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**The influence of mineral nutrition on synthesis of
growth regulators in the host plant *Pinus
sylvestris* L. and in the mycorrhizal fungus
Suillus luteus (L. ex Fr.) S. F. Gray.***

Abstract

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Nitrogen in the ammonium form seems to be primarily responsible for the unfavorable effect of mineral nutrition on mycorrhizal infection. Higher doses of N in the growth medium decreased auxin and cytokinin production by the fungal symbiont *Suillus luteus* and increased the hormone levels in roots of the host plant *Pinus sylvestris*. These two effects of high doses of nutrients disturb the hormonal balance of both mycorrhizal partners and can lead to the disappearance of ectomycorrhizae in pines at higher fertility conditions.

Additional key words: ectomycorrhiza, auxin, cytokinin.

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INTRODUCTION

Associations between fungi and roots of higher plants to form mycorrhizae are common in nature and play an important part in plant nutrition. The fungus absorbs inorganic nutrients from the soil and passes some of them on to the host plant, while receiving carbon compounds from the host for its own nutrition. Mycorrhizal seedlings are generally larger and darker green than nonmycorrhizal seedlings and accumulate

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more inorganic nutrients (Greene 1980). These effects may be due to the increased absorptive surface in plants bearing mycorrhiza and to the production of beneficial metabolites by the fungus (Harley 1969, Slankis 1973). A number of ectomycorrhizal fungi produce auxins and cytokinins in pure cultures (Urlich 1960, Miller 1967, Crafts and Miller 1974, Ek et al. 1983), which implies that these two hormones play an important role in inducing and maintaining the mycorrhizal relationship. Application of auxin to excised roots of *Pinus sylvestris* L. results in the formation of an increased number of short laterals, which branch dichotomously and lack root hairs (Slankis 1973). Treated with kinetin, the roots also produced short, dichotomously branched laterals, closely resembling mycorrhizae (Barnes and Naylor 1959). On this basis one can suspect that factors which inhibit the development of mycorrhiza, such as mineral fertilization (Slankis 1967, Lister et al. 1968, Björkman 1970) may act through changes of auxin and cytokinin synthesis by the mycorrhizal fungi.

On the other hand there exists a strict dependence between mineral fertilization and the metabolism of growth regulators in higher plants. However this problem was studied mainly in herbaceous plants and the knowledge concerning the influence of mineral nutrition on the dynamics of growth regulators in conifers is very scarce (Michniewicz et al. 1976).

The effect of various levels and sources of mineral nutrition on growth regulator production by mycorrhizal fungus *Suillus luteus* and on the hormonal aspects of metabolism of the higher plant is the subject of this paper.

MATERIALS AND METHODS

Cultivation of pine seedlings. One year old Scots pine seedlings with abundant unidentified mycorrhiza were transferred from the nursery into the greenhouse and potted in forest soil. Over a 5 month period the seedlings were fertilized every second day with nitrogen or nitrogen and phosphorus supplied as ammonium tartrate and phosphoric acid solution. By the end of the experiment the seedlings were examined under a binocular (5X) and the presence of mycorrhizal tips was estimated.

Cultures mycorrhizal symbiont. Common mycorrhizal symbiont of young Scots pine stands *Suillus luteus* was used throughout this work. Pure mycelium of *S. luteus* was grown in a liquid synthetic medium (SM) (Rudawska 1982), as a surface culture in Roux flasks at 23°C. For the experiments the levels of nitrogen and phosphorus

were modified in the SM using variable quantities of ammonium, nitrate or phosphoric ions as described previously (Rudawska 1983). After 30 days of growth in darkness, the mycelium dry weight, auxin and cytokinin content in fungal filtrate were determined.

Auxin assay. Auxin was estimated in the culture filtrate and root samples, using cold ether extraction according to Larsen (1955) and assayed in the standard *Avena* coleoptile curvature test (Larsen 1955). Cytokinins were extracted by the modified method of Biddington and Thomas (1973). Extracts from roots of Scots pine seedlings (equivalent of 5 g of fresh weight) or filtrates of *Suillus luteus* cultures (equivalent of 1 Roux flask) were streaked onto filter paper sheets. The sheets were chromatographed in water-saturated sec-butanol. Ten equal strips according to Rf value were cut and eluted directly into 100 ml Erlenmayer flasks containing 50 ml of medium and planted with soybean tissue (*Glycine* Max. L. Mevrii var. Acme) as described by Miller (1968).

