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# Auxin Relations between Mycorrhizal Fungi and their Partner Trees

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Auxin relations between mycorrhizal fungi and their partner plants are of interest at least from two points of view. Firstly, the morphologic transformation of short-roots into ectotrophic mycorrhizae could surely be demonstrated to be the effect of raised auxin levels in these organs (Slankis, 1951, Subba-Rao and Slankis 1959). Secondly, the high auxin levels will induce intensified translocation of organic compounds to the mycorrhizae, thus securing sufficient nutrient supply to the symbiotic fungi, since the plant organs of high auxin and cytokinin contents were found to be accumulation centres of carbohydrates and amino acids (Cleland 1961, Pohl 1961, Mothes 1961). The resulting effect would thus be in accordance with the carbohydrate theory of Björkman (1942) and with the results of Meyer (1962).

It was concluded by Slankis (1948, 1951) that hyperauxiny in mycorrhizae is due to auxin production by the fungi. The results of Moser (1959, 1962), Ulrich (1960) and Horak (1963, 1964) seemed to prove this theory because a number of mycorrhizal fungi were found to be capable to synthesize indole-acetic acid (IAA) when tryptophan was added to the culture medium. Without tryptophan, however, only very weak auxin synthesis was observed. But a tryptophan content of about 2000 ppm in the surroundings of the mycelium must be regarded as very unphysiological because tryptophan or related indole precursors will hardly be available in such amounts to the fungal symbionts in the soil or in the root tissues. Therefore it seems questionable whether indole synthesis by the fungi alone will be sufficient to allow the formation of mycorrhizal structures under natural conditions and to secure continually a high auxin level in the root system. This is supported by the fact that the auxin content in plant organs is enzymatically regulated by auxin oxydases so that exogeneously applied IAA

in lower concentrations may be broken down. Inactivation of these auxin oxydases is thus another possibility leading to raised auxin levels within the plant tissues (Gortner and Kent 1953, 1958, Mumford, Smith and Castle 1961, Sacher 1961, 1962, 1963). The so far known inhibitors of auxin oxydases are e.g. phenolic compounds, chlorogenic acid, ferulic acid, coffeic acid and flavone derivatives.

Of special interest is the fact that certain fungi and bacteria pathogenic to plants cause strong hyperauxiny in the host tissues by inhibition of auxin oxydases (e.g. Pilet 1960, Sequeira 1964).

Therefore we tested the inhibitory effect of crude and purified culture solutions of various mycorrhizal fungi on auxin oxydase preparations from pine, birch and bean roots.

## MATERIALS AND METHODS

Auxin oxydase preparations: root ends of *Pinus silvestris* L. (3-years-old) without and with mycorrhizae of *Suillus luteus* (L. ex Fr.) Gray, respectively (Ritter 1963), young roots of *Betula pendula* Roth (2-years-old) and root systems of *Phaseolus vulgaris* L. (grown in sand for 18 days) were used: 80 g of frozen roots were ground to powder and suspended in 300 ml of 0.02 M phosphate buffer pH 6,2 for 3 h at 2°C. After centrifugation, cold acetone was added to the clear supernatant 1:1 (v:v) and the mixture was again centrifuged. The precipitate was resuspended in 40 ml of buffer for 15 h at 2°C. After centrifugation, the supernatant fluid was diluted with buffer up to 200 ml. This enzyme preparation could be stored frozen without appreciable loss of IAA oxydase activity for at least 3 weeks.

Reaction mixtures: they consisted of 3 ml enzyme solution, 1 ml $5 \times 10^{-4}$ M 2,4-dichlorophenol, 1 ml $10^{-3}$ M $MnCl_2$, 1 ml $2 \times 10^{-3}$ M IAA (each dissolved in 0.01 M phosphate buffer), 2 ml of 0.01 M phosphate buffer and 2 ml of the inhibitor solution to be tested. Through the mixtures a constant stream of air was passed by means of a pump for 2—3 h at room temperature. Every 30 min. 2 ml were removed and the oxydative degradation of IAA was measured with Salkowski reagent (3 ml) in a Pulfrich colorimeter at 533 mμ. The optical density of controls without enzyme added was taken as unit (100% IAA) and the values of the other measurements were compared with it.

