Phyton (Austria) Special issue: "Plant Physiology"	**Vol. 39**	Fasc. 3	(225)-(229)	30. 11. 1999

Influence of Endogenous Terpenes on Growth of Three Endophytic Fungi from the Needles of *Pinus nigra* Arnold

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K e y w o r d s : *Pinus nigra* needles, terpenes, endophytic fungi.

S u m m a r y

JURC D., BOJOVIĆ S. & JURC M. 1999. Influence of endogenous terpenes on growth of three endophytic fungi from the needles of *Pinus nigra* Arnold. - Phyton (Horn, Austria) 39 (3): (225) - (229).

The influence of crude terpenoid extract of needles from three subspecies of *Pinus nigra* Arn.(*P. nigra* ssp. *laricio* from Corsica - France, *P. nigra* ssp. *salzmannii* from Cevennes - France and *P. nigra* ssp. *austriaca* from Bled - Slovenia) on growth of endophytic fungi (isolated in Slovenia) was investigated. Growth experiments were conducted with a latent pathogen (*Cenangium ferruginosum*), with a facultative pathogen (*Sphaeropsis sapinea*) and with a saprob (*Phialophora hoffmannii*) which regularly occurr as endophytes in Austrian pine needles.

The growth of *S. sapinea* and *P. hoffmannii* in different concentrations of terpenoid extract from all three subspecies of pine is not inhibited. The growth of *C. ferruginosum* is stimulated in high concentration of terpene extract of *P. nigra* ssp. *austriaca*, from which it was isolated, but it is not stimulated in extracts from the other subspecies of pine. The terpenoid extract of *P. nigra* ssp. *austriaca* is characterized by higher amount in α-Pinene and Germacrene-d, which are probably responsible for the stimulatory effect.

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Introduction

In previous work endophytic fungal population of *Pinus nigra* Arn. needles in Slovenia was investigated (JURC 1996). In the same time the population diversity of *P. nigra* in the Mediterranean region by genetic analysis using terpenes as a primary tool was investigated (BOJOVIĆ 1995). The aim of the work presented here was to find out wheather there is an influence of terpenoid compounds from the needles of *P. nigra* from different geographical locations (having specific terpene profiles and here treated as subspecies) on growth of three endophytic fungi: *Cenangium ferruginosum* Fr., *Sphaeropsis sapinea* /Fr./ Dyko et Sutton and *Phialophora hoffmannii* (Beyma) Schol-Schwarz. The fungi were chosen for the following reasons: *C. ferruginosum* was found the second most frequent endophyte in healthy needles of Austrian pine (15,4% of all isolations belonged to this species), it is designated as facultative parasite, which kills the bark and cambium of branches weakened by environment, pests and pathogens (SINCLAIR & al. 1987). *S. sapinea* is the most obvious disease of Austrian pine in Slovenia causing tip blight and it is severely damaging to plantations of Austrian pine. As an endophyte it is rare being isolated in 0.8% of all isolations. The ecological status of *Ph. hoffmannii* is not clear and it might be mutualist in Austrian pine. It was isolated in 7.8% of all isolations and 75% of these isolations were located in the base segments of needles (JURC 1996).

The hypothesis that variable endogenous terpene contents of black pine needles from three subspecies differently influence the growth of endophytic fungi with different ecological status was postulated.

Materials and Methods

Sampling of *P. nigra* ssp. *austriaca* /Höss/ Vid. needles for isolation of endophytic fungi was performed in Kobjeglava (N: 45° 50,' E: 13 ° 47', Slovenia,) in January 1996. Sampling of pine needles for terpene extractions was performed at two locations in France (*P. nigra* ssp. *salzmannii* /Dunal/ Franco, Cevennnes, N: 44° 02', E: 3 ° 45'and *P. nigra* ssp. *laricio* /Poir./ Schwarz, Corsica, N: 42° 38', E: 6 ° 58') and at one locaton in Slovenia (*P. nigra* ssp. *austriaca* /Höss/ Vid., Bled, N: 46° 25', E: 14 ° 08') in 1995. Details about fungal isolation are given by JURC 1996 and JURC & JURC 1997. Crude terpene extract was made from 50 needles which were macerated and extracted overnight with 15 ml n-pentane. Decanted extract was dried with sodium sulfate and analysed by gas chromatography (BOJOVIĆ 1995).

100 ml of molten tap water agar was poured into 19 cm Petri dishes where three 7 cm Petri dishes with 25 ml of 2% malt extract agar medium (MEA, pH=6.2-6.5) were placed. In each of the small Petri dishes one of the endophytic species tested was inoculated with 2mm agar plugs taken from the growing margin of 14-days old colonies of listed species of fungi. They were grown in the air with different concentrations of crude terpene extract of the needles (control without terpenes; 32.5 ml^{-3}; 130 ml^{-3}; 260 ml^{-3} terpenes diluted in n-pentane in the air volume of 1075 cm^3) at 22°C for 21 days. In all experiments the diameter of the colonies was measured in two directions at right angles to each other, every two days for up to the 21st day. All experiments were carried out in three replicates.

