FOLIA OECOLOGICA – vol. 38, no. 1 (2011). ISSN 1336-5266

Response of walking spruce bark beetles *Ips typographus* to host odours

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Abstract

Jakuš, R., Blaženec, M. 2011. Response of walking spruce bark beetles *Ips typographus* to host odours. *Folia oecol*., 38: 38–45.

A two-choice walking bioassay olfactometer was used to asses the response of walking *Ips typographus* (L.) towards the odours from logs of Norway spruce (*Picea abies* [L.] Karst.) subjected to various treatments. The odour coming from fresh log from standing, unstressed trunk was unattractive or slightly repellent to males over clean air in bioassay. The same odour was neutral or slightly attractive to females. Storing of a log for one month led to increased attractiveness to both sexes. The odour from log after bark beetle breeding was slightly more attractive to both sexes over clean air, excepting very low level of source contact in males. Results on overall attraction are more unclear for females. The level of source contact was the main difference between the males and females. The females manifested higher levels of source contact than males.

Key words

Ips typographus, Norway spruce, primary attraction

Introduction

Two general theories were used to explain the host searching strategies in bark beetles. Pioneer beetles are either attracted to find susceptible hosts in response to olfactory stimuli from stressed trees (primary attraction), or they land on trees at random and select the tree to attack in response to gustatory stimuli (Rudinsky, 1962; Wood, 1982; Gries et al., 1989; Byers, 1996). Both ways of host location can be seen as subsequent steps after habitat (stand) selection, a behaviour which is likely a balance of positive input from host (conifer) kairomone and negative input from non-host (angiosperm broad-leafs) volatiles, NHV (Zhang and Schlyter, 2004). Führer et al. (1997) demonstrated that attacks of *I. typographus* occur on vigorously growing trees suffering seriously from a sudden stress. In endemic conditions, *I. typographus* utilises downed trees (Jakuš, 1995). According to Raffa et al. (2008), the tree-killing bark beetle species display flexible host-selection strategies. When the populations are low, avoiding healthy trees is adaptive. Vigorous trees pose a risk to beetles because of their superior defence. Physiologically compromised trees pose less risk, but are sparsely distributed in space, ephemeral in time, and nutritionally suboptimal. In the first stage of the outbreak in mountainous conditions (incipient epidemic population), *I. typographus* utilises predominantly wind thrown trees – mostly on fresh forest edges (spot initialisation), like in endemic conditions. Once populations increase, the discriminating behaviour becomes less adaptive because of the greater likelihood of recruiting enough beetles to overcome healthy trees. Once beetles have successfully killed a tree, because of low tree resistance and/or high beetle population, they may rapidly switch to the closest trees (Anderbrant et al., 1988). Such trees may likely become foci of aggregation and could be killed regardless of their resistance level. Originally vigorous trees provide a largely vacant resource, and in general they are the most suitable nutritionally for the beetles – because of their thick phloem. The beetles are more likely to attack trees adjacent to the already initialized spots (Jakuš et al., 2003).

In Norway spruce (*Picea abies* [L.] Karst.), it appears that disturbances of the water balance or storing of cut log per se bring about the emanation of volatile substances to which *I. typographus* (L.) shows olfactory responses (Merker, 1956). Most of the European entomologists state that these materials are not known

(Rudinsky, 1962). Mathematical simulations showed that in random search, maintenance of the population required a flight capacity, population size, and host tree abundance which are unlikely to occur at sub-outbreak levels in nature (Gries et al., 1989). The aim of this study is to test the response of *I. typographus* to various host odours in a laboratory bioassay.

Material and methods

Plant material

Five logs were cut from middle parts of trunks of two Norway spruce trees from Romefåsen in southern Sweden. The trees were dominant ones in an about 50-year-old commercial spruce plantation. The diameter at breast height was about 20 cm. The trunk was sawn into logs 20 cm high. Each log was packed into a polyethylene bag. Both ends of the logs were sealed with paraffin. The logs had diameter about 15 cm. The first pair of logs was cut one month before the experiment (May) and stored at +4 °C. The first log (Nr. 1) had an undamaged surface, while the second one (Nr. 2) was cut with a knife. About 10 strips about 1 cm wide and 10 cm long were peeled from the bark surface to allow free release of volatiles. The second pair of logs was tested immediately after the cutting (June). Log Nr. 3 was used as undamaged and log Nr. 4. as cut in the similar way as the stored logs (Nr. 2). The log Nr. 5 was a log after bark beetle breeding, coming from the continuous bark beetle culture.