Inhibitor solutions: Pure cultures of the following fungal species were grown on the synthetic medium of Melin and Das (1954) for 7 weeks at 25°C:

| | | |
|---|---|---|
| *Amanita muscaria* (L. ex Fr.) Hooker | strain | 3a |
| *Amanita rubescens* (Pers. ex Fr.) S. F. Gray | strain | 5b |

| | | |
|---|---|---|
| *Amanita citrina* (Schaeff.) S. F. Gray | strain | 7b |
| *Paxillus involutus* (Batsch) Fr. | strain | 6b |
| *Boletus edulis* Bull. ex Fr. | strain | 9a |
| *Leccinum scabrum* (Bull. ex Fr.) S. F. Gray | strain | 10a |
| *Suillus bovinus* (L. ex Fr.) Kuntze | strain | 12c |
| *Suillus luteus* (L. ex Fr.) S. F. Gray | strain | 11d |
| *Suillus variegatus* (Sow. ex Fr.) Kuntze | strain | 103b |
| *Xerocomus subtomentosus* (L. ex Fr.) Quél. | strain | 126c |

Though the crude culture solutions were mainly used as inhibitors, separation of the active substances was performed chromatographically. Of each solution 100 ml were concentrated *in vacuo* to 10 ml and extracted 5 times, each time with 50 ml of diethyl ether. The ether-soluble constituents were solved in 10 ml ethanol after evaporation of the ether. Of these preparations 5 ml were separated by paper chromatography with butanol — acetic acid — water (4:1:5) as solvent. Different numbers of fractions were obtained which were visible as slightly coloured zones or could be detected as fluorescent areas. These zones and the test areas between them were eluted with water (10 ml) and each was tested for inhibitory activity. Parallel samples were run with the noninoculated culture medium stored together with the fungal cultures as described.

## RESULTS

Preliminary tests showed that IAA oxydase activity of bean root preparations was most effective. After an incubation period of 120 min. only 19% of the initial IAA was left. Enzyme solutions of pine and birch roots also caused IAA destruction but exhibited a different activity. While the preparation of pine roots without mycorrhizae converted about 25% IAA, the destruction by the preparation of mycorrhizal roots was only about 6% IAA after 120 min. (Fig. 1). Culture solutions of *Suillus bovinus* added to the reaction mixtures gave total inhibition of the auxin oxydases thus indicating a strong inhibitory effect of this fungal symbiont at least in vitro.

As it seemed likely that there are only quantitative differences between pine, birch and bean root IAA oxydase preparations, the latter was used mainly in the following experiments because of its greater activity and easier availability.

Culture solutions of the majority of the test fungi gave total inhibition of bean root IAA oxydase activity similar to that by *Suillus bovinus* (Fig. 2). Only *Amanita muscaria* and *A. rubescens* produced smaller effects. With pine and birch root preparations, however, all test species gave total inhibition and may thus be regarded as suf-

ficiently effective to prevent oxydative auxin destruction in tree roots. In order to decide whether the ability to inhibit auxin oxydase is restricted to mycorrhizal symbionts, a number of wood-destroying fungi were tested. They were grown on a 3% liquid malt medium for 20 days and the culture solution was used as inhibitor. Strong inactivation of

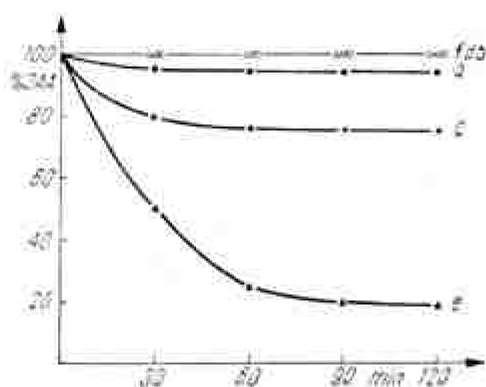

Fig. 1

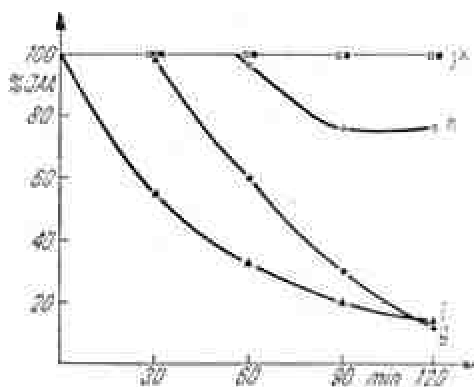

Fig. 2

Fig. 1. Destruction of IAA by various auxin oxydase preparations and inhibitory effects of culture solutions of *Suillus bovinus*.

