# Host Selection in *Tomicus piniperda* L.: Composition of Monoterpene Hydrocarbons in Relation to Attack Frequency in the Shoot Feeding Phase

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The aim of this study was to investigate the host selection capacity of the pine shoot beetle, *Tomicus piniperda,* in the shoot-feeding phase and analyze the chiral and non-chiral host volatiles by means of GC-MS and 2D-GC in five *Pinus* species originating from France (*Pinus sylvestris, P. halepensis, P. nigra laricio, P. pinaster maritima, P. pinaster mesogeensis*). Dominating monoterpenes were (–)-$\alpha$-pinene, (+)-$\alpha$-pinene, (–)-$\beta$-pinene and (+)-3-carene. The amounts of the enantiomers varied considerably within and among the species. In a principal component analysis-plot, based on the absolute amounts of 18 monoterpene hydrocarbons, separation of the pine species into two groups was obtained. *P. halepensis* and *P. sylvestris* were grouped according to the amount of (+)-$\alpha$-pinene and (+)-3-carene, while *P. nigra laricio*, *P. pinaster maritima* and *P. pinaster mesogeensis* were grouped according to (–)-$\alpha$-pinene and (–)-$\beta$-pinene. *P. nigra laricio* was the species most attacked and *P. halepensis* the one least attacked by *T. piniperda*.

*Key words:* Host Preference, *Tomicus*, (–)-$\alpha$-Pinene

## Introduction

Bark beetles are important in natural ecosystems as they attack weakened or dead trees, thus contributing to the decomposition and mineralization in the forest, but some species are tree killers and can cause extensive damage (Berryman *et al.,* 1984; Christiansen *et al.,* 1987). Most tree killing bark beetles are vectors of blue stain fungi (*Ceratocystis* sp., *Leptographium* sp. or *Ophiostoma* sp.) which are inoculated in the host during the bole-attack. They can be important for the beetle establishment and contribute in killing the tree. In Scandinavia and elsewhere in Europe *Tomicus piniperda* (L.) causes huge growth losses due to the shoot-feeding in the crowns of the pines. However, the attacks on the bole usually kill only weakened trees and do not cause extensive damage. In Asia, on the contrary, *T. piniperda* seems to be able to attack and kill healthy trees over large areas.

Pine trees contain large amounts of terpenes, a few of which are known to be important for conifer bark beetle interactions. The sensitivity towards different kinds of monoterpenes differs within populations of bark beetles, depending on whether the composition of the terpenes represents their respective host trees or not (Byers, 1995). *T. piniperda* is strongly attracted by the host monoterpenes released through an injury on the tree or through the holes made by the pioneer beetles in the trunk. This can in fact induce a mass attack comparable to that induced by the aggregation pheromones in other species (Långström *et al.*, 1992; Byers, 1995).

In the shoot-feeding phase, *T. piniperda* can attack healthy trees and the mechanisms of tree and shoot choices are unknown.

The aim of this study was to investigate the host-finding preferences of *T. piniperda* in the shoot-feeding phase, and to determine whether or not the monoterpene compositions of five *Pinus* species could explain the host-finding preferences observed.

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## Material and Methods

### *Plant material*

The beetle choice experiments were conducted in France in 1999 in five *Pinus* species, and the chemical analyses were carried out in Sweden. Five pine species were used: *Pinus sylvestris* Linnaeus (Scots pine), *Pinus halepensis* Miller, *Pinus nigra laricio* (Poiret) Maire (from Corsica), *Pinus pinaster maritima* Aiton, and *Pinus pinaster mesogeensis* Aiton (5–6 years old).

### *Collection of beetles*

Four populations of *T. piniperda* were used in the tests, namely: *T. piniperda* from Orléans captured on *P. sylvestris, T. piniperda* from Bordeaux captured on *P. pinaster maritima, T. piniperda* from Comps (southeastern France) captured on *P. sylvestris* and *T. piniperda* from Lubéron (southeastern France) captured on *P. halepensis.*

In each natural habitat of the species used, the trees were cut down in December 1998 and divided into one-metre long logs. The logs were covered with paraffin at both ends to avoid drought and left in the forest. In January–February 1999 the logs were attacked by *T. piniperda*. After being attacked, the logs were left in the forest for 10–20 d (to ensure attack), brought to the laboratory and stored at 3 °C. 2 d before the experiment the logs were moved to a room with a temperature of 18–24 °C, each in a screened bag. Emerging offspring leaving the logs was used in the experiments.

