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Effects of fertilizers and arbuscular mycorrhizal fungi on the *post-vitro* growth of micropropagated strawberry

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Summary — A 5-wk factorial experiment was conducted to examine the effects of osmocote (18N:11P:10K; commercial controlled-release fertilizer), rock phosphate and 3 species of arbuscular mycorrhizal fungi on the growth of the strawberry cultivar Senga Sengana in a peat–sand–vermiculite substrate. Three *Glomus* species, G98 (probably *G intraradices*), G128 (an undescribed species) and *G geosporum* were compared. G98 and G128 significantly increased shoot dry weights compared with the non-mycorrhizal control. Levels of root colonization by G98 and G128 were significantly greater than by *G geosporum*. The addition of both fertilizer types significantly increased shoot dry weights. Neither osmocote (0.5–2.0 g/l) nor rock phosphate (1.0–5.0 g/l) addition had a significant effect on colonization by the 3 fungi. An osmocote fertilizer applied at 25% of the minimum recommended commercial rate to mycorrhizal plants was sufficient to produce equivalent dry matter yields as non-mycorrhizal plants receiving the full application of osmocote. All 3 fungal species significantly increased the stolon number per plant when compared with the non-mycorrhizal controls. Results clearly show that healthy mycorrhizal strawberry plants can be produced at weaning using commercial or reduced rates of osmocote or a rock phosphate formulation.

phosphate / fertilizer / arbuscular mycorrhizal fungus / strawberry / micropropagation

Résumé — Effet d'engrais et de champignons mycorhiziens arbusculaires sur la croissance *post-vitro* de fraisiers micropropagés. *Un essai factoriel de 5 semaines a été fait pour examiner les effets de l'osmocote, (engrais commercial à diffusion contrôlée 18:11:10), du phosphate naturel, et de 3 espèces de champignons AM sur la croissance du cultivar de fraisier Senga Sengana sur un substrat tourbe : sable : vermiculite. On a comparé 3 espèces de* Glomus *G98 (probablement* G intradices*), G128 (espèce non décrite), et de* G geosporum. *G98 et G128 ont augmenté significativement les poids sec de tiges, par rapport aux témoins non inoculés. Les niveaux de colonisation des racines par G98 et G128 étaient plus élevés que celui de* G geosporum. *L'addition des 2 engrais a significativement augmenté le poids sec des tiges. Ils n'ont aucun effet sur la colonisation par les 3 champignons. L'osmocote appliqué à 25% de la dose minimale conseillée commercialement suffit pour produire les mêmes rendements en matière sèche sur les plantes mycorhizées que la dose complète sur les témoins non mycorhizés. Les 3 espèces de champignon augmentent significativement le nombre de stolons par plante, par rapport au témoin. Les résultats montrent clairement que l'on peut produire des plantes mycorhizées saines de fraisier au sevrage en utilisant des doses commerciales ou réduites d'osmocote ou de phosphate naturel.*

phosphate / engrais / champignon mycorhizien arbusculaire / fraisier / micropropagation

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INTRODUCTION

Strawberry production in the UK involves outplanting of nursery plants, usually into soils sterilized with methyl bromide and/or chloropicrin to control root diseases such as *Verticillium* wilt, and red core caused by *Phytophthora fragariae* (Wilhelm and Paulus, 1980). Unfortunately, soil fumigation also eliminates any indigenous arbuscular mycorrhizal fungi (AMF) which are of vital importance for efficient uptake of nutrients, especially phosphorus (P), by plants growing in many natural and agricultural ecosystems (Krikun *et al,* 1990).

The presence of mycorrhizal fungi in strawberry planting material has been implicated in a significant increase in fruit production in a fumigated field (Robertson *et al,* 1988) and in increases in dry weight following inoculation with mycorrhizal fungi (Holevas, 1966; Koomen *et al,* 1987). More recently Hrselova *et al* (1989) demonstrated a 17% increase in stolon length and increased P content in all plant parts in strawberry plants colonized by AMF. Dunne and Fitter (1989) demonstrated that the P-inflow during strawberry flower and fruit development exceeded the calculated maximum potential inflow *via* both the old and the new roots. They concluded, therefore, that this excess P requirement could only be met if nutrient uptake was assisted by AMF.

