Crypt. Bot. 4, 8–13 (1993)

Cryptogamic Botany

Effect of plant growth regulators on growth of ectomycorrhizal fungi

A. Pokojska, E. Strzelczyk, C. Y. Li[1], H. Różycki and M. Szablewska

Institute of Biology, Laboratory of Microbiology, Nicolaus Copernicus University, ul. Gagarina 11, 87–100 Toruń, Poland

[1] *U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station, 3200 SW Jefferson Way, Corvallis, OR 97331, USA*

SUMMARY

Studies were carried out on the effect of auxins (IAA, IAN, IBA, IPrA), gibberellic acid (GA_3) and kinetin on the growth of ectomycorrhizal fungi: *Laccaria laccata, Hebeloma crustuliniforme* and *Rhizopogon vinicolor*. Differences in the effects of plant growth regulators on mycorrhizal fungi depended upon the kind of hormone, its concentration, the kind of fungus and the type of medium (liquid and agar media). The influence of GA_3 on the growth of the fungi studied was not statistically significant. Kinetin inhibited biomass production by *Laccaria laccata* in a liquid medium, but it did not inhibit the linear growth of this fungus on agar medium. Reverse results were observed with *Rhizopogon vinicolor*. Auxins did not affect the growth of *Laccaria laccata* but some of them exhibited both inhibitory and stimulatory effects on the growth of *Hebeloma crustuliniforme* and *Rhizopogon vinicolor* depending upon the concentration and type of the medium.

Introduction

Studies initiated by Slankis (Slankis 1950) had shown that auxins as well as cytokinins are necessary for the formation of mycorrhizal structures. Auxins added to the synthetic media inhibited elongation of pine seedlings roots. The roots became thicker and dichotomically branched, devoid of root hairs and caps – structures characteristic for the non-mycorrhizal roots (Slankis 1973). Cytokinins are responsible for the translocation of carbohydrates to the mycorrhizal roots. They act also indirectly on the activity of auxins (Gogala 1991).

The ability of mycorrhizal fungi to produce plant growth regulators, mainly auxins and cytokinins, is well established (Rudawska 1982; Gay 1986; Frankenberger and Poth 1987; Hanley and Greene 1987; Ho 1987a, b; Gay et al. 1989; Kampert and Strzelczyk 1989; Pokojska and Strzelczyk 1988). It's assumed that hormones of fungal origin may take part in formation and functioning of mycorrhizae (Gogala 1991). In the plant root zone there are also plant growth regulators elaborated by microorganisms accompanying mycorrhizae (Kampert and Strzelczyk 1984; Strzelczyk and Pokojska-Burdziej 1984) and those originating from the root exudates (Gogala 1991). Little is known about the direct effects of these compounds on mycorrhizal fungi. Therefore these studies on the effects of auxins, gibberellic acid and kinetin on the growth of *Laccaria laccata, Rhizopogon vinicolor* and *Hebeloma crustuliniforme* – ectomycorrhizal fungi used in practice as inoculants in forest nurseries – were performed.

0935-2147/93/0004-0008 $ 3.50/0

Materials and methods

Fungi

Three mycorrhizal fungi isolated from ectomycorrhizae of Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco): *Laccaria laccata* (Scop. ex Fr.) Berk and Br. (S-238), *Rhizopogon vinicolor* Smith (7534) and *Hebeloma crustuliniforme* (Bull. ex St. Am.) Quel (7650) were used in this study. The isolates of fungi were obtained from Dr. C. Y. Li of the U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station, Forestry Sciences Laboratory, Corvallis, Oregon, U.S.A.

Effect of plant growth regulators on the growth of mycorrhizal fungi in liquid medium

Preparation of inoculum

The fungi were grown at 26 °C in Petri dishes on Potato Dextrose Agar (Difco), *Lac. laccata* for 7 days, *Rh. vinicolor* and *H. crustuliniforme* for 14–21 days. Discs (1 cm in diameter) were cut from the edges of the mycelium. One disc served as inoculum for 100 ml of the medium in 300 ml Erlenmeyer flask.