*a* — enzyme from pine roots with mycorrhizae of *Suillus luteus*, without inhibitor; *b* — the same but with inhibitor (*S. bovinus*); *c* — enzyme from nonmycorrhizal pine roots, without inhibitor; *d* — the same but with inhibitor; *e* — enzyme from bean roots, without inhibitor; *f* — the same but with inhibitor.

Fig. 2. Inhibition of oxydative destruction of IAA by culture solutions of some mycorrhizal fungi (IAA oxydase preparation from bean roots).

*g* — control (without inhibitor); *h* — with culture solutions of *Amanita muscaria*; *i* — *A. rubescens*; *j* — *Leccinum scabrum*; *k* — *Xerocomus subtomentosus*, as inhibitors.

bean root auxin oxydase was caused by *Fomes marginatus* (Fr.) Gill., *Gloeophyllum sepiarium* Karst., *Cereomyces albus* Corda, *Lentinus lepideus* Fr., *Schizophyllum commune* Fr. and *Collybia velutipes* Curt., while *Trametes betulina* Pil., *Trametes versicolor* Pil. and *Pholiota mutabilis* Schaeff. ex Fr. had little or no effect. It is remarkable that the first, active group mainly consists of brown rot fungi which lack extracellular oxydases. Only *Schizophyllum commune* and *Collybia velutipes* cause white rots, but their laccase production is weak as compared to that of the species of the second group (L y r 1958). According to F a h r a e u s (1961), IAA is easily destroyed by laccase in vitro. Therefore it is possible that a strong laccase production effaces the possible presence of IAA oxydaseinhibiting substances and supports the activity of auxin oxydase in this special case.

Noninoculated culture media either of the mycorrhizal or of the wood-destroying species showed no inhibitory effect on the auxin oxydase preparations used.

In further experiments the effect of inhibitor concentration on auxin oxydase activity was tested. As example, Fig. 3 shows that there exists a linear correlation between the inhibitor content and IAA destruction after an incubation period of 90 min.

Heat stability of the inhibitors could be demonstrated by keeping culture solutions at 100°C for 20 min. before use for the oxydase inactivation tests.

As the active compounds proved to be ether-soluble, further concentration and purification seemed practicable. Table 1 summarizes

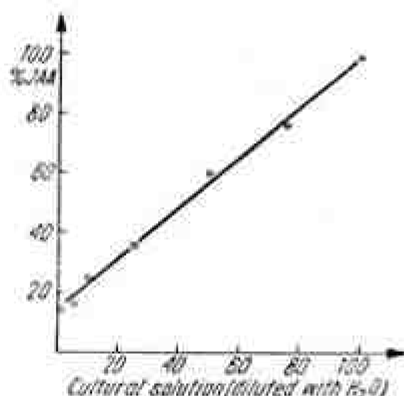


Fig. 3. Effect of inhibitor concentration on degradation of auxin oxydase from bean roots. Inhibitor: culture solution of *Suillus bovinus*; reaction time: 90 min.

$R_f$-values, inhibitory effects and color characteristics of fractions obtained by chromatographic separation of culture solutions as described. Obviously, the inhibitory activity is not restricted to one fraction but is caused by a varying number of compounds produced by the mycelia. When comparing the $R_f$-values it should be noted that the active compounds were mainly found in the $R_f$-ranges of 0.84—0.63 and 0.32—0.24. Though it will not be easy to interpret these results because of further differences in colour even at equal $R_f$-values it may be that phenolic derivatives are, at least in part, responsible for the inhibitory effects. For instance, the already mentioned ferulic and coffeic acids show $R_f$-values of 0.84 and 0.78, respectively in the solvent system used. Because of the considerable variety of inhibiting components observed no certain chemical identifications could be achieved up till now, so that further work has to solve this promising question.

