EFFECT OF AN IAA OVERPRODUCER MUTANT
OF THE FUNGUS *HEBELOMA CYLINDROSPORUM* ROMAGNESI
ON THE EARLY STAGES OF ECTOMYCORRHIZAL INFECTION
AND CARBOHYDRATE CONTENT IN SEEDLINGS OF *PINUS PINASTER* (AIT.) SOL.

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ABSTRACT

Ectomycorrhizal and control seedlings of *Pinus pinaster* were cultured on a synthetic Melin-Norkrans medium in Petri dishes. Seedlings were inoculated with a mycelial slurry of an indole-3-acetic acid (IAA) overproducer *Hebeloma cylindrosporum* mutant 331. The wild strain *H. cylindrosporum* h1 was used as a reference. Medium was supplemented or not with glucose. The mycelial slurry appeared to be very effective for mycorrhizal inoculation even on the medium without glucose. In such culture conditions ectomycorrhizal ability of the IAA overproducer mutant 331 was significantly higher than of the comparable wild type. The highest content of soluble sugars was found in stems and roots of plants mycorrhizal with the mutant followed by mycorrhizal plants with the wild type and then by the uninoculated control. Sucrose practically disappeared from roots of mycorrhizal plants. Starch content in roots of mycorrhizal plants with the IAA overproducer mutant was lower as compared with other treatments. Fungal auxin in mycorrhizal symbiosis seems to be responsible for maintaining the source-sink relationship. This is revealed by higher sugar level in the host's photosynthetic tissue (source) and the rise of soluble sugar content in roots (sink) due to enhanced translocation of sugars to the roots and auxin stimulated conversion of carbohydrates (sucrose, starch) of the host.

KEY WORDS: Indole-3-acetic acid, IAA overproducer, *Hebeloma cylindrosporum*, *Pinus pinaster*, ectomycorrhizae.

INTRODUCTION

The mechanism by which the symbiotic relationship between ectomycorrhizal fungi and tree roots is regulated has been a subject of controversy since 1940 s. This topic was reviewed by Nylund (1988) and Gogala (1991). The main theories emphasize the role of carbohydrates (Björkman 1942) and auxins (Slankis 1973) in the regulation of mycorrhizal association. Although the ability of ectomycorrhizal fungi to synthesize auxins (IAA-Indole-3-acetic acid) is now well established (Moser 1959, Ulrich 1960, Ek et al. 1983, Rudawska 1980, Gay and Debaud 1987) the physiological significance of this metabolite in ectomycorrhizas remains unclear. Slankis' (1973) hormone theory states that the fungal production of IAA is essential for mycorrhizal formation. This theory is based on findings that treatment of pine roots with

exogenous auxin stimulates dichotomous branching while inhibiting the formation of root hairs. This change in root morphology is similar to that induced by mycorrhizal colonization (Slankis 1973). Fungal IAA may also promote establishment of the Hartig net by inducing host cell wall loosening (Tomaszewski and Wojciechowska 1974, Dudridge and Read 1984) and could also affect host cell membrane permeability providing organic compounds necessary for the fungal partner to grow and achieve its life cycle. It has been estimated that 15-30% of the total assimilated carbon in a coniferous forest is allocated to ectomycorrhizal fungi (Fogel and Hunt 1983). Although an increase of carbohydrate exudation during ectomycorrhiza formation is possible as an effect of hormonal substances released by fungus and acting upon the host cells, its operation has not been experimentally confirmed. To clarify this unsolved problem in the area of hormonal relationships in ectomycorrhiza, a very useful, biological model of an IAA overproducer monokaryotic mutant of *H. cylindrosporum* Romagnesi was utilised (Durand et al 1992). This mutant was selected in earlier experiments be-

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Fig. 1. Dynamics of ectomycorrhiza formation on *P. pinaster* seedlings inoculated with the IAA overproducer mutant 331 and the wild type (h1) of *H. cylindrosporium*. (culture medium with glucose $1\text{ g} \cdot \text{L}^{-1}$).

cause of its capacity to metabolize endogenous tryptophan and in consequence cause IAA-overproduction (Durand et al. 1992). Structural observations of ectomycorrhizas formed by this mutant with *Pinus pinaster* seedlings revealed a large mantle and an overdeveloped Hartig net which reached even the endodermis, whereas the wild type mycorrhiza had a thin mantle and a uniseriate Hartig net which reached the second layer of cortical cells as generally described (Gea et al. 1995, in press). It has been hypothesized that fungal IAA may evoke these morphological changes via alterations in the metabolism of the host tree favourable for the mycelial partner. Because mycorrhizal fungi are for their source of carbon almost entirely dependent on soluble carbohydrates produced by the host plant, carbohydrates contents in seedlings mycorrhizal with the IAA overproducer mutant and the wild type were determined.

