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**Original article**

# Preliminary study of the monoterpene response of three pines to *Ophiostoma clavigerum* (Ascomycetes : Ophiostomatales) and two chemical elicitors

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**Summary** — The monoterpene response of phloem and sapwood of individual pines belonging to 3 species (*Pinus contorta*, *Pinus ponderosa* and *Pinus monticola*) to inoculation with *Ophiostoma clavigerum* and injection with chitosan, a proteinase inhibitor-inducing factor and a control buffer, was investigated quantitatively and qualitatively. The total quantity of monoterpene in the reactive tissues increased with each treatment but to different levels. In each tree, the monoterpene composition of the reactive tissues differed from that of the unwounded tissues, but was the same whatever the treatment, even in the case of an injection with buffer control. In addition, phloem and sapwood responses were qualitatively identical although constitutive compositions differed greatly. The composition of reactive tissues was not very different from that of unwounded sapwood. The direction of variation of each monoterpene from unwounded to reactive tissues differed according to the particular tree. Only phellandrene + limonene reacted consistently. From these results we cannot conclude that chitosan is a natural elicitor, and the non-specificity of the response for the aggression favors the hypothesis that an elicitor originates from the tree itself. Because of this non-specificity, and the fact that the three trees responded in a qualitatively different manner, we suggest that the qualitative monoterpene response of the tree is not adapted to any specific aggressor even though these trees are usually hosts of the same bark beetle-fungus complex. Thus, the role of monoterpenes in the induced defensive response is very likely a quantitative and dose-dependent relationship.

**monoterpenes / *Pinus contorta* / *Pinus ponderosa* / *Pinus monticola* / *Ophiostoma clavigerum* / chemical elicitors / defense reaction / gas chromatography**

**Résumé — Étude préliminaire de la réponse monoterpénique de trois pins à *Ophiostoma clavigerum* (Ascomycètes : Ophiostomatales) et à deux éliciteurs chimiques.** *La réponse monoterpénique du phloème et de l'aubier de 3 arbres appartenant aux espèces* Pinus contorta, Pinus ponderosa *et* Pinus monticola *a été étudiée d'un point de vue quantitatif et qualitatif, après des inoculations du champignon* O clavigerum *et des injections de chitosane, d'un facteur induisant une inhibition de protéinase (PIIF) et d'une solution tampon témoin. La quantité totale de monoterpènes (hydrocarbures) mesurée après 3, 7 ou 14 j dans les tissus réactionnels augmente après chaque traitement, mais atteint des niveaux différents, le plus élevé étant obtenu après inoculations du champignon. Dans le cas du chitosane, la réponse est quantitativement proche de celle dirigée contre* O clavigerum *chez* P ponderosa, *mais ne diffère pas de celle dirigée contre le PIIF et la solution tampon chez les 2 autres arbres (tableau I). Dans chaque arbre, la composition monoterpénique des tissus réactionnels diffère de celle des tissus non altérés, mais s'avère semblable quel que soit*

*le traitement, même avec les inoculations de tampon témoin (tableau II et fig 1). De plus, les réponses du phloème et de l'aubier sont qualitativement identiques, bien que leur composition initiale soit très différente. La composition des tissus réactionnels n'est en outre pas très différente de celle de l'aubier inaltéré (fig 1). Le sens de variation de chaque monoterpène entre le tissu inaltéré et le tissu réactionnel varie selon l'arbre considéré; seul le groupe phéllandrène + limonène réagit toujours dans le même sens (fig 2).*

*Il n'est pas possible de conclure de ces résultats que le chitosane est un éliciteur naturel, et la non-spécificité de la réponse vis-à-vis de l'agresseur est en faveur d'une hypothèse qui situerait dans l'arbre lui-même l'origine de l'éliciteur. À cause de la non-spécificité de la réponse et du fait que les 3 arbres réagissent différemment d'un point de vue qualitatif, il est suggéré que la réponse monoterpénique qualitative d'un arbre n'est pas adaptée à un agresseur particulier, bien que ces arbres soient des hôtes habituels du même couple scolytide-champignon. Ainsi, le rôle des monoterpènes dans la réaction de défense induite est très probablement de nature quantitative et dépendrait de la dose accumulée.*