Field inoculation of crop plants, however, is impractical due to the technical difficulties and the large amount of inoculum required. Inoculation of the nursery plant may be an alternative strategy for improving plant growth (Gianinazzi *et al,* 1989). In both the UK and in Finland, many varieties of strawberry are micropropagated prior to planting in the propagation beds. Inoculation of micropropagated strawberry with AMF has increased plant height at weaning by up to 343% (Chavez and Ferrera-Cerrato, 1987). Kiernan *et al* (1984) showed that strawberry tissue-cultured plants increased their shoot growth in a peat–perlite substrate when inoculated with 2 *Glomus* species. As plant death is often a problem when weaning micropropagated material there is also potential for increasing survival rates and for controlling root pathogens (Caron, 1989).

This study was aimed at integrating the use of AMF into strawberry plant weaning by adapting current techniques to produce healthy mycorrhizal planting material. The effect of 2 different fertilizers on plant and mycorrhizal development during the plant weaning stage was examined. It was intended to find a fertilizer regime which would permit good mycorrhizal colonization and promote efficient plant growth while comparing 3 AMF species, 2 from Finland with 1 from the UK. With the increasing interest in reducing chemical/inorganic fertilizer inputs, techniques for reintroducing these beneficial fungi into strawberry production, at appropriate fertilizer levels, requires investigation.

MATERIALS AND METHODS

Plant material

In vitro strawberry plants (*Fragaria* x *ananassa* Duch cv Senga Sengana) were propagated on a modified Boxus agar medium (Boxus, 1974). They were transferred to rooting medium for 4 wk prior to being transplanted into the experimental substrata. In an earlier comparison of substrata mixes (data not presented), it was found that although high levels of mycorrhizal root colonization occurred in all substratum types, only peat–sand–vermiculite (8:1:1; v:v:v) supported good plant growth. A standard plant growth substratum of peat–sand–vermiculite (8:1:1; v:v:v), with the addition of 10 g/l dolomite lime to adjust pH to 5.5, was thus used for all treatments.

Mycorrhizal fungi

More than 80 AMF have been isolated into pure pot culture from various locations around Finland (Vestberg, unpublished data). This collection had been screened for induction of a beneficial growth response in the strawberry cultivar under test and 2 Finnish species were selected which gave the best growth response. G98 was isolated from a grassland with sandy soil, pH 5.35, from western Finland. It has been tentatively identified as *Glomus intraradices*. G128 was isolated from a grassland with sandy soil, pH 5.40, from central Finland. It is similar to *G maculosum* but is probably a new species (Walker, personal communication). A third species, *G geosporum* (from under wheat in an agricultural soil in Kent, southeast England, pH 7.2), was chosen as a broad range species for comparison in this experiment. The inoculum was prepared from pure pot-cultures and consisted of finely chopped root fragments, and pot-culture substratum (peat–sand–vermiculite; 8:1:1; v:v:v) containing extraradical spores and mycelium.

Fertilizer treatments

Two sources of P were compared: i) rock phosphate (16% P), a slow P-releasing fertilizer, known to promote good mycorrhizal development and healthy plant growth (Sieverding, 1991); and ii) osmocote (8–9 month, 18 N:11 P: 10 K, Sierra UK Ltd), a commercially available controlled-release fertilizer. Each fertilizer type was applied at 4 levels: osmocote at 0, 0.5, 1.0 and 2.0 g/l and rock phosphate at 0, 1.0, 3.0 and 5.0 g/l. Rock phosphate treatments had an additional 846 mg/l NK micronutrient fertilizer (Kemira Oy, Finland, containing: 13% N; 13% N; 9.2% S; 3.4% Ca; 3.0% Mg; 0.15% B; 0.1% Cu; 0.7% Mn; 0.1% Zn) to avoid any possibility of nutrient deficiency. All media were hand-mixed for 10 min to ensure an even distribution of all constituents.

Experimental details

Four mycorrhizal treatments were compared: i) control (no AMF); ii) inoculation with *G geosporum*; iii) inoculation with G98; and iv) inoculation with G128. The control inoculum consisted of a mixture of inoculum of all 3 AMF autoclaved 3 times for 20 min. Washings of fresh AMF inoculum were used for the initial watering of the control treatment to ensure that a similar bacterial population was applied to all treatments.

The substrata were placed in 250-ml square plastic pots, watered and allowed to equilibrate for 24 h before adding 2.5 g mycorrhizal inoculum to each planting hole. Treatments were replicated 6 times and arranged in a randomized complete block design. Plants were removed from their *in vitro* containers, the agar removed with water, and planted. For the first 5 d plants were covered with a double layer of "frost protection" fibre cloth to maintain a high humidity, and for the first 2 wk sprayed when necessary with water. Guard plants were arranged around the perimeter of the experimental layout. Plants were raised in a growth chamber with 70% humidity for the first 2 wk, and 50% for the remaining 3 wk. A 16-photoperiod of 205–273 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetic active radiation was supplied by 58 W warm white super fluorescent tubes. A mean temperature of 22 °C was maintained throughout the experiment.

