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Infectivity and effectiveness of *Glomus intraradices* on micropropagated plants

Abstract Colonization by *Glomus intraradices* takes place very early within the root system of micropropagated plantlets of strawberry (var. avanta, elsanta), raspberry (var. himboqueen, Zeva I), and hortensia (var. leuchtfeuer). The arbuscular mycorrhizal fungus (AMF) did not colonize roots of the different hosts to the same extent, and considerable differences were observed between the varieties. The results reported here confirm that endomycorrhizal root colonization is affected by the host-fungus combination. The effects ranged from mutualistic (hortensia), through neutral (strawberry var. avanta, raspberry var. Zeva I) to negative (raspberry var. himboqueen and strawberry var. elsanta). Non-mycorrhized (control) plants of strawberry produced more runners than mycorrhized plants under controlled growth conditions (phytotron). Transfer of the potted plants to the field resulted in drastic alterations in overall growth and development within 4 weeks. Mycorrhized plants became healthy, and mycorrhized strawberry plants produced many stout runners. The number of the runners and their biomass were almost the same (var. avanta) and treated plants produced even more runners than the controls (var. elsanta). The authors have demonstrated the need to determine the specific effects of each species of AMF on individual prospective host plants prior to their utilization in the micropagation of plantlets.

Key words Arbuscular mycorrhizal fungus
Micropropagated plants

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Introduction

Mycorrhizas are mutualistic associations occurring between most plant species and several groups of fungi. It has been postulated by several workers that the earlier the arbuscular mycorrhizal fungi (AMF) develop, the sooner the host micropropagated plants benefit from the symbiosis (Branzanti et al. 1992; Fortuna et al. 1992; Gianinazzi et al. 1990; Ravolanirina et al. 1989). AMF penetrate the living cells of plant roots without harming them, and their hyphae at the same time range far into the bulk soil (Sieverding 1991; Varma 1994), establishing equally intimate contacts with microbiota of soil aggregates and microsites. Thus, these fungi link plants with the soil and its biota. Micropropagation is an excellent tool for the production of homogeneous, genetically improved orchard and ornamental crops. However, this technique completely eliminates or at least drastically reduces the population of beneficial organisms present in the soil (Lovato et al. 1994), e.g. symbiotic endomycorrhizal fungi and plant growth-promoting rhizobacteria. The fungus may heavily colonize the roots without a proportional promotion of plant growth. A high level of infection may thus result in a drain of photosynthates without a compensatory improvement in the mineral nutrition of the host plant (Clapperton and Read 1992; Koide 1985; Lovato et al. 1992). Consequently, arbuscular mycorrhizas may influence the flow rate and the composition of root exudates, and a substantial proportion of root-derived organic C in the soil will be located in the mycorrhizal network (Bethlenfalvay 1992). A high level of root colonization is in principle desirable, since it means that sites for the entrance of microorganisms into the roots are already occupied; this is the basis of potential resistance to pathogenic microorganisms (Gianinazzi et al. 1990).

The effectiveness of AMF on micropropagated plants has been studied previously (Gianinazzi et al. 1986; Morandi et al. 1979; Pons et al. 1983; Ravolanirina et al. 1989). To the authors' knowledge, no one has

ever tested the effect of early mycorrhization of micropropagated plants in pots under controlled growth conditions (phytotron) and after their subsequent transfer to the field. Here we report the influence of early mycorrhization on micropropagated plantlets of small fruits, (strawberry, raspberry and the ornamental plant hortensia) in the phytotron and in a field situation. The positive role of mycorrhizal fungi in sustainable horticulture is discussed.

Materials and methods

Host plantlets

Micropropagated plantlets of raspberry (var. himboqueen), strawberry (var. avanta, early maturing, and elsanta, late maturing), and hortensia (var. leuchtfeuer) were obtained from Proplant AG, Mikrovermehrung, Neukirch-Egnach, Switzerland, and plantlets of raspberry (var. Zeva I) from Biotechnology Laboratory, Swiss Federal Research Station, Wädenswil, Switzerland. Raspberry and strawberry plantlets were obtained on agar rooting medium while hortensia plantlets were obtained 2 days after transfer onto the substratum (turf).

Turf (growth substrate)

Gamma-irradiated (Co^{60}, 10.5 kGy) turf substratum (Brill Erde, Torfwerke Brill, Georgsdorf, FRG) was used for the pot experiments. The nutrient contents were: (M^3/g): N, 210; P_2O_5, 240; K_2O, 270; $CaCO_3$, 7000; pH 5.5.

