Mycorrhiza (1992) 1:63-69

Mycorrhiza

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The effect of tree-root exudates on the growth rate of ectomycorrhizal and saprotrophic fungi

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Summary. The object of this investigation was the promotion by root exudates of the growth rate of ectomycorrhizal fungi, discovered by Elias Melin in 1954. Eight ectomycorrhizal and ten non-mycorrhizal species were used as test fungi in the experiments. Different species often reacted differently: none of the eight isolated strains of *Suillus luteus* were promoted by pine-root exudate, whereas the growth rates of all seven strains of *S. granulatus* were increased. Among the other ectomycorrhizal species, *S. variegatus*, *Laccaria bicolor*, *Pisolithus tinctorius* and *Thelephora terrestris*, each represented by only one, two or three strains, usually reacted to the pine root exudate with an increased growth rate; *S. bovinus* and *Paxillus involutus* did not respond at all. Hitherto, studies of root-exudate effects on fungi have been based exclusively on the responses of ectomycorrhizal species; in the present study saprotrophic fungi were also used as test organisms. Seven out of ten saprotrophic species reacted with markedly accelerated growth when exposed to a pine-root exudate. Melin's assumption that a constituent of the root exudate, the "M-factor", could replace the exudate growth-promoting activity was verified. By means of TLC fraction it was found that the fatty acid palmitic acid alone caused an increase in growth rate equal to that of the pine-root exudate. In line with previously published data by Gogala (1970), we also showed that certain cytokinins, especially isopentenylaminopurine, could act as substitutes for the total root exudate. Thus both palmitic acid and isopentenylaminopurine are able to function as M-factors equivalent to a root exudate.

Key words: M-factor - Palmitic acid - Sterols - Cytokinins

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Introduction

Elias Melin found that exudates from roots, especially tree roots, increased the growth rate of ectomycorrhizal fungi in vitro (Melin 1954). Exudates from the roots of some herbaceous species possess a similar capacity (Melin and Rama Das 1954). The effective compound(s) in the exudates was called "M-factor" or "factor M". Its chemical identity was not determined.

The organic content of root exudates has been studied by many researchers during the last decades (e.g. Rovira 1965; Smith 1969; Krupa and Fries 1971; Bowen and Theodorou 1973; Vancura et al. 1977; Hale and Moore 1979). In general carbohydrates form a small part of the exudate content, amino acids form a larger part and other organic acids constitute by far the largest component. Later studies (Fries et al. 1985) showed the latter components to include various fatty acids, most of them unsaturated and with 16-20 carbon atoms.

As the basic nutrient medium for his experiments with root exudates Melin used a "maximum nutrient solution", which besides the usual chemical ingredients also contained ten water-soluble vitamins and 19 amino acids in "biological" proportions (Schmidt 1944). Consequently, growth-stimulating effects of added root exudates must be caused by "other organic acids" or as yet unknown compounds in the exudate.

Attempts to solve the M-factor problem are justified for at least two reasons. If exudates from tree roots stimulate the growth rate of soil fungi, particularly ectomycorrhiza-formers, as in Melin's experiments (Melin 1963), this reaction may play an important and interesting role in forest soil ecology. Furthermore, when the chemical constitution of the active factor in the exudate is identified, this should lead to an improvement in the composition of synthetic nutrient media for the axenic culture of fungi.This might also lead to a deeper insight into the physiology of fungi grown in vitro.

Despite Melin's extensive studies, the response of several groups of soil fungi to root exudates remains unknown. An insight into the composition of root exu-

dates is also highly desirable, in particular for the purpose of identifying the growth-stimulating component(s), the M-factor.

Materials and methods

Fungi and media

The species of ectomycorrhizal and saprotrophic fungi used in the experiments are listed in Table 1. Most of them were isolated from living material collected in the forests near Uppsala, Sweden. Some were obtained through spore germination, the others were tissue cultures from fruit bodies. The axenic mycelia were maintained as stock cultures on N6:5 medium solidified with 1.5% agar (Fries 1978) but without malt extract. The nutrient medium employed in most of the experiments was N6:5 medium, but a further three media, BAF (Moser 1960), Hagem (Modess 1941) and Melin's "maximum nutrient solution" (Melin and Rama Das 1954) were used in some experiments. Homogeneous inocula were necessary for laboratory experiments. The inocula (4 mm in diameter) were floated on the liquid surface, one inoculum per flask, in 100-ml Erlenmeyer flasks containing 20 ml liquid medium and incubated stationary at 25° C in darkness. *L. bicolor* did not float but grew submerged.