### *Host selection experiments*

Host selection experiments took place in a greenhouse at the Arboretum des Barres (Nogent-sur-Vernisson, France). The greenhouse was divided into two equal chambers, 6 m × 4 m × 2 m (48 $m^3$), where two experiments were performed simultaneously. The trees were cultivated in pots and 5–6 years old. In total, 135 trees were used during four tests. For characteristics of the trees see Table I. The trees were brought to a merchandiser from their natural habitat and then to a nursery, where they were placed in groups of three from the same species on the southern side of the chamber on a metal bench, 70 cm above the ground level and exchanged for each test. One test consisted of two assays, one per chamber. During one assay, a group of 20 newly emerged insects from the same origin were used. The beetles were allowed to choose between five pine species. Five assays were made for each population of beetles. At each assay the place of the three species changed according to a schedule, so that one species was never twice in the same place. The insects were placed in small boxes and released in the middle of the chambers. The trees were checked for attacks 2 d later.

### *Odour collection*

Odour collection was made using 100 mg of Porapak Q (mesh 50/80) packed in a glass vial (50 × 19 mm). The polymer was fixed with deactivated glass wool at the top and bottom in the plug. During the odor collection, the plugs were connected to a Laboport air pump Type N86 KN18 (KNF Neuberger, Freiburg, Germany) with plastic tubes that had no odour. The airflow was adjusted to 30–40 ml/min with a Varian digital flow meter. The tree odours were collected for 24 h with one plug in the middle of each group of trees of the same species. The background air (control) in the greenhouse was also collected. During the odour collection the trees were covered by cotton tissue in order to enrich the monoterpenes. The control was also covered with cotton tissue, showing

Table I. Morphological characteristics of the pine trees used in host selection experiments. ANOVA followed by Scheffé tests at the 0.05% level. In a given row, numbers with the same letter do not differ significantly.

| | *Pinus halepensis* | *Pinus sylvestris* | *Pinus pinaster maritima* | *Pinus pinaster mesogeensis* | *Pinus nigra laricio* | *P* |
|---|---|---|---|---|---|---|
| Height [cm] | 116.5 ± 7.6 (a) | 122.9 ± 5.6 (a) | 130.8 ± 6.9 (a) | 64.1 ± 5.7 (b) | 120.0 ± 7.6 (a) | 0.0468 |
| Diameter of leader shoot [cm] | 4.5 ± 0.6 (b) | 7.8 ± 0.6 (b) | 5.6 ± 0.7 (b) | 5.2 ± 0.6 (b) | 10.2 ± 0.6 (a) | 0.0314 |
| Total number of shoots | 156.2 ± 73.1 (ab) | 259.8 ± 57.7 (a) | 69.3 ± 32.3 (ab) | 21.2 ± 12.7 (b) | 193.9 ± 59.4 (ab) | 0.0029 |

whether there were any odours released from the cotton itself that would interfere with the collections from the trees. The cotton tissues were removed after 24 h, at the same time as the beetles were released, to give them free access to the shoots.

*Extraction*

After 24 h the volatiles were desorbed by extraction with hexane (p.a., Merck). During the extraction, the plug was filled with hexane and 200 and 400 $\mu$l, respectively, were collected in separate vials at each extraction. The samples were stored in a freezer at −18 °C.

*Regeneration of Porapak Q*

Before each collection the plug was rinsed by hexane followed by dichloromethane. The same amount of each solvent (3.8 ml) was used. After the regeneration, the plugs were dried during 2 min with pure nitrogen and covered with aluminum in order to protect from UV light. Porapak Q was covered with aluminum during the odour collection, extraction and regeneration.

*Chemical analyses*

All of the samples were analyzed on a Varian 3400 GC by using a DB-WAX fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 $\mu$m; J & W Scientific™). Helium was used as the carrier gas with 2 ml/min. The temperature program was as follows: 42 °C – 1 min – 3 °C/min to 70 °C – 0.1 min – 10 °C/min to 160 °C – 0.1 min – 20 °C/min to 200 °C – 20 min. Injector temperature was 180 °C and detector temperature was 200 °C. *n*-Decane was used as internal standard; 6 $\mu$l (1/50 000) were added to each sample.