Natural microflora were rejuvenated by sprinkling unsterile tap water onto the sterile turf substratum and incubating it spread on plastic trays in the greenhouse for 72 h. The intention was to cultivate a diverse microflora in the substrate in order to avoid a microbial vacuum and to minimize microbial imbalance (Allen et al. 1993). Soil free of AMF was mixed with experimental soil to provide homogeneous rhizospheric microorganisms.

Inoculum

The crude AMF inoculum consisted of inoculated expanded clay obtained from maize (*Zea mays* L.) pot cultures and inoculated with *Glomus intraradices* Schenck and Smith in a greenhouse for 3–4 months. Roots were cut into segments of 2 cm length and then mixed with sand and expanded clay containing spores and hyphae. Mycorrhizal treatments consisted of 20 g inoculum per 200 g turf substratum (each inoculum dose contained hundreds of spores, a substantial amount of infected root fragments plus an unknown amount of external hyphae) mixed uniformly with soil substratum. Control treatments received an equal amount of autoclaved inoculum to provide similar physical and chemical conditions in all experiments but excluding indigenous AMF.

Growth conditions

Acclimatization of strawberry and raspberry plantlets was done by washing the rootlets with sterile deionized water to remove rooting nutrient agar medium and then potting on to growth substratum in washed (2% sodium hypochlorite for 20 min) rectangular plastic trays (300 ml). For the first 5 days, plantlets were grown in a greenhouse with day-night temperatures ranging from 16–21° C. Day length was extended to 16 h by cool white fluorescent lamps at 2000 lx. Plantlets were watered (deionized water) on alternate days. For the first 5 days, the trays were covered with glass to maintain adequate humidity (see flow diagram in Fig. 1).

In one set of strawberries (128), the roots were allowed to grow for 5 days in sterile substratum without AMF inoculum. The roots were then shortened to 1 cm, and the plants replanted into the respective acclimatization trays described above with active AMF inoculum. Plantlets after acclimatization (2 weeks) were transplanted individually into sterile clay pots (one plant per pot containing 250 g of turf) and allowed to grow in a phytotron (day 20.5° C, night 16° C; light 16 h/day, 320 $\mu E/m^2\ s^{-1}$; 65–70% relative humidity). Each pot was watered uniformly with deionized water (40 ml per day) by means of a Tropf Blumat device (Weininger KG, Austria). Pot plants were fertilized every 15 days with a 100-fold diluted Hewitt nutrient solution without phosphate (Hewitt 1966). After 12 weeks, the potted plants (except hortensia and Zeva I) were planted into the field. The field soils were "*Braunerde*", rich in silts and scattered small stones, pH 5.8–6.0.