Animals

The beetles were provided from a continuous laboratory culture on natural material in Alnarp as described earlier (Anderbrant et al., 1985 and Schlyter and Anderbrant, 1993). For bioassays, the beetles were collected every 12 hours and stored immediately at 98% relative humidity, +4 °C, in plastic cups with pieces of towel paper, exposed in a flight chamber (Schlyter and Löfquist, 1986) for periods of 48 hours. Then they were sex-separated (Schlyter and Cederholm, 1981). Five groups of males and five groups of females were used in the experiment. Each group consisted of 15 beetles.

Bioassay

The results reported here concern the use of a two-choice walking bioassay olfactometer in June. The two-choice walking bioassay olfactometer was made by adaptation of an open area walking bioassay olfactometer. Earlier pilot studies with an open area walking bioassay olfactometer (Byers and Wood, 1981) could not be considered as valid because of low male response to the attractants (Schlyter et al., 1995). This bioassay is modified for an open area walking bioassay olfactometer (Schlyter et al., 1995). It includes a two-choice platform (Fig. 1), two glass stimulus containers, plastic tubes and glass joints. The new two-choice platform was made of 5 mm bright and 3 cm high plastic walls. In order to achieve uniform light conditions, it was covered by transparent plastic plate covered by white paper. Each glass stimulus containers had a volume of 3 l. The experiments were performed in an environmental chamber at 22–23 °C. The air used in treatment and control, was drawn from an environmental chamber into a charcoal filter, through stimulus containers, through plastic tubes and was exhausted in glass tubes at bioassay. The balance of flows in treatment and control was controlled by flowmeters. The wind speed was about 1 m s^{-1}. The approximately laminar flow of the pure air was due to charcoal filtering of reduced-pressure compressed air let into baffle with spaced 2 mm holes. The complete two choices platform and plastic tubes were washed in 96% ethanol before each series of tests. Glass stimulus containers were washed in ethanol and heated to 150 °C. Paper parts were replaced. A mix of synthetic pheromone components, MB & cV at ratio 150 : 1 were used as a neat solution evaporating from 50 µl Microcaps ® (Inner Ø 0.80 mm). The release rate (g min^{-1}) was estimated based on the retreat of meniscus over time (1.7×10^{-6}).

Test procedure

One hour before the experiment, the beetles were placed in an experimental chamber in plastic Petri dishes with wet filtering paper. In the test, the beetles were released in groups in centre of the area opposite to the odour source (Fig. 1). Then, the platform was covered with a plastic cover. The duration of test was 10 minutes. The beetles leaving the platform at the part belonging to treatment or control were collected and counted. At the end of the test, the beetles in different parts of the apparatus were counted. The beetles reaching the line of the middle wall were scored as responding. The contact of beetles with the odour source was considered as source contact. When all the groups were tested, the bioassay was washed. Then all the groups of separate sexes were joined together and tested on clean bioassays.

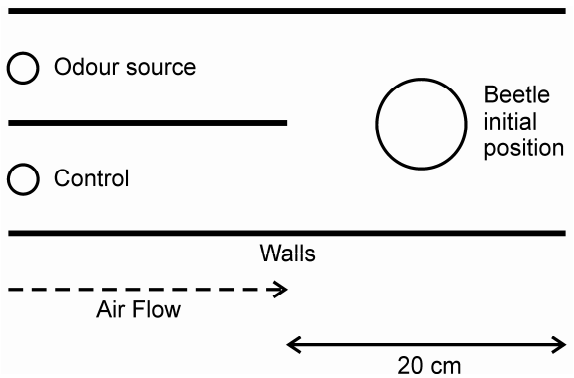

Fig. 1. Two-choices platform.

