Plant constituents as signals for aggregation and attack of pest insects

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Abstract - The compositions of volatile wood constituents of healthy (untreated) and top-cut trees of Scots pine, *Pinus sylvestris* L., have been studied and correlated to the attack rates of scolytids, *Hylurgops palliatus* (Gyll.), *Trypodendron lineatum*, and *Tomicus piniperda* (L.). The mean proportions of ethanol and acetaldehyde compared to other constituents were higher in top-cut trees than in healthy ones. Some differences in the monoterpene composition also contributed to the difference between the two groups of trees. Only top-cut trees were attacked. The importance of ethanol and and some of the monoterpene for the recognition and attack is discussed.

The enantiomeric composition of various monoterpenes in Scots pine (*P. sylvestris*) and in common spruce [*Picea abies* (L.) Karst.] has been determined using a recently developed GC-method based on α-cyclodextrin as a chiral phase. The importance of the chirality in host specific compounds in insect/plant interactions will be discussed in view of recent findings regarding the aggregation pheromone of the spruce bark beetle [*Ips typographus* (L.)].

The title of this presentation is rather broad and covers a large field, which has been investigated from different points of view (for references see reviews cited in ref. 1). Reasons for aggregation and attack of pest insects on host plants are that the host can be a good source for feeding or the ultimate choice for breeding. In this paper we will present two separate investigations in which host plant constituents play an important role for recognition and aggregation.

Most bark and ambrosia beetles breed in dead or weakened parts of host trees. The reason for this behavior may be due to the ineffective defense mechanisms of the host tree. Thus, the probability for successful breeding is larger in dead or weakened trees than in healthy trees. Volatile constituents released from an injured host tree may be significant for host recognition. This was the starting-point of an extensive investigation on the variation of the composition of volatile constituents in Scots pine (*Pinus sylvestris* L.) (ref. 2).

The attacks by the bark beetles *Tomicus piniperda* (L.) and *Hylurgops palliatus* (Gyll.) and the ambrosia beetle *Trypodendron lineatum* (Oliv.) on healthy (untreated) trees and on top-cut trees were studied using flight barrier traps and by inspection of the trees. The composition of volatile constituents in wood samples from healthy (untreated) trees was compared with those of top-cut trees. Wood samples were taken from top-cut trees and from untreated trees in early spring during the flight period of *T. piniperda*. For control of seasonal variations samples were also taken during other times of the year. The experiments were repeated over three years. The samples were analyzed for the composition of volatiles using gas chromatography (GC). Both headspace analysis and analysis of pentane extracts of the wood samples were performed. The GC data [normalized according to Johansson *et al.* (ref. 3)] were evaluated by multivariate data analysis using principal components analysis (PCA) and "projections to latent structures" discriminant analysis (PLS-DA) (ref. 4 and 5). In the latter method the modelling was performed analogous to that in PCA and in combination with a discriminant analysis. The experimental details and a detailed presentation of the treatment of the GC data have recently been presented (ref. 2).
Our results provide information about the very large variations in the composition of volatile constituents between individual trees. To our disappointment, this large variation made it difficult to determine any differences in composition between volatile constituents of untreated trees and top-cut trees. However, the treatment of the GC data using PCA and PLS-DA revealed the differences. A typical PLS-discriminant plot is shown in Fig. 1. The plot illustrates the relationship between untreated and top-cut trees. Each point represents a tree, and the closer the points are to each other the more similar is the chemical composition of the volatile constituents.

![Figure 1. PLS-discriminant plot showing the relationships in chemical compositions of volatile constituents between untreated (U) and top-cut trees (TC).](image)

Significant differences were observed in headspace data for the first and the third years. However, only a small part of the variance in the data was explained by these differences. Some part of the variance should be due to the known high variation in the composition of monoterpene hydrocarbons in Scots pine (ref. 6). Ethanol, acetaldehyde, 3-carene/myrcene (not separated by the GC-column used in this investigation), α-phellandrene, γ-terpinene, p-cymene and terpinolene (incl. unknown) are constituents that make an important contribution to the differentiation between the two groups of trees. The differences are based on a combination of several constituents rather than on single ones. The first and third year there is a considerable increase in the amounts of ethanol and acetaldehyde in the top-cut trees. It has previously been shown that these two compounds are released by stressed Pinus seedlings (ref. 7) and that flooding results in increased levels of ethanol (ref. 8).