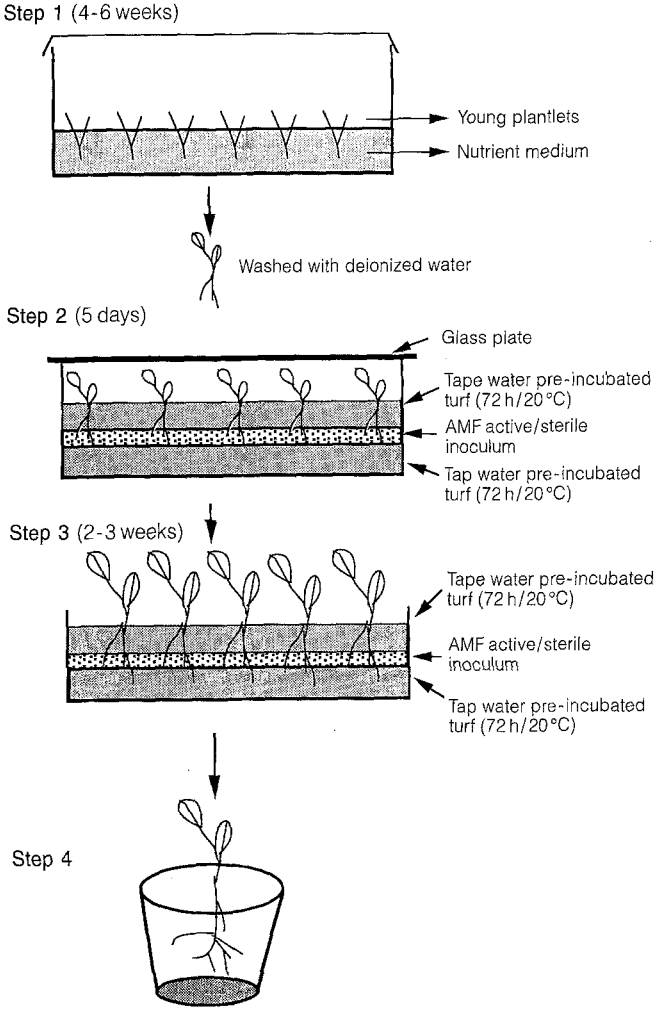

Fig. 1 Flow diagram showing the acclimatization of plantlets regenerated from tissue culture. Well-washed plantlets were transferred to sterile trays and allowed to grow in the greenhouse. After 5 days the glass sheet was removed in several steps to maintain adequate moisture and prevent sudden exposure to direct nonfiltered light. After transplanting to clay pots, the plants were cultured in the phytotron

Table 1 The effect of AMF inoculation (*Glomus intraradices*) on host plant height (cm, mean ± SD). The pot experiments were conducted in the phytotron. Plant height for strawberries was measured as the length of the longest leaf, and for raspberries as shoot apex

	Strawberry				Raspberry				Hortensia ($n=41$)	
	Avanta ($n=61$)		Elsanta ($n=63$)		Himboqueen ($n=62$)		Zeva I ($n=39$)			
	6 weeks	10 weeks	6 weeks	10 weeks	6 weeks	10 weeks	4 weeks	8 weeks	6 weeks	10 weeks
Control (sterile)	9.1±1.0	11.9±1.8*	9.8±0.5*	12.0±1.6*	19.1±2.0*	44.0±5.7*	11.4±1.4*	28.0±2.8	10.1±2.0	14.1±1.4*
Active inoculum	8.2±0.8	10.5±2.5*	8.1±0.67*	9.6±4.0*	13.6±1.5*	34.0±7.7*	12.8±1.2*	27.2±3.1	13.8±1.9	21.9±1.5*

* Significant difference between the treatments at each harvest time, $P=0.05$; n number of plants

Assessment of AMF colonization

A portion of the rootlets was harvested and stained with 0.01% trypan blue in lactic acid (modified from Phillips and Hayman 1970). Infected root pieces selected under the dissecting microscope (Wild Photo Makroskop, M-400, Heerbrugg, Leitz) were mounted on a slide and the morphology was examined using a Leitz Diaplan microscope. AMF colonization (as percent total root length) was determined by the gridline intersect method (Ambler and Young 1977) by observing about 200 root intersections to reduce the error to below 3 perent (Giovannetti and Mosse 1980). Samples in which root colonization was difficult to quantify due to inadequate staining were treated with 0.05% trypan blue and mounted on a slide for more detailed observation using a compound microscope (Dodd and Jeffries 1986).

Biomass estimation

The growth of hortensia and raspberry plant heights was determined as the height up to the apex and that of strawberry as the length of the leaves. Runners were counted, removed from the mother plants, dried at 90° C for 48 h and weighed.

Statistical treatment

Means were analysed in pairs by applying the t-test.

Results

The proportion of plants surviving acclimatization was 100%. No differences were apparent in the growth of control and inoculated plants at the time of transplanting to clay pots; shoot apices were active. No differences were noted between strawberry plantlets with roots shortened to 1 cm and those with untreated roots. Therefore the data are presented as the means of all plantlets measured.

Whereas strawberry var. avanta showed no significant difference in the heights of plants between the treatments, a significant difference was recorded for var. elsanta (Table 1). Control plants of the later variety (elsanta) attained a greater length than those which received active AMF inoculum. Raspberry var. himboqueen was severely retarded as a result of mycorrhization, but no such effect was observed for var. Zeva I. Identical growth was observed in the 4-week-old hortensia in both sets, but mycorrhized plants considerably improved by 10 weeks. Leaf counts were not markedly different between the treatments (data not given). Apparently, the growth of some hosts was depressed by heavy colonization pressure (Tables 1 and 4).

On transfer of the potted plants to the field, strawberry plants recovered considerably in height, with the differences recorded earlier in the phytotron between treated plants and the controls eliminated within 4 weeks (Table 2). The effect on raspberry var. himboqueen was particularly marked. AMF-inoculated plants became healthier and grew as tall as control plants (Table 2).

Runner formation showed some very interesting trends (Table 3). They appeared after 5–6 weeks (in the phytotron), and the number per plant was much higher for nonmycorrhized plants (5–11 per plant) than those which received active AMF inoculum (0–3 per plant).