The main study was preceded by methodological attempts testing the mycelial slurry for mycorrhizal synthesis and mycorrhizal synthesis in the absence of glucose in the medium.

MATERIALS AND METHODS

Fungal material

Hebeloma cylindrosporium wild type monokaryon h1 was isolated from single spore germination after laboratory fruiting of the wild dikaryon HC1 (Debaud and Gay 1987). The IAA overproducer monokaryotic mutant h1 FIR 4 F1 331 (shortened as 331) used in this work was fluorindole resistant (= FIR) and represents a progeny of an IAA overproducer mutant h1 FIR 4 obtained by Durand et al. (1992).

Plant material

Seedlings of *Pinus pinaster* (Ait) Sol were obtained from seeds collected in Medoc (SW of France). The seeds were imbibed in distilled water for 60 h and subsequently surface sterilized for 3 min with 0.02 % HgCl_2 and 30 min with 30% H_2O_2 . The seeds were germinated at $18 \pm 1^\circ\text{C}$ under photope-riodic illuminations, as previously described (Debaud and Gay 1987).

Fig. 2. Root system of *Pinus pinaster* seedlings inoculated with a mycelial slurry of *H. cylindrosporum* mutant 331 (A) and wild type h1 (B) on a medium with glucose (Exp. No. 1).

Ectomycorrhizal synthesis

Mycorrhizas were obtained under axenic conditions in large, round Petri dishes (140 mm in diameter) filled with 80 ml Melin's nutrient solution as modified by Norkrans (1949) (MN) and supplemented with Heller's (1953) micronutrients, 120 nM thiamine and 1.6 nM biotin. Depending on the experiment the medium was supplemented, or not, with glucose ($1 \text{ g} \cdot \text{L}^{-1}$).

The surface of the agar was covered with an autoclaved disc of Whatman filter paper No 1 (130 mm in diameter). Four weeks old *P. pinaster* seedlings obtained as described above were laid flat onto the filter paper of each Petri dish.

A new method for synthesizing ectomycorrhizae was employed using mycelial slurry for seedling inoculation. The slurry was obtained as follows: 2-3 weeks old fungal cultures grown on Oddoux agar medium (1957) were cut carefully into very small pieces and transferred into the liquid Oddoux medium in Petri dishes. After 7 days the mycelium was transferred into 30 ml of the fresh Oddoux liquid medium in small Erlenmeyer flasks (100 ml), homogenized in sterile conditions, transferred onto Petri dishes and left to grow for the next 7 days. After that time the abundant, compact mycelial mat was transferred into a new half-strength MN medium without glucose, homogenized and left to grow for 24 h. After that time the mycelium was ready for seedlings inoculation, taking 400 l of the slurry for the inoculation of 1 seedling.

The inoculated plants were placed at $22 \pm 1^\circ\text{C}$ under photo-periodic illumination (16h) provided by General Electric Cool White fluorescent lamps. The energy fluence rate at the culture level was 1200 mW^{-2} . The number of mycorrhizas formed in Petri dishes was estimated using a stereomicroscope during an approximately one-month culture period. The mycorrhizal activity of mycelia was expressed as total number of mycorrhizal root tips per plant. The number of replicates was 15-30 seedlings per mycelium. Data are given $\pm$ standard deviation of the mean ($p < 0.05$).

Sugar analysis

For carbohydrate analysis, the plant tissue (roots, stems, needles, lateral roots, main roots and mycorrhizas respectively) was ground in a mortar, extracted 2h in water (60°C), centrifuged (15000 g, 20 min) and analyzed enzymatically using Boehringer glucose, fructose and sucrose and starch kits for food analysis.

RESULTS

Seedlings of *P. pinaster* inoculated with the mycelial slurry on a medium with glucose.