Each Erlenmayer flask was planted with three pieces of soybean tissue, maintained for 28 days and fresh weight of each piece was measured.

RESULTS AND DISCUSSION

The results presented here showed that nitrogen nutrition in ammonium form, increased the level of auxin and cytokinin in the roots of pine, simultaneously at higher concentrations completely arresting mycorrhizal infection (Fig. 1 a, Fig. 2).

Phosphorus used together with ammonium was able to some extent to reduce the negative effect of nitrogen fertilization on mycorrhizal

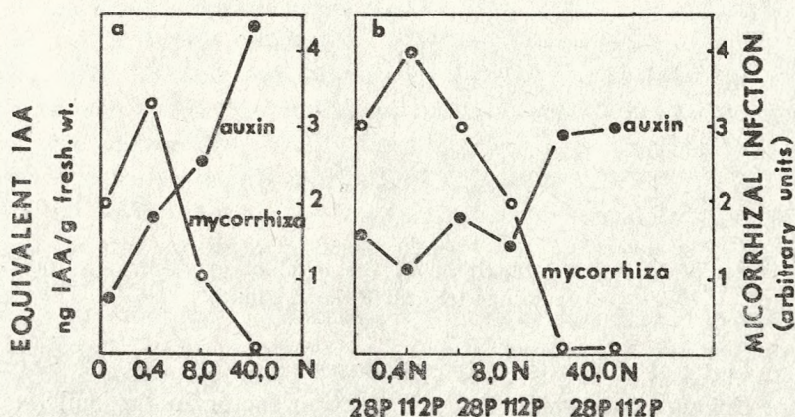


Fig. 1. Effect of N (a) and NP nutrition (b) on the mycorrhizal infection and the level of auxins in roots of pine seedlings. (N and P as mg/l given in solution of ammonium tartrate and phosphoric acid every second day during 5 months)

