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CONTROLLED RHIZOGENESIS AND MYCORRHIZATION OF HAZELNUT (*Corylus avellana* L.) CUTTINGS WITH BLACK TRUFFLE (*Tuber melanosporum* Vitt.)

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ABSTRACT

Hazelnut (*Corylus avellana* L.) is commonly used as mycorrhizal host for *Tuber melanosporum* Vitt. (black truffle). Black truffle orchards have been established in and outside its natural distribution area, currently also in Chile. Hazelnut can be propagated rapidly by cuttings, which, however, may not easily form mycorrhizal symbiosis under laboratory conditions. We successfully inoculated hazelnut cuttings with *T. melanosporum* and studied the effects of culture conditions, plant material and inoculum age on rhizogenesis and mycorrhization. Cuttings from sucker shoots showed the highest rooting rate (88.5%). Perlite and vermiculite as substrate increased root length (9.6 cm). Treatment with auxin significantly increased root yield (up to 27 roots per cutting). Larger containers increased the mycorrhization rate (33.6%). Up to 2 year storage of sporal inoculum did not affect mycorrhization. We conclude that root induction in *C. avellana* cuttings and mycorrhization with black truffle using up to 2-yr-old inoculum, do not present problems when performed under optimized conditions, allowing fast production of plants for commercial and reforestation purposes.

Key words: *Tuber melanosporum*, *Corylus avellana*, cuttings, rhizogenesis, mycorrhizae, culture conditions.

INTRODUCTION

The edible fruiting bodies *of Tuber melanosporum* Vitt. (Pezizales, Ascomycota), an ectomycorrhizal fungus commonly known as black truffle or Perigord truffle, are traded at high prices on the international market, having reached up to US$1000 per kilogram fresh weight in the recent past (Kaffsack, 2006). Black truffles are naturally distributed in the Mediterranean zones of Spain, France and Italy, however, a decline of truffle production in natural areas has been observed in these countries during the past decades (Barchfield, 2008). Consequently, techniques have been developed and applied successfully to cultivate the fungus in host tree plantations (Reyna, 2000; Smith and Read, 2008). Lately, truffle culture has also been introduced in the United States, New Zealand and Australia, where fruiting body production has recently shown its first results (Hall *et al*., 2001; Pruett *et al*., 2008). Around 2003, culture techniques for *T. melanosporum* have been introduced in Chile (Ramírez *et al*., 2003; 2004; Santelices *et al*., 2004; Pérez *et al*., 2007). Truffle production in the Southern Hemisphere is economically attractive, given that it can contribute to the international market when traditional supplies from the Northern Hemisphere suffer a seasonal shortfall (Smith and Read, 2008).

Among common host plants for *T. melanosporum* cultivation, European hazelnut (*Corylus avellana* L.) is especially suitable, due to its vigorous growth, its tendency to form a well developed root system and its well-known capacity to form ectomycorrhizae (Lefevre and Hall, 2001; Smith and Read, 2008). Previous studies carried out in Chile have demonstrated the feasibility of producing mycorrhizal plants of *C. avellana* inoculated with black truffle, matching the highest quality standards demanded on the European markets (Ramírez *et al*., 2004). The cited authors observed mycorrhization levels of about 50% when plants were inoculated with spores dispersed in talcum powder. Spores obtained from dried and ground fruiting bodies are an efficient inoculum, easy to handle, however, the effect of spore storage time upon inoculum viability of black truffle is still largely unknown.

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Seeds of *C. avellana* germinate well after few months of cold stratification (Bärtels, 1989; Hartmann and Kester, 1998), however, germination rates become irregular after longer storage (Williams *et al*., 1973), and seed viability is substantially reduced after 1 year or more (Mehlenbacher, 1991). Another limitation is the increasing, world-wide demand of hazelnuts for food production which may affect their availability as propagules, especially in recently emerging nut-producing countries like Chile (Merlo, 2006). In this context, asexual multiplication is an attractive alternative for the production of mycorrhizal plants. Inducing rooting in cuttings is a technique commonly used, due to its low cost and easy implementation (Dirr and Heuser, 1987; Bärtels 1989; Hartmann and Kester, 1998).