Statistics

The following indices were used to quantify the response: The percentage of responding beetles preferring the treatment (% T), calculated as % T = (T/(T+C)) ×100, where T = number of beetles responding to treatment and C = number of beetles responding to control. The percent of beetles contacted the odour source (% SC) calculated as % SC = (TSC/(TO + CO)) ×100, where TSC = source contact at treatment, TO = number of beetles leaving platform at treatment part, CO = number of beetles leaving platform at control part.

Statistical analysis of these indices was done by a parametric test (ANOVA) and Duncan's multiple range test of arcsin $p^{0.5}$ transformed data (SPSS/PC procedure ONEWAY).

In order to compare the treatment and control at separate runs of tests, the following indexes were calculated: % T, % C = (C/(T+C)) × 100, % SC, % SC − C = SC = (CSc/(TO + CO)) × 100, where CSc = source contact at control. These indices were tested by paired t-test of arcsin $p^{0.5}$ transformed data (SPSS/PC procedure t-test).

Results

The results are shown in Figs 2, 3, 4, 5. The males responded more significantly to stored and damaged log with or without pheromone than to the control (Fig. 2). The stored and damaged log without pheromone showed a slightly higher attraction than stored and damaged log with pheromone (Figs 2, 3). The lowest response was to the fresh log. The difference between the damaged stored log and the fresh log was statistically significant. The response to the fresh undamaged log was slightly lower than to a blank. The response and the percentage of source contact were lower in the fresh undamaged log than in the fresh damaged log. The percentage of source contact to the fresh damaged log was slightly higher than to the pheromone, to the stored damaged log with pheromone and to the stored log. A log after breeding (old) showed higher response than the fresh log. The percentage of source contact was the lowest at the log after breeding.

The females were significantly more attracted by stored and damaged logs with or without the pheromone than by the blank (Figs 4, 5). The stored and damaged log with the pheromone showed slightly higher attraction than the stored and damaged log without the pheromone. In the case of source contact, the pheromone alone was slightly less attractive than the stored log without or with pheromone. The females responded significantly less to the fresh log than to the control (pairs treatment and control; Fig. 4). The lowest response was in case of the fresh damaged log. The difference between the stored and damaged logs and the damaged fresh log was statistically significant. The response in case of the fresh damaged log was slightly lower than to the blank (clear air in both parts of platform). The response and

