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THE INFLUENCE OF ROOT EXUDATE AUXINS AND GIBBERELLINS ON THE GROWTH OF *SUILLUS VARIEGATUS* MYCELIUM

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Introduction

Higher plants with their hormones, which they exudate into the rhizosphere, influence the growth of their mycorrhizal partners. The role playd by cytokinin in the growth of the fungus *Suillus variegatus* is already quite clear, as is the role of cytokinin and auxin together (Gogala, Pohleven, 1976; Pohleven, Gogala 1977). However, how natural auxins and gibberellins influence the growth of the *Suillus variegatus* mycelium has not been determined yet. Results are available only for *Boletus pinicola* (Gogala 1971). Since these data are significant for the understanding of the hormonal regulation of the development of mycorrhiza, we determined the influence these growth substances have upon the growth of the fungus *Suillus variegatus.*

Materials and Methods

Seedlings of the pine tree *Pinus sylvestris* L. were raised from the seeds of 30 — 40 year old pines from Novokračine in the vicinity of Ilirska Bistrica. The seeds were from the 1975 crop. (ground: limestone, altitude : 600 m).

The fruiting body of *Suillus variegatus* (Swartz ex Fr.) O. Kuntze was from Polica near Grosuplje. The mycelium was isolated in 1975 and was cultivated on potato agar.

12 day old seedlings were taken from the vermiculite into bidistilled water, so that only the roots were in the water. The seedlings were grown in natural light at 6°C. In the following 6 days, the root exudate was evaporated until dry. Auxins were extracted with methanol, taken up on

80% methanol and separated with paper chromatography (Whatman 1). The solvent was isopropanol : water (8 : 2). The chromatograms were tested using the *Lepidium sativum* L. root growth test (Moewus 1949; cited by Linser, Kiermayer 1957). The auxins were eluated from the chromatograms with 80% methanol and evaporated till dry. They were taken up in absolute methanol and warm water and then added to the fungal medium.

Gibberellins were extracted with methanol and ethylacetate (Oegema, Fletcher 1972). They were taken up in ethylacetate and separated with paper chromatography (Wathman 1). The solvent was a mixture of isopropanol : water (8 : 2). The chromatograms were tested for gibberellins using barley endosperm, *Hordeum vulgare* L. (a modified test according to the authors Coombe, Cohen, Paleg 1967 after Moore 1974).

Gibberellins were eluated from the chromatograms using ethylacetate, evaporated till dry, isolated with warm water and added to the medium.

MNM medium was used for the cultivation of the mycelium (Marx 1969s. Hormones (β-indolacetic and gibberellic acid) in concentrations of 10^{-10} to 10^{-4} g/l, and natural hormones in concentrations of 10^{-4} to 10^{2} units of "M" factor were added to the medium. "M" factor is the amount of substance which a mg of root secretes in 6 days at 6°C (Melin 1963). The medium containing the hormones was autoclaved for 10 min at 1,5 at. The mycelium was grown in the dark, on slant agar at 26 (±1)°C. After 16 days fresh weight was determined and following liophylisation dry weight was also measured.

The data were processed in a Hewlett Packard 9820A computer with plotter 9862. The statistical significancy was carried out to 10 and 5% with the Cochran-Cox test.

Results

In the root exudate of the pine tree *Pinus sylvestris* L., we determined (with biotests) 5 auxin-like substances (fig. 1) and 6 gibberellin-like substances (fig. 2). Rf values are the following:

natural auxins:	A : 0,00 — 0,05
	B : 0,10 — 0,25
	C : 0,35 — 0,50
	D : 0,65 — 0,70
	E : 0,85 — 1,00
natural gibberellins:	a : 0,00 — 0,15
	b : 0,20 — 0,30
	c : 0,35 — 0,40
	d : 0,45 — 0,60
	e : 0,65 — 0,85
	f : 0,90 — 1,00

According to the R_f value β-indolacetic acid (IAA) corresponds to substances C and gibberellic acid (GA_3) to substance d. Substance a seems to be composed of several gibberellic esthers (Gogala 1971).