Previous publications about rhizogenesis of *Corylus* cuttings report principally on the use of indole-3-butyric acid (IBA) for stimulation of adventitious root growth and on the importance of the season and the type of plant material. Valenzuela (1988), Kantarci and Ayfer (1994), Ercisli and Read (2001) and Erdogan and Smith (2005) obtained high rates of root production in soft-wood cuttings of *C. avellana*. However, there may be other factors affecting root formation which should be studied more profoundly.

At present, culture methods of black truffle are based on the generation of mycorrhizal plants under controlled greenhouse conditions with techniques that are frequently kept as business secrets (Reyna, 2000). Thus, generating and disseminating more detailed information about successful inoculation of *C. avellana* with *T. melanosporum* and optimized production of mycorrhizal roots is a pending task.

The objectives of this study were to evaluate the effect of planting substrate, indole-3-butyric acid (IBA) application and origin of plant material in rhizogenesis of *C. avellana* cuttings, harvested in spring, as well as to analyze the effect of inoculum storage time and container volume on mycorrhization of hazelnut plants inoculated with black truffle.

MATERIAL AND METHODS

The experimental setup included two independent assays: a) the aim of the first experiment was to induce rhizogenesis in *C. avellana* cuttings and, b) plants taken from the treatments were subsequently tested for mycorrhiza formation with *Tuber melanosporum*.

Plant material

For the rhizogenesis experiment, cuttings of *C. avellana* var. Barcelona were used. This plant material was obtained from a commercial plantation located at El Toqui in the Central Valley of Chile, County of Pelarco, Maule Region (35°24' S, 71°30' W, 137 m.a.s.l.). In early January 2004, homogeneous cuttings from post-pruning sprouts and sucker shoots developing on 9-yr-old trees were selected.

For mycorrhization, uniformly developed plants were selected among those which had performed best in terms of root quantity and length in the previous rooting experiments (Figure 1); they were subsequently grown for another 4 mo in the greenhouse to increase their root systems before inoculation with *T. melanosporum*.

Experimental setup

The rooting treatments were performed in a polycarbonate-covered greenhouse at the Universidad Católica del Maule, Talca (35°26' S, 71°37' W, 126 m.a.s.l.), Chile. Temperature-regulated rooting beds were adjusted to maintain the substrate temperature between 21 and 25 °C. Irrigation was performed by an automatic misting system (Mistamatic®, E.C. Geiger Co., Harleysville, Pennsylvania, USA), maintaining relative ambient humidity constantly above 75%. Frequency and duration of misting varied according to the weather conditions.

The hazelnut cuttings were cut from the parent tree before 09:30 h, stored in a cooler and taken to the greenhouse within approx. 30 min, and washed with tap water to remove dust. Subsequently, they were cut

Figure 1. Four-months-old cutting of *Corylus avellana* obtained from a sucker shoot with abundant root growth. Bar = 5 cm.

down in sections of 15 cm standard length. Cuttings of homogeneous diameter and with at least two visible leaf buds were selected. The basal ends were cut down at right angles and then submerged 2.5 to 3 cm deep for about 4 s in an IBA solution. Concentrations of IBA, diluted in 65% ethanol, were as follows (treatments): 0 mg L^{-1} (control, 65% ethanol only), 1000, 2000, and 3000 mg L^{-1}. Immediately after application, cuttings were inserted in the rooting bed substrate to a depth of 5 cm approximately.

Rooting chambers were assembled from metal and wooden elements, where the bottom was thermically insulated by a layer of polystyrene panels, covered with polyethylene foil, both perforated to provide drainage. Substrate temperature was maintained constant by an electric resistance controlled by a thermostat adjusted to 21 °C. To ensure that temperature remained below 30 °C, ambient and substrate temperature were monitored periodically with a thermometer.

Mycorrhization studies were performed in the same greenhouse after spraying it with a solution of sodium hypochlorite (10%) for disinfection.