Results

The terpene profile of *P. nigra* ssp. *austriaca* from Bled, Slovenia is characterised by higher amount in α-Pinene and Germacrene, that of *P. nigra* ssp. *salzmannii* from Cevennes, France by γ-Cadinene and δ-Cadinene, and the terpene profile of *P. nigra* ssp. *laricio* from Corsica, France is characterised by higher amount in Caryophyllene, Myrcene, trans-Ocimene, Linalyl acetate and α – Humulene.

Growth of *Sphaeropsis sapinea* and *Phialophora hoffmannii* in the presence of terpene extracts from all three subspecies of Austrian pine did not differ from the control. The difference in growth appeares only in *Cenangium ferruginosum* which is stimulated by the highest concentration of terpene extract from *P. nigra* ssp. *austriaca*. For the growth of *C. ferruginosum* the most favourable conditions are: higher amount in α-Pinene and Germacrene-d which are typical for *Pinus nigra ssp. nigra* from Slovenia, and the most unfavourable conditions are: higher amount in Caryophyllene, Myrcene, trans-Ocimene, Linalyl acetate and α-Humulene which are typical for *Pinus nigra ssp. laricio* from Corsica.

The growth of *C. ferruginosum* in different concentrations of terpene extract from *P. nigra* ssp. *nigra* is presented in Fig. 1.

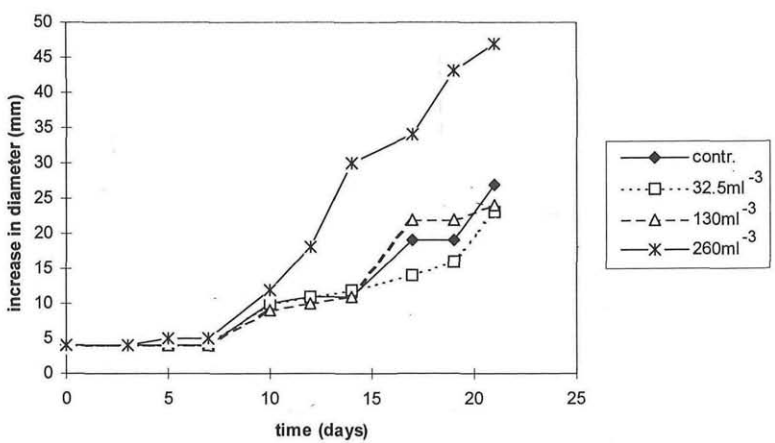

Fig. 1. Growth of *C. ferruginosum* in different concentrations of terpene extract from the needles of *P. nigra* ssp. *austriaca*.

Discussion

Endogenous terpenes may inhibit the growth of fungi in high concentrations especially in those which are near saturation. In low concentrations they can stimulate the growth of fungi. Endogenous content of terpenes vary with age of plant tissues and it is lower in scenescent or dying off tissues (COBB & al. 1968, FLODIN & FRIES 1978, THIBAULT-BALESDENT & DELATOUR 1985, ESPINOSA-GARCIA & LAGENHEIM 1991a, b). Low concentrations of terpenes used in presented growth experiments did not have any effect on facultative pathogen *S. sapinea* and on saprob *P. hoffmannii*, but significant stimulatory effect on latent pathogen *C. ferruginosum.* This fungus invade only stressed tissues of its host, fructifications appear in short time after necrotisation of branches. Lowering the terpene concentrations in host tissues during the dying off process may be the reason for the enhanced growth activity of the fungus. Ability to tolerate or even to be stimulated by inhibitory secondary substances of the host is of great selection advantage for the pathogen. Its frequent endophytic presence in healthy needles of the host enables this fungus to invade quickly the nearby bark tissues when the concentration of the inhibitory substances falls.

Terpene composition of phenotypes of certain species vary and it can have different effect on endophytes. In *Pinus nigra* population the terpene composition of phenotypes can be used to group the population in several subpopulations (designated here as subspecies). Only the terpene extract from *P. nigra* ssp. *austriaca* exhibit stimulatory effect on growth of *C. ferruginosum*, which was isolated from the same subspecies. This fact can be explained by the adaptation of the fungus to the specific terpene composition of one subspecies. ESPINOSA-GARCIA & LAGENHEIM 1991a hypothesized that strains of the endophyte would be adapted to specific terpene phenotypes, but their results did not support the hypothesis. Our results support their hypothesis although the differences in composition of terpene profiles of subspecies of pine used in our experiments were greater than differences of terpene profiles of four trees used in their experiments.

Acknowledgements

The work was funded by The Ministry of Science and Technology of the Republic of Slovenia (No. S34-0404-001/2491/93) and The British Council of Slovenia (ALIS link No.28 1996/99). We thank Dr. N. MAGAN for constructive comments and V. RAJH for her help in the laboratory work.

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