***monoterpène* / Pinus contorta / Pinus ponderosa / Pinus monticola / Ophiostoma clavigerum / *éliciteur chimique* / *réaction de défense* / *chromatographie en phase gazeuse***

## INTRODUCTION

The fungus *Ophiostoma clavigerum* (Robinson–Jeffrey and Davidson) Upadhyay plays a decisive role in the mechanisms of establishment of the bark beetle *Dendroctonus ponderosae* Hopk in North American pines, particularly *Pinus contorta* var *latifolia* Engelmann, *Pinus ponderosa* Lawson and *Pinus monticola* Douglas (Reid *et al,* 1967; Safranyik *et al,* 1975; Shrimpton, 1978; Raffa and Berryman, 1983). During bark beetle attacks, this fungus stimulates host parenchymal cells to produce resin which impregnates the tissues located around the site of attack (Reid *et al,* 1967; Berryman, 1969; Lieutier and Berryman, 1988). This induced reaction is the main line of tree defense against the attack of the bark beetle and its associated fungus. However, the nature and the origin of the chemical elicitor responsible for the stimulation of the parenchyma cells is not clear.

In a previous paper, we reported the histological changes induced in the reactive tissues of these 3 pine species by artificial inoculations of *O clavigerum* and injections of 2 chemical elicitors, chitosan and a proteinase inhibitor-inducing factor (PIIF) (Lieutier and Berryman, 1988). Here we demonstrate both qualitative and quantitative changes in monoterpenes induced in the same tissues by the same inoculations and injections. Note that chitosan is a mixture of β-(1,4) glucosamine polymers which are constituents of arthropod integuments and of most fungal cell walls (Hadwiger and Beckman, 1980). PIIF is composed of pectic oligomeric fragments derived from plant cell walls, the most active being α-(1,4) galacturonic acid polymers and oligomers (Ryan *et al,* 1985). Both chitosan and PIIF are possible elicitors of induced responses in plants naturally attacked by insects and fungi (Hadwiger *et al,* 1981; Walkers-Simons *et al,* 1984; Green and Ryan, 1972).

Quantitative and qualitative monoterpene modifications in response to the attack of bark beetles and associated fungi have been reported in conifers by several authors. Shrimpton (1973), Raffa and Berryman (1982a), Schuck (1982) and Delorme and Lieutier (1990) noted an increase of the total monoterpene content of phloem and sapwood in the induced reactions of *P contorta*, *Abies grandis* (Lindley),

*Picea abies* Karst and *Pinus sylvestris* L, respectively. Miller *et al* (1986) reported a greater increase in the total monoterpene content of Lodgepole pine phloem in response to chitosan than to either PIIF or *O clavigerum*. Qualitative changes in the monoterpene fraction of the phloem were observed by Russel and Berryman (1976) and Raffa and Berryman (1982a) in *A grandis*, by Raffa and Berryman (1982b) in *P contorta*, by Cook and Hain (1985) in *Pinus taeda* L and by Delorme and Lieutier (1990) in *P sylvestris*. In the last 2 cases, the qualitative changes were the same for a given tree for all treatments (*ie*, 2 different strains of the same fungus in *P taeda*, 3 different fungus species and 1 beetle in *P sylvestris*). Shrimpton (1973) was unable to observe any qualitative changes in *P contorta* sapwood, with the exception of β-phellandrene after natural attacks by *D ponderosae*. However, Schuck (1982) reported changes in some monoterpene components of *P abies* sapwood after wounding.