Medium

The liquid medium was Lamb's solution: 10 g of glucose, 0.5 g of NH_4Cl, 0.5 g of $MgSO_4 \times 7H_2O$, 0.5 g of KH_2PO_4, 1 cm^3 5 ppm Fe solution of FeEDTA, 1 mg of thiamine HCl, 0.01 mg of biotin per litre of distilled H_2O. The medium was adjusted to pH 5.8.

Plant growth regulators

The sterile media before inoculation were supplemented with filter sterilized (Millipore 0.45 μm pore size) plant growth regulators to give the final concentrations: 0.01, 1.0 and 10.0 μg/ml. The following plant growth rtegulators were used: indole-3-acetic acid (IAA), Schuchardt; β-indolylacetonitrile (IAN), Sigma; γ-3-indolyl-butyric acid (IBA), Roth OHG; indole-3-propionic acid (IPrA), Schuchardt; gibberellic acid (GA_3), Sigma; kinetin, Serva.

Fungus culture

Mycorrhizal fungi in the presence of plant growth regulators were grown at room temperature (22±2 °C), in darkness, *Lac. laccata* for 14 days, *Rh. vinicolor* and *H. crustuliniforme* for 21 days. Five replicates were used for each combination. After incubation the mycelium was removed by filtration and oven-dried at 80 °C to constant weight.

Data analysis

Data were processed by 1-factor analysis of variance (Anova) and the Newman-Keuls test ($p \leq 0.05$). Pair comparisons were also made by a Student's t-test ($p \leq 0.05$). Analysis of data was carried out using "Stats+" and CSS: Statistica procedures (StatSoft, Tulsa, Oklahoma, USA).

Effect of plant growth regulators on the growth of mycorrhizal fungi on solid medium

Culture conditions

Petri dishes with agar Lamb's medium containing IAA, GA_3 and kinetin in three concentrations (0.01, 1.0, 10.0 μg/ml) and control plates were inoculated – in five replicates – with pieces (about 3 mm) of mycelium cut from the young cultures of fungi incubated on Potato Dextrose Agar. Every two days within 14 days of incubation at the temperature of 22±2 °C, the diameter of Lac. laccata colony was measured. Rhz. vinicolor and H. crustuliniforme were cultured for 21 days. The measurements of the colony diameter were started after 8–10 days of incubation and were taken every two days.

Data analysis

At the selected time points colony diameter increments in control were compared with those of the fungi cultured in the presence of plant growth hormones, using Student's t-test for independent samples ($p \leq 0.05$).

Results

The results of our studies on the effect of plant growth regulators on biomass production by mycorrhizal fungi in liquid Lamb's medium are presented in Tables 1–2 and on Fig. 1. Figure 2 illustrates the data concerning the effects of IAA, GA_3 and kinetin on linear growth of these fungi (on agar Lamb's medium).

Differences in the effects of plant growth regulators on mycorrhizal fungi depending on the kind of hormone, its concentration, the kind of fungus and type of medium were found. None of the fungi studied responded significantly on GA_3 in concentrations 0.01, 1.0 and 10.0 μg/ml (Table 2, Fig. 1–2).

The effect of auxins (IAA, IAN, IBA, IPrA) both on biomass yield and linear growth of *Lac. laccata* was not

Table 1. Effect of auxins on biomass production by ectomycorrhizal fungi in liquid Lamb's medium (mg of dry mycelium/100 ml of medium ± standard error).