The incubation time varied with the species studied. Each harvest comprised five flasks per series. The mycelia were harvested on Munktell Number 1 filter papers by one wash and oven-dried for 4 h at 104° C. The average yields of mycelium were calculated. The data in the tables represent the mean dry weight of mycelium (mg/flask) and the standard errors of the mean. The significances of the differences from the control values at the different probability levels (* $P<0.05$, ** $P<0.01$ and *** $P<0.001$) are also shown.

Table 1. Ectomycorrhizal and saprotrophic fungi used

Species	No. of strains	Origin of cultures
Ectomycorrhizal		
Suillus luteus (L. ex Fr.) S. F. Gray	8	Tissue culture
Suillus granulatus (L. ex Fr.) O. Kuntze	6	Monosporous
	1	Tissue culture
Suillus variegatus (Schwartz ex Fr.) O. Kuntze	2	Tissue culture
Suillus bovinus (L. ex Fr.) O. Kuntze	1	Tissue culture
Laccaria bicolor (Maire) Orton	1	Tissue culture
Paxillus involutus (Batsch) Fr.	1	Tissue culture
Pisolithus tinctorius (Pers) Coker & Couch (Pt)	2	Tissue culture
Telephora terrestris (Ehrb.) Fr.	1	Polysporous
Saprotrophic		
Lentinellus omphalodes (Fr.) P. Karst.	1	Tissue culture
Flammulina velutipes (Curt. ex Fr.) Sing.	1	Tissue culture
Schizophyllum commune Fr. ex Fr.	1	Tissue culture
Coprinus comatus (Mull. ex Fr.) S. F. Gray	1	Tissue culture
Stereum hirsutum (Willd.) Pers.	1	Tissue culture
Fomes annosus Fr.	1	Tissue culture
Stereum sanguinolentum (Alb. et Schw.) Fr.	1	Tissue culture
Trametes serialis Fr.	1	Tissue culture
Polyporus versicolor (L.) Fr.	1	Tissue culture
Coniophora cerebella (Pers.) Duby	1	Tissue culture

Exudate from nursery pine roots and from axenic pine seedling roots

Pine root exudates were obtained from young Scots pines (*Pinus sylvestris* L., 2 or 3 years old) grown in a forest tree nursery near Uppsala. These 20-30 fresh pine trees were cleaned and kept in 500 ml distilled water in 1-l beakers for 3 days. The exudate solution was concentrated by rotatory evaporation to 1 ml and added to the liquid medium before autoclave sterilization.

The pine seedling root exudate was obtained from aseptically grown pine seedlings. The seeds, supplied by Eksjö Nursery, Dömänverket, Sweden, were surface-sterilized in 70% ethanol for 30 s and soaked in 30% (v/v) hydrogen peroxide for 20 min, followed by one wash in sterile distilled water. The seeds were germinated on water-agar plates at 20° C in the light. When the roots were 2–3 cm long, the seedlings were fixed aseptically onto glass slides by means of double-sided, adhesive tape. Each slide carried 20 seedlings. The slides were placed in square, glass staining jars, three slides per jar. Each jar contained about 70 ml sterile distilled water, in which the seedling roots remained immersed for exudation during 4 days. The exudate solution was then concentrated by evaporation for further use as an addition to the medium in culture experiments.

In some experiments, small (ca. 3 cm long) pieces of living, sterile seedling roots were added to the culture flask instead of prepared exudates and kept there during the whole experiment.

Thin-layer chromatograms of the exudates

Exudate solutions were separated into lipophilic and non-lipophilic fractions using hexane/isopropanol/water 10:10:9 v/v/v. The lipophilic fraction was analysed by TLC according to the method described by Dutta and Appelquist (1989). Aluminium plates (20 × 20 cm) coated with silica gel G60 (0.25 mm, Merck) without fluorescence indicator were used. A reference lipid mixture was applied at the same time. The separated lipid fractions were scraped off and extracted with methanol/chloroform 2:1 v/v overnight at 4° C, dried in liquid nitrogen and dissolved in alcohol for later use.

Putative components of the exudates tested as pure chemicals

Putative components of the root exudates, such as palmitic and stearic acids (Fries et al. 1985), were obtained from Sigma. Cytokinins (Gogala 1970) from Apex Organics Ltd., Oxford, were also employed in some experiments.