The mycorrhizal inoculum was imported from the Center for Environmental Studies of the Mediterranean (Centro de Estudios Ambientales del Mediterráneo), Valencia, Spain, and stored at the Universidad Católica del Maule, Talca, for up to 2 years in a conventional refrigerator at 4 ºC. It consisted of dried and pulverized fruiting bodies of *T. melanosporum*, harvested during the 2003-2004 season and homogeneously mixed with talcum powder (hydrated magnesium silicate) at a 1:4 ratio (v/v).

The substrate consisted in an unsterilized mixture of peat (Sunshine Nº 6, pH 6), vermiculite (pH 8) and perlite (pH 6.4) at a ratio of 5:4:1 (v/v/v). With the purpose to obtain a substrate pH of 7.5 to 8.0, 20 g L^{-1} fine-grained calcium carbonate ($CaCO_3$) was added. Subsequently, fertilizer (18-6-12; Osmocote, Scotts, Geldermalsen, The Netherlands) was added at a proportion of 3 g L^{-1}.

Inoculation of cuttings was performed in April 2004 in the desinfected greenhouse: plants were carefully extracted from the substrate and their roots submerged in tapwater-filled trays to remove substrate particles and keep the fine roots moist before inoculation. To reduce stress to a minimum, total time of extraction, inoculation and transplantation did not exceed 15 min. For dry inoculation, the roots of 24 plants at a time were placed on a layer of 11 L of homogeneously spread substrate over a desinfected table. With the help of a medium-sized salt shaker, filled with 12 g of inoculum, approx. 1 g was evenly dispersed on each root system. The substrate with the scattered surplus inoculum was later incorporated in the planting containers. Further cultivation was performed in multi-plant plastic trays (Quickpot™ 12 T/18) consisting of 12 plant containers of 650 mL volume each, or in individual, foldable plastic containers (Fullpot™) of 450 mL volume. Both container types have vertical ridges on the inside to avoid root spiralling and holes in the bottom which allow root self-pruning and drainage.

For monitoring of mycorrhization after 8 months, the non-destructive method proposed by Reyna (2000) was applied: a horizontal root core was extracted with a cork borer (1.5 cm diameter) from the middle of the root bale. At the laboratory, each core was rinsed and decanted several times in water to remove substrate particles from roots. Cleaned roots were then poured on a 1 mm sieve and retrieved to a Petri dish with distilled water. Sticky substrate remnants were removed with an ultrasonic bath (Transsonic T310, Elma, Singen, Germany). All mycorrhizal fine roots of the sample were accounted for under a dissecting stereomicroscope (SMZ 143 Zoom 1/4x, Motic, Xiamen, China). The root count from the core was then extrapolated for the whole plant according to the container volume.

After pre-classification of the mycorrhizal roots under the dissecting microscope, based on their color and morphology, the identity of *T. melanosporum* as mycobiont was confirmed under a compound microscope (B3 220, Motic) by diagnostic details like the cellular pattern of the fungal mantle, presence, ramification and size of cystidia and cell wall ornamentation, following keys and reference descriptions by Zambonelli *et al*. (1993), Sáez and De Miguel (1995), De Miguel and Sáez (1997) and Etayo and De Miguel (1998).

Experimental design and data analysis

For the rhizogenesis experiment, a factorial design of sites subdivided in randomized complete blocks with three replications and 12 cuttings per experimental unit was used. The three analyzed factors and their respective levels were as follows:

Factor 1. Substrate (A): a_1, peat-perlite (4:1 v/v); and a_2, perlite-vermiculite (7:1 v/v).

Factor 2. IBA concentration (B): b_1, 0 mg L^{-1}; b_2, 1000 mg L^{-1}; b_3, 2000 mg L^{-1}; and b_4, 3000 mg L^{-1}.

Factor 3. Origin of cuttings (C): c_1, post-pruning resprouts; and c_2, sucker shoots.

After 2 months in the greenhouse, the following parameters of the cuttings were evaluated: survival rate, callus formation rate, rooting rate and root yield (root number and length). A cutting was classified as rooted when it produced at least one root of 1 cm length and as dead when there were visible symptoms of necrosis.