(Experiment No. 1., Tables 1 and 2, Figs 1 and 2)

One month old *P. pinaster* seedlings, inoculated with a slurry of the mycelium *H. cylindrosporum* and cultured on the medium with glucose produced 85 mycorrhizal tips per plant with mutant 331 and 49 with the wild type h1 (Fig. 1). Both fungi cause very similar effects on the shoot growth. Root fresh weight however was higher in the plant mycorrhizal with the IAA-overproducer mutant (Tab. 1). Growth of the mycelial mat on the surface of roots of *P. pinaster* was much more abundant in case of mutant 331 than with the wild type h1 (Fig. 2). In roots of mycorrhizal plants there was a tendency to have lower individual and total soluble sugars content in relation to the uninoculated control. A very low

TABLE 1. Effect of inoculation with the 331 IAA overproducer mutant and the h1 wild type of *H. cylindrosporum* on the mycorrhizal activity and host growth on a medium with glucose ($1\text{g} \cdot \text{L}^{-1}$) of *P. pinaster* seedlings inoculated with a slurry, recorded after a 39 days culture period. Data are given standard deviation of the mean.

Mycelium	Number of laterals per plant	Number of mycorrhizal root tips per plant	Stem f.w. (mg)	Root f.w. (mg)
331	50 ± 4	88 ± 11	352 ± 10	111 ± 6
h1	42 ± 3	50 ± 7	363 ± 23	92 ± 7
uninoculated control	29 ± 7	—	268 ± 15	65 ± 3

level of sucrose was observed in roots of highly mycorrhizal plants with the mutant 331 (Tab. 2).

Seedlings of P. pinaster inoculated with the mycelial slurry on a medium without glucose.

(Experiment No. 2, Tables 3 and 4, Fig. 3 and 4)

This experiment revealed the possibility of ectomycorrhiza formation on a medium without the addition of external sugars. When glucose was absent in the culture medium, mycorrhiza formation on roots of seedlings inoculated with both fungi was quite satisfactory. Like in the previous experiment the IAA overproducer mutant 331 was characterized by very abundant growth of the mycelium on the surface of pine roots compared with the wild type. (Fig. 4.). IAA overproducer mutant 331 proved to be three times as efficient in mycorrhiza formation (estimated as the number of mycorrhizal root tips

per plant) as the wild type (Tab. 3, Fig. 3). However there was no difference in the effect of both fungi on root and stem fresh weight of seedlings. Root and stem fresh weight of both mycorrhizal treatments differed however significantly from the uninoculated control (Tab. 3). In roots and stems of seedlings in ectomycorrhizal symbiosis with mutant 331, a higher concentration of glucose, fructose and a total pool of soluble sugars was found than in seedlings mycorrhizal with the wild type and in the uninoculated control (Tab. 4). Generally a very low level of sucrose was observed in all samples. As in previous experiments there was no sucrose in roots forming mycorrhizae with the IAA overproducer mutant (Tab. 4). Starch was not observed in roots of seedlings mycorrhizal with the mutant, a very low level was present in roots mycorrhizal with the wild type but it was easy to find in uninoculated plants (Tab. 4).

TABLE 2. Effect of mycorrhiza on root and stem sugar concentration in seedlings of *P. pinaster* inoculated with slurry and growing on medium with glucose ($1\text{g} \cdot \text{L}^{-1}$). Roots (r) or stems (s) of 5 pooled seedlings were extracted after 39 days of culture. Data are given $\pm$ standard deviation of the mean.

Mycelium		Glucose	Fructose	Sucrose	Total sol. sug.
(mg $\cdot$ g $^{-1}$) f.w.					
331	r	2.0 ± 0.20	1.3 ± 0.20	0.02 ± 0.10	3.32 ± 0.20
	s	1.8 ± 0.10	1.5 ± 0.10	0.26 ± 0.10	3.56 ± 0.10
h1	r	1.7 ± 0.20	1.2 ± 0.20	0.14 ± 0.40	3.04 ± 0.20
	s	2.0 ± 0.04	1.8 ± 0.03	0.51 ± 0.01	4.31 ± 0.03
control	r	2.8 ± 0.50	1.9 ± 0.40	0.10	4.80 ± 0.07
	s	2.0 ± 0.20	1.7 ± 0.10	0.26 ± 0.1	4.01 ± 0.13

TABLE 3. Effect of inoculation with the 331 IAA overproducer mutant and the h1 wild type of *H. cylindrosporum* on the mycorrhizal activity and host growth on a medium without glucose of *P. pinaster* seedlings inoculated with a slurry. Data are given $\pm$ standard deviation of the mean.