Auxins	Concentrations of auxins – μg/ml			
	0 (control)	0.01	1.0	10.0
		Laccaria laccata		
IAA	46.1± 4.7a	45.2± 5.7a	41.4±2.9a	50.6±4.6a
IAN	41.6± 4.5a	43.4± 3.8a	46.7±4.2a	42.5±5.6a
IBA	27.1± 7.8a	35.8± 6.7a	31.9±2.9a	30.6±2.1a
IPrA	26.9± 2.8a	36.3± 6.3a	29.5±5.8a	25.6±2.3a
		Rhizopogon vinicolor		
IAA	57.3± 8.7b	50.1± 5.5b	44.1±4.9b	20.4±3.1a
IAN	42.9± 7.0a	46.8±10.4a	43.0±5.8a	44.2±6.5a
IBA	63.5± 9.5b	56.6± 5.5b	38.7±7.3a	39.9±4.3a
IPrA	44.3± 4.3b	43.4± 7.4b	31.8±2.8b	24.7±2.6a
		Hebeloma crustuliniforme		
IAA	45.4± 8.9a	54.8± 5.4a	36.7±5.0a	28.4±7.3a
IAN	62.1±11.1a	49.3± 7.3a	53.0±6.4a	46.0±8.5a
IBA	44.9± 7.0a	41.8± 7.5a	37.6±3.4a	33.7±6.1a
IPrA	24.4± 6.2a	31.5± 4.0a	23.8±9.5a	20.6±5.3a

Note: means within a given row followed by different letters are significantly different ($p \leq 0.05$).