The mycorrhization experiment was designed as a factorial experiment in randomized complete blocks with four replications and 20 plants per experimental unit; studied factors were container volume and inoculum storage time, which levels are listed below:

Factor 1: Container volume (A): a_1: 650 mL; and a_2: 450 mL .

Factor 2: Inoculum storage time (B): b_1: 1 year; and b_2: 2 years.

Plants mycorrhization rate was evaluated 8 months after inoculation (December 2004). Three fine root categories were established, viz. non-mycorrhizal tips, mycorrhizal tips colonized by *T. melanosporum,* and mycorrhizal tips spontaneously colonized by other fungi.

In both experiments, each block was considered as a replication and was identified by its position in the greenhouse. Analysis of variance (ANOVA) and comparison of means were performed using the General Linear Model (GLM) of the software SPSS v. 15. Percentage values were normalized previous to performance of ANOVA using the formula $y' = \text{arc sen } \sqrt{p}$, where p is the percentage (Ostle, 1992). When significant differences were detected, means were separated using the Tukey multiple range test (5%).

RESULTS

Rhizogenesis of *Corylus avellana* cuttings

In all treatments, induction of adventitious roots in cuttings of *C. avellana* was successful (Figure 1). As no positive interaction could be observed between the studied factors, they were analyzed separately.

During the process of rhizogenesis, the applied substrate had a significant effect on mean root length per cutting (Table 1), which was about 20% longer in plants grown in perlite-vermiculite than in the peat-perlite mixture. The other parameters were not statistically different between both groups.

When looking at the effect of the cutting origin, significant differences could be observed in survival rate, rooting rate and number of produced roots (Table 2) with all three parameters being significantly higher in cuttings obtained from sucker shoots in comparison with those obtained from resprouts (15%, 86% and 52%, respectively).

Although it was possible to obtain rooting without auxin application, IBA concentration had a significantly positive effect on some of the studied variables (Table 3): auxin-treated cuttings showed an almost four-fold higher yield in root numbers compared to control plants, without major differences between the three concentrations. Mean root length was significantly higher in cuttings treated with 1000 mg L^{-1} IBA and the same group also showed the highest rooting values although not at a statistically significant level.

In all treatments, the rate of callus formation was higher than 99%, without observing statistically relevant differences or correlation with other variables.

Mycorrhization of *Corylus avellana* cuttings

The ectomycorrhizal structures formed by *Tuber melanosporum* and *C. avellana* (Figure 2) were clearly identified by the characteristic color, mantle pattern and cystidia. No presence of other mycorrhiza-forming fungi was observed.

Table 1. Effect of substrate on the rooting capacity of *Corylus avellana* cuttings, after 2 months under greenhouse condition.

Substrate	Survival rate	Callus formation rate	Rooting rate	Root yield	
				Number	Length
	%			Nº	cm
Peat-perlite (4:1 v/v)	91.6a	99.6a	69.1a	21.7a	8.0b
Perlite-vermiculite (7:1 v/v)	85.8a	100.0a	67.0a	24.1a	9.6a

Note: all values are means; values with different letters are significantly different according to Tukey multiple range test ($p < 0.05$).

Table 2. Effect of cutting type on the rooting capacity of *Corylus avellana* after 2 months under greenhouse conditions.

Cutting type	Survival rate	Callus formation rate	Rooting rate	Root yield	
				Number	Length
	%			Nº	cm
Sucker shoot	94.8a	100.0a	88.5a	27.6a	8.6a
Resprout	82.6b	99.6a	47.6b	18.2b	8.9a

Note: all values are means; values with different letters are significantly different according to Tukey multiple range test ($p < 0.05$).