After 5 wk all plants were placed at 4 °C. Shoot and root fresh and dry weights (60 °C for 24 h) were recorded. Roots were cleared and stained in 0.05% trypan blue in acidified glycerol (modified from Phillips and Hayman, 1970) and AMF colonization determined under the steromicroscope (x 20) using a grid-line intersect method (Giovanetti and Mosse, 1980). Samples in which root colonization was difficult to quantify due to poor staining were mounted on a slide for more detailed observation under the compound microscope (x 400).

Statistical treatment

The experiment was set up in a randomized complete block design and was statistically analyzed as a factorial. Data were subjected to analysis of variance using Genstat 5 (Rothamsted Experimental Station, Harpenden, UK). The statistical significance of differences between means was determined using the least significant difference (LSD) at $P = 0.05$ (Steel and Torrie, 1960).

RESULTS

The percentage root colonization by both G98 and G128 within the 5-wk time period was significantly greater than for *G geosporum* (table I). Approximately half of the root system had been colonized by these 2 isolates at the end of the experiment whereas only 25% of the root system was colonized by *G geosporum*. The type and amount of fertilizer did not affect the relative amount of root colonization (table II).

Microscopic observation of roots revealed differences in the staining of fungal structures depending on the AMF used. In roots of plants inoculated with *G geosporum* typical AMF vesicles and arbuscules could be clearly observed. No intraradical colonization was discernable in roots of plants inoculated with G98 and G128, when examined under the stereomicroscope, even though stained extraradical mycelium, appressoria and spores were clearly visible. More detailed inspection of these roots under a compound microscope, however, revealed that

Table I. Mean percentage root colonization of plants after 5 wk growth colonized by 3 arbuscular mycorrhizal fungi under 2 fertilizer regimes. Data for all osmocote and rock P treatments combined.

Fertilizer	*Arbuscular mycorrhizal fungi*		
	G geosporum	*G98*	*28*
Osmocote	23	46*	52*
Rock P	28	48*	53*

* Significant different from *G geosporum* within fertilizer treatments at $P = 0.05$ (LSD values for osmocote = 8 and for rock phosphate = 7).

Table II. Mean percentage arbuscular mycorrhizal root colonization of plants after 5 wk growth given different levels of fertilizer. Data for all fungal inoculations combined.

No P control	*Osmocote*			*Rock P*		
	0.5 g/l	*1.0 g/l*	*2.0 g/l*	*1.0 g/l*	*3.0 g/l*	*5.0 g/l*
44	36	41	41	40	43	44

unstained vesicles and fungal mycelium containing characteristic lipid droplets were present inside the roots. Larger vesicles of G98 and G128 did, however, stain effectively and could be clearly seen with stereomicroscope. In the results reported here, levels of colonization for G98 and G128 have been determined from a combination of low and high-power microscope examination.

Table III shows the effects of combinations of AMF and fertilizer on the shoot dry weights of plants at harvest. Plants colonized by G98 and G128 and receiving osmocote fertilizer showed significant increases in dry weight compared with non-mycorrhizal plants. Plants colonized by *G geosporum* and supplied with osmocote fertilizer also out-yielded non-mycorrhizal plants but this was not quite significant at the $P = 0.05$ level. Shoot dry weights of plants receiving rock phosphate fertilizer were not increased significantly by colonization with any of the AMF (table III).

Table III. Mean shoot dry weights (mg) of plants after 5 wk growth colonized with 3 arbuscular mycorrhizal fungi under 2 fertilizer regimes. Data for all osmocote and rock P treatments combined.

Fertilizer	*Uninoculated control*	*Arbuscular mycorrhizal fungi*		
		G geosporum	*G98*	*G128*
Osmocote	505	637	741*	700*
Rock P	580	593	681	664

* Significant difference from the control within fertilizer treatments at $P = 0.05$ (LSD values for osmocote = 137 and for rock phosphate = 132).