Results

The influence of unfractioned pine root exudates on the growth of ectomycorrhizal fungi

The data in Table 2 show that four species of the same genus *Suillus* exhibited quite different responses to the whole root exudates. In *Suillus*, the mycelial growth of *S. granulatus* and *S. variegatus* was significantly stimulated in terms of dry weight by the exudates at three concentrations. On the other hand, all eight strains of *S.*

Table 2. Effect of whole root exudates on the growth of ectomycorrhizal fungi. * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Species and strain number		Exudate concentration [mg/l]			
		15.0	5.0	1.5	Control
S. luteus	905	30.8±1.2	31.0±2.0	32.9±1.5	31.8±2.9
	906	25.2±1.2	35.6±1.4	36.6±1.2	33.6±0.7
	909	12.8±1.2	34.0±2.1	34.5±1.7	34.8±0.6
	324:A9	22.5±1.3	25.9±0.6	24.7±0.5	23.9±1.4
	304:B1	24.1±1.8	25.9±0.9	24.2±0.7	25.6±0.9
	306:C2	21.2±0.6	21.6±1.3	23.0±1.0	21.0±0.6
	896:D	21.1±1.2	21.7±1.2	21.9±1.6	21.6±1.1
	326:E7	25.7±0.6	25.3±1.1	22.4±0.6	23.7±0.4
S. granulatus	134:1	35.1±0.5**	35.9±1.4	35.0±1.3	32.0±0.6
	134:4	38.8±0.6***	35.4±1.0***	34.6±1.2**	27.3±1.2
	134:6	30.9±0.7*	30.1±1.3	31.2±1.2*	27.4±0.6
	134:8	35.4±0.4***	31.9±1.2**	33.9±0.9***	26.9±0.7
	396:1	34.5±1.2**	35.3±0.9***	33.2±1.3*	28.0±1.3
	177:7	34.4±1.7***	34.6±1.2***	25.0±0.9	25.0±0.9
	1089	32.2±1.0**	34.6±1.0***	31.7±1.2**	26.1±0.8
S. variegatus	842	32.1±1.1***	33.6±1.9***	33.3±0.8***	25.0±0.9
	1090	33.4±0.4***	22.4±1.2**	24.3±1.0***	16.4±1.0
S. bovinus	1091	7.7±0.3	7.8±0.1	21.9±1.1	23.1±2.8
L. bicolor	360	19.7±0.5***	15.5±2.0***	14.5±1.4***	4.9±1.0
P. involutus	65	27.2±1.3	25.1±0.4	24.4±1.6	24.3±1.2
P. tinctorius	900	22.2±2.0***	17.7±0.4***	16.3±0.6***	12.4±0.6
	899	36.9±2.2***	42.2±1.5***	36.1±2.5***	19.1±2.0
T. terrestris	886	20.8±0.8	24.4±2.0*	27.7±2.3**	16.6±2.2

luteus gave a weak or zero response. These results agree with the earlier findings of Melin (1962) that a weak response to the exudates occurs in some *Suillus* species. Six monosporous isolates and one tissue culture of *S. granulatus* responded positively to the exudate at the concentrations tested. It is obvious that different monosporous isolates and other strains of the same fungal species show a similar response to the root exudates. Such observations were not reported earlier. Even more striking responses appear in the show-growing species *L. bicolor* and the fast-growing species *P. tinctorius*. The stimulation by the exudates at particular concentrations varied with the species studied. In *L. bicolor*, a strong effect was obtained with a relatively high exudate concentration of 15.0 mg/l, while *T. terrestris* reacted contrarily.

The influence of pine-root exudates on non-mycorrhizal fungi

The effect of root exudates on the mycelial growth rate of ectomycorrhizal and saprotrophic fungi in four nutrient media is shown in Tables 3 and 4. Four out of six ectomycorrhizal fungi, one coprophile and six out of nine saprotrophic fungi were markedly stimulated in the N6:5 medium. Thus, the promoting effect of root exudates is not specific to ectomycorrhizal fungi but is also effective on some saprotrophic fungi. Growth stimulation by exudates at a concentration of 15.0 mg/l was also seen in the other nutrient media. The growth-rate response of some fungi varied with the medium. For example, although *P. tinctorius* showed a significant growth-rate increase ($P<0.001$) in all four media, *L. bicolor* differed from the control in N6:5 and BAF media ($P<0.001$) and Melin's medium ($P<0.01$) but not in the Hagem medium. Thus, the effect of root exudate, and probably also of the M-factor, depends to some extent on the composition of the basal nutrient medium.