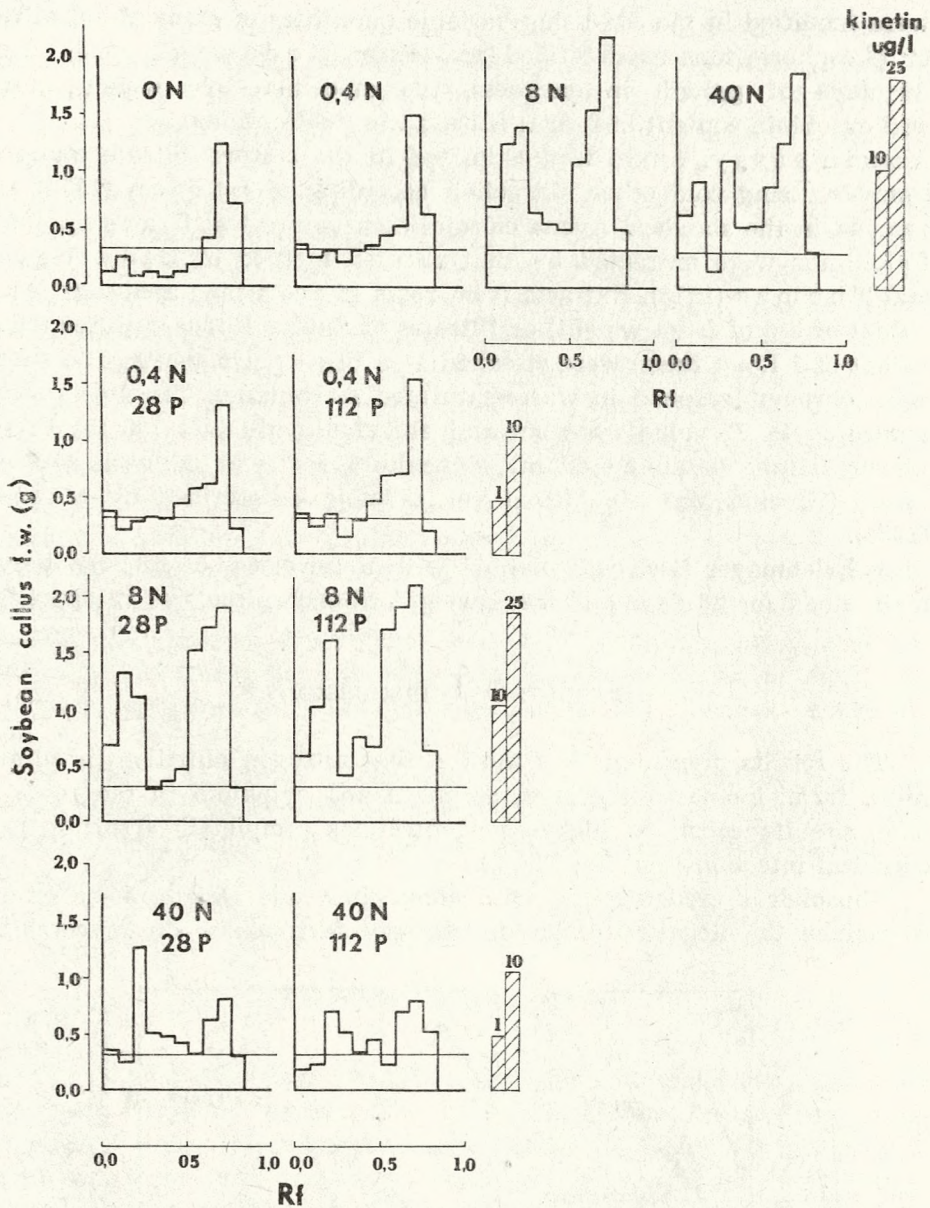


Fig. 2. Effect of N and NP nutrition on the level of cytokinins in roots of pine seedlings (N and P as in Fig. 2)

infection but not at higher nitrogen doses (Fig. 1 b, Fig. 2). Phosphorus may act through increasing the nitrification factor in the soil (Nowotny-Mieczyska 1976) and thus can decrease ammonium availability for the fungal symbiont. The results also emphasize the importance of a proper balance between the essential elements, principally N and

P for conifer seedlings growth and mycorrhiza formation in particular. Whether the increased auxin and cytokinin levels in the roots have a negative effects on the mycorrhizal relationship remains an open question. The distribution and frequency of lateral roots is of great importance for mycorrhiza formation. The physiology of lateral root branching in mycorrhizae is complex, but it seems likely that some balance between auxins and other substances, possibly cytokinins, is involved (Crafts 1973). Nitrogen at high concentration decreased the length of roots and the number of lateral roots (Rudawska 1981). Blakely et al. (1972) found that cytokinin inhibited lateral root initiation. Physiologists also believe that auxin controls root cell elongation. Some authors (Torrey 1976) adhere to the model proposed by Burström (1957) that auxin acts in a 2-phase system, one primarily stimulatory, the other inhibitory. However probably, no basis exists for this case in mycorrhizal relationship under higher doses of fertilizers. More work is needed to answer these questions.

An effect of mineral nutrients on the synthesis of the growth regulators by the host plant tissue and by the fungal symbiont differed significantly. When *S. luteus* was grown in a liquid medium at ammonium levels ranging from 0 to 3 g/l of N, dry weight increased up to the level of 0.3 g/l and only the highest amount of N in the medium limited growth of the fungus (Fig. 3a). But the highest auxin (Fig. 3 b) and cytokinin (Fig. 3 c) activity was exhibited by *S. luteus* at a low levels of ammonium N and increased doses of N inhibited auxin and cytokinin release despite the excellent growth of mycelium. These results confirm the earlier reports (Tomaszewski and Wojciechowska 1974, Rudawska 1982, 1983) about unfavourable effects of ammonium nutrients on growth regulator release by mycorrhizal fungi. Nitrate appeared to be a very poor nitrogen source for growth of *S. luteus* which is in agreement with other findings (Lundeborg 1970, Trappe 1967). A very low level of auxin was observed in the presence of nitrate ions in the medium (Fig. 3 b) and no cytokinin activity was shown (Fig. 3 c). These findings may suggest that nitrate fertilizers which are hardly utilized by the mycorrhizal symbiont can result in smaller changes in the fungal metabolism and finally be better tolerated by the mycorrhizal association than the very readily utilized ammonium form of nitrogen and urea. Phosphorus used even at very high concentrations did not decrease the growth and cytokinin production of *S. luteus*. Intense phosphate accumulation in the mycorrhizae and in the fungal mantle (Bowen 1973) in the form of a nonmetabolic pool of orthophates can explain this phenomenon.