Table 2 Host plant height (cm, mean ± SD) after transfer of the potted plants (phytotron) to the field, measured after 4 weeks. Plant height for strawberries was measured as length of the longest leaf, and for raspberries as shoot apex

	Strawberry		Raspberry
	Avanta ($n=62$)	Elsanta ($n=62$)	Himboqueen ($n=57$)
Control (sterile)	14.5±3.2	17.2±2.2	57.3±13.5
Active inoculum	15.4±2.3	16.8±2.2	61.4± 9.1

Table 3 The effect of AMF inoculation on the total runner biomass (g dry wt) of the host strawberry plants). Assessments were made from the pot experimental plants (phytotron) and after transfer to the field. Runners were removed from the mother plants, counted and dried for 48 h at 90° C

	Phytotron		Field	
	Avanta ($n=30$) 12 weeks	Elsanta ($n=30$) 12 weeks	Avanta ($n=30$) 4 weeks	Elsanta ($n=30$) 4 weeks
Control (sterile)	34.3	25.3	131.0	27.9
Active inocolum	14.8	7.9	109.1	64.8

Table 4 Mean (±SD) percent AMF (*Glomus intraradices*) root colonization of host plants. Infection analysis made 6 weeks after inoculation, observed with trypan blue (*a* % arbuscule formation, *m* % hyphal infection along with vesicles, *n* total root segments counted)

	Strawberry				Raspberry				Hortensia ($n=238$)	
	Avanta ($n=173$)		Elsanta ($n=191$)		Himboqueen ($n=273$)		Zeva I ($n=194$)			
	m	a	m	a	m	a	m	a	m	a
Control (sterile)	0	0	0	0	0	0	0	0	0	0
Active inoculum	41.2±13.7	15.9±11.5	96.7±44.8	28.4±33	91.9±12.1	29.8±27.1	52.2±18.1	22.3±14.2	81.4±18.7	33.8±29.7

This difference in number (data not given) and in the total biomass were much greater for var. avanta than for var. elsanta (Fig. 2; Table 3). Runner biomass weight and number per mother plant in phytotron conditions correlated significantly with root colonization by AMF. The higher the percent root colonization the lower the runner biomass for var. elsanta; however, the reverse was observed for var. avanta (Tables 3, 4).

After transfer of the potted plants to the field, runner formation underwent interesting changes. Within 4 weeks, there was considerable production of runners by each mother plant and the differences between controls and mycorrhized plants observed in the phytotron decreased considerably (Table 3). Mycorrhized plants produced more runners per plant (data not given) and increased in total biomass (Fig. 2). Plants inoculated with AMF produced many larger and stouter runners than the controls and also showed a higher capacity for runner production relative to plant size. The very large differences in runner production observed in the phytotron between the different treatments of var. avanta were greatly reduced. However, mycorrhized plants of var. elsanta exceeded the total biomass of control (Table 3). Runners showed a distinct tendency to establish in the soil and rooting early without any sign of mycorrhizal infection. With strawberries, flowers failed to appear in the phytotron; however, on transfer to the field, uniform flower and fruit setting (about 40–50%, middle June-early July) commenced in both treatments of var. avanta. Plants of Zeva I and hortensia were not transferred to the field.

Two weeks after inoculation, *G. intraradices* had already infected the roots, and many appressoria were clearly visible on the surface of strawberry var. elsanta, raspberry and hortensia roots (Fig. 3a–c). The number of appressoria per millimetre of root was high except in var. avanta (data not given). Percent mycorrhizal infection varied from high for strawberry var. elsanta, raspberry var. himboqueen and hortensia through moderate for raspberry var. Zeva I to relatively low for strawberry var. avanta (Table 4). A progressive increase in mycorrhizal infection at 10 weeks was recorded for all the hosts (data not given). Roots of elsanta, himboqueen and hortensia produced a large number of distinctly stained vesicles (Fig. 3d), most of which transformed into spores after 10–12 weeks. More detailed inspection of these roots under a compound microscope revealed unstained vesicles and fungal mycelium containing characteristic lipid droplets inside the roots. Roots of strawberry var. avanta showed less development of intercellular hyphae and retained the ability to stimulate preinfection stages as well as appressorium formation. Appressoria frequently formed in the furrows between epidermal cells (Fig. 3b). Penetration of the epidermal cells occured via a narrow hypha that developed beneath the appressorium (arrowed in Fig. 3b, c). This hypha regained its normal diameter within the cell but narrowed again before penetrating the outer wall of the hypodermal layer. Hyphal coils occured in the subepidermal cells of the hypodermis (exodermis). An abundance of vesicles were observed in the roots of raspberry and hortensia (Fig. 3d). Hyphal transformation into vesicles was considerably delayed in the strawberry varieties. In general, arbuscule formation was limited in all the hosts. It is important to note that roots of control plantlets were free of AMF hypha (Fig. 3e). However, their root surfaces were invariably colonized by other fungi, notably species of *Rhizopus* and *Phialophora*, and occasionally *Rhizoctonia*. Nonmycorrhizal infections on the root surfaces of AMF-treated plants were rare and were only seen on the lower portion of the heavily coiled roots produced by the physical limitations to expansion and growth in the clay pots.