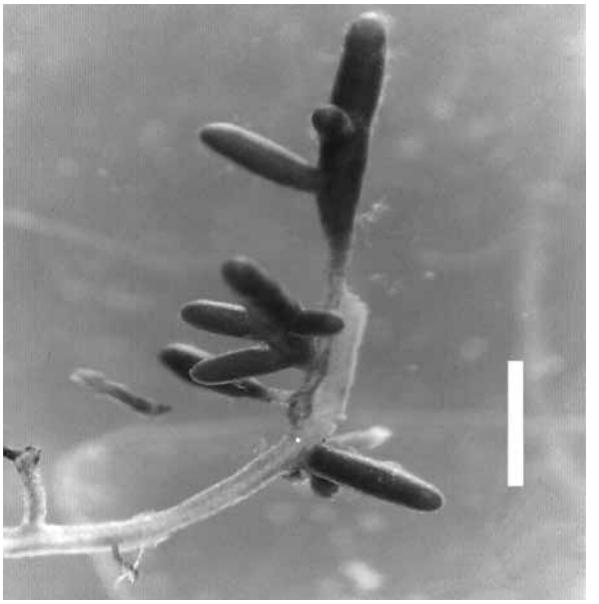

Figure 2. Mycorrhizal fine root system formed by *Tuber melanosporum* and *Corylus avellana*. Bar = 1 mm.

No positive interaction between the studied factors was observed, consequently, they were analyzed separately.

Container volume had a significant effect on the number of total root tips and especially on the mycorrhization rate (Table 4). The percentage of root tips colonized by *T. melanosporum* in 650 mL containers, more than doubled the corresponding value of plants grown in 450 mL containers.

No significant differences in the mycorrhization level were observed as an effect of inoculum storage time (Table 5). Mean values were approximately 25% of mycorrhization with *T. melanosporum* in both treatments.

DISCUSSION

Rhizogenesis in cuttings

The general success in adventitious root induction in *C. avellana* cuttings in all treatments should encourage the use of this technique which has been previously reported to be problematic in this species (Gautam and

Table 3. Effect of indolbutyric acid (IBA) on the rooting capacity of *Corylus avellana* cuttings, after 2 months under greenhouse conditions.

IBA concentration	Survival rate	Callus formation rate	Rooting rate	Root yield	
				Number	Length
mg L^{-1}	——— % ———			Nº	cm
0	100.0a	100.0a	64.6a	7.4b	8.0b
1000	89.6ab	99.3a	77.2a	27.3a	10.2a
2000	80.5b	100.0a	60.4a	29.2a	9.1ab
3000	84.7ab	100.0a	68.1a	27.7a	7.9b

Note: all values are means; values with different letters are significantly different according to Tukey multiple range test ($p < 0.05$).

Table 4. Effect of container volume on mycorrhization of *Corylus avellana* cuttings with *Tuber melanosporum* after 8 months under greenhouse conditions.

Container volume	Mycorrhizal root tips per plant		Total root tips per plant	
mL	Nº	%	Nº	%
650	12 649a	33.6a	25 710a	66.4a
450	2 689b	16.1b	14 977b	83.9b

Note: all values are means; values with different letters are significantly different according to Tukey multiple range test ($p < 0.05$).

Table 5. Effect of *Tuber melanosporum* storage time on mycorrhization of *Corylus avellana* cuttings after 8 months under greenhouse condition.

Inoculum storage time	Mycorrhizal root tips per plant		Total root tips per plant	
	Nº	%	Nº	%
2003 (2 yr)	7 293a	24.7a	17 324b	75.3b
2004 (1 yr)	8 046a	25.0a	23 363a	75.0a

Note: all values are means; values with different letters are significantly different according to Tukey multiple range test ($p < 0.05$).

Howard, 1994; Hartmann and Kester, 1998). Our results demonstrate the feasibility of this quick propagation method which, in general terms, corresponds with previous findings of Valenzuela (1988), Kantarci and Ayfer (1994) and Erdogan and Smith (2005).