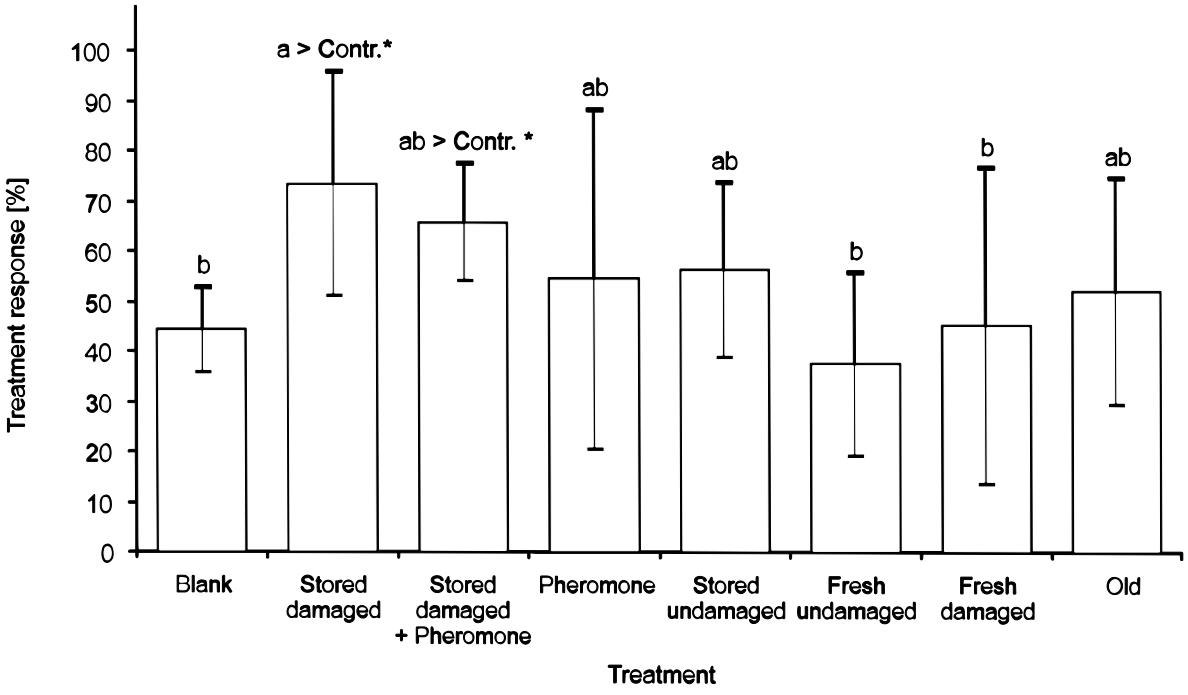

Fig. 2. Response of males to logs. Bars (+ standard error) with the same letters are not different by Duncan's multiple range test. " >Contr.* " denotes pairs treatment and control statistically different by t-test at P = 0.05.

the percentage of source contact were lower at the fresh damaged log than the fresh undamaged log. The log after breading (old) showed higher attractiveness than the fresh damaged log and lower attractiveness than the fresh undamaged log.

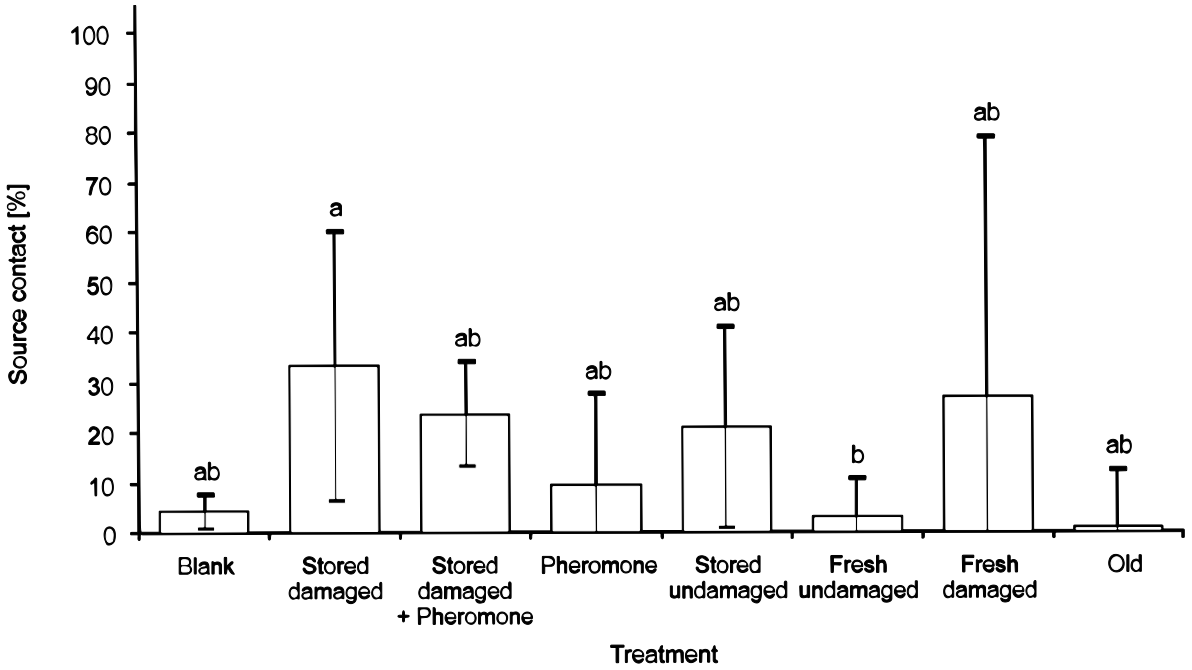

Fig. 3. Percentage of source contact of males to logs. Bars (+ standard error) with the same letters are not different by Duncan's multiple range test.