## MATERIALS AND METHODS

The experimental devices and techniques were previously described by Lieutier and Berryman (1988). One specimen of each tree species (*P contorta, P ponderosa, P monticola,* ≈ 30 cm diameter breast height from an even-aged mixed conifer stand) received a total of 12 inoculations (4 treatments replicated thrice) in July 1985 at breast height using the cork-borer technique (Wright, 1933; Wong and Berrryman, 1977). The first treatment was inoculation with *O clavigerum,* the second with chitosan, and the third with PIIF. Fungal cultures were 10–15 d old. The chemical solutions consisted of a nitrous acid cleaved crab shell chitosan and a raw PIIF extract from tomato plants dissolved in 0.05 M sterile phosphate buffer (pH 7) at the rate of 1 mg/ml. The fourth treatment was an injection of the sterile buffer alone. All inoculations consisted of 100 µl of chemical solution or a 5-mm plug of agar containing fungal mycelia. On each sampling occasion (3, 7 and 14 d after treatments), one sample of each treatment was taken on each tree. Reactive phloem (with cambium) and sapwood were removed and cut longitudinally in half. One half was immersed in a cupric acetate solution for histological observations (Lieutier and Berryman, 1988) and the other was immediatly placed on dry-ice and stored at –60 °C. Two wk after treatment, samples of unwounded phloem and sapwood were also collected and stored in the same manner.

Monoterpene analyses were performed on samples collected after 3 and 14 d. Samples collected 14 d after treatment were divided into 3 20-mm pieces, starting at the point of inoculation and working away from the wound, giving sub-samples at 0–20 mm, 20–40 mm, and 40–60 mm Three-d-old samples consisted of a single 0–20 mm piece. Each phloem sub-sample was finely chopped and then shaken in 10 ml pentane for 24 h. The extracts were filtered by flash chromatography in silica-gel G which was rinsed thrice in pentane. They were concentrated under a stream of nitrogen to 0.5, 1 or 2 ml according to the richness in total monoterpenes. Analyses were performed on a Perkin–Elmer Sigma-3 gas chromatograph equipped with a flame ionization detector and a 30 m x 0.2 mm capillary column (Supelco SE-30). The carrier gas was helium at 1.1 ml/min at 18 psi. The column temperature program was 80 °C for 14 min, a rise of 20° per min to 100 °C, then 100 °C for 40 min. The injector and detector temperatures were constant at 250 °C. Three replicates were performed for each sub-sample. Peaks were identified by comparison with the retention times of pure monoterpenes added to the samples and by enhancement after these additions. For *P contorta,* comparisons were also made with mass spectrography results from Raffa and Berryman (1982b). The quantitative values were determined by means of an electronic digital integrator using *p*-cymene as an internal standard (this terpene was found to be lacking in preliminary chromatograms).

The monoterpene compositions of the samples were compared by principal component analysis (PCA), for each tree separately, and considering only monoterpenes which were present at levels of 0.5% or greater in each sample. This analysis was carried out with SAS software (SAS Institute).

In the present study, each tree species was represented by only one individual. However, our aim was not to define the qualitative re-

sponse of these species but rather to compare the terpene composition of responding tissues with that of unwounded tissues of the same tree. Although there is a great deal of variation in the monoterpene composition of conifer species (see, for example, Cates and Alexander, 1982), variations between species are much greater to the extent that they can be used as taxonomic characteristics (Zavarin *et al*, 1977). Our study was designed to examine the extremes of variability in the defensive reaction to a pathogen and 2 elicitors.

## RESULTS

As the extracts were filtered on silica gel, oxygenated compounds were probably lost from the samples. Thus, in the following, the word "total monoterpene" refers only to hydrocarbides which in fact correspond to most of the monoterpene compounds.