The importance of the composition of the rooting substrate for cutting propagation of broad-leaved species has been widely acknowledged (Tchoundjeu and Leakey, 2001; Tchoundjeu *et al*., 2002; 2004). Different substrates may have a potential effect on the root system quality (Hartmann and Kester, 1998) by way of differences in water holding capacity (Mesén *et al*., 1997), among other factors. However, the results presented in this study show that the capacity of *C. avellana* to generate adventitious roots was similar in all used substrates. Statistically relevant differences were only found in root length, with higher values in cuttings planted in a mixture of perlite and vermiculite (7:1 v/v). This may probably be due to a larger macropore size or higher pH compared to the peat-perlite mixture. Larger pore size of the substrate has been shown to increase root length in pine seedlings (Lehto, 1986) and it is also known that a neutral pH favors rhizogenesis (Hartmann and Kester, 1998). Although the used mixture of peat and perlite (4:1 v/v) had a weakly acidic pH (around six) compared to the perlite-vermiculite substrate (between 7 and 8), the high water holding capacity of the peat component (Hartmann and Kester, 1998) probably provided a compensating beneficial effect, which could explain the slightly higher rooting rate in this treatment.

Root formation was, on average, higher than 68%, which is matching recommendations given by Easley and Lambeth (1989) for an acceptable operational level at commercial scale. The high quantity of roots produced, on average more than 22 per cutting, allows positive predictions of a satisfactory establishment of these plants in the field. Survival rates in this experiment ranged between 85 and 92%, matching well the proportion of surviving cuttings of more than 90% obtained by Erdogan and Smith (2005).

The use of softwood cuttings in the propagation of *C. avellana* has been emphasized by various authors (Kantarci and Ayfer, 1994; Ercisli and Read, 2001), who obtained a higher root-forming rate compared to hardwood cuttings. Our results show that there are clear advantages of using sucker shoots instead of post-pruning sprouts. The observed rooting percentage, which was higher than 88% comes close to the results obtained by Erdogan and Smith (2005), who also worked with sucker shoots of the var. Barcelona, obtaining over 90% of rooting.

The use of auxins to stimulate rhizogenesis in cuttings is well established (Dirr and Heuser, 1987; Bärtels, 1989; Hartmann and Kester, 1998). Some authors have shown an increase in the rooting capacity of *C. avellana* after exogenous application of auxins (Valenzuela, 1988; Mehlenbacher, 1991; Kantarci and Ayfer, 1994; Ercisli and Read, 2001; Cameron *et al*., 2003; Erdogan and Smith, 2005). Although after application of IBA in our assay, the survival rate diminished in comparison to the control plants, root production per plant, expressed as total root number, increased almost four-fold.

It is a common observation that with increasing auxin concentration, root induction also increases to a maximum and subsequently declines with higher concentrations (Wasser and Ravetta, 2000). This follows a typical dose-response curve, where an optimal concentration can be observed displaying an optimum curve (Barceló-Coll *et al*., 2001). In our assay, this curve was not observed. Comparison of our results with previously published data shows varying effects of IBA application: Erdogan and Smith (2005) obtained in some cases 100% of rooting with 750 mg IBA L^{-1}, using cuttings obtained in summer (June), whereas Kantarci and Ayfer (1994) only got 20% root formation after applying 1000 mg L^{-1} IBA in softwood cuttings, harvested in the same season. These differences may be due to the cultivar used, given that Erdogan and Smith (2005) propagated the cv. Barcelona, whereas Kantarci and Ayfer (1994) used different Turkish varieties. In this context, Ercisli and Read (2001) found that *C. americana* x *C. avellana* softwood cuttings performed best when harvested in June and subsequently treated with 1500 mg IBA L^{-1}, which comes close to our results, although the cited authors observed genotypic differences. In an assay performed in Chile, Valenzuela (1988) also obtained a significantly higher rooting percentage (91% after 2 months) with 1000 mg IBA L^{-1}, compared to control plants without auxin treatment, using cuttings harvested in December (early summer in the Southern Hemisphere); however, the average root number was about one-third lower than in our assay.

