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THE UPTAKE OF CADMIUM, ZINC, PHOSPHORUS, AND PLANT HORMONE KINETIN BY ECTOMYCORRHIZAL FUNGI

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Introduction

There are relatively few data in the literature on the absorption and translocation of trace elements in ectomycorrhizal fungi. Some more information is available e. g. for phosphorus uptake and its role in the metabolism of higher plants, while investigations on toxic trace elements (Cd, Hg, Pb), often demonstrate only their inhibitory effects on photosynthesis, transpiration, gas exchange (Bazzaz et al. 1974, Bazzaz et al. 1975, Carlson et al. 1975) etc.

The application of labelled compounds in plant biochemistry has facilitated investigations into the role of organic plant substances (hormones, enzymes) in plant metabolism. Among the plant hormones, cytokinins seem to play a very important role because of their ability to promote water uptake in ectomycorrhizal fungi (Gogala 1971). It probably means that a higher plant influences the water uptake as well as the growth of ectomycorrhizal fungus via cytokinin action. On the other hand the question is, whether cytokinins have any influence on ion uptake too. In this context the use of radioactive istopes in comparison to classical methods represents an advantage in research into toxic as well as essential trace elements. The advantage is in the ability to follow radioactive isotopes of these elements, which often appear and act in very small amounts, in any organ or tissue of the fungus or a higher plant.

In our work the uptake of cadmium, zinc, phosphorus and a synthetic plant hormone kinetin, from culture media to mycelia of ectomycorrhizal fungi *Amanita muscaria* and *Suillus variegatus* was investigated, using radioactive isotopes and ^{14}C-labelled kinetin.

Materials and Methods

Fungi: For following the uptake of cadmium, zinc and phosphorus, as well as the synthetic hormone kinetin, *Suillus variegatus* was used because of its rapid and effective growth. *Amanita muscaria*, which can accumulate cadmium in the fruit-body (Byrne et al. 1976), was included in the experiment on cadmium uptake from the culture media to the fungal mycelia.

Culture of fungi: The fungi used were grown on the following culture media: potato-dextrose agar gels (3.9% aqueous solution pH 5.6) and MNM (Melin-Norkraus-Marx) gel, pH 5.8 (Marx 1967). The starts of all the fungi were obtained from the Centralbureau voor Schimelcultures, Baarn, Netherlands, or isolated from fruit-bodies from the generative fungal tissue. Both culture media were prepared in the same way, by dissolving the components in distilled water, which was then made up to 1 litre. The solution was heated to 80 °C and immediately distributed into experimental tubes, as shown in Fig. 1A. The translocation of Zn and P isotopes from the point of application to the lateral parts of the mycelia of *Suillus variegatus* was followed using Conway cells (Fig. 1B).

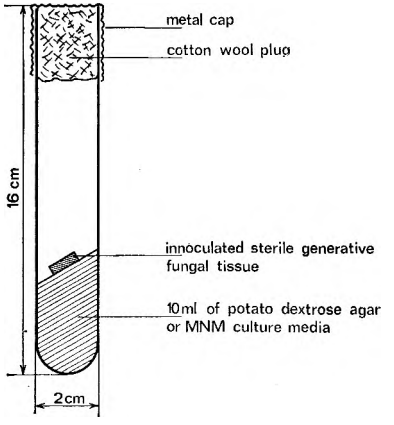

Fig. 1A. Experimental tube with culture media

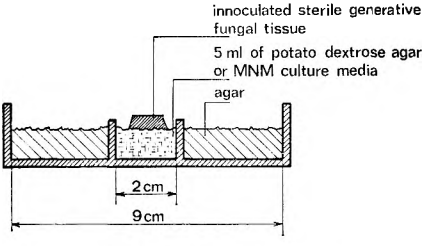

Fig. 1B. Conway dish.