Concentrations of total monoterpenes (hydrocarbides) in the different phloem and sapwood samples are presented in table I. As we have only 1 tree per species, between-tree comparison of absolute values is not possible. We therefore compare values between treatments within trees. *O clavigerum* generally induced a higher accumulation of monoterpenes than the chemical treatments. In *P ponderosa* however, the quantitative response to chitosan was often close to the response to the fungus. The terpene accumulations induced by PIIF and buffer alone were always less than that induced by the fungus. They were also less than that induced by chitosan in *P ponderosa* (phloem and sapwood, 14 d after injury) and in the phloem of *P monticola.*

Seventeen different peaks (not always present) were obtained by gas chromatography when reactions were compared with unwounded tissue. One peak was heptane, 12 were monoterpenes, and 4 (probably monoterpenes) unidentified peaks were labelled T1 to T4. β-phellandrene and limonene made up a single peak, but *P contorta* contains mainly β-phellandrene (Raffa and Berryman, 1982b; Smith, 1983) and *P ponderosa* mainly limonene (Smith, 1966). As an example, table II gives the monoterpene percentages for the response of the trees to fungus after 14 d in comparison with unwounded tissues. In this table, some major differences can be noticed between reactive and unwounded tissue. Figure 1 allows a general qualitative comparison between treatments, dates, tissues, and distance from the inoculation point for each tree.

The first axis of the PCA (fig 1) explained 58.6, 72.5 and 55.6%, the second axis 20.8, 13.1 and 21.8% and the third axis 7.2, 6.9 and 9.9% of the variability, respectively in *P contorta, P ponderosa* and *P monticola.* The first axis compared unwounded phloem to reactive tissues and can be called the reaction axis. In *P ponderosa* and *P monticola*, the second axis, with the first, separated unwounded sapwood from reactive tissues. In *P contorta*, unwounded sapwood was separated from reactive tissues by the third axis. Thus, 3 main types of monoterpene composition were identified: unwounded phloem, unwounded sapwood, reactive tissues (phloem and sapwood together) (fig 1; table II).

In all trees and in all 3 axes, reactive phloem could not be separated from reactive sapwood. In addition, the composition of reactive tissue did not appear very different from that of unwounded sapwood, with only small differences occurring in the concentration of some monoterpenes (table II). The changes in phloem composition induced by treatments are summarized in figure 2, the response to fungus inoculation after 14 d being chosen as being representative of all treatments (*cf*, fig 1). Monoterpene fractions changed differently

**Table I.** Total quantities of monoterpenes (in µg per 100 mg fresh tissue), at different distances (in mm) from the point of injury.

| | *3 d after injury* | | | | *14 d after injury* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Unwounded* | O clav | *Chit* | *PIIF* | O clav | | | *Chitosan* | | | *PIIF* | | *Buffer alone* | |
| | | *(0–20)* | *(0–20)* | *(0–20)* | *(0–20)* | *(20–40)* | *(40–60)* | *(0–20)* | *(20–40)* | *(40–60)* | *(0–20)* | *(20–40)* | *(0–20)* | *(20–40)* |
| Phloem | | | | | | | | | | | | | | |
| PC | 37 | 332 | 126 | 175 | 1 304 | 1 346 | 998 | 189 | 29 | – | 236 | 45 | 231 | 57 |
| PP | 39 | 142 | 110 | 103 | 768 | 479 | 6 | 658 | 499 | 7.5 | 274 | 58 | 69 | 7.1 |
| PM | 9 | 174 | 116 | 55 | 804 | 805 | 466 | 104 | 7.3 | – | 38 | 3 | 46 | 4.8 |
| Sapwood | | | | | | | | | | | | | | |
| PC | 15 | 326 | 220 | 243 | 868 | – | – | 445 | – | – | 193 | – | 414 | – |
| PP | 5 | 435 | 420 | 543 | 1 050 | – | – | 587 | – | – | 150 | – | 371 | – |
| PM | 2 | 153 | 185 | 145 | 535 | – | – | 135 | – | – | 113 | – | 91 | – |

PC = *Pinus contorta*; PP = *Pinus ponderosa*; PM = *Pinus monticola*; O clav =*O clavigerum*; Chit = chitosan.