To determine the enantiomeric composition of the chiral monoterpene hydrocarbons, the samples were analyzed using a two-dimensional Varian 3400 GC instrument (Borg-Karlson *et al.,* 1993). The GC program for chiral analyses was: 55 °C for 15 min, followed by 1 °C/min to 77 °C and kept at this level for 15 min. Injector temperature was 50 °C and the detector temperature was 160 °C. The columns were (1) Cyclodex-B (permethyl-$\mu$-cyclodextrin/DB-1701, length 30 m, 0.25 mm i.d., film thickness 0.25 $\mu$m; J & W Scientific™) and (2) Chirasil-DEX CB (permethyl-$\mu$-cyclodextrin, chemically bound with a polydimethyl siloxane, length 25 m, 0.25 mm i.d., film thickness 0.25 $\mu$m; Chrompack™).

*Data analyses*

Terpene results were based on the values of 18 compounds [(+)/(−)-$\alpha$-pinene, (+)/(−)-$\beta$-pinene, (+)/(−)-limonene, 3-carene, (+)/(−)-$\beta$-phellandrene, myrcene, (+)/(−)-camphene, *p*-cymene, tricyclene, (+)/(−)-sabinene, terpinolene and *cis*-ocimene] analyzed on 20 samples (one sample = one group consisting of three trees). The enantiomeric composition was not determined for 3-carene, as in earlier work only the (+)-enantiomer had been found in *P. sylvestris* (Hiltunen and Laakso, 1995; Sjödin *et al.,* 1996; Wibe *et al.,* 1998).

The areas of the GC-peaks from non-enantiomeric analyses were divided by the internal standard peak area by volume [($X$/0.3 ml), where $X$ was the volume of extracted effluent from the Porapak vial]. The chiral monoterpenes were multiplied by the ratio between (+)- and (−)-enantiomers obtained from the GC-data with the chiral column.

Absolute amounts (calculated by the use of an internal standard from GC-data) of the host monoterpenes were subjected to principal component analysis (PCA) using Codex® as an add-in software in Excel™. The significance of each principal component was judged by cross validation (Wold *et al.,* 1989). Comparison between means were made with SAS software (SAS Institute Inc., 1989–1996) by ANOVA (GLM procedure), followed by the Sheffé test. Each mean is presented by its standard error.

## Results and Discussion

*Beetle attacks*

The four populations of *T. piniperda* used in the tests showed significant differences in levels of attack ($p < 0.0001$). The beetle population of *T. piniperda* from Bordeaux was more aggressive (mean number of attacks per tree 2.120.42; $n = 25$) than the three other populations, which did not differ significantly between each other, although the least active population under these circumstances seemed to be *T. piniperda* from Comps (mean number of attacks per tree for *T. piniperda* from Orléans 1.00.24, $n = 25$; from Lubéron 0.600.22, $n = 25$; and from Comps 0.400.13, $n = 15$). Beetles originating from one population (developed in a given pine species) did not attack this pine species

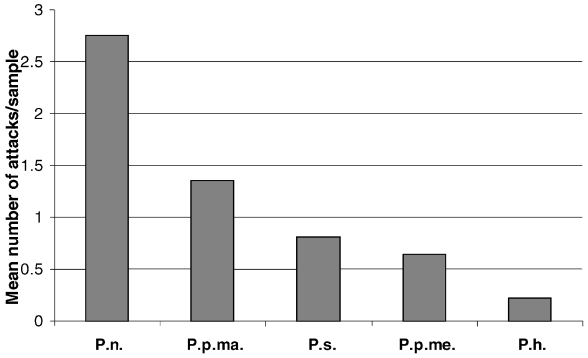


Fig. 1. Mean number of attacks in each species ($N = 90$). One sample ($N$) equals one group of three trees. P.n., *P. nigra laricio*; P.p.ma., *P. pinaster maritima*; P.s. , *P. sylvestris*; P.p.me., *P. pinaster mesogeensis*; P.h., *P. halepensis.*

with preference (mean comparison by ANOVA and LSD-tests). Comparing all populations together, the number of attacks on the different pine species was significantly different ($p < 0.0001$). *P. nigra laricio* was significantly more attacked than *P. pinaster mesogeensis* and *P. halepensis. P. halepensis* was significantly less attacked than *P. nigra laricio* and *P. pinaster maritima* (Fig. 1). The number of attacks on the trees was shown to correlate to the proportion of (−)-$\alpha$-pinene for each of the three populations of beetles from Lubéron, Orléans, and Bordeaux (Fig. 2) This is in accordance with previous published results on the attractiveness of (−)-$\alpha$-pinene for *T. piniperda* (Schröder and Eidmann, 1987). In other investigations larger amounts of (−)-$\alpha$-pinene, (−)-camphene and (+)-$\beta$-pinene were found in attacked pines than in non-attacked ones from Cuba (Valterová *et al.,* 1995).