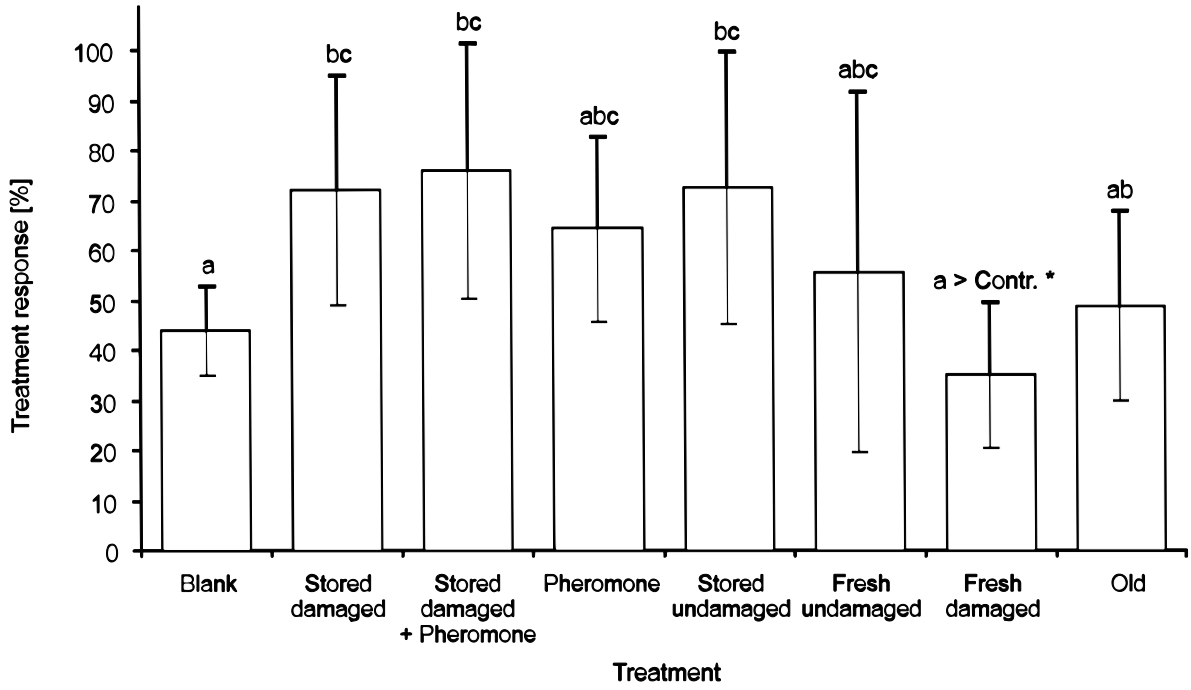

Fig. 4. Response of females to logs. Bars (+ standard error) with the same letters are not different by Duncan's multiple range test. " <Contr.* " denotes pairs treatment and control statistically different by t-test at P = 0.05.

