Application of endomycorrhizae to commercial production of Rhododendron microplants

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Application of endomycorrhizae to commercial production of *Rhododendron* microplants

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Summary — *Rhododendron* plants produced *in vitro* are weaned under greenhouse conditions but ≈ 10% of the microplants either die or do not grow during this stage. A series of experiments was carried out to determine whether production could be increased through controlled mycorrhization. Of 7 fungi isolated from roots of plants sampled in a commercial nursery (Derly France, SA), 1 was mycorrhizal and 6 caused significant damage, showing the need to disinfect substrata before outplanting. Of 5 combinations of pH and substrata tested, only 1 was suitable for both plant growth and mycorrhization. Screening of different strains of ericoid mycorrhizal fungi from our laboratory against microplants of 9 cultivars of *R hybrida* indicated a physiological specificity between fungi and plants. These results show that the use of defined disinfected substrata, combined with specific mycorrhizal fungal strains, is essential for guaranteeing an optimal production of outplanted *Rhododendron* microplants at the nursery level.

endomycorrhiza / *Rhododendron* / microplants / nursery substratum

Résumé — Application de l'endomycorhization à la production commerciale de plants de *Rhododendron* micropropagés. *Les plants de* Rhododendron *issus de culture* in vitro *subissent environ 10% de pertes lors de leur acclimatation en serre. Des essais de mycorhization contrôlée ont été effectués pour tenter d'améliorer le système de production. L'examen de racines de plantes prélevées en pépinières a révélé la présence de 7 champignons différents parmi lesquels 1 est endomycorhizogène et 6 sont défavorables à la culture, d'où la nécessité de désinfecter le substrat avant le repiquage des jeunes vitroplants. L'utilisation de 5 combinaisons différentes de substrats et de pH a permis de déterminer des conditions de cultures optimales. Un criblage entre plusieurs isolats de champignons endomycorhiziens et différents cultivars de* Rhododendron *a indiqué l'existence d'une spécificité physiologique entre les 2 partenaires. Ces résultats montrent que l'utilisation d'un substrat sélectionné et désinfecté associée à une endomycorhization controlée serait essentielle à l'optimisation de la production en pépinière de* Rhododendron *issus de vitroplants.*

endomycorhizes* / Rhododendron / *micropropagation* / *pépinière

INTRODUCTION

Ericaceous plants constitute an important part of the ornamental horticulture business. About 50% of the production is based on micropropagated plants, but at weaning ≈ 10% of plants either die or do not attain market standards, causing significant losses at the commercial level. Mycorrhizal infection of *Rhododendron* has been studied (Peterson *et al,* 1980; Duddridge, Read, 1982; Moore-Parkhurst, Englander, 1981, 1982; Douglas *et al,* 1989) and has frequently been shown to enhance mineral nutrition and growth of ericaceous plants such as *Calluna vulgaris* L, *Vaccinium* or *Rhododendron* species (Gianinazzi-Pearson, Gianinazzi, 1981; Beaujard, 1982; Read, Bajwa, 1985; Straub, 1988). A series of experiments was therefore carried out to test the efficiency of controlled mycorrhization for survival of *Rhododendron* clones and therefore for improvement of the production system of this ornamental plant.

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MATERIALS AND METHODS

Rhododendron hybrida microplants, provided by "Pépinières Derly France", were outplanted and weaned under growth chamber conditions (23 °C, 16-h day, 90% relative humidity) for 3 months. The different nursery substrata used for experimentation were sterilized by γ-irradiation (10 KGy). Nutrient solution (Liquo Plant FD2, Plantin) was applied twice a week and plant fresh mass was measured after 8 wk growth. Endomycorrhizal infection was estimated as percent root colonization. Ten replicate plants were used per treatment and data were analysed by ANOVA and Newman–Keuls test.

Fungal isolates

Indigenous fungi were isolated from 4-3-month-old nursery *Rhododendron* plants growing in bf3 substratum (Hydroazote, Lyon). Roots were washed in sterile water and disinfected with antibiotics according to Pearson and Read (1973). Small root pieces were incubated in water agar and the fungi growing out were isolated. Seven isolates were obtained. These and 5 isolates of known ericoid endomycorrhizal fungi from our laboratory (Ipae 9, 13, 15, 25, 42) were grown for 14 d in Norkrans liquid medium with 20 ppm phosphorus, mycelium was filtered, washed, macerated and suspended in water; each plant received 1 ml of this inoculum suspension. Isolates Ipae 25 and 42 were identified as *Hymenocyphus ericae* Korf and Kernan, whilst the others were unidentified ascomycetes.

Substrata tested

Five nursery substrata were tested for growth and mycorrhization (Ipae 25) of 4 clones (124, 206, 209, 282) of *Rhododendron* microplants: bf3 at pH 4.5, bf3 at pH 6.0, Stekmedium (Klassman), Hortipro (Wogesal) and Floratorf (Floragard).