The chemical components of tree-root exudates and their effects on fungal growth

Earlier studies demonstrated that the growth-promoting constituents of root exudates are found in the lipophilic fraction (Melin 1963; Fries et al. 1985). We performed an experiment in which a lipophilic fraction was tested for growth-supporting activity using five ectomycorrhizal fungi as test organisms (Table 5).

After three weeks culture, it was evident that the lipophilic fraction was less active than either the total exudate or pieces of living pine seedling roots as a growth-rate promoter. As expected, *S. luteus* did not react to any addition but the other four test species responded very strongly to both the total exudate and the pine seedling roots. Only *S. granulatus* and *L. bicolor* showed a clear, positive reaction to the lipophilic fraction (Table 5).

Attempts were then made to separate fractions of root exudate lipids on TLC and to evaluate their growth-rate-promoting capacity (Table 6). Five fractions were obtained of which fraction no. 4 contained mainly free fatty acids, nos. 1–3 sterols and no. 5 sterol esters.

Table 3. The effect of pine seedling root exudate on the growth of ectomycorrhizal fungi in different nutrient media. * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Species and strain number		Medium[a]			
		N6:5	BAF	Hagem	Melin
S. luteus 906	Test	23.3±0.4	59.9±1.0	12.7±0.6	—[b]
	Control	23.6±0.3	55.6±1.7	9.7±0.6	—[b]
S. granulatus 1089	Test	24.6±0.6***	76.4±1.3*	13.5±1.1	23.3±2.2**
	Control	19.8±0.6	67.8±2.3	8.0±0.5	13.9±0.4
S. variegatus 1090	Test	30.6±0.9	67.8±0.4***	32.5±1.3*	70.7±2.4***
	Control	31.1±0.7	46.1±2.2	27.9±0.8	38.2±1.1
L. bicolor 360	Test	7.4±0.4***	7.7±1.2***	11.5±0.8	7.1±0.2**
	Control	3.3±0.7	3.9±0.1	11.5±0.5	5.2×0.5
P. tinctorius 899	Test	23.1±1.7***	17.7±0.4***	21.6±1.1***	20.3±1.1***
	Control	12.5±0.7	9.7±0.1	11.7±0.7	10.8±0.4
T. terrestris 886	Test	22.5±0.5***	No growth	8.5±0.3	19.0±0.3
	Control	14.2±1.2	No growth	8.2±0.6	18.0±0.8

[a] Concentration 15.0 mg/l
[b] Not tested

Table 4. The effect of pine seedling root exudate on the growth of saprotrophic fungi in different nutrient media. * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Species and strain number		Medium[a,b]			
		N6:5	BAF	Hagem	Melin
L. omphalodes 210	Test	11.4±0.1***	16.6±0.4	10.2±1.3	—[a]
	Control	8.0±0.2	15.9±1.1	11.1±1.0	—[a]
F. velutipes 855	Test	13.1±0.4***	19.0±0.9	8.3±0.3	—[a]
	Control	5.9±0.1	17.5±0.9	5.0±0.2	—[a]
S. commune 552	Test	17.1±1.3	41.5±2.3	15.4±0.5	—[a]
	Control	17.9±1.3	39.8±0.2	14.8±0.8	—[a]
C. comatus 418	Test	13.6±0.4***	11.7±0.7**	11.3±0.6***	—[a]
	Control	5.2±0.3	8.8±0.4	7.2±0.2	—[a]
S. hirsutum 506	Test	11.2±0.6**	—[a]	—[a]	—[a]
	Control	8.2±0.5	—[a]	—[a]	—[a]
F. annosus 384	Test	9.7±1.0	—[a]	—[a]	—[a]
	Control	13.7±0.6	—[a]	—[a]	—[a]
S. sanguinolentum 393	Test	8.3±0.9***	—[a]	—[a]	—[a]
	Control	5.1±0.6	—[a]	—[a]	—[a]
T. serialis 546	Test	4.5±0.2**	—[a]	—[a]	—[a]
	Control	3.7±0.1	—[a]	—[a]	—[a]
P. versicolor 501	Test	8.8±0.4	—[a]	—[a]	—[a]
	Control	8.1±0.3	—[a]	—[a]	—[a]
C. cerebella 498	Test	14.2±1.1**	—[a]	—[a]	—[a]
	Control	9.8±0.4	—[a]	—[a]	—[a]