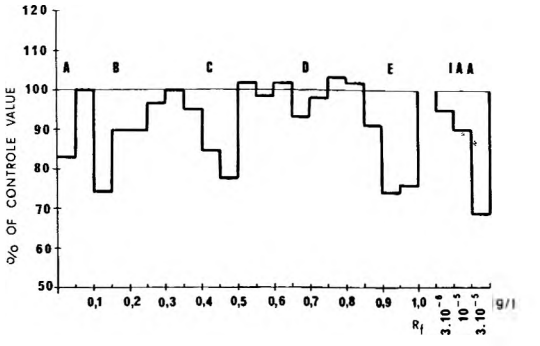

Fig. 1. Root exudate of *Pinus sylvestris*-test for auxins. Equivalent 40 of »M« factor. Abscissa: position on the chromatogram. Ordinate: length of roots expressed in % of the controls. Right: influence of ß-indolacetic acid (IAA).

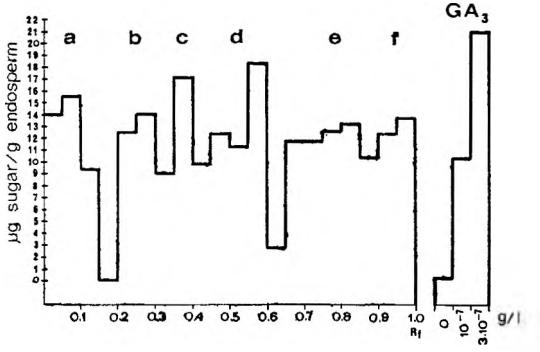

Fig. 2. Root exudate of *Pinus sylvestris*-test for gibberellins. Equivalent 20 of »M« factor. Abscissa: position on the chromatogram. Ordinate: Amount of reductive sugars in g of barley endosperm. Right: the control and the influence of giberellic acid (GA_3).