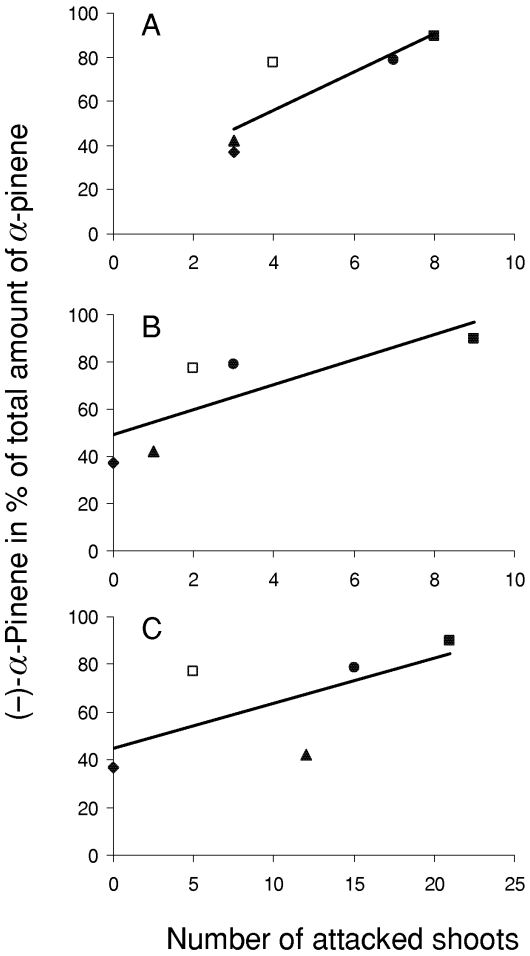


Fig. 2. Correlation between the enantiomeric composition of $\alpha$-pinene and the number of attacks by three populations of *Tomicus piniperda*. All populations were less attracted to *P. halepensis* which emitted less (−)-$\alpha$-pinene and most attracted to *P. nigra laricio* emitting most (−)-$\alpha$-pinene. Romb, *Pinus halepensis*; cross, *P. pinaster mesogeensis*; triangle, *P. sylvestris*; round, *P. pinaster maritima*; square, *P. nigra laricio.* A, Bordeaux; B, Lubéron; C, Orléans.

*Chemistry*

Monoterpene hydrocarbons were the overall dominating constituents in the pines analyzed. The main monoterpenes were (+)- and (−)-$\alpha$-pinene, (−)-$\beta$-pinene and in certain species (+)-3-carene (Fig. 3). The amounts of the enantiomers varied considerably within and among species. In a PCA-plot (Fig. 4) based on absolute amounts, including the enantiomeric ratios of $\alpha$-camphene, $\beta$-pinene, sabinene, limonene, and $\beta$-phellandrene, a separation of the pine species into two groups was obtained. *P. halepensis* and *P. sylvestris* were grouped due to the amounts of (+)-$\alpha$-pinene and (+)-3-carene, while *P. nigra laricio*, *P. pinaster maritima* and *P. pinaster mesogeensis* were grouped according to their higher proportions of (−)-$\alpha$-pinene and (−)-$\beta$-pinene.

The most attacked species *P. nigra laricio* contained more (−)-limonene than the least attacked species *P. halepensis.* However, the attacked *P. halepensis* trees contained more (−)-limonene than non-attacked ones, and based on the PCA-plot (Fig. 4), (−)-limonene has no influence on the grouping of trees. Little is known about the role

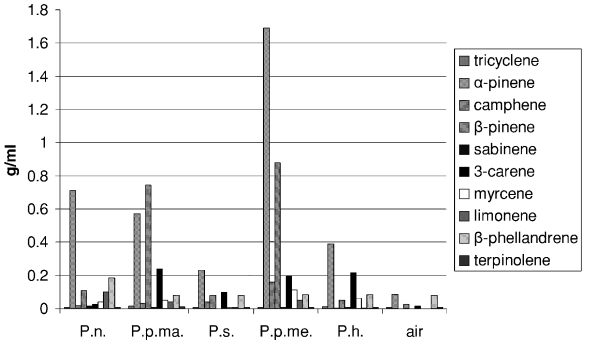


Fig. 3. Absolute amounts of monoterpenes released from five species of pines in decreasing order of attack by *Tomicus piniperda*. P.n., *P. nigra laricio*; P.p.ma., *P. pinaster maritima*; P.s. , *P. sylvestris*; P.p.me., *P. pinaster mesogeensis*; P.h., *P. halepensis*.