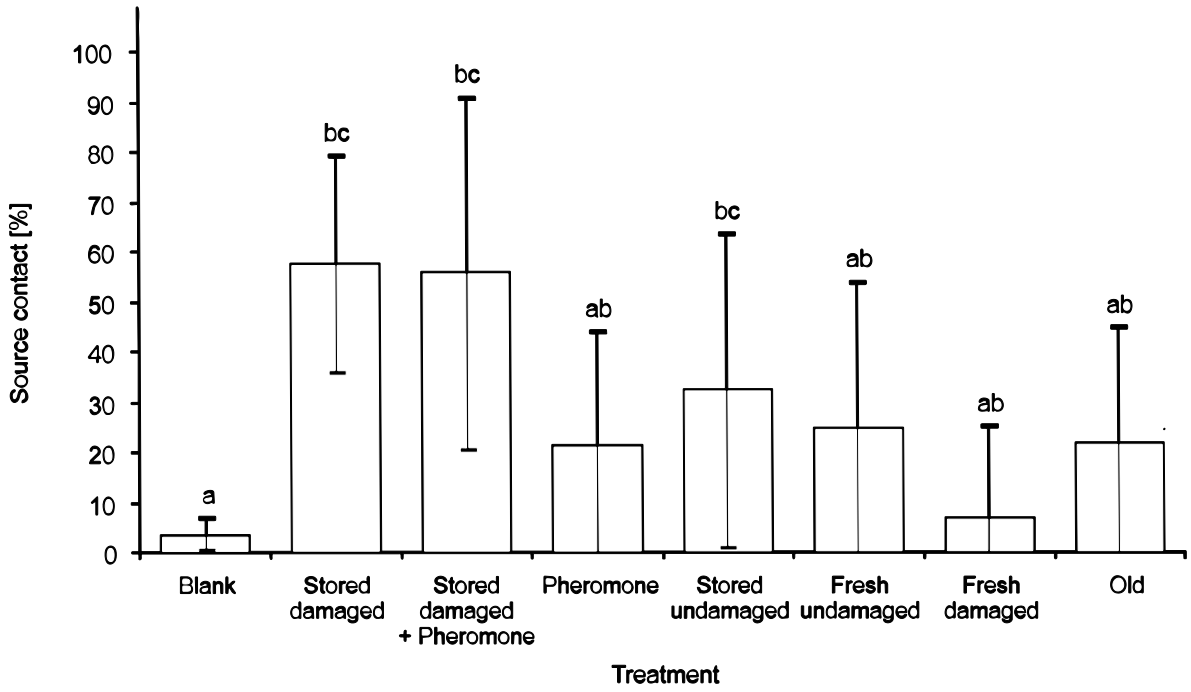

Fig. 5. Percentage of source contact of females to logs. Bars (+ standard error) with the same letters are not different by Duncan's multiple range test.

Discussion

The results show, that the odour coming from a standing, unstressed trunk (fresh log) was unattractive or slightly repellent to males in the bioassay. The same odour was neutral or slightly attractive to females. Mechanical damage to standing, unstressed trunk (damaged fresh log) led to a slight increase of attraction in males and to repellence in females. Storing of log for one month increased attractiveness for both sexes. The damaged stored log was slightly more attractive than the undamaged to both sexes. The stored log simulates a trap tree or a downed tree.

According to Merker (1956), healthy turgid trees are low attractive or repellent to *I. typographus*. Gossenauer-Marohn (1988) showed that the odour from a fresh cut log is not statistically significantly attractive to walking *I. typographus* in bioassay. Our results are in agreement with both of these authors. The avoidance of healthy conifers by bark beetles is also shown by Führer et al. (1997) and Raffa et al. (2008).

Damaging of relatively fresh spruce logs led to increased number of bark beetle attacks (Johann, 1986a). At our bioassay, damaging of the fresh log caused a slight increase of attraction to males. The observed repellence to females is not in disagreement with the Johann's findings (1986a).

Cut spruce trunks are attacked by bark beetles after 6–8 weeks. This traditional forestry knowledge is the background for the method of trap trees (Johann, 1986a). The odour from the stored log was attractive to both sexes in our bioassay. Damage to the stored log led to a slight increase of attraction to both sexes. Johann (1986a) showed that damage to a stored log led to decreased number of bark beetle attacks. This disagreement with our results may be explained by possible different timing of experiments.

The odour from the log after bark beetle breeding was slightly attractive to both sexes, with very low level of source contact of males. This positive response may be explained either by habitat preference of bark beetles or by trace of pheromone remains. The odour from the old log was more attractive than the clear air.

A synthetic pheromone alone is several times more attractive than spruce logs in field conditions (Bakke, 1977). Several authors (Bakke 1970, 1977; Rudinsky et al., 1971; Švihra, 1972) showed several times higher levels of *I. typographus* attraction to bark-beetle-invaded logs than to logs alone in field conditions. Much higher attraction than pheromone or than spruce log separately was achieved with the combination of fresh logs or logging debris with a synthetic pheromone (Austrara et al., 1986; Johann 1986a, 1986b). The relative disagreement of results from our bioassay with the results from field experiments could be explained by the differences in the odour situation between the bioassay and a field conditions. In case of bioassay, the atmosphere and the control were a clean air. The atmosphere of spruce forest contains monoterpenes in considerable concentrations (Steinbrecher et al., 1990).