The potato-dextrose agar and/or MNM culture media were sterilized for 10 min at 120 °C and 1.5 atm before the innoculation. Innoculates were applied on the surface of culture media under sterile conditions (UV light). Mycelia, grown in the dark, at 25 °C and 70% relative humidity, were taken for analysis every 3rd or 5th day.

Isotopes: The following isotopes were used in the experiments: ^{115m}Cd (half life 44.8 days) is very suitable for shorter experiments (Decker et al. 1957, Gunn et al. 1957) in vitro, while ^{109}Cd (half life 453 days) is often used in longer experiments (Miller et al. 1969, Shaikh et al. 1972, Suzuki et al. 1971). These isotopes, of different specific activities, were purchased from the Radiochemical Centre, Amersham, Great Britain. ^{115m}Cd was always added to the culture media

as $CdCl_2$ in 0.01N HCl, so that the final concentration of Cd was in the range 1.1 to 3.9 μg/ml of culture media. ^{65}Zn (half life 245 days), which was purified on an ion-exchange column (Dowex X-8) after irradiation of elemental zinc in the nuclear reactor at Vinča, Belgrade, was added to culture media as $ZnCl_2$ in 0.002N HCl to a final concentration of 2.8 μg Zn/ml of culture media.

^{32}P, in contrast to cadmium and zinc, a short-lived (half life 14.3 days) β-emitter, was prepared from ammonium monohydrogen phosphate. Irradiations were performed in our Triga Mark II reactor for 40 hours at a flux of 2×10^{12} n. cm^{-1} sec^{-1}. After irradiation, phosphorus was oxidised by nitric acid to $^{32}PO_4^{3-}$, which was then diluted with water and added to culture media in the concentration range from 50 to 170 μg $^{32}PO_4^{3-}$/ml of culture media.

Kinetin, ^{14}C labelled 6-furfurylaminopurine, was obtained from the Radiochemical Centre, Amersham, dissolved in distilled water and added to the culture media of *Suillus variegatus*. The final activity was 0.025 μCi/ml; this value corresponds to 0.03 μg KIN/ml of culture media.

Counting: γ-activites of ^{115m}Cd and ^{65}Zn found in mycelia were measured in a NaI/Tl well-type scintillation crystal, connected to a single or multichannel analyser. Before counting, a complete digestion of each isolated mycelium was made on a sand bath, using concentrated nitric acid.

For ^{14}C labelled kinetin, liquid scintillation counting was performed, using a NE LSC 1 liquid scintillation counter connected to a SR 5 scaler--ratemeter. Aqueous aliquots of completely digested fungal mycelia were mixed with liquid scintillator NE 250 (1,4-dioxane based) in the ratio 1 : 10 or 2 : 10. For final counting, plastic vials were used.

Results and Discussion

Fig. 2 shows the cadmium uptake from culture media (1.1 μgCd/ml) to mycelia of *Suillus variegatus* and *Amanita muscaria*.

The uptake is expressed in terms of activity of the isotope per weight unit of mycelia (cpm/mg). Experimental data for both fungi showed the highest uptake of cadmium during the most intensive growth of mycelia. The uptake stopped around the 20th day after innoculation, when about 50% of Cd had been taken up. This indicates the active regulation of the cadmium uptake by the fungi used in the experiment. A comparison between cadmium uptake in *Amanita muscaria* and *Suillus variegatus* showed approximately 4 times higher uptake of this element by the latter fungus, which is probably due to physiological differences between *Amanita* and *Suillus* species. A similar pattern of cadmium uptake was observed at any concentration of Cd in the culture media (inside the limits of 1.1 to 3.9 μgCd/ml). The results were repeated several times with good agreement and reproducibility.

The uptake of zinc (Fig. 3) and phosphorus in *Suillus variegatus* (Fig. 4), was the highest within the first 10 days after innoculation.

This uptake of the essential elements zinc and phosporus also in the period of most intensive growth of mycelia, was more rapid than in the case of toxic cadmium. After 30 days the uptake of Zn was complete. The hormones (KIN and β-IAA), added to the culture media together with the isotopes, according to the present evidence, had no influence on the ion uptake by the mycorrhizal fungi used.