## DISCUSSION

According to Slankis (1951, 1958) a continuous high auxin content in the mycorrhizae has to be maintained, about 50 to 100 times

Table 1

$R_f$-values, inhibitory effects and colour characteristics of chromatographically separated culture solution fractions of the test fungi

| Fungus | $R_f$ | Inhibition | Colour | |
|---|---|---|---|---|
| | | | visible light | UV |
| *Amanita muscaria* | 0.97 | O | light yellow | O |
| | 0.82 | ++ | light yellow | light brown |
| | 0.77 | +++ | ligh grey | light brown |
| | 0.73 | +++ | light brown | light brown |
| | 0.68 | ++ | light brown | yellow |
| | 0.60 | O | O | yellow |
| | 0.53 | O | brown | reddish brown |
| | 0.46 | O | brown | violet |
| | 0.31 | O | O | light yellow |
| | 0.21 | O | O | light yellow |
| *Amanita rubescens* | 0.96 | O | light yellow | bright |
| | 0.90 | O | light yellow | light yellow |
| | 0.80 | O | light yellow | bright |
| | 0.75 | O | light yellow | grey |
| | 0.71 | ++ | light yellow | bright |
| | 0.57 | O | O | light blue |
| | 0.51 | O | O | brown |
| *Amanita citrina* | 0.98 | O | light yellow | bright |
| | 0.90 | O | light yellow | light yellow |
| | 0.84 | +++ | light yellow | bright |
| | 0.80 | +++ | O | light brown |
| | 0.74 | +++ | O | bright |
| | 0.68 | O | O | brown |
| | 0.63 | O | O | bright |
| | 0.56 | O | O | yellow |
| | 0.35 | O | O | bright |
| *Paxillus involutus* | 0.97 | O | light yellow | bright |
| | 0.90 | O | light yellow | yellow |
| | 0.83 | +++ | light brown | yellow |
| | 0.74 | +++ | O | yellow |
| | 0.63 | +++ | O | bright |
| | 0.58 | O | light yellow | brown |
| | 0.50 | + | O | light yellow |
| | 0.34 | O | O | light brown |
| | 0.25 | +++ | light yellow | O |
| | 0.16 | O | O | light brown |

| Fungus | $R_f$ | Inhibition | Colour | |
|---|---|---|---|---|
| | | | visible light | UV |
| *Boletus edulis* | 0.97 | O | light yellow | bright |
| | 0.91 | O | light yellow | light yellow |
| | 0.80 | ++ | light yellow | brown |
| | 0.76 | + | light yellow | bright |
| | 0.64 | O | O | bright |
| | 0.35 | O | O | light brown |
| *Suillus bovinus* | 0.92 | O | O | light brown |
| | 0.83 | +++ | ligth grey | blue |
| | 0.73 | + | O | light grey |
| | 0.60 | O | O | light blue |
| | 0.54 | O | light yellow | O |
| | 0.30 | +++ | light yellow | blue |
| | 0.16 | O | light yellow | light blue |
| *Suillus luteus* | 0.97 | O | light yellow | bright |
| | 0.89 | O | yellow | greenish |
| | 0.78 | ++ | light brown | brown |
| | 0.72 | O | O | bright |
| | 0.61 | + | O | bright |
| | 0.56 | + | O | light brown |
| | 0.21 | O | O | light blue |
| | 0.12 | O | O | light brown |
| *Suillus variegatus* | 0.97 | O | light yellow | bright |
| | 0.91 | O | light brown | light yellow |
| | 0.86 | O | light brown | greenish |
| | 0.80 | +++ | yellow | blue |
| | 0.75 | ++ | yellow | yellow |
| | 0.65 | ++ | light yellow | light yellow |
| | 0.58 | +++ | light yellow | light yellow |
| | 0.53 | ++ | light brown | violet-brown |
| | 0.48 | O | O | light yellow |
| | 0.44 | + | O | violet-brown |
| | 0.38 | ++ | green | bright ochre |
| | 0.24 | +++ | O | dark blue |
| | 0.20 | + | light green | light yellow |
| *Xerocomus subtomentosus* | 0.90 | O | O | light yellow |
| | 0.82 | +++ | light grey | blue |
| | 0.74 | ++ | light yellow | light brown |
| | 0.63 | ++ | greenish | light brown |
| | 0.54 | O | light brown | O |
| | 0.44 | O | O | yellow |
| | 0.39 | O | O | yellow |
| | 0.32 | +++ | grey | light blue |
| | 0.17 | + | light brown | light brown |

greater than that of nonmycorrhizal roots. Theoretically three ways might be possible to realize such high auxin concentrations:

1. intensive and steady auxin synthesis by the mycorrhizal fungi;
2. induction of auxin formation in the roots by the fungi;
3. inactivation of IAA oxydase in the roots by inhibitors produced by the fungal symbionts.