The catches of *I. typographus* are the highest in pheromone traps placed in clearcut areas in spruce forests logged in the previous winter and gradually drop with the time after the felling. Agricultural areas manifested the lowest catches (Bakke 1985; Sanders 1987). These findings are in agreement with our results. In case of a pheromone trap situated on a clear-cut in spruce forest logged the previous winter, flying beetles are probably exposed to an odour similar to the odour coming from a stored log combined with pheromone odour in a bioassay. The response of a flying bark beetle to a pheromone trap in agricultural land is similar to the response to an odour coming from a pheromone source in a bioassay. The concentration of primary attractants in suitable spruce forest air is probably much higher than the concentration of secondary pheromones. *I. typographus* probably needs primary attractants mainly for habitat selection and attraction of pioneer beetles. Therefore, the mixture of primary and secondary attractants and avoidance of NHV (Zhang and Schlyter, 2004) leads to colonization of a particular host substrate. The pheromone alone provides beetles with confusing information.

The catches of *I. typographus* are relatively low in old clear-cuts (Bakke, 1985) or in pheromone traps near to old dry spruce log (Johann, 1986b). Our results from testing the odour of the old log formerly colonised by bark beetles correspond with the previous results. A slightly higher response of males to odour coming from the old log may be explained as the habitat selection.

The level of the source contact was the main difference between the males and females. The females displayed higher level of source contact than males. Males are a pioneering sex, they need to find a suitable site for a nuptial chamber. A male needs semiochemicals for broader orientation. A female needs to find a gallery to enter. The higher level of female source contact perhaps suggests that the females are orienting towards short-range chemical signals released by males at the entrance holes (Paytner et al., 1990). The response of males to the damaged stored log was slightly higher than to the damaged stored log with pheromone. On the other hand, the response of females to the damaged stored log was slightly lower than to the damaged stored log with pheromone. This difference could be explained by the difference in behaviour of pioneer males and females searching for galleries with males.

Conclusions

The odour coming from a fresh log was unattractive or slightly repellent to males over a clear air in the bioassay. Storing of the log for one month led to increased attractiveness for both sexes. The odour from a log after bark beetle breeding was slightly attractive to both sexes over clear air. The level of source contact was the main difference between the males and females. The females had higher level of source contact than males.

Acknowledgement

This publication is the result of the project implementation: Centre of Excellence: Adaptive Forest Ecosystems, ITMS 26220120006, supported by the Research & Development Operational Programme Supported by the ERDF. The authors wish to thank professors Löfquist and Schlyter (Swedish University of Agricultural Sciences Alnarp) for enabling experiments at the Department of Chemical Ecology and the European Environmental Research Organization for enabling the study stay in Sweden.

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Reakcia lezúceho lykožrúta smrekového *Ips typographus* (L.) na vône šíriace sa od hostiteľa

Súhrn

Pomocou dvojcestného rozhodovacieho olfaktometra sme skúmali reakciu lezúceho lykožrúta smrekového *Ips typographus* (L.) na vône z rôzne pripravených a upravených sekcií kmeňa smreka (*Picea abies* [L.] Karst.). Vôňa čerstvej sekcie zo zdravého smreka bola neatraktívna, alebo až mierne repelentná pre samcov. Tá istá vôňa bola neutrálna, alebo mierne atraktívna pre samičky. Vôňa zo sekcie kmeňa, skladovanej jeden mesiac, bola atraktívna pre obe pohlavia. Vôňa z kmeňa po skončení vývoja lykožrúta smrekového bola atraktívnejšia ako čistý vzduch. Výsledky pre samičky boli menej jasné. Hlavným rozdielom medzi samcami a samičkami bol výrazne väčší kontakt samičiek so zdrojom vône.

Received January 18, 2011
Accepted March 8, 2011