Mycelium	Number of laterals per plant*	Number of mycorrhizal root tips per plant*	Stem** f.w. (mg)	Root** f.w. (mg)
331	44 ± 5	57 ± 8	388 ± 22	120 ± 10
h1	46 ± 4	19 ± 3	390 ± 18	110 ± 8
control (uninoculated plants)	13 ± 3	—	247 ± 21	66 ± 7

* Recorded after 36 days of culture

** Recorded after 40 days of culture

TABLE 4. Effect of mycorrhiza on root and stem sugar concentration in seedlings of *P. pinaster* inoculated with slurry and growing on medium without glucose. Roots (r) or stems (s) of 5 pooled seedlings were extracted after a 40 days culture period. Data are given $\pm$ standard deviation of the mean.

Mycelium		Glucose	Fructose	Sucrose	Total sol. sug.	Starch**
		(mg $\cdot$ g ⁻¹) f.w.			(μg $\cdot$ g ⁻¹) $\cdot$ f.w.	
331	r	3.8 $\pm$ 0.1	2.7 $\pm$ 0.2	0.00	6.5 $\pm$ 0.2	0.0
	s	4.2 $\pm$ 0.3	3.6 $\pm$ 0.2	0.34	8.1 $\pm$ 0.3	48 $\pm$ 5
h1	r	3.1 $\pm$ 0.1	2.4 $\pm$ 0.1	trace	5.5 $\pm$ 0.1	36
	s	3.3 $\pm$ 0.4	2.9 $\pm$ 0.3	0.16	6.3 $\pm$ 0.3	31
control	r	2.7 $\pm$ 0.1	2.0 $\pm$ 0.1	0.09	4.7 $\pm$ 0.1	422 $\pm$ 52
	s	2.8 $\pm$ 0.1	2.5 $\pm$ 0.1	0.32	5.6 $\pm$ 0.1	161 $\pm$ 14

** Recorded after 40 days of culture.

TABLE 5. Effect of mycorrhiza formation with the 331 IAA overproducer mutant and the h1 wild type of *H. cylindrosporum* on growth on a medium without glucose of different parts of *P. pinaster* seedlings inoculated with slurry, recorded after a 30 days culture period. Data are given $\pm$ standard deviation of the mean.

Mycelium	Needles	Stem	Main root	Lateral roots	Mycorrhizae
(mg fresh weight per plant)					
331	595 $\pm$ 47	70 $\pm$ 9	107 $\pm$ 16	110	22
h1	548 $\pm$ 42	57 $\pm$ 4	62 $\pm$ 6	62	9
uninoculated control	280 $\pm$ 30	47 $\pm$ 5	55 $\pm$ 5	26	—

TABLE 6. Effect of ectomycorrhiza formation with the IAA overproducer mutant 331 and the wild type h1 of *H. cylindrosporum* on sugars concentration in different parts of *P. pinaster* seedlings cultured on a medium without glucose.

Mycelium	Needles	Stem	Main root	Lateral roots	Mycorrhizae
GLUCOSE content*					
331	2.7 $\pm$ 0.1	4.4 $\pm$ 0.3	4.1 $\pm$ 0.4	2.6 $\pm$ 0.2	3.0
h1	3.3 $\pm$ 0.2	5.3 $\pm$ 0.2	3.3 $\pm$ 0.6	2.3	2.9
control	3.4 $\pm$ 0.3	3.5	3.4 $\pm$ 0.1	2.3	—
FRUCTOSE content*					
331	3.1 $\pm$ 0.4	3.8 $\pm$ 0.30	3.3 $\pm$ 0.40	1.8 $\pm$ 0.1	1.5
h1	2.9 $\pm$ 0.1	4.5 $\pm$ 0.04	2.9 $\pm$ 0.50	1.7	1.9
control	2.9 $\pm$ 0.2	2.8	2.5 $\pm$ 0.03	1.5	—
SUCROSE content*					
331	0.8 $\pm$ 0.3	0.3 $\pm$ 0.1	0.3 $\pm$ 0.20	0.00	0.3 $\pm$ 0.1
h1	0.1 $\pm$ 0.3	0.0	0.2	0.06	0.2
control	0.3 $\pm$ 0.2	0.5	0.4 $\pm$ 0.04	0.00	—
TOTAL SOLUBLE SUGARS content*					
331	6.5 $\pm$ 0.3	8.5 $\pm$ 0.2	7.7 $\pm$ 0.3	4.3 $\pm$ 0.15	4.8
h1	6.3 $\pm$ 0.2	9.8 $\pm$ 0.1	6.3 $\pm$ 0.4	4.1	4.9
control	6.6 $\pm$ 0.2	6.8	6.3 $\pm$ 0.1	3.8	—
STARCH content**					
331	0.0	321 $\pm$ 83	146 $\pm$ 69	136	0.0
h1	0.0	250 $\pm$ 77	61 $\pm$ 32	95	0.0
control	0.0	148	393 $\pm$ 19	196	—