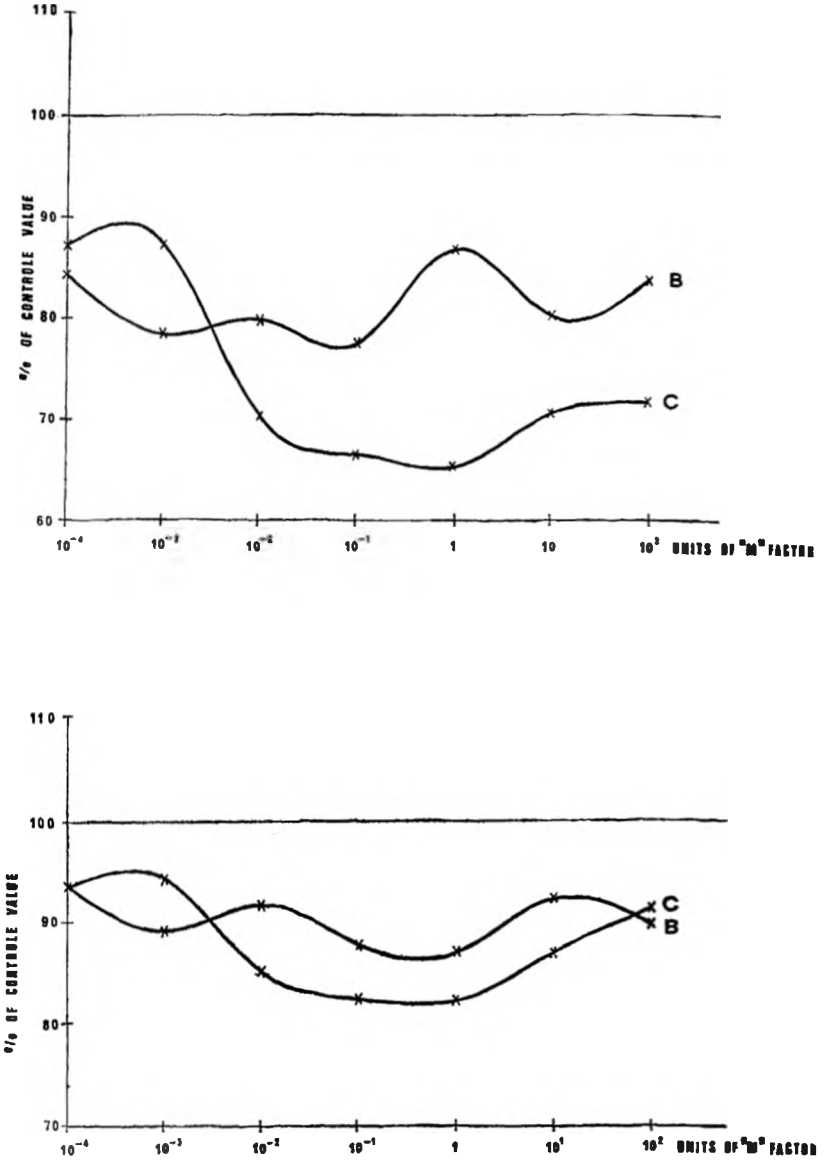

Fig. 3. The dependency of the weight of the *S. variegatus* mycelium upon the concentrations of auxins B and C isolated from the root exudate of *Pinus sylvestris*. Above: fresh weight, below: dry weight.

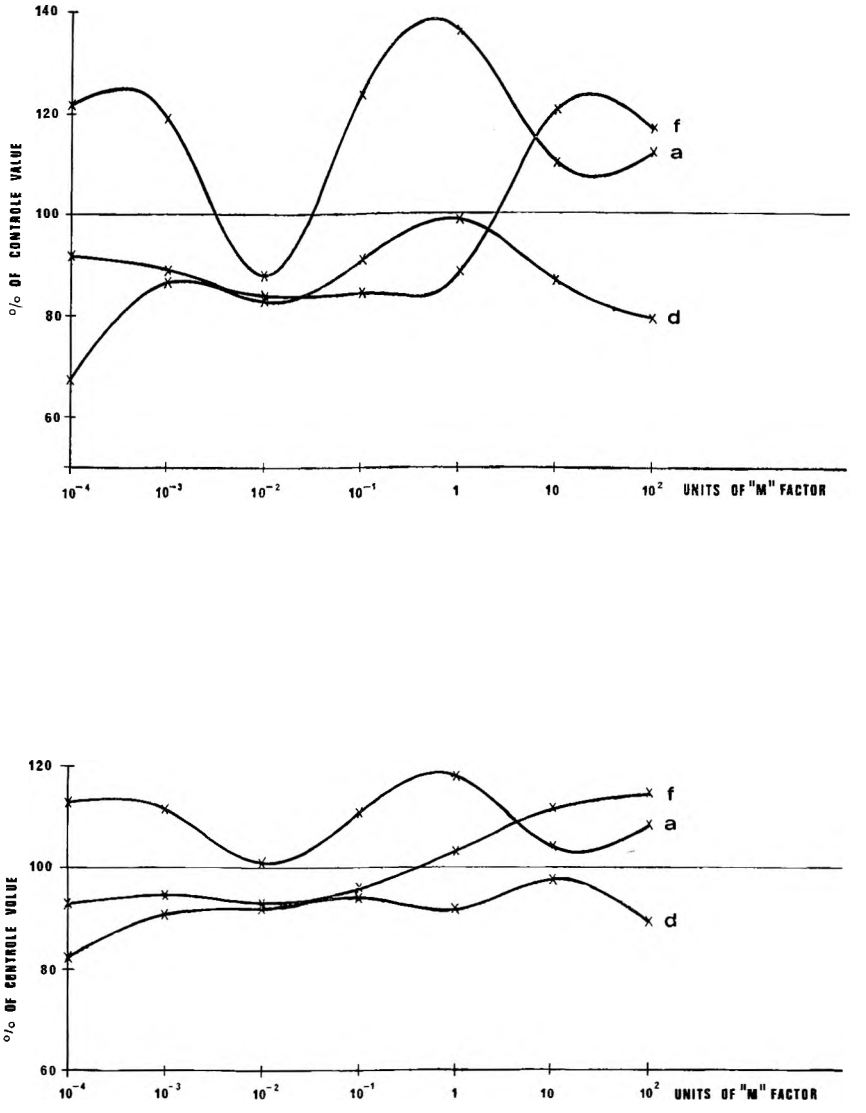

Fig. 4. The dependency of the *S. variegatus* mycelium weight upon the concentrations of gibberellins a, d, f, isolated from the *Pinus sylvestris* root exudate.
Above: fresh weight, below: dry weight.