Discussion

A literature survey shows that the effect of mycorrhizal inoculation on the overall growth of a large number of micropropagated host species was either stimulatory (Chavez and Ferrera-Cerrato 1987) or had no effect (Kiernam et al. 1984) or a negative effect (Hrselova et al. 1988). A positive growth response to mycorrhizae in micropropagated strawberry has been reported by several authors (Holevas 1988; Vestberg 1992; Vosatka et al. 1992). However, in other experiments an adverse effect of inoculation by a *Glomus* sp. was found, although an increase in runner formation by inoculated plants was observed (Hrselova et al. 1989). In the present study, a similar range of positive (hortensia), no effect

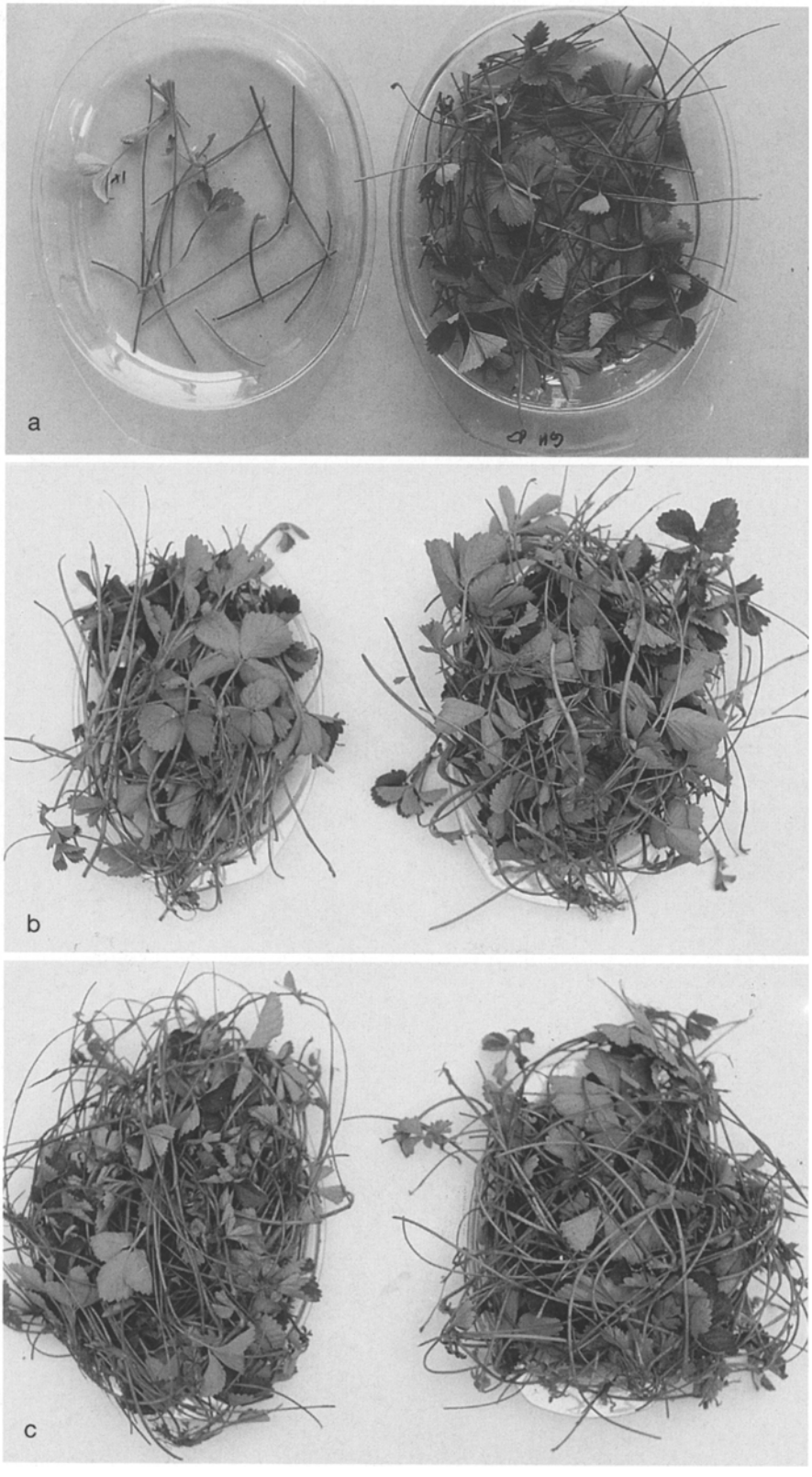

Fig. 2a–c Runner production. The plants on the left received the active inoculum (*AMF+*) and those on the right received sterilized inocula (*AMF−*). The upper photographs show the total runners detached from the mother plants whilst in the phytotron; the middle and lower photographs show the total runners 4 weeks after transfer to the field. **a, b** Elsanta; **c** avanta

(strawberry var. avanta and raspberry var. Zeva I), or negative effect (strawberry var. elsanta and raspberry var. himboqueen) was recorded in controlled growth conditions (phytotron). Several authors claimed that the technique of in vitro propagation, where plants are grown in sterile media and then transplanted into artificial substrata lacking AMF, strongly reduced or did not allow the formation of mycorrhizas. Inoculation with AMF appears to be critical for the survival and growth of microplants (Branzanti et al. 1991, 1992).