* in mg $\cdot$ g⁻¹ fresh weight

** in μg $\cdot$ g⁻¹ fresh weight

Fig. 3. Dynamics of ectomycorrhiza formation on *P. pinaster* seedlings inoculated with the IAA overproducer mutant 331 and the wild type h1 of *H. cylindrosporum* (culture medium without glucose).

Effect of mycorrhizae with the overproducer mutant 331 and the wild type h1 H. cylindrosporum on the growth and carbohydrate content in different parts of P. pinaster seedlings.

(Experiment No. 3., inoculation with mycelial slurry on a medium without glucose, Table 5 and 6).

This experiment was conducted according to the scheme of experiment No. 2. and devoted to a more detailed sugar analysis in particular parts of the cultured seedlings *i.e.*: needles, stem, lateral roots, main root and mycorrhizas.

In this experiment mycorrhiza formation by mutant 331 and the wild type h1 of *H. cylindrosporum* was slightly lower than in experiment No. 3 (data not shown). Similarly as in previous experiments both isolates caused considerable increase in growth parameters of their host plant *P. pinaster* in comparison with the uninoculated control. Impact of the IAA overproducer mutant 331 on such growth parameters as needles, stem, main root, lateral roots and mycorrhizas fresh weight was significantly higher than that of the wild type (Table 5). Glucose and fructose concentration in needles, lateral roots and mycorrhizas of seedlings from different treatments did not differ considerably (Table 6). However glucose concentration was higher in stems and roots of plants mycorrhizal with mutant 331. Sucrose concentration was much higher in needles of 331 mutant inoculated plants than in uninoculated controls and in plants inoculated with the wild type. Total soluble sugar content was very similar in needles of mycorrhizal and control plants but higher in stems and roots of the mycorrhizal plants. There was no starch found in needles of all plants, however a significantly higher level was found in stems of 331 mutant inoculated plants than in stems of plants

mycorrhizal with the h1 wild type and in control plants. In roots on the other hand starch content was highest in the uninoculated control and lower in mycorrhizal plants (Table 6).

DISCUSSION

In our studies we demonstrated the possibility of using a mycelial suspension for seedling inoculation on Petri dishes, even on a medium without glucose (Exp. No. 2 and 3). Fungal development and mycorrhiza formation depended in this condition completely on sugars obtained from the host plant. Effectiveness of mycorrhiza formation was in such circumstances much higher in the case of the IAA overproducer mutant than in the case of the wild type. (see Fig. 2 and 4). This may imply an effect of IAA overproduction on the initial steps of mycorrhiza formation. Involvement of auxin in the early moments of ectomycorrhizas nascency is also suggested by the findings of Unestam and Stenstöm (1989). They found a greater number of mycorrhizal roots on *P. sylvestris* seedlings inoculated with *Amanita muscaria* when agar blocks with IAA were placed in close contact with the roots. Very similar observation was also made by Kieliszewska and Rudawska (1995) using selected naturally high and low IAA producing strains of *Paxillus involutus* and *Pinus sylvestris* seedlings in a sterile peat-perlite medium. Isolates characterized as having higher IAA-synthesizing activity appeared to be more abundant mycorrhiza formers in pure culture conditions. Existence of a correlation between the capability of auxin production and mycorrhizal activity of symbiotic fungi was already indicated by Tomaszewski (1974). He found that fungi able to synthesize IAA