Screening of fungal isolates

Nine clones of *Rhododendron* (110, 117, 119, 136, 200, 201, 209, 212, 232) were outplanted into substratum bf3 and inoculated either with 1 of the 5 different isolates of ericoid endomycorrhizal fungi or with a mixture of the 5 isolates. The 7 fungi isolated from nursery plants were tested individually by inoculating one *Rhododendron* clone (126). Controls were uninoculated plants.

RESULTS AND DISCUSSION

Indigenous fungi

In spite of intensive fungicide treatments (2 or 3 fungicides* and insecticides** were applied twice weekly at the nursery stage) seven fungi were isolated (A–G) from *Rhododendron* roots (table I). Among these 7 isolates, only one (A) was mycorrhizal and presented a typical infection pattern (intracellular hyphal coils in 90% of the roots). Plants inoculated with this isolate showed good growth, similar to that obtained with isolate Ipae 25, both isolates giving more homogenous populations. The other 6 isolates (B–G) caused significant damage to the microplants, which in some cases died, and only a few plants showed normal growth. These results indicate that fungicide treatments were not sufficient to eliminate all the fungi from the substrata and that it is advisable to disinfect the substrata before outplanting *Rhododendron* microplants.

Table I. Effects of fungi isolated from roots of *Rhododendron* microplants on growth of clone 126.

Fungal strains	*Fresh mass (mg)*	*% coefficient of variation*
A	2 161[a*]	11.38
B	507[b]	37.31
C	377[b]	57.55
D	14[c]	16.76
E	625[b]	15.49
F	94[b]	36.36
G	539[b]	37.00
Ipae 25	1 817[a]	8.53

*Values for each clone followed by different letters are significantly different at 95% confidence levels.

* Procymidone: Sumisclex; vinchlozoline: Ronilan; zirame: Pomarsol; iprodione: Rovral; prothiocarb: Previcur; benomyl: Benlate.

** Deltamethrine: Decis; dichlorvos: Dedevap; methomyl: Lannate.

Nursery substrata

There was a significant effect of the different substrata on growth of the 4 tested clones of *Rhododendron* microplants inoculated with strain Ipae 25 (table II). Substratum bf3 used at pH 4.5 gave best results for weaning of all *Rhododendron* plantlets, and significant mycorrhizal growth responses were observed for clones 124 and 206. For each of these clones, the coefficient of variation indicated that the mycorrhizal plant population was more homogenous than the non infected one. Stekmedium had good early effects but plant growth slowed down by 8 wk. In general, mycorrhization caused an increase in plant growth of clones on Stekmedium, whilst clones were generally less responsive on Floratorf and Hortipro. There was an effect of pH for substratum bf3, plant growth being generally lower at pH 6.0 than pH 4.5. Infection was high (50–80%) in bf3 and Floratorf, and low (10%) in the other substrata. The former was therefore used for screening of endomycorrhizal fungal isolates.

Table II. Effect of five nursery substrata on growth of 4 clones of mycorrhizal (+M) (Ipae 25) and non-mycorrhizal (NM) *Rhododendron* microplants.

Rhododendron clones	*Substrata*	*Fresh weight (mg)*		*% coefficient of variation*	
		NM	*+M*	*NM*	*+M*
124	bf3 pH 4.5	28^{b*}	73^{a}	54.28	27.04
	Hortipro	8^{b}	9^{b}	11.02	27.32
	Floratorf	7^{b}	10^{b}	6.95	7.71
	Steklmedium	8^{b}	26^{b}	5.67	29.61
	bf3 pH 6	9^{b}	24^{b}	3.30	21.17
206	bf3 pH 4.5	45^{b}	123^{a}	37.28	20.05
	Hortipro	12^{c}	17^{c}	18.34	13.80
	Floratorf	19^{c}	27^{c}	28.16	21.88
	Stekmedium	19^{c}	91^{a}	18.68	9.18
	bf3 pH 6	10^{c}	22^{c}	3.44	31.14
209	bf3 pH 4.5	106^{a}	118^{a}	29.47	17.45
	Hortipro	36^{b}	39^{b}	28.92	9.53
	Floratorf	21^{b}	75^{ab}	17.88	12.86
	Stekmedim	30^{b}	64^{ab}	23.11	13.78
	bf3 pH 6	42^{b}	82^{ab}	36.20	16.66
282	bf3 pH 4.5	33^{ab}	48^{a}	38.92	25.70
	Hortipro	11^{b}	15^{b}	14.62	9.93
	Floratorf	10^{b}	21^{ab}	22.03	23.56
	Stekmedium	14^{b}	34^{ab}	9.23	11.49
	bf3 pH 6	20^{ab}	31^{ab}	34.25	18.15

* Values for each clone followed by different letters are significantly different at 95% confidence levels.