The first possibility mentioned has already been discussed to some extent. In the experiments of Moser (1959, 1962), Ulrich (1960) and Horak (1963, 1964), only a limited number of mycorrhizal fungi were able to form IAA in media without tryptophan. Furthermore it is difficult to believe that the small amounts recorded (about 0.05 ppm) could be sufficient to yield the morphogenic effects, as 1—2 ppm IAA are necessary for mycorrhizal-like root forking, 5—10 ppm for coralloid forms and 10—20 ppm for tuberculous forms, respectively (Slankis 1958). Besides, auxin synthesis by mycorrhizal fungi depends not only on the species involved, but even differs in certain strains of the same species (Moser 1959). Therefore it is not clear whether auxin production by the fungi themselves is of considerable relevance for mycorrhizal symbiosis.

Fungal induction of auxin synthesis in the roots has not been ascertained as yet. A mechanism of this kind, however, would demand a highly specific interaction between fungi and enzymatic processes in the root cells, which from indole precursors lead to IAA.

The results described show a less complicated way to realize the experimentelly proved hyperauxiny in myccorrhizal roots. They too may give an explanation for the observations of Levisohn (1952, 1960), according to which aqueous extracts or percolates of soils containing fungal symbionts or mycorrhizae induce root forking in pine seedlings without fungal infection. In these *in vivo* experiments a direct action of auxin may be excluded, firstly because of the small IAA amounts to be expected and secondly because auxin is readily destroyed in the soil (Scheffer and Ulrich 1959). The more stable extracellular inhibitors of IAA oxydase, however, are more likely to be responsible for the morphogenic effects described.

If we draw conclusions of this kind, the question arises, why nonmycorrhizal fungi also exert an inhibitory effect on auxin oxydase preparations as is the case with a number of the wood-destroying species. The answer may be that probably inhibitor formation is primarily an unspecific effect, which in certain cases favours the existence of a microorganism within its host. Parallels may be seen in inhibitor-induced hyperauxiny in the plant-parasitic fungi and bacteria already mentioned, e.g. *Uromyces pisi, Endophyllum sempervivi* and *Pseudomonas solanacearum*. Surely, wood-destroying fungi are in most cases

saprophytes or facultative parasites unable to inhabit living tissue without injuring it. But the borderline between saprophytism, parasitism and symbiosis is by no means clear when considering the fungal species involved. This can be demonstrated by examples such as *Armillaria mellae*, *Polystictus circinatus*, *Marasmius* sp. and *Fomes* sp. which are in general wood-destroying fungi but may establish mycorrhizal symbiosis with adequate host plants. Therefore, besides the hyperauxiny induced, other properties are surely necessary to keep a fungus as a symbiont in the mycorrhizal state. The most important of these will be the ability to maintain a physiological equilibrium between the fungus and the inhabited host tissue without injuring it as most of the facultatively parasitic wood-destroying species do.

## SUMMARY

Mycorrhizal fungi induce raised auxin contents in the roots of their partner plants. Experiments in vitro were performed to decide whether the increased auxin levels might be due to an inactivation of auxin oxydase in the roots.

1. Mycorrhizal fungi in pure culture are able to stop the oxydative destruction of indole-acetic acid by exudation of inhibitors into the nutrient solution. The inhibiting substances are active against auxin oxydases form pine, birch and bean roots.

2. Chromatographic analyses revealed that species of the genera *Amanita, Paxillus, Boletus, Leccinum, Suillus* and *Xerocomus* form several inhibitors each.

3. Inhibitor formation must not be considered as specific for mycorrhizal fungi as culture solutions of wood-destroying fungi also inactivate auxin oxydase preparations.

4. It is concluded that the inhibiting substances are the cause of hyperauxiny of mycorrhizal roots *in vivo*. This mechanism is regarded necessary for an increased translocation of organic nutrients to the roots which serve as most important food base for the symbiotic fungi. Besides, surely other qualities are required to keep a fungus as a symbiont in the mycorrhizal state.

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## *Stosunek auksyn u grzybów mikoryzowych i ich partnerów*

### Streszczenie

Grzyby mikoryzowe powodują zwiększanie się zawartości auksyny w korzeniach ich partnerów. Doświadczenia in vitro przeprowadzone z borowikowatymi oraz sosną, brzozą i fasolą pozwoliły na wyjaśnienie, jaki stopień nagromadzenia auksyny może doprowadzić do inaktywacji oksydazy w korzeniach.