The bark beetle, *H. palliatus*, and the ambrosia beetle, *T. lineatum*, only attacked top-cut trees during the first and the third years. During these two years the top-cut trees differed significantly from the untreated trees in the composition of volatile constituents. The mean proportion of ethanol was also higher in the attacked than in the unattacked top-cut trees. These two forest insects have also been shown to be strongly attracted by ethanol alone, and by a combination of ethanol and α-pinene (ref. 9). This observation has been useful for the development of lures for trapping these insects, mainly for monitoring purposes in field tests.

The bark beetle, *T. piniperda*, attacked top-cut trees during all three years. Monoterpene hydrocarbons as well as ethanol or combinations were shown to be attractive to this insect in field tests (ref. 9). Furthermore, attack on healthy trees could be induced when monoterpene hydrocarbons in combination with ethanol or ethanol alone were applied to such trees (ref. 9).

This extensive investigation of the chemical differences between healthy trees and injured or weakened trees was rather frustrating for a natural product chemist. We expected to find some new biologically active compounds. Instead, very simple well known compounds were found to be responsible for the significant biological activities. However, the investigation was rewarding from a scientific point of view and provides useful results for forest entomologists and ecologists.
The second part of this presentation will deal with another aspect of insect/plant interaction. In this case, a plant constituent plays an important role as a precursor for the production of an aggregation pheromone constituent.

![Figure 2](image)

**Figure 2.** Possible microbial transformations of host compounds in the bark beetle, *Ips typographus*.

It is known that (-)-α-pinene (1, Figure 2) of the host plant is converted by the spruce bark beetle, *Ips typographus* (L.), to the aggregation pheromone constituent (S)-cis-vebenol (2). This conversion is probably a microbial transformation (Figure 2). If the beetle is exposed to (+)-α-pinene (3) the corresponding trans-verbenol (4) is formed. This latter compound is not an aggregation pheromone constituent and its biological significance has not yet been settled. We have shown that the enantiomeric composition of α-pinene varies considerably between spruce trees of different genetic origins (ref. 10). There is also a strong correlation between the chirality of α-pinene in the host tree and the cis/trans ratio of verbenol produced by the bark beetle, which has infested the tree (ref. 10). We are now investigating if there are clones of Norway spruce that only produce (+)-α-pinene (3). Such trees may be more resistant to spruce bark beetle attacks because these trees lack the substrate for the production of the essential aggregation pheromone constituent (ref. 11).

Our research on this topic depended very much on methods for the determination of the enantiomeric purity of α-pinene. When we started our investigation no analytical methods suitable for this purpose were available. Therefore, we developed a GC method that is based on a chemical transformation of the monoterpene hydrocarbon to an alcohol by hydroboration/oxidation followed by derivatization to a carbamate (ref. 10). This carbamate can be separated on a Chirasil-Val column according to König *et al.* (ref. 12). We have now developed a GC method that performs the separation of enantiomers of hydrocarbons without derivatization (ref. 13). The method is suitable for several common monoterpene hydrocarbons and is based on an observation by Koscielski *et al.* (ref. 14), that the enantiomers of α- and β-pinenes can be separated by GC using α-cyclodextrin in formamide as a stationary phase.

![Figure 3](image)

**Figure 3.** GC separation of (+)- and (-)-α-pinene on an α-cyclodextrin phase. Column: 1.8 m x 0.8 mm packed with α-cyclodextrin (20 % w/w) in formamide/water on Celite (45 - 60 mesh); column temp. 35° C.
We have shown that water plays an important role for the efficiency of the GC system using \( \alpha \)-cyclodextrin in formamide (ref. 13). Therefore, a simple device for the saturation of the carrier gas with water was developed. The separation of the enantiomers of \( \alpha \)-pinene is shown in Fig. 4. The short retention times and the large separation factors of this GC system make it suitable for preparative purposes. Some successful preliminary experiments have been carried out. Thus, we have prepared pure reference samples of (+) and (-)-camphene from a racemic mixture (ref. 13).

Very good enantiomeric separations have been obtained on this GC system. However, the GC peaks of the various monoterpene hydrocarbons overlap. Therefore, when analyzing a complex mixture, the individual monoterpenes have been separated by a simple off-line GC procedure (ref. 13).

We have used our GC method for investigations of the enantiomeric compositions of monoterpene hydrocarbons in Norway spruce, \textit{Picea abies}, and in commercial turpentine from the wood of this conifer (ref. 15). We have also used this method in a study of the defense secretions of termite soldiers (ref. 16). In addition, the access to a simple GC method for the determination of enantiomeric compositions of monoterpenes will be most valuable for biogenetic studies since chirality is a useful label for such investigations.

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\textbf{REFERENCES}