**Table II.** Monoterpene composition of unwounded and reactive phloem and sapwood. Numbers are percentages of the total quantity of monoterpenes.

| | | Hep | T1 | Tri (2) + α-thu | α-pi | Cam | Sab | β-pi | Myr | T2 Bor | Car | α-ter | phell + lim | γ-ter | Terp | T3 | T4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Unwounded | Phloem | – | – | 0.15 | 5.1 | 0.3 | 0.9 | 12.1 | 2.2 | 1.3 | 0.8 | 0.25 | 76.9 | tr | 0.15 | – | – | – |
| *P contorta* | Sapwood | – | – | tr | 8.0 | 0.4 | 1.0 | 28.5 | 2.1 | 1.1 | 3.2 | – | 55.5 | – | 0.3 | – | – | – |
| Reactive (1) | Phloem | – | – | 0.2 | 7.5 | 0.5 | 1.1 | 26.4 | 2.0 | 1.2 | 2.0 | 0.4 | 57.8 | tr | 0.55 | – | 0.3 | – |
| *P contorta* | Sapwood | – | – | 0.1 | 7.3 | 0.5 | 1.1 | 26.9 | 2.0 | 1.2 | 2.1 | 0.35 | 57.7 | tr | 0.5 | – | 0.3 | – |
| Unwounded | Phloem | – | – | tr | 18.6 | tr | 1.1 | 0.7 | 3.0 | tr | 43.9 | – | 18.9 | 0.2 | 13.7 | – | – | – |
| *P ponderosa* | Sapwood | – | – | tr | 11.4 | tr | 1.5 | 2.9 | 6.7 | tr | 69.2 | tr | 2.7 | 0.4 | 5.2 | – | – | – |
| Reactive (1) | Phloem | – | – | 0.25 | 9.2 | 0.3 | 1.6 | 1.5 | 6.9 | 0.1 | 72.6 | 0.1 | 1.4 | 0.5 | 5.2 | – | 0.8 | – |
| *P ponderosa* | Sapwood | – | – | 0.25 | 8.7 | tr | 1.6 | 1.4 | 7.0 | 0.1 | 72.8 | tr | 1.5 | 0.5 | 5.3 | – | 0.9 | – |
| Unwounded | Phloem | 0.9 | – | 0.45 | 39.5 | 8.9 | tr | 22.0 | 2.4 | 0.4 | 0.05 | – | 23.0 | – | 1.5 | 0.4 | – | – |
| *P monticola* | Sapwood | 1.3 | 0.6 | tr | 58.5 | 1.3 | tr | 23.3 | 1.9 | 0.4 | tr | – | 11.9 | – | tr | 0.9 | – | – |
| Reactive (1) | Phloem | 1.1 | 0.7 | 0.1 | 65.8 | 1.3 | tr | 22.9 | 1.7 | 0.1 | 0.05 | – | 3.8 | – | 0.4 | 1.9 | – | 1.1 |
| *P monticola* | Sapwood | 0.9 | 0.7 | 0.1 | 63.8 | 1.2 | 0.1 | 23.1 | 1.7 | 0.15 | 0.1 | – | 5.8 | – | 0.4 | 1.9 | – | 1.1 |

tr = traces; Hep = heptane; tri = tricyclene; α-thu = α-thujene; α-pi = α-pine; Cam = camphene; Sab = sabinene; β-pi = β-pine; Myr = myrcene; Car = Δ3-carene; α-ter = α-terpinene; phell = β-phellandrene; Lim = limonene; γ-ter = γ-terpinene; Terp = terpinolene; Bor = bornyl acetate; T1, T2, T3, T4 = undetermined compounds. (1): Reactions to fungus after 14 d and close to the point of inoculation have been chosen as examples of reactive tissues representative from reactions to any treatment (*cf* fig 1). (2): Only α-thujene in *P ponderosa*.