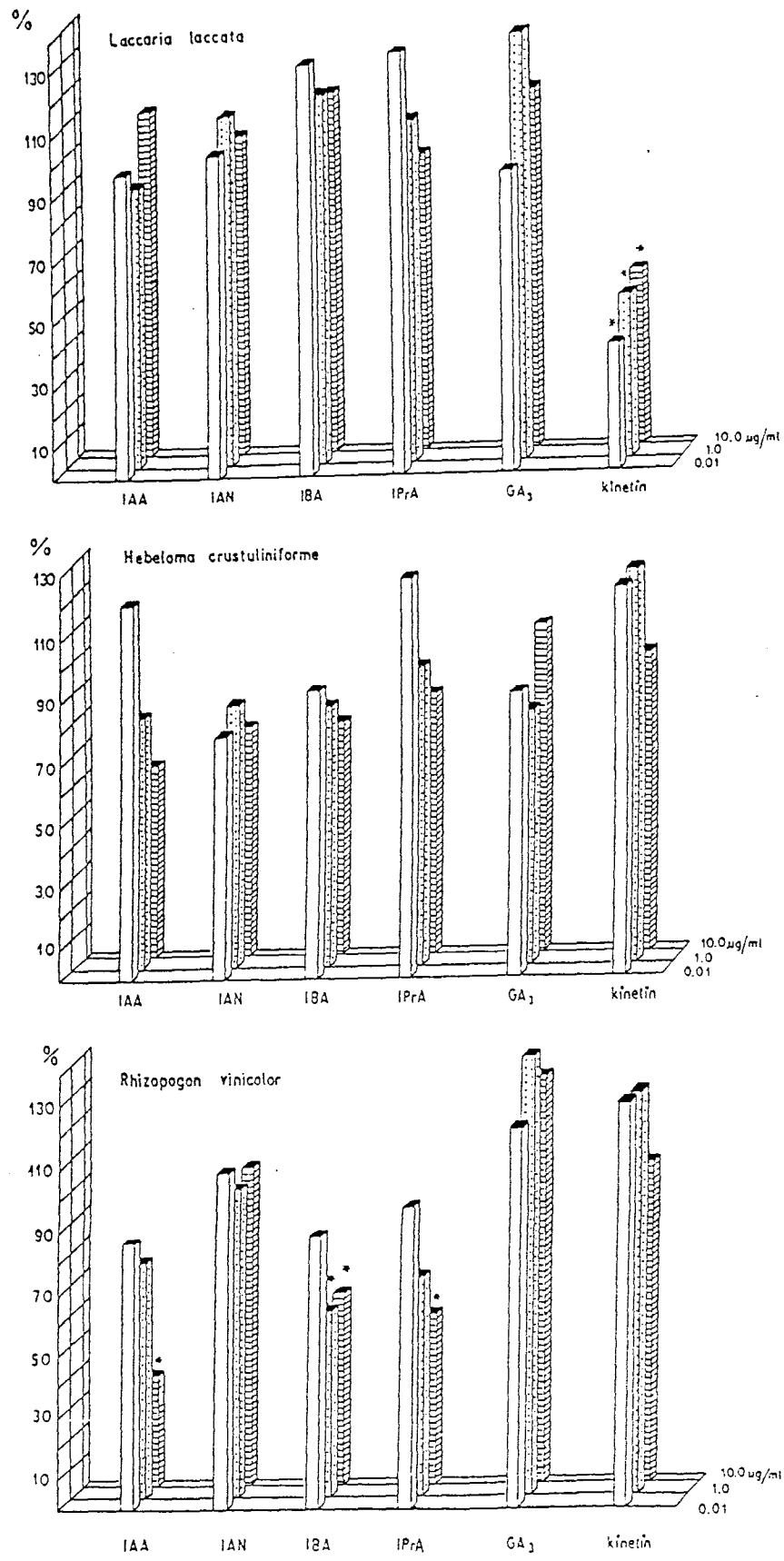

Fig. 1. Effect of plant growth regulators on biomass production by mycorrhizal fungi in liquid Lamb's medium (in % of control growth). Asterisks indicate significant differences as compared with control (t-test, $p \leq 0.05$).

Table 2. Effect of GA_3 and kinetin on biomass production by ectomycorrhizal fungi in liquid Lamb's medium (mg of dry mycelium/100 ml of the medium ± standard error).

Plant growth regulators	Concentrations of GA_3 and kinetin – µg/ml			
	0 (control)	0.01	1.0	10.0
		Laccaria laccata		
GA_3	21.7 ± 4.7a	21.0 ± 7.0a	29.7 ± 6.7a	25.2 ± 5.6a
kinetin	47.9 ± 4.9b	19.6 ± 4.2a	25.4 ± 3.2a	27.2 ± 2.6a
		Rhizopogon vinicolor		
GA_3	23.2 ± 6.3a	28.6 ± 6.6a	32.8 ± 5.3a	30.4 ± 5.1a
kinetin	21.8 ± 5.6a	28.5 ± 9.7a	28.4 ± 8.3a	23.4 ± 11.9a
		Hebeloma crustuliniforme		
GA_3	44.3 ± 4.9a	41.0 ± 6.0a	35.9 ± 7.2a	47.2 ± 8.9a
kinetin	31.9 ± 8.3a	39.9 ± 11.0a	40.3 ± 9.2a	30.6 ± 10.5a

Note: see Table 1.

observed (Table 1, Fig. 1–2). However the effect of IAA on growth of *Rh. vinicolor* and *H. crustuliniforme* was noted. IAA at the highest concentration (10 µg/ml) inhibited significantly the growth of *Rh. vinicolor* in liquid medium (dry mass of mycelium reached in this experimental combination only 36% of the control mycelium. A similar response was observed in *H. crustuliniforme*. The linear growth of both fungi under the influence of the highest concentration of IAA was inhibited too. However in liquid medium *H. crustuliniforme* was inhibited stronger than *Rh. vinicolor* (Fig. 2). Stimulation of the linear growth of *H. crustuliniforme* with IAA at concentration 0.01 µg/ml