Fig. 4. Root system of *P. pinaster* seedlings inoculated with a mycelial slurry of *H. cylindrosporum* mutant 331 (A) and wild type h1 (B) on a medium without glucose (Exp. No. 2)

without external tryptophan addition (as *Suillus luteus*, *S. variegatus*, *S. bovinus*, *Rhizopogon luteolus*) may have some advantage in mycorrhiza formation in relation to species which produce IAA taking tryptophan only from an external source; for example *Amanita* sp., *Lactarius* sp.). Similarly in our experiment the IAA overproducer mutant, showing an altered regulation of the tryptophan biosynthetic pathway, able to accumulate free tryptophan and metabolize it to IAA formed mycorrhizas to a much higher extent than a wild type, the lower producer of IAA. As a consequence of mycorrhiza formation a better growth of mycorrhizal seedlings was observed compared to the uninoculated controls which is in agreement with classical experiments (see Harley and Smith 1983). There was a very small difference in shoot growth (measured as fresh weight per 1 seedling) between seedlings inoculated with the mutant and with the wild type and a slightly better root growth was observed in case of seedlings bearing mycorrhizas with mutant 331. This may be a result of the greater ability to absorb nutrients from the culture medium due to higher mycorrhizal abundance.

The main purpose of this paper was to compare soluble sugars and starch content in different parts of seedlings of *P. pinaster*, being in the early stages of ectomycorrhizal association with both fungi and to indicate the possible effect of IAA overproduction.

In the first experiment glucose was present in the culture medium. In such culture conditions the content of glucose and fructose in stems and roots of both mycorrhizal treatments (331 mutant and h1 wild type) was differentiated, with a tendency for lower amounts in the case of seedlings mycorrhizal with the IAA overproducer mutant 331. It was rather difficult to find any distinct effect of IAA overproduction. It seems that glucose present in the culture medium could be, at least partly, responsible for this. Duddridge and Read (1984) showed that sugar added in synthesis experiments drastically increased the infectivity of the mycobiont, which consequently does not have to obtain its energy supply from the host. Duddridge (1986) also mentioned that the availability of an external C source to the fungus upsets the balance of symbiosis in favour of the fungal partner, causing changes in the host cell wall and the host-fungus interface. Presence of free glucose even in the concentration used in our experiment ($1\text{g} \cdot \text{L}^{-1}$) is hardly possible in natural conditions and evidently influenced the carbohydrate status of the host plant and fungus metabolism. IAA as a secondary metabolite is produced in cultures of mycorrhizal fungi in inverse ratio to fungal growth (Rudawska 1987). It was also shown by the results of Gay (1986) that glucose-rich nutrient solution stimulates fungal growth but inhibits IAA production by the ectomycorrhizal fungus *H. hiemale* in pure culture. Finally the most interesting fact from this experiments with glucose was that the mycobiont still colonizes the root at all, which implies that it must obtain from symbiosis something more than only carbohydrate energy.

A reverse situation was observed in the next experiment (No. 2) when glucose was absent in the culture medium. In such circumstances, when the mycorrhizal fungus was completely dependent for C source on the host plant (as in nature), a significant effect of symbiosis was observed on the carbohydrate status of upper and lower parts of the plant mycorrhizal with mutant 331 and with the wild type (Table 3 and 4). Formation of mycorrhizas with an IAA-overproducer mutant was reflected in the highest soluble sugar content in roots and stems of pine seedlings, followed by plants mycorrhizal with the wild type and then by the uninoculated control. Nyland (1988) summing up the present knowledge concerning

theories explaining the regulation of mycorrhiza formation suggested: "the fungus draws carbohydrate from the host, at least partly by means of an exudable regulatory metabolite, which increases carbohydrate concentrations in roots and causes them to leak. Some fungi do this more strongly than others, more likely leading to a more intense infection". Our results from the second experiment lend support to this statement. The exudable regulatory metabolite is probably IAA of fungal origin. Higher carbohydrate content was found in plants mycorrhizal with mutant 331 capable of IAA overproduction than in plants mycorrhizal with the weaker producer of IAA, wild strain of *H. cylindrosporum*. As a consequence mutant 331 was always characterized by more intense mycorrhizal infection than the wild type.