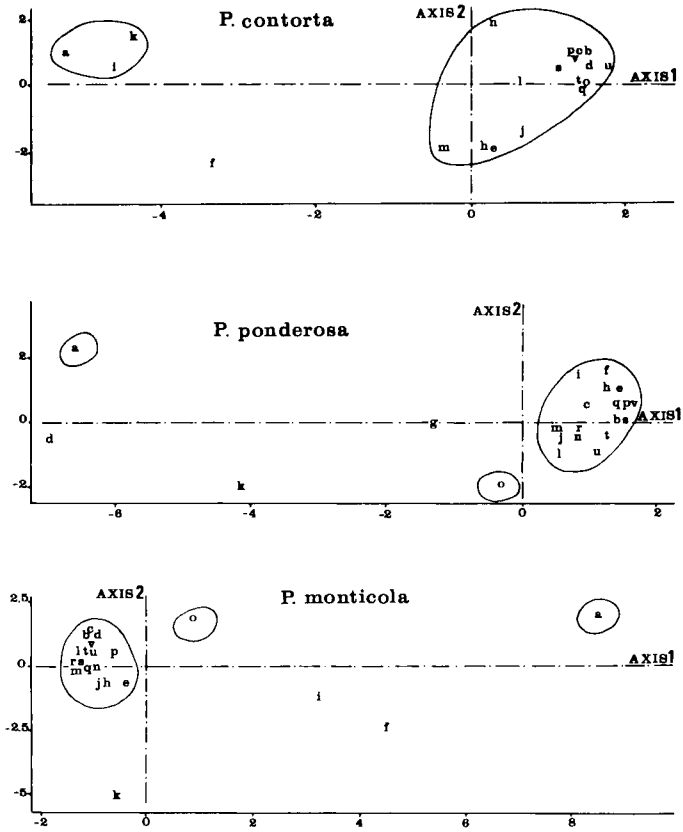


**Fig 1.** Comparison of the monoterpene composition of the different samples by principal component analysis (PCA). Only monoterpenes with a maximum ratio of at least 0.5% were considered. Circles around "clusters" were drawn by hand. a = unwounded phloem; b, c, d, = reactive phloem 14 d after treatment by *O clavigerum* at respectively 10–20, 20–40 and 40–60 mm from the point of injury; e, f, g = *idem* after treatment by chitosan; h, i = reactive phloem 14 d after treatment by PIIF at respectively 0–20 and 20–40 mm from the point of injury; j, k = *idem* after treatment by buffer control; l, m, n = reactive phloem at 0–20 mm from the point of injury 3 d after treatment by respectively *O clavigerum*, chitosan and PIIF; o = unwounded sapwood; p, q, r, s = reactive sapwood 14 d after treatment by respectively *O clavigerum*, chitosan, PIIF and control buffer; t, u, v = reactive sapwood 3 d after treatment by respectively *O clavigerum*, chitosan and PIIF. Note: in *P contorta*, unwounded sapwood was separated from reactive tissues by the third axis.

in different tree species, except for β-phellandrene + limonene which always decreased. Some reactive phloem samples had a monoterpene composition similar to unwounded phloem (fig 1), but always in parts of the reaction most distant from the point of inoculation or injection; *eg*, PIIF (20–40 mm after 14 d) and buffer (20–40 mm after 14 d) from Lodgepole pine phloem. In addition to the 3 main types of

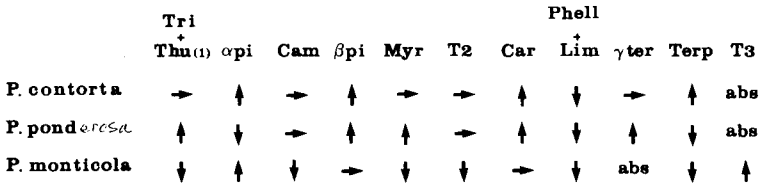


**Fig 2.** Comparative changes in monoterpene compositions of the phloem induced in 3 pine trees by a fungus inoculation after 14 d, as an example of reactive tissues representative from reactions to any treatments (*cf* fig 1). This table is limited to those monoterpenes which varied in at least 1 tree. The monoterpene abbreviations are the same as those in table II. (1) Only α-thujene in *P ponderosa*. Abs = absent.