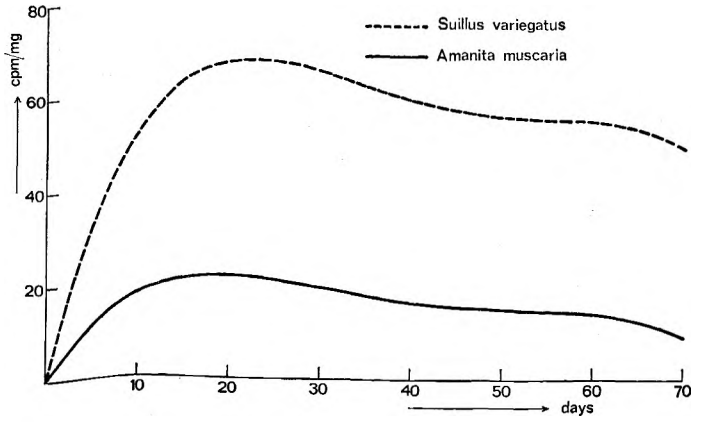

Fig. 2. ^{115m}Cd uptake by *Amanita muscaria* and *Suillus variegatus*.

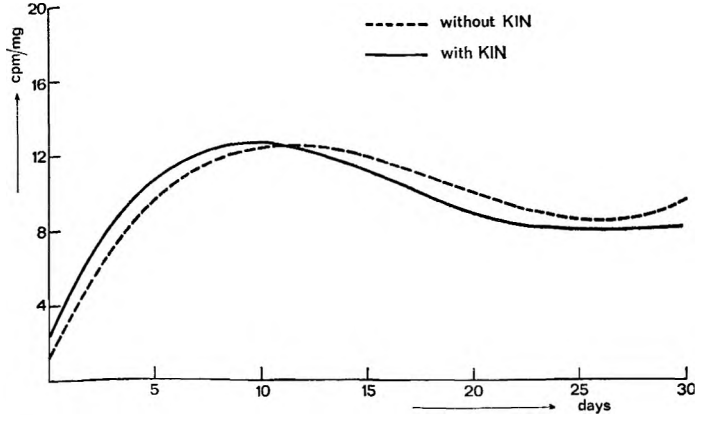

Fig. 3. ^{65}Zn uptake by *Suillus variegatus*.

In the case of phosporus, loss of the element was found during the mature stage of mycelia of *Suillus variegatus* (after the 22th day, Fig. 4). This observation was additionally supported by the translocation of ^{32}P from the point of application via the peripheral parts of mycelia into the initially non-active culture medium, while in the case of zinc no activity was detected in an identical medium (Fig. 5).

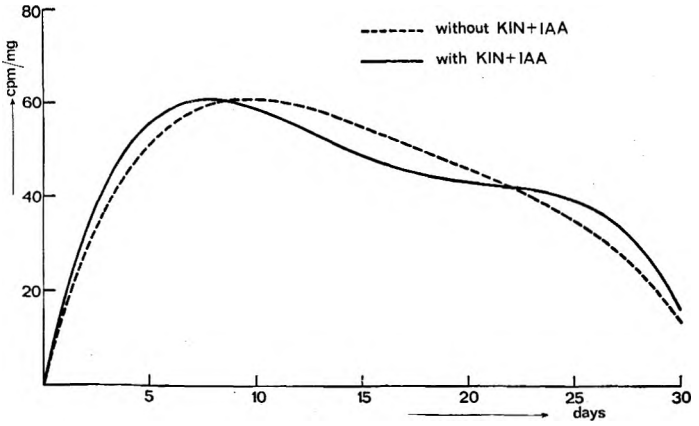

Fig. 4. ^{32}P uptake by *Suillus variegatus*.