[a] Not tested
[b] Concentration 15.0 mg/l

Table 5. Comparison of the effects of exudate components on the growth of ectomycorrhizal fungi. * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Species and strain number		Living pieces of seedling root 3.0 mg (dry wt.)/flask	Whole exudate 5.0 mg/l	Lipophilic fraction 5.0 mg/l	Control
S. luteus	906	24.5±1.0	27.8±1.4	24.1±0.8	25.3±0.4
S. granulatus	1089	23.5±1.9**	24.0±1.0***	23.5±1.1***	17.1±0.5
S. variegatus	1090	39.5±1.3***	34.0±0.9**	28.5±1.4	28.2±1.2
L. bicolor	360	24.7±1.3***	15.5±1.0***	11.1±1.0***	4.9±0.3
T. terrestris	886	16.6±0.9***	15.5±2.1*	5.6±0.5	10.4±0.6

Table 6. The effect of lipid fractions separated by TLC on the growth of ectomycorrhizal fungal species. * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Species and strain number	Fraction[a]					
	Sterols			Free fatty acids,	Sterol esters,	Control
	No. 1	No. 2	No. 3	No. 4	No. 5	
S. granulatus 1089	30.1±1.3	33.1±1.4*	34.7±1.1**	31.1±1.0	34.2±1.4**	27.9±1.2
S. variegatus 1090	25.0±1.6	27.9±1.6**	27.6±1.4**	24.4±1.0	26.9±1.5*	21.7±0.9
L. bicolor 360	8.0±0.5	7.9±0.6	11.3±0.9*	8.0±0.8	12.5±1.0**	8.0±0.6
T. terrestris 886	14.1±1.0	—[b]	—[b]	13.5±2.4	22.4±1.0***	14.7±0.8

[a] Concentration 150 mg/l
[b] Not tested

Table 7. The effect of two fatty acids (as sodium salts) on the growth of ectomycorrhizal fungi. * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Species and strain number		Palmitic acid ($1.5 \cdot 10^{-4}$ M)	Stearic acid ($3.0 \cdot 10^{-5}$ M)	Mixture (1:1)	Control
S. luteus	906	18.4±1.1	19.0±1.0	20.9±1.4	29.8±0.7
S. granulatus	134:1	36.9±0.6***	23.8±1.3*	10.7±1.1	20.5±0.4
	134:4	32.3±1.4***	20.9±0.7	26.3±1.3**	21.5±0.7
	1089	42.3±0.4***	34.8±0.7*	32.4±1.4	30.0±0.6
S. variegatus	842	30.8±1.4*	32.6±0.9***	28.2±1.5	26.1±0.8
	1090	36.1±1.3***	37.5±1.1***	24.7±0.8	27.4±1.1
L. bicolor	360	22.8±0.2***	8.0±0.8	7.0±0.4	8.7±1.0
T. terrestris	886	43.0±0.6***	32.6±1.4	33.3±0.8	34.7±1.1

The separated free fatty acids (fraction 4) had a weak or negligible influence on the growth of four ectomycorrhizal species (Table 6). However, since some fatty acids, notably palmitic and stearic acid, are known to occur in root exudates (Fries et al. 1985), their possible growth-stimulating effect on five ectomycorrhizal fungi was investigated (Table 7).

Palmitic acid at the single tested concentration of $1.5 \cdot 10^{-4}$ M stimulated the growth rate in all fungi except *S. luteus*, whereas stearic acid at $3.0 \cdot 10^{-5}$ M increased the growth rate of only two out of five test species (Table 7). Surprisingly, these two fatty acids had little growth-stimulating capacity when tested together.

Abietic acid, which is known to induce spore germination in *Suillus* spp. (Fries et al. 1987), is also able to promote the growth of *S. granulatus* (Fries 1989). However, repeated experiments with abietic acid (unpublished results) showed only occasional and minor positive effects with *S. granulatus*. None of the other ectomycorrhizal species tested responded to abietic acid.

Effects of cytokinins

The cytokinins are hormones produced in various parts of the plant, including the root system (Gogala 1970; Letham 1978; Letham and Palni 1983). Gogala (1970, 1989) found that low concentrations of kinetin (10^{-7} g/ml) stimulated the growth rate of *Boletus edulis* var. *pinicola* in liquid culture.

Our experiments with cytokinins at $2.4 \cdot 10^{-7}$ M (Table 8) showed that kinetin and zeatin increased the growth rate in two out of six species of ectomycorrhizal fungi, whereas isopentenylaminopurine increased the growth rate of five out of six ectomycorrhizal species and three out of five saprotrophic fungi. Thus, in agreement with the reports of Gogala (1970, 1973), certain cytokinins exuded from roots increase the growth rate of ectomycorrhizal basidiomycetes.