monoterpene composition, some phloem samples appeared intermediate between unwounded phloem and reactive tissues (fig 1). These also originated from parts of the reaction distant from the point of inoculation or injection; *eg*, chitosan (20–40 mm after 14 d) from Lodgepole pine, chitosan (40–60 mm after 14 d) and buffer (20–40 mm after 14 d) from Ponderosa pine, chitosan (20–40 mm after 14 d) and PIIF (20–40 mm after 14 d) from Monticola pine. In these intermediate samples, the ratio of some monoterpenes was similar to that of unwounded phloem, the ratio of others was similar to that of reactive phloem, while others had a ratio intermediate between the 2 categories of phloem.

The fungus sample (40–60 mm after 14 d) in *P ponderosa* and the buffer sample (20–40 mm after 14 d) in *P monticola* were atypical, not being separated from unwounded or reactive phloems by axis 1 but by axis 2. In fact, these 2 samples differed from their respective group by 1 terpene (β-pinene in *P ponderosa* and T3 in *P monticola*) which had an "abnormally" high concentration.

In the case of *P contorta*, it was possible to recognize 3 subgroups inside the reactive samples (fig 1). One consisted of all reactive sapwood and phloem samples resulting from fungus inoculation after 14 d; these samples had the highest values along axis 1. The 2 others included reactive phloem after 3 d or phloem treated by buffer, PIIF or chitosan, the value on axis 1 being lower than the previous subgroup. It was not possible, however, to recognize such subgroups in *P ponderosa* and *P monticola*.

## INTERPRETATION AND DISCUSSION

### *Comparison between treatments*

The increase in total monoterpenes (hydrocarbides) after treatment is in agreement with all previous results of phloem and sapwood reactions in different conifer species (Shrimpton, 1973; Russel and Berryman, 1976; Raffa and Berryman, 1982a, b; Schuck, 1982; Miller *et al*, 1986; Delorme and Lieutier, 1990). We observed differences in the responses of a given tree to fungus, chitosan, PIIF or buffer, the former quantitative treatment inducing a higher accumulation of resins. Chitosan induced a quantitative reponse comparable with that

induced by the fungus, or higher than that induced by PIIF and buffer, only in some cases. Our results are thus not in a complete agreement with those of Miller *et al* (1986) and with our previous histological observations in suggesting a possible role of chitosan as natural elicitor of defensive metabolism in conifers (Lieutier and Berryman, 1988).

In considering the qualitative response, we note that situations where the monoterpene composition of the reactive tissues was similar to that of unwounded tissues or was intermediate, were all found in samples collected far from the point of aggression. This allows us to consider these situations as either outside the reaction or being an incomplete reaction. This opinion is strengthened by the fact that the total concentration of monoterpenes in these cases was similar to that of unwounded tissues. On the contrary, in situations close to the point of aggression, all reactions clearly differed qualitatively from unwounded phloem. Moreover, they all had the same qualitative composition. Each of the trees responded in a different way. However, we can conclude that the responding tissues of a given tree all have the same monoterpene composition irrespective of treatment, and that this composition differs from the unwounded tissues of the same tree.

The conclusion that the reaction is nonspecific for the agression supports the results of Cook and Hain (1985), with Loblolly pine and 2 strains of *Ophiostoma minus*, and of Delorme and Lieutier (1990) with Scots pine and 3 different fungi and 1 beetle species. In his histological studies, Mullick (1977) suggested that response to injury is not in defense but rather to restore tissues and block sapwood conduction, processes which are inherent, and not specific as to the incitant. However, we need more information to suggest if such a hypothesis is the case after beetle- fungus attack.