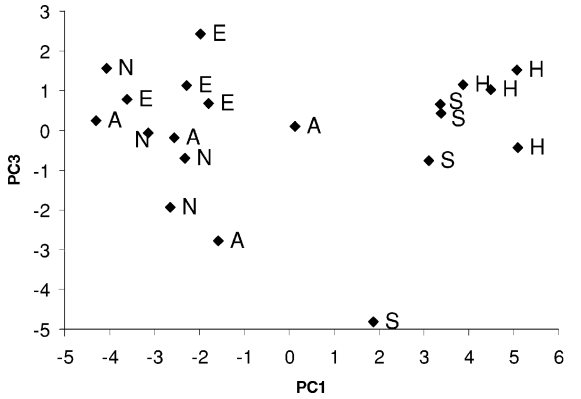


Fig. 4. PCA-plot based on absolute amounts of 18 monoterpenes: (+)- and (−)-$\alpha$-pinene, (+)- and (−)-$\beta$-pinene, (+)- and (−)-limonene, (+)-3-carene, (+)- and (−)-$\beta$-phellandrene, myrcene, (+)- and (−)-camphene, *p*-cymene, tricyclene, (+)- and (−)-sabinene, terpinolene and *cis*-ocimene. Two components (PC1 and PC3) were found to be significant by cross validation and 58% of the variance in GC-data was explained. A, *P. pinaster maritima*; E, *P. pinaster mesogeensis;* H, *P. halepensis*; N, *P. nigra laricio*; and S, *P. sylvestris.* (−)-$\alpha$-Pinene and (−)-$\beta$-pinene seemed to be responsible for the grouping, whereas (−)-limonene seemed not to have any influence on the grouping.

of limonene in beetle attraction or repellency. Limonene was experimentally observed to be the most repellent terpene for *Scolytus ventralis* (Bordasch and Berryman, 1977). It has also been reported to have a toxic effect on this beetle (Raffa *et al*., 1985) as well as on *T. piniperda* (Delorme and Lieutier, 1990). However, the effects of its enantiomers are unknown.

Morphological characteristics of the trees used in the assays could not explain the differences between beetle preferences, since the tree heights and the total numbers of shoots did not differ significantly between the most attacked species (*P. nigra laricio*) and the least attacked one (*P. halepensis*), although the former had the biggest leader shoots (Table I). Consequently, (−)-$\alpha$-pinene seems to play an important role in the interaction between the beetles and their hosts and (−)-limonene might have also an influence on the host selection process. The biological activity of the enantiomers has been reported to be different for $\alpha$-pinene and limonene (Karlson *et al*., 1996; Sjödin *et al.*, 1996; Wibe *et al.*, 1998; Roten *et al.*, 2002). Earlier, (+)-3-carene and the enantiomers of $\alpha$-pinene have been tested on *T. piniperda* in the premating phase (Byers *et al.*, 1985), but these samples had an uncertain enantiomeric purity, and there was no evidence that the beetle was able to distinguish between those volatiles. It would be interesting to compare the biological functions of both the enantiomers of $\alpha$-pinene, $\beta$-pinene and limonene in the shoot-feeding phase of *T. piniperda*, to see if they have either an attractive or repellent effect. It would also be of interest to test the effect of (+)-$\alpha$-pinene.

The quantity of the volatiles released might have an effect on bark beetle attacks. *P. pinaster mesogeensis* emitted large amounts of volatiles during the assays. These trees were not at all the most attacked species. The reason for that could be their small size (average height 64.1 cm, Table I). *P. halepensis* emitted higher quantities of monoterpenes but was the least attacked species when compared to *P. sylvestris* that emitted a smaller quantity of monoterpenes and had a higher number of attacks. *P. halepensis* possibly contained some repellent compounds in addition.

The comparison between *P. sylvestris* and *P. pinaster mesogeensis* raised a problem when a possible role of terpenes (including enantiomers) in beetle preferences was considered. Indeed, these two species were almost equally attacked by beetles (Fig. 1), although they differed both in the total quantity of volatiles released (Fig. 3) and in their monoterpene composition (Fig. 4). They also differed greatly regarding certain morphological characteristics (Table I). Some parameters not taken into account in our study might interfere in the beetle choice of shoot, but, if considering the correlation found between the enantiomeric com-

position of $\alpha$-pinene [the proportion of (−)-$\alpha$-pinene related to the sum of both (−)- and (+)-$\alpha$-pinene] and the number of attacks by *T. piniperda* (Fig. 2), it seems that the relative amount of (−)-$\alpha$-pinene in the pine volatiles may explain the preferences observed.

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