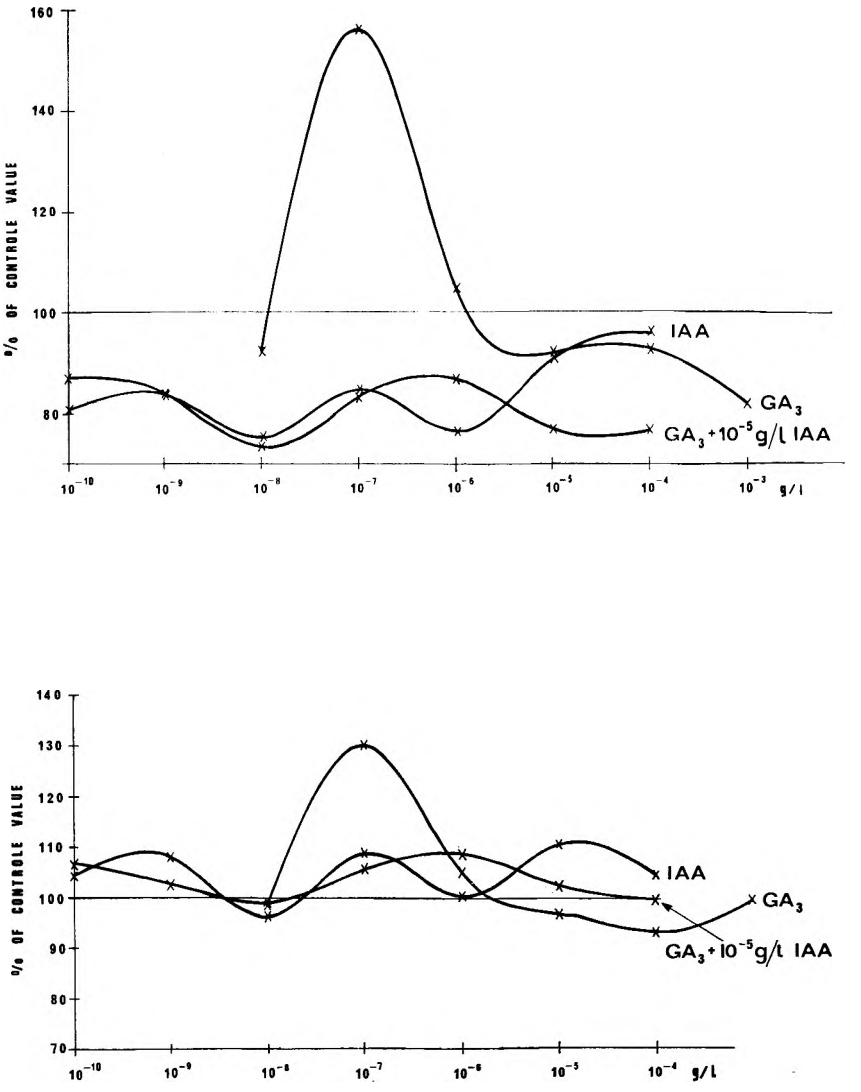

Fig. 5. The dependency of the *S. variegatus* mycelium weight, upon the concentrations of ß-indolacetic acid (IAA), gibberellic acid (GA_3) and a combination of both, where the concentration of IAA is 10^{-5}g/l. Above: fresh weight, below: dry weight.

Natural auxins A, B, C, and D in the concentrations of 10^{-4} to 10^{2} »M« factor, inhibited the growth of *Suillus variegatus* mycelium. They particularly inhibit the uptake of water (fig. 3). Growth was inhibited most by auxins C and D, the minimal fresh weight of the mycelium was only 65% of the control value, while the dry weight was 83% of the controls. Auxin E did not influence the growth of the mycelium.

We compared the effect of natural auxins with the effect of IAA. IAA in concentrations of 10^{-10} to 10^{-4} g/l inhibited the uptake of water, the dry weight being approximately equal to that of the control (fig. 5).

Natural gibberellins of the root exudate in concentrations of 10^{-4} to 10^{2} »M« factor, were added to the fungal medium. A statistically significant acceleration of growth occurred only in the presence of gibberellin a and higher concentrations of gibberellin f. Gibberellins b and e slightly accelerate growth while gibberellin d inhibited growth of the mycelium (fig. 4).

Gibberellic acid GA_3 stimulated the growth of *Suillus variegatus* mycelium only within narrow concentration limits (at concentrations of 10^{-7} to 10^{-6} g/l), at higher and lower concentrations growth was inhibited (fig. 5). At optimal concentrations the fresh weight reaches 150% and the dry weight 130% of controls.