This non-specificity, together with the fact that we found sterile phosphate buffer inducing the same qualitative response, make it difficult to prove the role of chitosan and PIIF as natural elicitors. Moreover, it favors the hypothesis that the elicitor originates from the tree. Nevertheless, Raffa and Berryman (1982a) found that monoterpene composition of the reaction of *Abies grandis* induced by inoculations with *Trichosporium symbioticum* Wright differed significantly from uninjured phloem in terms of many compounds, while the composition of the reaction to mechanical wounding differed significantly from unwounded phloem by only one compound. Thus the reaction to fungal inoculation was qualitatively different than to mechanical wounding. As a consequence, our results in pines do not agree with those of Raffa and Berryman (1982a) in firs.

### *Comparison between tissues*

The monoterpene composition of reactive tissue was similar for phloem and for sapwood in all 3 species, but the composition of constitutive tissues was different. Thus, the reaction state of tissues can be characterized by a well-defined monoterpene composition in a particular tree, and this does not depend on the initial composition of the tissue. Shrimpton (1973) did not find significant qualitative changes in the sapwood of *P contorta* in response to attacks by *D ponderosa*. This is explained by the fact that reactive sapwood had a composition close to that of unwounded sapwood. Shrimpton (1973) only found an increase in β-phellandrene, which is contrary to our results, but this may be due to between-tree variation in the qualitative response, as observed by Schuck (1982) in the sap-

wood of *P abies* and by Delorme and Lieutier (1990) in the phloem of *P sylvestris*.

The existence of a defined monoterpene composition of reactive tissues for a given tree, whatever the tissue, fits the hypothesis that neosynthesis is from cells different from those responsible for the synthesis of constitutive resin. This is in agreement with the ideas of Reid *et al* (1967), Berryman (1969), Cheniclet *et al* (1988) and Lieutier and Berryman (1988), who suggested that parenchymal cells were responsible for neosynthesis. Our results can be explained by the intervention of an elicitor whose "message" could be read by any target cell. Indeed, Cheniclet *et al* (1988) suggested that the neosynthesis against a beetle-associated fungus in *Pinus pinaster* is preceded by the reactivation of previously inactive cells.

### *Comparison between trees*

In response to aggressors, each tree responded in a different manner. There were no between-tree similarities in the monoterpene response. Indeed, only one terpene varied in the same direction (decrease) in the 3 trees. The modification of the monoterpene ratio in response to *O clavigerum* was thus different in each tree although they are all hosts of *D ponderosae* and *O clavigerum*.

Russel and Berryman (1976), Bordasch and Berryman (1977) and Raffa and Berryman (1982a) have reported that the defense reactions of *A grandis* to *T symbioticum* contain a higher relative concentration of the terpenes which are least favorable to *Scolytus ventralis* LeConte, the beetle associated with *T symbioticum*. Conversely, the monoterpenes least repellent to this beetle decline in the defense reaction (Bordasch and Berryman, 1977). In our experiments, however, the 3 pines did not exhibit a consistent differential response to *O clavigerum*. Moreover, resistance of *P ponderosa* to *Dendroctonus brevicomis* LeConte seems to be associated with limonene and myrcene concentrations (Smith, 1966); limonene is the most toxic monoterpene to this beetle, followed by Δ3-carene and myrcene (Smith, 1965). In our *P ponderosa* samples, however, myrcene and Δ3-carene increased while limonene decreased. Raffa and Berryman (1982b) found that the percentages of α-pinene and limonene increase while Δ3-carene decreases in the response of *P contorta* to *O clavigerum* while in our experiment concentrations of α-pinene and Δ3-carene both increased. These results suggest that between-tree variability in monoterpene composition is the rule in the response of *P contorta*, as is also true for *P abies* (Schuck, 1982) and *P sylvestris* (Delorme and Lieutier, 1990).

Consequently, the qualitative monoterpene response of conifers does not seem to be adapted to the species of aggressor. Instead, the role of monoterpene in the induced reponse of conifers to aggression is probably quantitative and dose-dependent, as previously suggested (Raffa and Berryman, 1982a, b; Delorme and Lieutier, 1990).

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