Screening of fungal isolates

Some physiological specificity was observed between the different *Rhododendron* cultivars and the fungal isolates (table III). Significant positive effects on plant growth, as compared to non-inoculated controls, were only observed for isolates Ipae 9 with clone 212 and Ipae 13 with clone 232, although significant differences between isolates regarding effect on plant growth were also observed for clones 119 and 200. Isolate Ipae 25 tended to improve growth and decrease heterogeneity (lower coefficients of variation) of the *Rhododendron* clones 119 and 200, whilst positive effects of isolates Ipae 9 and 15 were observed with clones 117 and 209; in the latter, growth stimulation was also observed with isolate Ipae 42. With clone 201, the control became infected with mycorrhizal fungi, so there was no response. The mycorrhization by certain isolates was inclined to decrease heterogenity with smaller variations between plants (lower coefficient of variation) being related to positive growth effects for *Rhododendron* clones 117, 119, 136, 200 and 232. Improved homogeneity of the plantlet populations should constitute an important parameter for choice of the fungal strain. The mixture of isolates did not give better results than an isolate alone, indicating that it was sufficient to choose one specific fungal strain and that interactions between strains were not synergistic. Plant growth increase and root infection were not necessarily correlated. Some fungal isolates had negative effects on growth and homogeneity of certain *Rhododendron* clones (*eg* Ipae 25/clone 201), underlining the necessity to screen for beneficial combinations.

CONCLUSIONS

The results reported here clearly show that defined culture procedures should be adopted to ensure successful production of microplants of *Rhododendron*. It is essential to carefully choose substrata with an appropriate pH (4.5) and this should be disinfected before outplanting of microplants, in order to eliminate

Table III. Growth of 5 clones of *Rhododendron* microplants outplanted into disinfected bf3 substratum and inoculated with different endomycorrhizal fungi.

Rhododendron clones	*Fungal strains*	*Fresh mass (mg)*	*% Coefficient of variation*
110	Ipa9	136[a*]	11.70
	Ipa 13	197[a]	12.64
	Ipa 15	134[a]	6.64
	Ipa 25	122[a]	12.33
	Ipa 42	160[a]	12.65
	Mixture	173[a]	9.80
	Control	174[a]	13.53
117	Ipa 9	178[a]	6.32
	Ipa 13	141[a]	20.23
	Ipa 15	175[a]	9.48
	Ipa 25	116[a]	23.08
	Ipa 42	109[a]	16.76
	Mixture	132[a]	11.06
	Control	109[a]	13.60
119	Ipa 9	67[ab]	23.40
	Ipa 13	48[b]	22.50
	Ipa 15	40[b]	28.60
	Ipa 25	115[a]	16.09
	Ipa 42	83[ab]	22.01
	Mixture	48[b]	22.60
	Control	65[ab]	16.38
136	Ipa 9	326[a]	41.41
	Ipa 13	303[a]	13.43
	Ipa 15	345[a]	17.31
	Ipa 25	410[a]	16.19
	Ipa 42	345[a]	16.55
	Mixture	257[a]	11.20
	Control	283[a]	16.61
200	Ipa 9	54[ab]	13.54
	Ipa 13	45[ab]	49.18
	Ipa 15	47[ab]	22.38
	Ipa 25	82[a]	16.47
	Ipa 42	41[b]	15.97
	Mixture	46[ab]	16.90
	Control	50[ab]	17.22
201	Ipa 9	97[a]	17.99
	Ipa 13	60[ab]	12.99
	Ipa 15	49[b]	19.37
	Ipa 25	106[a]	17.74
	Ipa 42	124[a]	22.12
	Mixture	99[a]	10.62
	Control	124[a]	22.26
209	Ipa 9	1 181[a]	12.64
	Ipa 13	803[a]	15.74
	Ipa 15	1 230[a]	10.00
	Ipa 25	886[a]	19.83
	Ipa 42	1 177[a]	7.50
	Mixture	1 190[a]	15.96
	Control	860[a]	15.50
212	Ipa 9	441[a]	19.07
	Ipa 13	340[ab]	24.39
	Ipa 15	295[abc]	14.78
	Ipa 25	294[abc]	13.29
	Ipa 42	283[abc]	7.03
	Mixture	202[bc]	14.38
	Control	134[bc]	18.55
232	Ipa 9	210[bc]	14.34
	Ipa 13	450[a]	12.99
	Ipa 15	353[ab]	10.69
	Ipa 25	169[c]	13.01
	Ipa 42	217[bc]	16.15
	Mixture	281[bc]	13.73
	Control	305[bc]	19.70

* Values for each clone followed by different letters are significantly different at 95% confidence levels.

harmful fungi. It would also be advantageous to inoculate microplants with an appropriate endomycorrhizal ericoid fungus adapted to both the substratum and plant clone, in order to ensure optimal growth and homogenous production of microplants.

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