These data demonstrate that nitrogen nutrition alters hormonal balance of the host plant and the fungal symbiont. The importance of a

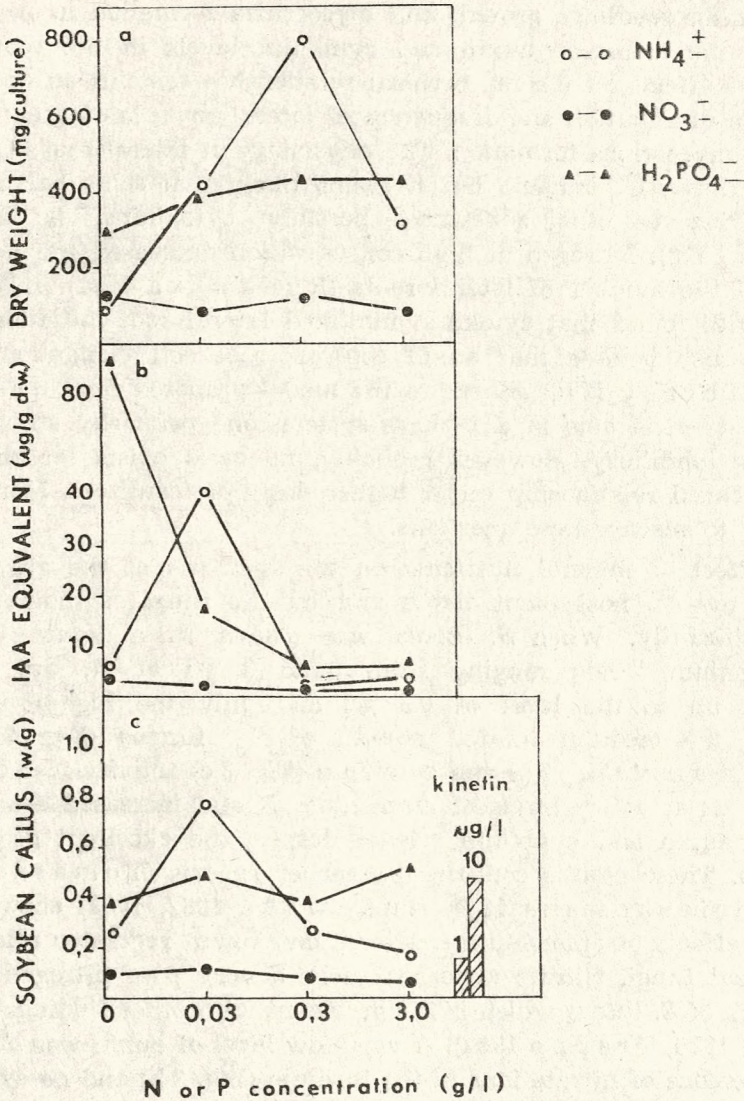


Fig. 3 a, b, c. Effect of different levels of ammonium (o-o) and nitrate (●-●) nitrogen and phosphorus (▲-▲) on the mycelium dry weight (a) and auxin (b) and cytokinin (c) release by pure cultures of *Suillus luteus*. (The levels of N and P as g/l in ammonium tartrate, potassium nitrate and potassium phosphate monobasic respectively)

proper hormonal balance in mycorrhizae was stressed by Allen (1984). Thus mineral nutrition must be considered as an important factor regulating mycorrhizal symbiosis.

SUMMARY

One year old Scots pine (*Pinus sylvestris* L.) seedlings with abundant unidentified mycorrhiza were transferred from the nursery into the greenhouse and potted in forest soil. Over a 5 month period the seedlings were fertilized every second day with nitrogen and nitrogen and phosphorus nutrient solution. The results showed that nitrogen in ammonium form increased level of auxin and cytokinin in the roots of pine, simultaneously at higher concentration (8 and 40 mg/l) completely arresting mycorrhizal infection. Phosphorus used together with ammonium was able to some extent to reduce the negative effect of nitrogen fertilization on mycorrhizal infection and did not cause so high increasing of auxin and cytokinin amount in the roots. *Suillus luteus* growing in liquid medium on ammonium as nitrogen source released auxin and cytokinin only at low level of N and increased doses of N inhibited auxin and cytokinin release despite the excellent growth of mycelium. Nitrate appeared to be a very poor nitrogen source for growth of *S. luteus* and very low level of auxin and no cytokinin activity was shown. Phosphorus used even at very high concentration did not decrease the growth and cytokinin activity of *S. luteus* but the highest auxin activity was observed in the medium without phosphorus. These data demonstrate that nitrogen nutrition alters hormonal balance of the host plant and the fungal symbiont. Proper hormonal balance between both mycorrhizal symbionts seems to be of great significance for mycorrhizal relationship and mineral nutrition belongs to important factors disturbing this balance.