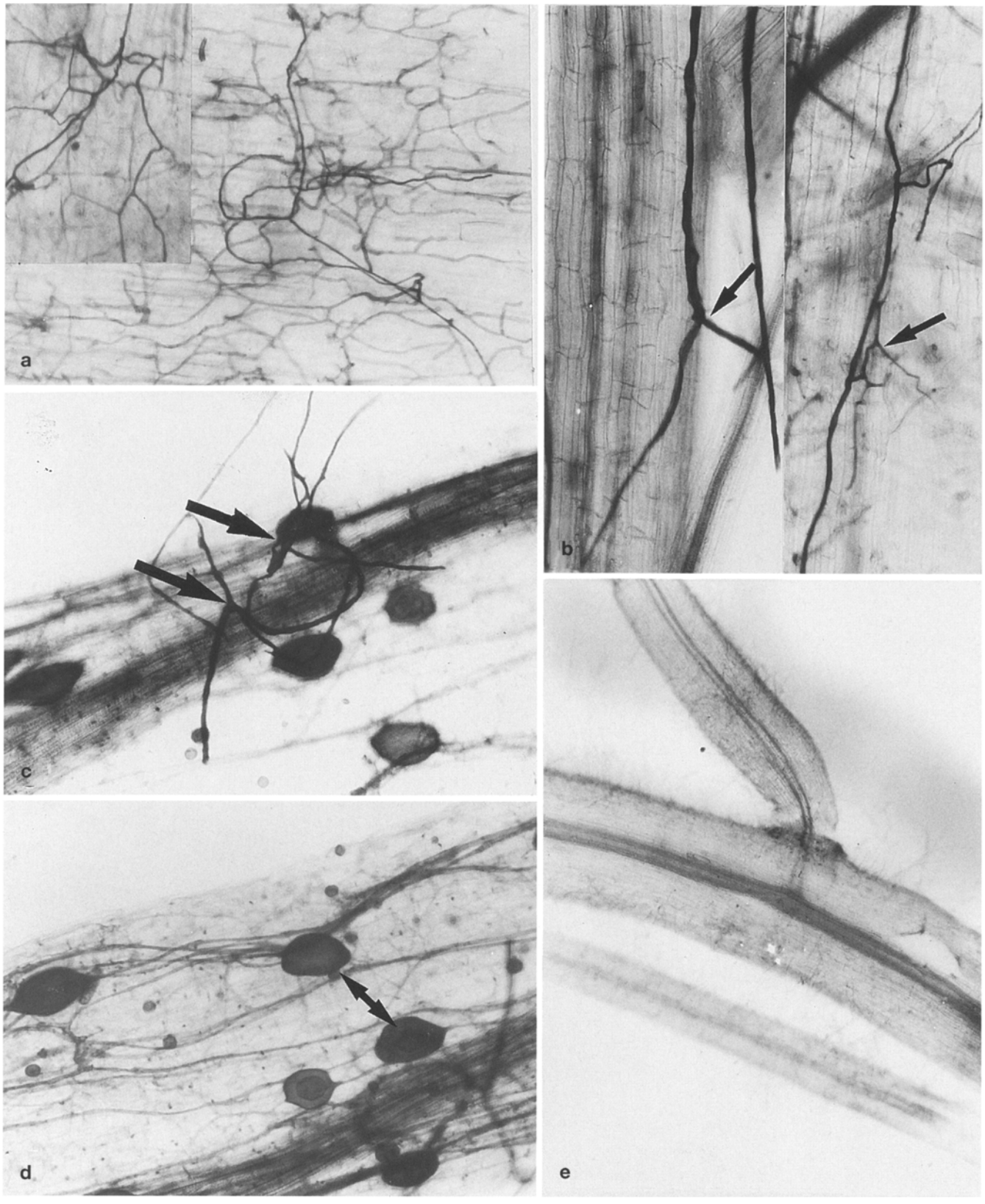

Fig. 3a–e Stages of root infection by *Glomus intraradices* of micropropagated plantlets after 6 weeks. **a** The early stages of root colonization (raspberry var. himboqueen). Preinfection branching and development of coils in the sub-epidermal cell layers. **b** Appressoria formation and penetration of epidermal and hypodermal cell walls (*arrows*). Note intercellular hyphae. **c** Formation of a coil in the hypodermal cell layer (*arrows*). **d** Longitudinal squash of a root of hortensia showing development of vesicles and intercellular hyphae. **e** Longitudinal squash of roots of raspberry var. himboqueen; no fungal colonization by AMF; stained with trypan blue

Fortuna et al. (1992) reported that control (non-mycorrhized) plum plants showed blocked apical growth just after transplanting, and shoot apices failed to resume activity ("transplant shock") during the entire experiment. Micropropagated plantlets died on transfer to the substratum (Caron 1989; Williams et al. 1992); however, these authors did not observe the reported blocked apical growth phenomenon in any of the host plantlets investigated, possibly because of initial rejuvenation of natural microflora in growth substrata during acclimatization (see Allen et al. 1993).

Most of the published results suggest that mycorrhizas enhance plant growth at low soil nutrient levels and that this effect is lost with higher nutrient availability (Schubert et al. 1992). In the present study, the fertilization levels of the substratum, the fungal inoculum and other growth conditions were identical for all the test hosts. However, carbon drain was considered to occur as a function of *Glomus* species and variety of hosts in controlled conditions. It seems, therefore, that substratum nutrient level is not the only critical factor for plant growth. A better insight into the physiological events in the plant during acclimatization, such as growth of roots and photosynthesis, will considerably increase