Discussion

Earlier studies on growth promotion by root exudates were restricted to a few species of higher fungi, all of them ectomycorrhizal fungi (Gogala 1970, 1973; Gogala and Pohleven 1976; Rudawska 1982). Therefore, this interaction has often been interpreted as merely one of the procedures leading to ectomycorrhiza formation. In the present report, we show that several saprotrophic fungi, e.g. wood decomposers and coprophiles, are also positively influenced by root exudates. Thus it appears that some species can react to root exudates irrespective of ecological affiliation: only two out of six ectomycorrhizal fungi grown in the basal medium N6:5 did not react to the root exudate, and among the saprotrophs, only three out of ten species (Tables 3, 4).

The pin-root exudate contains many different compounds. Melin in 1954 assumed that one of these compounds, the M-factor, was responsible for the increase

Table 8. The effect of cytokinins on the growth of ectomycorrhizal and saprotrophic fungi. * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Species and strain number		Incubation time (days)	Kinetin[a]	Zeatin[a]	2 IP[a,b]	Control
Ectomycorrhizal						
S. luteus	906	14	26.2±0.8	27.9±0.5	29.8±0.7	28.2±0.5
S. granulatus	1089	11	27.5±0.5*	23.1±0.9	29.0±1.1*	24.3±1.4
S. variegatus	1090	14	22.5±0.2	20.7±0.3	24.8±0.8*	21.8±1.0
L. bicolor	360	21	4.4±0.6	6.5±0.5*	7.1±0.7***	4.6±0.5
P. tinctorius	899	11	19.0±0.3	14.3±1.2	28.9±1.0***	17.6±1.6
T. terrestris	886	14	12.7±0.2***	19.4±2.2***	15.8±0.1***	9.1±0.5
Saprotrophic						
L. omphalodes	210	11	—[c]	—[c]	4.6±0.2	5.6±0.1
S. commune	552	13	—[c]	—[c]	13.9±0.1*	12.4±0.5
C. comatus	418	14	—[c]	—[c]	3.7±0.1	3.7±0.1
S. hirsutum	506	13	—[c]	—[c]	35.3±0.7***	28.9±0.6
S. sanguinolentum	393	11	—[c]	—[c]	6.9±0.3*	5.6±0.2

[a] $2.4 \cdot 10^{-7}$ M [b] Isopentenylaminopurine [c] Not tested

in growth rate of test fungi. Our TLC experiments demonstrated that substances from all three fractions, containing fatty acids, sterols and sterol esters, were able to accelerate growth rate.

Some of the fatty acids in root exudates have been identified (Fries et al. 1985). Of these, palmitic acid tested separately was highly active and considerably enhanced the growth rate of various fungal species. The chemistry of the individual sterols and sterol derivatives will remain obscure until these compounds have been isolated.

In addition, some workers have discovered that root exudates contain cytokinins, a group of hormones much involved in the ontogeny of higher plants (Letham and Palni 1983) which are produced by both plants and various microorganisms. There are also indications, both in our present experiments and in published reports (Gogala 1970, 1973), that certain cytokinins increase the growth rate of ectomycorrhizal species. As is seen in Table 8, the cytokinin isopentenylaminopurine increased the growth rate of 8 out of 11 tested fungi.

Thus it seems justified to assume that palmitic acid and isopentenylaminopurine contribute to the M-factor effect. It is quite possible, however, that other, perhaps more powerful, but not yet identified compounds also play an important role in mycelial growth control. In cooperation with organic chemists, we are now trying to isolate and identify further growth-accelerating constituents of the pine-root exudate.

Finally, the possibility must be accepted that some compounds in the root exudate are adapted to a particular genus or species of fungi in a way similar to that of the spore germination-inducing compounds in the root exudate (Fries 1989). Whatever the case, it now appears unjustified to speak of *the* M-factor as there are probably several. The future will show whether they differ in taxon specificity, and mode of action.

Acknowledgements. This work was supported by grants from the Swedish Natural Science Research Council and Consul Faxe's Fund. The authors thank the staff of the Swedish National Forest Enterprise at the forestry nursery "Lugnet" for their generous donation of uniform pine plantlets for the root exudates. We are also indebted to Professor L.-Å. Appelqvist and his staff at the Department of Food Hygiene of the Swedish University of Agricultural Sciences for valuable advice on TLC and other separation techniques.

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