Table IV shows that addition of osmocote or rock phosphate significantly increased the shoot dry weight of strawberry plants. Increasing the amount of osmocote added, however, from 0.5 g/l–2.0 g/l or rock phosphate from 1.0 g/l to 5.0 g/l, did not result in any significant increases in plant size. In a more detailed analysis of the combined data for all mycorrhizal plants, when compared with their non-mycorrhizal counterparts, further differences were noted. Figure 1 shows the comparative shoot yields of plants receiving different amounts of osmocote fertilizer. For the non-mycorrhizal plants, the shoot dry weight increased proportionally with the amount of osmocote added. For mycorrhizal plants, however, the shoot dry weight of plants had reached a plateau at the lowest rate of osmocote applied, and this maximum yield was sustained across all concentrations of osmocote used. Even at the highest rate of osmocote used (2.0 g/l, which is equivalent to the minimum recommended commercial rate), the mycorrhizal plants outyielded the non-mycorrhizal plants, although this was not significant ($P = 0.05$).

All 3 AMF significantly increased the stolon number of plants receiving osmocote fertilizer but had no significant effect on stolon number when plants received rock phosphate (table V). Addition of both fertilizer types significantly increased stolon number compared with the control (table VI).

DISCUSSION AND CONCLUSION

The aim of this study was to find an optimum level of fertilizer which would allow good coloniza-

Table IV. Mean shoot dry weights (mg) of plants after 5 wk growth given different amounts of fertilizer. Data for all fungal inoculations combined.

No P control	*Osmocote*			*Rock P*		
	0.5 g/l	*1.0 g/l*	*2.0 g/l*	*1.0 g/l*	*3.0 g/l*	*5.0 g/l*
398	690*	678*	817*	712*	706*	702*

* Significant difference from the control within fertilizer treatments at $P = 0.05$ (LSD values for osmocote = 137 and for rock phosphate = 132).

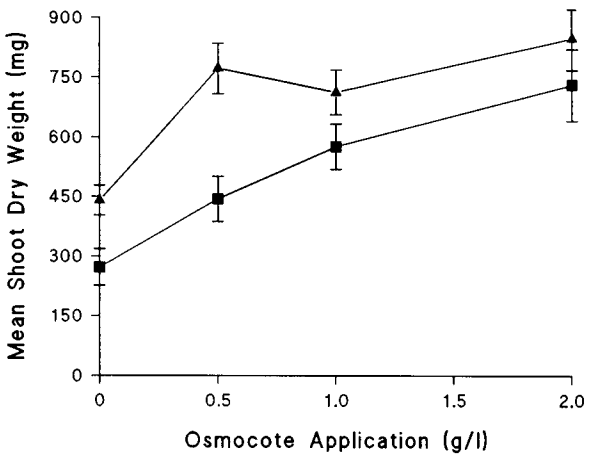

Fig 1. Mean shoot dry weight of mycorrhizal (▲) and non-mycorrhizal (■) strawberry plants, after 5 wk growth, given different amounts of osmocote. Data for all 3 mycorrhizal treatments combined (error bars = standard error of treatment means).

tion by AMF while stimulating healthy plant growth acceptable to the strawberry industry. We have shown that mycorrhizal colonization take place within the root system of strawberry plants receiving commercial rates of phosphorus fertilizers and that this results in improved growth. Furthermore, we have shown that fertilizer inputs can be reduced in mycorrhizal plants to levels considerably lower than those used in commercial practice and yet plant development can be maintained that is equivalent to that of non-mycorrhizal plants receiving full fertilizer inputs.

In these experiments it was noted that all 3 AMF did not colonize to roots to the same extent. G98 and G128 colonized more of the root system than *G geosporum.* Fertilizer application did not significantly decrease levels of colonization but plants colonized by G98 and G128 were significantly larger than those colonized by *G geosporum.* The improved growth of plants obtained following inoculation with G98 and G128 could have been due to either the higher levels of colonization or to differences in AM fungal efficiency in transporting P and other nutrients to the host plant. There was, however, no significant correlation between the percentage root colonization and shoot dry weight (data not shown). This indicates that the increased growth of shoots was probably related to the greater effectiveness of G98 and G128 in supplying the host plant with nutrients under these conditions. Differences in the ability of AMF to extract nutrients from the soil solution and to release them to the plant have been reported in the literature (Hayman and Tavares, 1985; Sieverding, 1991). Chavez and Ferrera-Cerrato (1990) measured the responses of 4 micropropagated strawberry cultivars to root colonization by AMF. They found that plant yields differed significantly depending on the AMF used and that yield was unrelated to percentage root colonization. Some plant cultivar/fungal combinations resulted in improved plant growth while others did not. Our results were similar and suggest that *G geosporum* would be the least suitable of the 3 AMF tested for integration into strawberry production under the conditions used. This is not surprising given the fact that G98 and G128 were chosen on the basis of a preliminary screening programme.

