

Depressed metabolic activity of VA mycorrhizal fungi by photosynthesis inhibitor herbicides

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INTRODUCTION

Vesicular-arbuscular mycorrhizas (VAM), as other mutualistic symbioses between plants and microorganisms, are biotrophic entities where the heterotrophic partner is supplied with carbohydrates from the photosynthate of the autotrophic host (HARLEY, 1975). Hence, any fact which modifies the photosynthesis products available for distribution would affect VAM development (HAYMAN, 1974; FURLAN & FORTIN, 1977; DAFT & EL-GIAHMI, 1978). Since these fungi have not yet been cultured axenically, knowledge of their biochemical abilities is scarce, however, the occurrence of key dehydrogenases linked to the respiratory pathways of VAM fungi has been histochemically demonstrated (McDONALD & LEWIS, 1978). The succinate dehydrogenase activity (SDH) was detected in the fungal mycelium, but not in the host cytoplasm. Thus, a specific staining reaction for SDH could therefore be used as indicator for a rapid and direct assessment of the mycelial development with this metabolic activity. The aim of this communication is to study the feasibility of this technique to examine if the operativity of SDH in VAM fungi is affected by foliar application of herbicides which inhibit the photosynthetic CO₂ assimilation (DIAZ *et al.*, 1980; MORELAN, 1980).

THE EFFECT OF PHOTOSYNTHETIC HERBICIDES ON VAM

The experiment was carried out in open pots of soil as was that previously described (BAREA *et al.*, 1980). This soil was steam-sterilized and then mixed with sterile sand (5:2 mixture). Two VA mycorrhizal host plants were tested: *Medicago sativa* L. cv. Aragon and *Triticum vulgare* var. Lozano. Seedlings were transplanted into pots (two per pot) containing 300 g of the soil/sand mixture. The VA mycorrhizal endophyte assayed was a form of *Glomus mosseae* from a stock plant culture. The alfalfa seedlings were also inoculated with the strain 203 of *Rhizobium meliloti* isolated in this laboratory. Twenty replicate pots for each host were prepared, and plants were grown in a glasshouse at 19-25°C. They were watered from below and given Long Ashton nutrient solution (HEWITT, 1952) (5 ml/week) without phosphate for the wheat and also without nitrogen for the alfalfa. After ten weeks' growth, plants were treated with the bis-carbamic herbicide phenmedipham, which is known to inhibit CO₂ assimilation (CHUECA *et al.*, 1980). Three phenmedipham concentrations were tested (0.025, 0.25 and 2.5 mg/ml) which were prepared in diluted aqueous solutions and 2 ml per pot sprayed to the foliage with a hand sprayer. The soil was covered with cotton wool during spraying. Five replicate pots for each treatment were prepared and there was also untreated controls.

After 48 h of the herbicide spraying, plants were harvested and the root system of each one of the replicate pots was divided into three aliquots to record: i) Total mycorrhizal infection. ii) Mycorrhizal fungus development with SDH activity and iii) Sugar content in root extracts.

Table 1. Effect of the photosynthesis inhibitor herbicide phenmedipham on the sugar content in root extracts and mycorrhizal infection of *Medicago* and *Triticum*.

Host plant	Amount of herbicide sprayed (mg/pot)	Total sugars (µg/g) fresh root	Reducing sugars (µg/g) fresh root	Total mycorrhizal root length (%)	VA mycelium with SDH activity (%)
Wheat	0	2230 ^a	745 ^a	64 ^a	56 ^a
	0.05	1280 ^b	493 ^b	67 ^a	45 ^b
	0.5	1382 ^b	462 ^b	65 ^a	38 ^c
	5	1635 ^b	556 ^b	63 ^a	14 ^d
Alfalfa	0	4311 ^a	758 ^a	85 ^a	79 ^a
	0.05	3851 ^b	673 ^{ab}	87 ^a	79 ^a
	0.5	2859 ^b	482 ^b	87 ^a	70 ^b
	5	2429 ^b	517 ^b	83 ^a	53 ^c

Data are given in a per pot basis and, for each host and record, values sharing a letter in common do not differ significantly at $P = 0.05$. Mycorrhizal infection was estimated by microscopically examining stained (PHILLIPS & HAYMAN, 1970) root samples (about 100 segments per pot) and given as percent of root length infected. Succinate dehydrogenase activity (E.C. 1.3.99.1) in the fungal mycelium was detected by the reduction of tetrazolium salts at the expense of added succinate (MACDONALD, 1980), and measured under a compound microscope, as the mycorrhizal infection is quantified. Total and reducing sugar content in fresh root was assessed as before (RATNAYAKE *et al.*, 1978; AZCON & OCAMPO, 1981).

It is apparent (Table 1) that the content of total and reducing sugars in root extracts from alfalfa and wheat is lowered by the application of the photosynthesis inhibitor herbicide, but there was no a dose-response relationship between the amount of phenmedipham supplied and the content of root sugars. Obviously, neither the total % root length infected nor the shoot dry weight of plants (data non included in Table 1) were affected by the herbicide concentration in such a short term exposure. In spite of that, the proportion of the mycorrhizal fungus with SDH activity was dependent on the concentration of herbicide applied. This indicates a rapid and specific effect of the photosynthesis inhibitor on the metabolism of the fungal symbiont itself.

As far as we know no information about the influence of these photosynthetic inhibitor herbicides on VAM development has been published. Is to be expected these agrochemicals reduce the flow of carbon compounds from the leaves to the heterotrophic parts of the systems, which is important regarding VAM development but it also of interest to know that the fungal mycelium, in some extent, became actually metabolically altered.

The transfer of carbohydrates from plants to their VA mycorrhizal fungi has been evidenced by allowing plants to photosynthesis with $^{14}\text{CO}_2$ (BEVEGE *et al.*,

1975; LOSEL & COOPER, 1979) and subsequent analysis of the distribution of radioactivity in mycorrhizal roots. In fact, $^{14}\text{CO}_2$ assimilated by photosynthesizing leaves is readily translocated to roots reaching the endosymbiont fungi. In addition to a "storage sink" for photosynthate, the active growth and metabolism of the fungus can be also an operative sink (HARLEY, 1975; SMITH, 1980). Our results suggest that the growth-sink could be evaluated by the SDH staining technique. Obviously the study of the incorporation of $^{14}\text{CO}_2$ labelled photosynthetic assimilate by endomycorrhizal fungus, or the measure of a short term uptake of ^{32}P from labelled soils (BOATMAN *et al.*, 1978), could be used to assess if growth or metabolism of the VAM fungi, or their active uptake of nutrient, are affected as a consequence of the herbicide activity. Techniques using radioactive materials are however more tedious and do not improve the direct evidence of the SDH staining technique which is easy to handle and quick.

CONCLUSIONS

The staining reaction for SDH demonstrates that the operativity of the tricarboxylic acid cycle in VAM fungi is depressed by inhibitor of the photosynthetic CO_2 assimilation. The reported results suggest an additional ecological cost of the indiscriminate use of herbicides via their effect on VAM. Beside, the use of photosynthesis inhibitor herbicides with different and selective mode of action could be an interesting tool, as helped by cytochemical techniques, for physiological studies on carbohydrate metabolism in VAM.

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