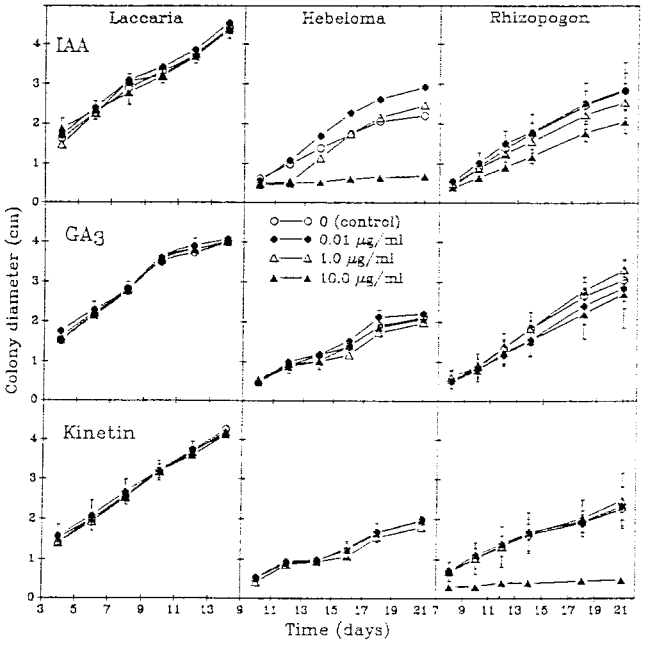

Fig. 2. Effect of plant growth regulators on linear growth of mycorrhizal fungi on agar Lamb's medium.

has been stated. Statistically significant stimulation appeared after 14 days of incubation and lasted to the end of the experiment (to the 21st day). Biomass production of *H. crustuliniforme* in medium supplemented with the lowest concentration of IAA was enhanced but these data were not statistically significant (Table 1, Fig. 1). Besides IAA other auxins did not affect the biomass yield of *H. crustuliniforme* but IBA and IPrA at higher concentrations inhibited growth of *Rh. vinicolor* (Table 1).

Kinetin added to the liquid medium independently of the concentration inhibited the biomass production of *Lac. laccata* (dry weight of mycelium reached only about 50% as compared to the control mycelium), Fig. 1. However the effect of kinetin on the linear growth of this fungus was not observed. Reverse results were obtained with *Rh. vinicolor*. This fungus was strongly inhibited by kinetin (at concentration 10 µg/ml) on agar medium but it did not respond to this growth regulator in liquid medium.

Discussion

The importance of auxins, gibberellins and cytokinins in plant growth and development is known. The role of these substances in microorganisms is not elucidated as yet. However some microorganisms were found to respond to plant growth regulators added to the culture media (Gruen 1959, Shklyar 1965). Many papers published concern the effect of auxins on microfungi such as *Saccharomyces, Penicillium, Aspergillus, Neurospora* (a review by Gruen 1959). The effect of plant growth regulators on pathogenic fungi and the process of infection was studied too (Atmar et al. 1976; Kriesel 1989; Michniewicz 1989, Strzelczyk and Reddy 1989). The data on the influence of plant hormones on mycorrhizal fungi are scarce. Gogala and Pohleven (1976) have shown that cytokinins promoted the mycelial growth of *Suillus variegatus* and affected the content of K, Ca, P and Na in the mycelium of this fungus (Pohleven and Gogala 1986). In the presence of kinetin the uptake of Cd, Zn, P by some ectomycorrhizal fungi increased significantly (Stegnar et al. 1978). Gogala (1989) studied the effect of jasmonic acid (JA), which is considered to be a new type of plant growth regulator, on biomass production in *Suillus variegatus*. She found that JA was a strong inhibitor of growth even at the lowest concentration (0.1 µg/l of medium). JA applied together with other hormones (kinetin, IAA, ABA, GA_3) showed an inhibitory effect too.

In our studies no significant effect of GA_3 on the growth of *Lac. laccata, Rh. vinicolor* and *H. crustuliniforme* was noted. Such fungi as *Aspergillus, Fusarium, Penicillium* also did not respond to GA_3 (Shklyar 1965). GA_3 in concentrations of 0.2 and 2.0 mg/l did not affect the growth of *Pythium myriotylum* but inhibited the growth of this fungus at concentration 200.0 mg/l (Hale et al. 1981). Enhancement of growth of pathogenic fungi like *Rhizoctonia solani* and *Fusarium culmorum* by GA_3 was observed (Michniewicz 1989, Strzelczyk and Reddy 1989). There are no data on the influence of gibberellins on the growth of mycorrhizal fungi.