The pH of the growth substratum is an important parameter affecting mycorrhizal efficiency and could also explain the varying abilities of the 3 AMF used in this experiment to stimulate a plant growth response. *G geosporum* was isolated from a Kent soil of pH 7.2, whereas the Finn-

Table V. Mean number of stolons per plant after 5 wk growth colonized by 3 arbuscular mycorrhizal fungi under 2 fertilizer regimes. Data for all osmocote and rock P treatments combined.

Fertilizer	*Uninoculated control*	*Arbuscular mycorrhizal fungi*		
		G geosporum	*G98*	*G128*
Osmocote	0.5	1.0*	1.0*	1.0*
Rock P	0.8	0.8	1.2	1.0

* Significant difference from the control within fertilizer treatments at $P = 0.05$ (LSD values for osmocote = 0.4 and for rock phosphate = 0.5).

Table VI. Mean number of stolons per plant after 5 wk growth given different amounts of fertilizer. Data for all fungal inoculations combined.

No P control	*Osmocote*			*Rock P*		
	0.5 g/l	*1.0 g/l*	*2.0 g/l*	*1.0 g/l*	*3.0 g/l*	*5.0 g/l*
0.2	1.0*	0.9*	1.4*	1.1*	1.2*	1.3*

* Significant difference from the control within fertilizer treatments at $P = 0.05$ (LSD values for osmocote = 0.4 and for rock phosphate = 0.5).

ish AMF were isolated from and maintained in substratum with a pH of ≈ 5.5. A substratum pH of 5.8 is optimum for strawberry plant growth (Lineberry, 1935) and the pH of the plant growth medium used in this experiment was 5.5. It may well have been unsuitable for the effective functioning of a *G geosporum* mycorrhiza. The effect of pH on the effectivity of different AMF has been noted by other workers. For example, Hayman and Tavares (1985) showed that the effectiveness of *Acaulospora laevis* in stimulating growth of alpine strawberry was reduced > pH 6, in contrast to *Glomus fasiculatum*, the effectiveness of which decreased gradually with increasing pH.

Differences in the ability of AMF to colonize root systems, the "colonization capacity" (Tommerup, 1992), may also have played a part in the lower percentage root colonization observed with *G geosporum*. By adjusting the quantity of inoculum added for each AMF, similar colonization levels might have been obtained by the end of the 5-wk experimental period. Differences in percentage root colonization could also have been due to problems experienced in the staining of fungal structures of G98 and G128 inside the roots. Merryweather and Fitter (1991) reported a similar problem in bluebells and suggested that non- or poorly-staining AMF are common in natural soils and that this has led to a gross underestimation of AMF colonization levels in previously published work. In such cases it is necessary to use the compound microscope to assess the presence of AMF in root systems (Dodd and Jeffries, 1986).

The addition of osmocote controlled-release fertilizer and rock phosphate significantly increase plant dry matter production, and this effect was enhanced when AMF were present. Increased amounts of applied fertilizer had no effect on root colonization by G98 and G128. Other workers have also reported the integration of controlled release fertilizers with AMF inoculation. Davies (1987) showed that an equivalent growth of *Rosa multiflora*, inoculated with 2 *Glomus* species in a composted bark–sand substrate, could be obtained using 29% of the recommended rate of osmocote (18 N:6 P:12 K). Our data show that mycorrhizal plants receiving 25% of the minimum recommended commercial rate of osmocote had a similar yield to non-mycorrhizal plants which received the full recommended rate of this fertilizer. Waterer and Coltman (1988) used osmocote (19 N:2.6 P:10 K) to produce mycorrhizal and non-mycorrhizal transplants of pepper and leek in a peat–vermiculite (1:1; v:v) medium. They concluded that it was possible to maintain significant mycorrhizal colonization in transplants given enough controlled-release P to be horticulturally acceptable. Our results confirm this finding as we also maintained high levels of AMF root colonization in plants supplied with the commercial rates of fertilizer commonly used in Finland and in the UK. Our results have also shown that mycorrhizal plants produced more stolons per plant within the experimental period. There could be several explanations for these observations, such as improved nutritional status or alterations in hormonal balance, but we do not have the data to interpret this effect in any more detail.

In conclusion, great potential exists for using AMF by the horticultural industry when used in combination with reduced quantities of an appropriate fertilizer. The field performance of this planting material compared with non-mycorrhizal material remains to be determined.

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