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Wpływ żywienia mineralnego na syntezę regulatorów wzrostu przez roślinę-gospodarza *Pinus sylvestris* L. i symbionta mikoryzowego *Suillus luteus* (L. ex Fr.) S. F. Gray

Streszczenie

Jednoroczne siewki sosny *Pinus sylvestris* L. z obfitą, niezidentyfikowaną mikoryzą przeniesiono ze szkółki do szklarni i umieszczono w doniczkach w glebie leśnej. Przez 5 miesięcy siewki zasilano co drugi dzień roztworem azotu lub azotu i fosforu. Stwierdzono, że azot użyty w formie amonowej zwiększał znacznie poziom auksyny i cytokininy w korzeniach siewek sosny, powodując jednocześnie przy wyższych dawkach (8 i 40 mg/l) całkowity zanik mikoryz. Fosfor użyty łącznie z nawożeniem azotowym powodował pewne złagodzenie negatywnego wpływu azotu na tworzenie mikoryz i nie tak znaczny wzrost poziomu auksyny i cytokininy w korzeniach. Hodowany w czystej kulturze popularny symbiont mikoryzowy sosny *Suillus luteus* syntetyzował auksynę i cytokininę tylko w warunkach niskiej dostępności azotu amonowego w podłożu. Najwyższa aktywność hormonalna tego grzyba nie była skorelowana z optymalnym wzrostem grzybni. Jon azotanowy był tylko w niewielkim stopniu wykorzystywany przez grzyb *S. luteus* i w filtratach pochodowlanych stwierdzono tylko niewielką produkcję auksyny oraz brak aktywności cytokininowej. Fosfor użyty nawet w wysokim stężeniu (3000 mg/l) nie hamował wzrostu grzybni *S. luteus* i syntezy cytokininy, lecz najwyższą aktywność auksyny obserwowano na pożywce bez fosforu.

Uzyskane wyniki wskazują, że żywienie mineralne może modyfikować syntezę hormonów zarówno przez symbionta grzybowego, jak i w roślinie-gospodarzu. Odpowiednia równowaga hormonalna pomiędzy oboma partnerami mikoryzowymi jest prawdopodobnie ważnym elementem regulującym istnienie symbiozy mikoryzowej, a nawożenie mineralne należy do istotnych czynników naruszających tę równowagę.

Влияние минерального питания на синтез регуляторов роста растением-хозяином *Pinus sylvestris* L. и микоризным симбионтом *Suillus luteus**

Резюме

Однолетние сеянцы сосны *Pinus sylvestris* L. с буйной, неидентифицированной микоризой перенесли в теплицу и поместили в горшках с лесной почвой. В течение 5 месяцев сеянцы подкармливали каждый второй день растворами азота или азота и фосфора. Отмечено, что азот примененный в аммонийной форме значительно увеличивал уровень ауксина и цитокинина в корнях сеянцев сосны, вызывая одновременно при более высоких дозах (8 и 10 мг/л) полное исчезновение микоризы. Фосфор примененный одновременно с внесением азотного удобрения вызывал некоторое уменьшение отрицательного действия азота на возникновение микоризы и не так значительный рост уровня ауксина и цитокинина в корнях. Выращиваемый в чистой культуре известный микоризный симбионт сосны *Suillus luteus* синтезировал ауксин и цитокинин лишь в условиях незначительной доступности аммонийного азота в субстрате. Самая высокая гормональная активность этого гриба не соответствовала с оптимальным ростом грибницы. Нитратный ион был в незначительной степени использован грибом *S. luteus* и в культуре найдено лишь незначительное количество ауксина и отсутствие активности цитокинина. Фосфор применяемый даже в больших количествах (3000 мг/л) не тормозил роста грибницы *S. luteus* и син-

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теза цитокинина, но самую большую активность ауксина наблюдали на субстрате без фосфора.

Полученные результаты указывают на то, что минеральное питание может модифицировать синтез гормонов так грибным симбионтом, как и растением-хозяином. Соответствующее гормональное равновесие между микоризными партнерами является по всей вероятности существенным элементом регулирующим существование микоризного симбиоза, а минеральное удобрение принадлежит к числу существенных факторов нарушающих это равновесие.