the potential for AMF inoculation of micropropagated plantlets (Bethlenfalvay and Linderman 1992). A more thorough understanding of the importance of organic carbon and rhizodeposition in the fungal network (external/internal) of treated plants in relation to non-hyphal carbon derived from roots will require further measurements. This is an important consideration with respect to mycorrhizal ontogeny and factors determining rates of turnover of fungal components (hyphae, arbuscules, vesicles) as a result of early mycorrhization in tissue culture-propagated plants.

To our knowledge there is no record of runner number and biomass from micropropagated strawberry plantlets within controlled (phytotron) conditions. However, in agreement with our results, Niemi and Vestberg (1992) and Williams et al. (1992) showed that mycorrhized plants produce more runners per plant in the field. There are several possible explanations of this phenomenon, such as improved nutritional status or alterations in hormonal balance in the field-grown plants and physiological stress and/or uneven photosynthate distribution for runner production in the mycorrhized plantlets. This change may be due to alterations in cytokinin level which can affect vegetative reproduction of strawberry. Nitrogen is known to influence the synthesis of cytokinins (Wagner and Michael 1971), and high levels of nitrogen application are routinely used to enhance runner production. AMF are known to increase the uptake of nutrients, mainly phosphorus but also nitrogen (Frey and Schüepp 1993), and have been shown to change the level and flux of cytokinins in different plants (see Allen 1992). The observed stimulation of runner formation in the inoculated strawberries may thus be a phytohormone-mediated mycorrhizal effect on reproductive physiology. The reduction in runner production in mycorrhized plants compared to the controls in the phytotron may be due to physical limitation of space for root expansion and the maintenance of optimum metabolism.

