et al.*, 1998). The

number of eggs laid per reproductive female was not affected significantly by crowding, but there was a significant effect on the distribution of eggs. Single reproductive females laid an increasingly large percentage of their eggs in the sand, outside the pine substrate, as the number of pre-reproductive female weevils increased. This finding could explain the seemingly conflicting results in the two long-term experiments, where the majority of eggs were laid in the sand in experiment I, but in the pine substrate in experiment II. In the first experimental set-up, a total of 220 weevils competed for the same combined food and oviposition substrate, while in the second experiment a single female or a female paired with a male shared the same resource. Similarly, in a study by Christiansen & Bakke (1968) higher *H. abietis* densities resulted in extended temperature ranges for both feeding and oviposition.

Hylobius abietis deprived food for a week feed more intensively when food becomes accessible again. Compensatory feeding following a period of starvation has also been shown in other species of weevils. For instance, when Mexican bean weevils *Epilachna varivestis* (Mulsant) (Coleoptera: Coccinellidae) were first starved and then given access to food in an investigation by Jones *et al.* (1981), their rate of feeding increased compared to that of a control group. The higher feeding rates were due to longer bouts of feeding rather than increased frequency of feeding bouts. It is not known if this also applied to the compensatory feeding by *H. abietis* reported here.

Larvae that hatch from eggs outside the larval food substrate are able to migrate through the soil and to find a food source by themselves (Nordenhem & Nordlander, 1994). Nordlander *et al.* (1997) found that in a peat soil eggs were laid in the soil rather than in the roots near stumps. Eggs laid at some distance from a root may be less exposed to predators searching the roots for prey, and avoid being destroyed by conspecific adults or larvae feeding nearby. The potential importance of the latter factor is indicated by the distribution of feeding marks and eggs in experiment II, since most feeding was done at the opposite side of the pine piece from that where the majority of eggs were laid. Furthermore, in some cerambycid beetles ovipositing females mark the oviposition scar in the bark with substances that deter egg-laying by other females (Anbutsu & Togashi, 2001).

Feeding and oviposition rates in the outdoor experiment should be close to those that occur in natural situations. Based on the results from the two long-term feeding experiments it is estimated that the daily average per capita feeding rate is 23 mm^2 under semi-natural conditions. This daily average was calculated by taking into account the differences between sexes, indoor and outdoor conditions and the temporal changes observed over 14 weeks. Applying similar calculations to egg-laying gives a daily average number of 0.8 eggs per female *H. abietis* outdoors. However, outdoor data were gathered during a period of fairly warm weather and weevils were confined to boxes with permanent access to abundant food and oviposition substrate. Therefore, it should be noted that the feeding and egg-laying rates estimated here might be lower under natural situations where conditions are less ideal.

Applications of results

The density of *H. abietis* weevils during the summer in a fresh clear-cutting in southern Sweden has been estimated to

be c. 14,000 individuals per hectare (Nordlander *et al.*, 2003a). Using the estimated consumption of 23 mm^2 (per individual per day) an area of 0.32 m^2 bark tissue per hectare would be consumed each day by these weevils. The bark area of a newly planted, containerized seedling suitable for feeding is about 1000 mm^2, and the planted seedling density commonly used in practice is about 2500 seedlings per hectare. Thus, the seedlings provide a food resource of 2.5 m^2 per hectare, which would be enough to feed the estimated weevil population for a maximum of eight days, provided the entire suitable bark area on all seedlings was consumed. In reality, seedlings are usually killed long before they are completely debarked (Örlander *et al.*, 1997), and a fraction of the seedlings almost always escapes damage (Nordlander, 1991). Thus, the food requirements of *H. abietis* at an average density during a breeding season will greatly exceed the total available seedling resources. When adult *H. abietis* arrive at a fresh clear-cutting they initially feed in the canopy of trees at the forest edge (Örlander *et al.*, 2000). During the main part of the season other food sources, such as roots of established coniferous trees, are required to feed them (Örlander *et al.*, 2001; Nordlander *et al.*, 2003a,b).

If it is assumed that there are 700 conifer stumps per hectare in a fresh clear-cutting, and that 70 eggs can be produced per female during a 10-week egg-laying season, then at the estimated population density of 14,000 weevils per hectare (sex ratio 1:1), the total number of eggs laid per hectare over 10 weeks will be approximately 490,000 eggs. The bark area of roots suitable for *H. abietis* larvae has been estimated to amount to approximately 3000 m^2 per hectare in a clear-cutting in southern Sweden (unpublished data). In late July, the density of eggs and larvae would then be 163 per m^2 root surface area. This estimated egg density corresponds well with the larval density of ~150 larvae per m^2 root surface area reported by Nordlander *et al.* (1997).

The ability to accurately forecast the risk of plant damage caused by *H. abietis* requires the development of reliable methods to estimate weevil densities and to integrate factors such as access to alternative food sources, weevil mortality and the actual consumption by weevils in a predictive model. The basic estimates of feeding and oviposition rates presented here should be useful in risk-prediction models when feeding habits, the survival of adult weevils and their progeny, and migration patterns of *H. abietis* have been further investigated.

Acknowledgements

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