Discussion

Melin (1963) has established, that the root exudate of higher plants influences fungal growth. Lower concentrations stimulate growth, while higher concentrations inhibit growth. He assumed, that either the roots secreted not only a stimulans but also an inhibitor, or that its mode of action depended upon its concentration. Gogala (1971) confirmed the second assumption by determining, that cytokinins isolated from the root exudate of the pine tree *Pinus sylvestris* L. influenced the growth of *Boletus pinicola* in the same manner as the rough exudate. *Suillus variegatus* reacted in the same way whether treated with a root exudate or with isolated cytokinins (Pohleven 1975).

The results we obtained on the influence IAA exerts upon the growth of the mycelium differ from those found in literature; Fortin (1967) determined that IAA inhibited the growth of several species of Basidiomycetes, while it stimulated *Suillus variegatus* when in concentrations up to $1{,}75\times10^{-3}$ g/l. Pohleven and Gogala (1977) also established that concentrations of 10^{-7} to 3×10^{-4} g/l stimulated growth of the mycelium. In our experiments (fig. 5) the growth conditions of the mycelium culture were the same as those in the 1977 experiments, the only difference being that the culture was isolated from a fruiting body of another locality. This indicates that the fungal reaction to IAA is highly specific.

Natural auxins inhibit the growth of *Boletus pinicola* (Gogala 1971) and *Suillus variegatus*.

GA_3 and giberellin d (R_f value corresponds to GA_3) in higher concentrations inhibit the growth of *Boletus pinicol*a (Gogala 1971). Gibberellin d also inhibits the growth of *Suillus variegatus*, while GA_3 inhibits its growth in higher and extremely low concentrations. Growth of the mycelium is greatly accelerated at concentrations of 10^{-7} g/l.

We determined that *Suillus variegatus* react to very low concentrations of GA_3 (10^{-10} g/l) and to gibberellin d (10^{-4} »M« factor). High concentrations of gibberellins a and f stimulate the growth of *Boletus pinicola* (Gogala 1971a) and *Suillus variegatus*. With gibberellin the weight fluctuates when concentrations differ probably because there are more gibberellic esthers on the chromatogram at R_f 0,00 — 0,15 (Gogala 1971).

Conclusions

In the exudate of the pine tree *Pinus sylvestris* we determined 5 natural auxins and 6 natural gibberellins. Auxins inhibit growth of the *Suillus variegatus* mycelium (with the exception of auxin E). However, IAA inhibits only the uptake of water by the mycelium. Gibberellins of the root exudate stimulate growth of the mycelium to a slight degree, except for gibberellin d which inhibits growth. GA_3 stimulates growth only within very narrow concentration limits, concentrations outside these limits inhibit growth. When GA_3 and IAA act together the effect of IAA predominates.

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SAŽETAK

UTJECAJ AUKSINA I GIBERELINA IZ EKSUDATA KORIJENA NA RASTENJE MICELIJA GLJIVE *SUILLUS VARIEGATUS*

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U eksudatu korijena bora našli smo 5 prirodnih auksina i 6 prirodnih giberelina. Auksini koče rastenje micelija gljive *Suillus variegatus* (uz iznimku auksina E). IAA inhibira u prvome redu primanje vode u micelij. Giberelini rastenje blago pospješuju, uz iznimku što giberelin d rastenje inhibira. GA_3 stimulira rastenje u vrlo uskom koncentracijskom području izvan kojega ga koči. Ako utječemo na rastenje s GA_3 i IAA zajedno, prevladava utjecaj IAA.

VSEBINA

VPLIV AVKSINOV IN GIBERELINOV KORENINSKEGA EKSUDATA NA RAST GOBE *SUILLUS VARIEGATUS*

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U eksudatu bora *Pinus sylvestris* smo našli 5 naravnih avksinov in 6 naravnih giberelinov. Avksini zavirajo rast micelija gobe *Suillus variegatus* (z izjemo avksina E). IAA inhibira predvsem sprejem vode v micelij. Giberelini rast rahlo pospešujejo, z izjemo giberelin d rast inhibira. GA_3 stimulira rast v zelo ozkem koncentracijskem območju, izven tega jo zavira. Če vplivamo na rast skupaj z GA_3 in IAA, prevlada vpliv IAA.

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