Niemi and Vestberg (1992) have reported fewer but larger runners in commercially grown strawberries inoculated with *G. mosseae* compared to controls. Contrary to their observations, in our experiments *G. intraradices* produced a large number of long and healthy runners on transfer to the field. Strawberry is routinely propagated from runners or by micropropagation, and from a commercial point of view such an effect of AMF on runner production after transfer to the field may provide a suitable technique for mass propagation of pathogen free, healthy mycorrhized plants, and assist strawberry farming.

Recent results suggest that there is specialization among AMF in functions affecting plant nutrition (Bethlenfalvay and Linderman 1992). AMF species of the genus *Gigaspora* appear to favour fluxes of C compounds from plants to soil biota, resulting ultimately in enhanced soil aggregation, while *Glomus* spp. tend to favour plant growth through improved mineral nutrition (Espinoza-Victoria et al. 1993; Miller and Jastrow 1992). Measurable effects of AMF on micropropagated plantlets, even in our short-term experiments, suggest that they eventually affect plant nutrition not only through the direct regulation of mineral uptake or exclusion but also through the modification of photosynthate utilization. The contribution of the fungi to soil organic matter plays a key role in rhizosphere biology (Bethlenfalvay 1992). Where the response is negative (raspberry var. himboqueen, strawberry var. elsanta), C lost by the host plant enters the soil, to the ultimate benefit of the plant-soil system as a whole (Quintero-Ramos et al. 1993).

Mycorrhizal infection with *G. mosseae* has been reported to be similar but rather low (10–15%) in several cultivars of commercially grown strawberries (Niemi and Vestberg 1992). This observation does not agree with the relatively high AMF infection obtained for all the hosts tested by the present authors (see Table 4). In another experiment, Vestberg (1992) reported higher root colonization and sporulation in four early cultivars (22–42%) than late cultivars (22–34%). Contrary to this observation, in our experiments early var. avanta had a lower response to AMF than late-maturing var. elsanta.

The intraradical component of the mycosymbiont may constitute up to 16% of root dry weight, and the mycosymbiont may consequently consume substantial amounts of host C (Jakobsen 1993). The use of C by the mycosymbiont is often compensated by increased photosynthesis. The lack of any compensation of this C drain would lead to serious growth depression (Eissenstat et al. 1993; Peng et al. 1993). The higher belowground C allocation in mycorrhized than in non-mycorrhized plant is used for growth and maintenance of root and mycosymbiont. Greater construction costs of

the lipid-rich roots and greater root biomass allocation could explain most of the additional C use of roots in strawberry var. elsanta and raspberry var. himboqueen and other experimental plant hosts with similar chemical and physical status. The efficiency of C production seems to be actually slightly lower in mycorrhizal than non-mycorrhizal plants of similar status because of greater C allocation to root biomass and below-ground respiration, which was not compensated by increased net assimilation. Net transfer of hexoses from plant to fungus is stimulated by rapid transformation into lipids, present in hyphae, vesicles and spores and constituting 95–100% of the C pool in spores (Jakobsen and Rosendahl 1990; Jakobsen 1994; Martins 1992). Our experiments have demonstrated that *G. intraradices* modifies the plant nutrient status regardless of the soil nutrients. Host plants to a great extent in turn modified the AMF effects both in the phytotron and in the field.

Micropropagation techniques have been applied to plants for use in revegetation programmes (Salamanca et al. 1992). It is envisaged that AMF act as mediators of nutrient exchange in the soil-plant system. In fact, the AMF mycelium acts as a key component in a close "cause and effect interchange" of mineral nutrients, C compounds, signals between the plant and rhizosphere populations, and soil aggregation (Linderman 1986). As these authors argued, the maintenance of an optimal biological balance in the soil is critical for sustainability. It is obvious that coordinated manipulation of AMF and sustainable microorganisms is the only way to restore the biological component of sustainability. The fast growth recovery and active runner production by mycorrhized strawberries and the accelerated shoot growth in mycorrhized raspberry var. himboqueen on transfer to field conditions are in complete agreement with the system proposed by several authors (Azcon-Aguilar et al. 1992; Barea et al. 1993; Bethlenfalvay 1992; Bethlenfalvay and Linderman 1992; Linderman 1992).

Highly mycotrophic plants are characteristic of stable, sustainable ecosystems and any attempts to convert non-sustainable ecosystems to sustainable ones must consider the mycorrhizal component. As the data show, AMF are an important component of the soil, since they colonize the roots of virtually all crop plants with diverse effects on plant nutrition. Because of the diversity of these effects, selection mechanisms need to be developed before AMF can be used effectively to promote biotechnologically developed plants to the soil.

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