The subsequent experiment (No. 3) performed according to the same scheme as the previous one but with more detailed analysis of sugar in different parts of the plant, generally confirmed data from Exp. No. 2. Differentiated content of tested sugars was found in particular parts of the seedlings, with the highest concentration in stems of mycorrhizal plants. In stems of seedlings forming mycorrhiza with the wild type, a higher level of total soluble sugars was revealed than in stems of plants mycorrhizal with the mutant. However similarly like in the previous experiment in roots of seedlings forming mycorrhiza with the mutant, a significantly higher level of sugars was found than in roots mycorrhizal with the wild type and in nonmycorrhizal controls. Some difficulties in obtaining consistent results on the level of soluble sugar concentration indicate that a number of uncontrolled factors influence the carbohydrate pool during mycorrhiza formation (Nylund and Wallander 1989, Wallander and Nylund 1991). A slightly less pronounced effect of IAA overproduction on soluble sugar content in Exp. No. 3 may be the result of lower frequency of ectomycorrhizas obtained in this trial with the mutant 331, which is the main sink of simple sugars produced by the upper part of seedlings. The very young age of pine seedlings and early moment of ectomycorrhiza establishing, tested in the present experiment, may be one of the reason why our results are not as strong expressed as in previous data where authors clearly indicated that activities of the symbiotic fungi cause faster and greater flow of assimilates to the infected roots than in plants without mycorrhizae (Nelson 1964; Schweyrs and Meyer 1970; Reid et al. 1983). A suggestion has been made that diversion of carbohydrates to the mycorrhizal fungus stimulates photosynthesis rate (Reid et al. 1983). Finally the sugar content of the mycorrhizal plant constitutes the resultant between increased photosynthetic rate due to the mycorrhizal symbiosis and increased drain of carbohydrates by the fungal partner. Difficulties in showing increased allocation of sugars into mycorrhizal roots is that rather than retaining photosynthate, the mycorrhizal roots export it immediately to the extramatrical mycelium, whereas non-mycorrhizal roots accumulate photosynthate to a greater extent (Gorissen et al. 1991). Dosskey et al. (1990) demonstrated a non-nutritional basis for the increased rate of photosynthesis caused by some ectomycorrhizal fungi, which can be explained by increased photosynthate sink generated by extensive fungal growth associated with the mycorrhizas. Some fungi increased the rate of photosynthesis to higher extent than others. Presently we know that even between strains of the same species there are significant differences in physiological reactions (Kieliszewska and Rudawska 1995).

The question arises, to what extent IAA produced by mycorrhizal fungus may be involved in assimilate partitioning of their host plant? Auxin-induced increase in growth rate (cell

division) lead to increase in the flow of carbon to the area of growth (Weaver and Johnson 1985). Auxin may directly influence phloem transport. It has been suggested that IAA is transported long distances in plants and enhances transport rates in the phloem increasing the availability of carbohydrates in the sink (Patrick and Steains 1987). Using low concentration of IBA and IAA applied to the roots of *P. sylvestris* Nylund and Wallander (1989) showed enhanced net photosynthesis and increased concentrations of root sugars. Tomaszewski and Wojciechowska (1974) observed effect of exogenous auxin on the turnover of cell wall materials in pine roots: IAA treated roots exuded more radioactive photosynthates than the controls growing on plain nutrient. Auxin may also enhance activity of sucrose-cleaving enzymes (e.g. invertase or sucrose synthase) in the sink tissue (Morris and Arthur 1987). IAA can lower pH by increasing proton extrusion into the apoplast to levels at which acid invertase is activated (Hager et al 1991, Offler and Patrick 1986). Via auxin action the fungus *H. crustuliniforme* was able to increase invertase activity within mycorrhiza by lowering the pH of the interface towards a pH optimum for the enzyme (Salzer and Hager 1993). This is manifested in our experiments (No. 2) by the disappearing of sucrose from roots of mycorrhizal plants probably as a result of enhanced hydrolytic activity of responsible enzymes. More detailed analysis of sucrose from exp. No. 3 – indicates significant increase of the sucrose levels in needles of plants mycorrhizal with mutant 331. This may be interpreted as a sign that photosynthesis, and as a consequence carbohydrate production, was stimulated to a higher extent in plants mycorrhizal with mutant 331 but a rapid absorption by newly formed ectomycorrhizas prevents to reveal it in higher extent in roots. Such findings are in agreement with papers of Harley and Lewis (as reported and discussed in Harley and Smith 1983) and Nylund and Wallander (1989) who also found a rapid hydrolysis of sucrose in roots of mycorrhizal plants.