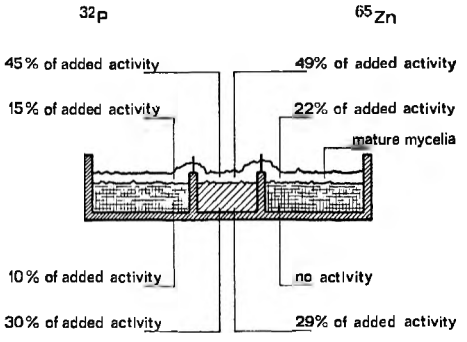

Fig. 5. Translocation and distribution of ^{65}Zn and ^{32}P between mycelia of *Suillus variegatus* and culture media 30 days after isotope application.

Fig. 6 shows the uptake of kinetin, a synthetic plant hormone, whose composition and the role in plant metabolism is similar to naturally occurring cytokinins. As in the case of Zn and P uptake, the highest uptake of kinetin from the culture media to mycelia was found during the most intensive growth (within the first 10 days after application) of *Suillus variegatus*.

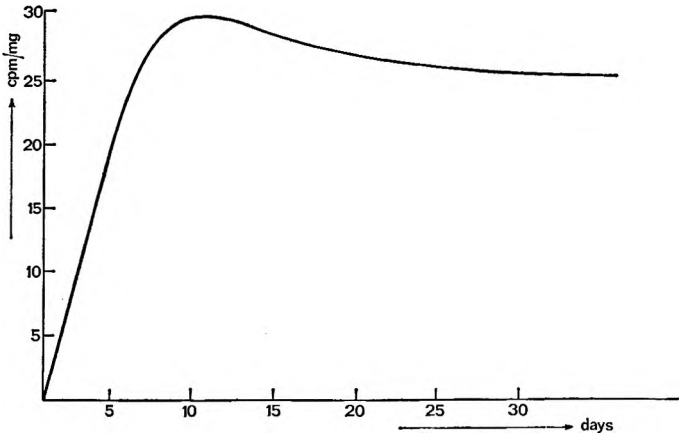

Fig. 6. KIN uptake by *Suillus variegatus.*

In general, radiotracer experiments showed that the highest uptake of trace elements cadmium, zinc, and phosphorus, as well as plant hormone kinetin, was during the most intensive growth of mycelia of *Amanita muscaria* and *Suillus variegatus,* and that the mycelium can absorb these elements very quickly without the influence of mycorrhiza of the higher plant.

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VSEBINA

PRIVZEM KADMIJA, CINKA, FOSFORJA IN RASTLINSKEGA HORMONA KINETINA PRI EKTOMIKORIZNIH GLIVAH

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V članku je prikazan privzem kadmija, cinka, fosforja ter rastlinskega hormona kinetina pri ektomikoriznih glivah rdeči mušnici *(Amanita muscaria)* in peščenki *(Suillus variegatus).*

Z uporabo radioaktivnih izotopov smo ugotovili, da je privzem kadmija, cinka, fosforja in tudi kinetina največji v fazi intenzivne rasti micelija pri obeh vrstah poskusnih gliv in da micelij brez mikorizne povezave z višjo rastlino lahko izredno hitro absorbira omenjene elemente in hormon iz gojišča.

SADRŽAJ

APSORPCIJA KADMIJA, CINKA, FOSFORA I BILJNOG HORMONA KINETINA U EKTOMIKORIZNIH GLJIVA

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U članku je prikazana apsorpcija kadmija, cinka, fosfora i biljnog hormona kinetina u ektomikoriznih gljiva *Amanita muscaria* i *Suillus variegatus.*

Primjenom radioaktivnih izotopa autori su utvrdili da je apsorpcija kadmija, cinka, fosfora i kinetina najveća u fazi intenzivnog rasta micelija u obih pokusnih vrsta gljiva i da micelij bez mikorizne veze s višom biljkom vrlo brzo apsorbira navedene elemente i hormon iz podloge.

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