The effect of kinetin on *Lac. laccata* and *Rh. vinicolor* depended upon the type of medium. Different reactions of microorganisms grown in liquid and on agar media with IAA were described earlier by Gruen (1959). Stimulation of fungal growth by kinetin was observed more often than inhibition (Barea et al. 1974; Atmar et al. 1976; Gogala and Pohleven 1976; Gogala 1989; Reddy and Strzelczyk 1989). Despite the agreement of many researchers that optimal concentrations of cytokinins are stimulatory for the fungi it is assumed that these compounds are not necessary for the growth of microorganisms (Greene 1980).

The reactions of the fungi studied on auxins were different. Stimulation of growth of *H. crustuliniforme* was noted at IAA concentration of 0.01 μg/ml but inhibition at 10.0 μg IAA/ml. Inhibition of *Rh. vinicolor* growth was caused not only by IAA but also by IBA and IPrA. *Lac. laccata* did not respond on any of the auxins used. Our results are in accordance with earlier observations that the effect of plant growth regulators on fungi depends on many factors such as the kind of hormone, its concentration, type of the medium and the fungus species (Gruen 1959; Gogala 1989).

According to the data obtained from the literature as well as obtained in this work it can be assumed that auxins, gibberellins and cytokinins do not play the role of hormonal factor in microorganisms.

Acknowledgements

This research was carried out under a joint program performed in cooperation with the United States Department of Agriculture and the Polish Ministry of Agriculture and Food Production under the Second M. Sklodowska-Curie Fund (Project No. PL-AES-139).

References

1 Atmar, V. T., Thorneberry, G. O., Kuhn, G. D. (1976): Effects of adenine and cytokinins on growth and protein kinase activity of *Verticillium albo-atrum*. Mycopathol. *59*, 171–174.

2 Barea, J. M., Navarro, E., Palomares, A., Montoya, E. (1974): A rapid microbiological assay method for auxins, gibberellic acid and kinetin using yeast. J. Appl. Bact. *37*, 171–174.

3 Frankenberger, W. T. Jr., Poth, M. (1987): Biosynthesis of indole-3-acetic acid by the pine ectomycorrhizal fungus *Pisolithus tinctorius*. Appl. Environ. Microbiol. *53*, 2908–2913.

4 Gay, G. (1986): Effect of glucose on indole-3-acetic acid production by the ectomycorrhizal fungus *Hebeloma hiemale* in pure culture. Physiol. Veg. *24*, 185–192.

5 Gay, G., Rouillon, R., Bernillon, J., Favre-Bonvin, J. (1989): IAA biosynthesis by the ectomycorrhizal fungus *Hebeloma hiemale* as affected by different precursors. Can. J. Bot. *67*, 2235–2239.

6 Gogala, N. (1989): Growth substances in root exudate of *Pinus sylvestris* – their influence on mycorrhizal fungi (Effects of jasmonic acid). Agric. Ecosystems Environ. *28*, 151–154.

7 Gogala, N. (1991): Regulation of mycorrhizal infection by hormonal factors produced by hosts and fungi. Experientia *47*, 331–340.

8 Gogala, N., Pohleven, F. (1976): The effect of cytokinins and auxins on the growth of mycorrhizal fungus *Suillus variegatus*. Acta Bot. Croat. *35*, 129–134.

9 Greene, E. M. (1980): Cytokinin production by microorganisms. Bot. Rev. *46*, 26–74.

10 Gruen, H. E. (1959): Auxins and fungi. Ann. Rev. Plant. Physiol. *10*, 405–440.

11 Hale, M. G., Moore, L. D., Orcutt, D. M. (1981): Effects of gibberellin A_3 and 2,4-D on plant and root exudate lipids and susceptibility to *Pythium myriotylum*. Soil. Biol. Biochem. *13*, 395–399.

12 Hanley, K. M., Greene, D. W. (1987): Gibberellin-like compounds from two ectomycorrhizal fungi and GA_3 response on Scotch pine seedlings. Hort. Sci. *22*, 591–594.