An addition of auxin to plants also enhances hydrolysis of starch into sugars (see Meyer 1974) and effects the translocation of sugar from the starch reservoir to those locations where the auxin is secreted (Cleland 1961, Thimann 1972). Starch analysis of plants forming mycorrhizas with the IAA overproducer mutant supports this hypothesis. The effect of IAA overproduction was strongest expressed on the starch level. According to the findings of Slankis (1971) auxin supplied externally to one part of the root system unfolds its action throughout the whole root system. This seems to be true also for our experiment where auxin effect exerted mostly on lateral roots is even stronger pronounced in the main root (much lower starch level than in the control). This decrease must be traced back to auxin secretion by both types of *H. cylindrosporum* used for mycorrhizal synthesis. Slightly higher level of starch in roots of seedlings mycorrhizal with the mutant may be ascribed to a generally increased sink effect in seedlings mycorrhizal with mutant compared with the wild type.

Several possible mechanisms were proposed for explaining how establishment of a carbon sink may be attained in ectomycorrhizal symbiosis (Martin et al. 1987). Effect of a mediator of fungal origin (IAA?) on host cell carbon metabolism was proposed by a few authors (Slankis 1973, Meyer 1974, Tomaszewski and Wojciechowska 1974, Nylund 1988). However so far no experimental research was available on intact mycorrhizal plants besides a paper of Nylund et al. (1994) who failed to show any IAA-overproduction effect due to a semi-hydroponic culture system not suitable for these purposes. Our studies involving seedlings mycorrhizal with an

IAA-overproducer mutant and cultured on a glucose free Petri dish system, provide preliminary data showing that at the early stages of ectomycorrhiza formation the mycobiont really affects its host carbohydrate metabolism via auxin action. Fungal auxin in mycorrhizal symbiosis seems to be responsible for maintaining a classical "source-sink" relationship. It reveals by higher sugar level in the host's photosynthetic tissue (source) and the rise of soluble sugar content in roots (sink) due to enhanced translocation of sugars and conversion of carbohydrates of the host (sucrose and starch decrease).

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WPLYW MUTANTA GRZYBA *HEBELOMA CYLINDROSPORUM*,
ZDOLNEGO DO NADPRODUKCJI AUKSYNY,
NA WCZESNE ETAPY INFEKCJI MIKORYZOWEJ
I ZAWARTOŚĆ WĘGLOWODANÓW W SIEWKACH *PINUS PINASTER*

STRESZCZENIE

Ektomikoryzowe i kontrolne siewki *Pinus pinaster* hodowano na syntetycznej pożywce Melin-Norkrans na szalkach Petriego. Siewki zaszczipiono zawiesiną grzybní dwóch szczepów *Hebeloma cylindrosporum*: nadproducentem auksyny, mutantem 331 oraz dzikim szczepem h1. Połowa siewek rosła na pożywce pozbawionej glukozy. Zawiesina grzybní, zastosowana jako źródło inokulum do szczepienia siewek sosny, okazała się bardzo skuteczna w formowaniu mikoryz, nawet na pożywce bez glukozy. Siewki zaszczipione grzybníą mutantu 331 tworzyły więcej mikoryz niż siewki ze szczepem dzikim. Najwyższą zawartość cukrów rozpuszczalnych stwierdzono w korzeniach i części nadziemnej siewek tworzących mikoryzę ze szczepem 331. W korzeniach siewek mikoryzowych wykazano zaledwie śladowe ilości sacharozy. Zawartość skrobiu była najniższa w siewkach zaszczipionych mutantem 331.

Wydaje się, że auksyna produkowana przez grzyby mikoryzowe może regulować zawartość i przemieszczanie się cukrów w poszczególnych częściach rośliny gospodarza. Działanie auksyny objawia się podwyższonym poziomem węglowodanów w tkankach części nadziemnej na skutek intensyfikacji procesów fotosyntezy oraz wzrostem zawartości cukrów w korzeniach w następstwie stymulowanego auksyną przemieszczania ich do mikoryz i enzymatycznego przekształcania sacharozy i skrobiu. Na etapie formowania się symbiozy auksyna grzybowa poprzez wpływ na metabolizm węglowodanów może spełniać ważną funkcję regulującą szybkość i intensywność tworzenia ektomikoryz.

SŁOWA KLUCZOWE: Kwas indolilo-3-octowy, nadproducent IAA, *Hebeloma cylindrosporum*, *Pinus pinaster*, ektomikoryza.