13 Ho, I. (1987a): Comparison of eight *Pisolithus tinctorius* isolates for growth rate, enzyme activity and phytohormone production. Can. J. For. Res. *17*, 31–35.

14 Ho, I. (1987b): Enzyme activity and phytohormone production of a mycorrhizal fungus, *Laccaria laccata* Can. J. For. Res. *17*, 855–858.

15 Kampert, M., Strzelczyk, E. (1984): Effect of pH on production of cytokinin-like substances by bacteria isolated from soil, rhizosphere and mycorrhizosphere of pine (*Pinus sylvestris* L.). Acta Microbiol. Polon. *33*, 77–85.

16 Kampert, M., Strzelczyk, E. (1989): Effect of amino acids on cytokinin-like substances production by mycorrhizal fungi of pine (*Pinus sylvestris* L.). Agric. Ecosystems Environ. *28*, 219–228.

17 Kriesel, K. (1989): The role of growth regulators in the host-parasite relationship between pine seeds (*Pinus sylvestris* L.) and isolates of *Cylindrocarpon destructans* Zins. Scholt. III Gibberellins. Bull. Polish Acad. Sci. *37*, 33–40.

18 Michniewicz, M. (1989): Growth regulators formed by Fusaria: Their significance for fungus growth, sporulation and pathogenicity towards the host plant. In: Chelkowski, J. (ed.). Fusarium mycotoxins, taxonomy and pathogenicity. Elsevier, Amsterdam, Oxford, New York, Tokyo, pp. 227–241.

19 Pohleven, F., Gogala, N. (1986): The influence of natural cytokinins on the content of K, P, Ca and Na in the mycelium of the mycorrhizal fungus *Suillus variegatus*. Biol. Vestn. *34*, 79–88.

20 Pokojska, A., Strzelczyk, E. (1988): Effect of organic acids on production of auxin-like substances by ectomycorrhizal fungi. Symbiosis *6*, 211–224.

21 Reddy, M. N., Strzelczyk, E. (1989): Influence of plant growth regulators on growth and virulence of *Rhizoctonia solani* Kuhn pathogenic to groundnut seedlings *Arachis hypogea* L. var. TMV 2. J. Phytopathology *125*, 187–191.

22 Rudawska, M. (1982): Effect of various organic sources of nitrogen on the growth of mycelium and content of auxin and cytokinin in cultures of some mycorrhizal fungi. Acta Physiol. Plant. *4*, 11–20.

23 Shklyar, M. S. (1965): Effect of gibberellins on microorganisms and on the enzymatic activity of barley during malt growth. A review. Prikladnaya biokhimiya i mikrobiologiya *1*, 102–109.

24 Slankis, V. (1950): Effect of α-naphthaleneacetic acid on dichotomous branching of isolated roots of *Pinus silvestris*. Physiol. Plant. *3*, 40–44.

25 Slankis, V. (1973): Hormonal relationships in mycorrhizal development. In: Marks, G. C., Kozlowski, T. T. (eds.). Ectomycorrhizae – Their ecology and physiology. Academic Press, New York, pp. 231–298.
26 Stegnar, P., Gogala, N., Pohleven, F. (1978): The uptake of cadmium, zinc, phosphorus, and plant hormone kinetin by ectomycorrhizal fungi. Acta Bot. Croat. *37*, 67–73.
27 Strzelczyk, E., Pokojska-Burdziej, A. (1984): Production of auxins and gibberellin-like substances by mycorrhizal fungi, bacteria and actinomycetes isolated from soil and the mycorrhizosphere of pine (*Pinus sylvestris* L.). Plant. Soil. *81*, 185–194.

Key words: Ectomycorrhizal fungi – Growth – Auxins – Gibberelic acid – Kinetin

A. Pokojska, Institute for Biology, Laboratory of Microbiology, Nicolaus Copernicus University, ul. Gagarina 11, 87–100 Torun, Poland