Mycorrhization of cuttings

In all assays realized in this study, mycorrhization of *C. avellana* with *Tuber melanosporum* was achieved. A clearly positive effect of container size was observed, with a significantly higher mycorrhization percentage in larger containers, possibly because the inoculated plants were able to develop a more extensive root system. The mycorrhizal percentage above 33% obtained in 650 mL containers can be considered excellent according to Reyna (2000) and matches quality standards of European producers. However, comparing the result to similar experiments realized in Chile with plants propagated from seeds (Ramírez *et al*., 2004), the mycorrhizal percentage of our cuttings was about one-third less, possibly because

differentiation of ectomycorrhizal structures formed by *T. melanopsorum* could not keep pace with development of fine root tips. On the other hand, absolute numbers of colonized root tips in our assays more than doubled those of the study cited above; this could be explained by the presence of more and longer fine roots in the cuttings in the moment of inoculation, compared to sexually propagated plants which are usually used for mycorrhization.

An important factor for successful mycorrhization is spore viability which, in turn, is dependent on inoculum storage time (Torres and Honrubia, 1994; Chen *et al*., 2006). In this study, no statistical differences in mycorrhization could be observed as an effect of 1 or 2 years storage time of the inoculum. Most spores seem to remain viable longer than 2 years when stored at 4 ºC, an observation which is important for the pre-productive phase of truffle culture in "out-of-area" countries like Chile, allowing a more efficient management of imported inoculum and production of mycorrhizal plants during several seasons. Chen *et al*. (2006) observed sustained viability of spore inoculum of *Scleroderma* spp. after 5 years storage at 4 ºC, obtaining similar rates of mycorrhization with two *Eucalyptus* species as with fresh spores, whereas spores stored at ambient temperature showed a clear loss of mycorrhization capacity. The same authors point out that the success of mycorrhization may depend on the quantity of spores applied to each plant, although Brundrett *et al*. (2005) report a poor correlation between spore density, of diverse ectomycorrhizal fungi, and mycorrhization success in *Eucalyptus*; this issue should be further studied in *T. melanosporum*.

Other factors not considered in this study (e.g. inoculum provenance and the type of lime used to adjust the substrate pH), can also affect the mycorrhization level as has been shown for the Burgundy truffle (*Tuber aestivum*) by Pruett *et al*. (2008).

Alternative methods of mycorrhization, especially inoculation with mycelium in axenic culture, have been successfully applied on *T. melanosporum* and *C. avellana* (Dubé, 2003). However, this method involves higher production costs but may be justified in countries where high pest and disease control standards do not allow the import of potentially "contaminated" spore inoculum.

CONCLUSIONS

Root induction in cuttings of *Corylus avellana* and mycorrhization with black truffle, according to our results, do not present major problems when performed under optimized conditions which imply, among others, thorough selection of vegetative propagules, auxin treatment of cuttings and an adequate container size. In this way, a fast production of *Corylus* mycorrhizal plants to be used in truffle orchards or other plantation types can be achieved.

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RESUMEN

Rizogénesis y micorrización controlada de estacas de avellano europeo (*Corylus avellana* L.) con trufa negra (*Tuber melanosporum* Vitt.) El avellano europeo (*Corylus avellana* L.) es uno de los simbiontes más comunes utilizados para inocular plantas con *Tuber melanosporum* Vitt. (trufa negra). Los huertos truferos han sido establecidos tanto dentro como fuera del área de distribución natural de este hongo micorrizógeno, actualmente también en Chile. El avellano europeo puede propagarse rápidamente por estacas. Sin embargo, no es fácil el desarrollo de órganos micorrícicos bajo condiciones de laboratorio. En este estudio se inocularon exitosamente estacas de avellano europeo con *T. melanosporum* y se estudió el efecto de algunos tratamientos culturales, del material vegetal y tiempo de almacenamiento del inóculo, en el proceso de rizogénesis y micorrización. Las estacas provenientes de chupones tuvieron una mayor capacidad de arraigamiento (88,5%). El uso de perlita y vermiculita como sustrato incrementó la longitud de las raíces (9,6 cm). Los tratamientos con auxinas aumentaron significativamente la producción de raíces (más de 27 raíces por estaca). Por otra parte, el uso de contenedores más grandes permitió incrementar el porcentaje de micorrización (33,6%) y fue posible almacenar hasta 2 años el inóculo esporal sin que se afecte la micorrización. Se concluye que es posible la inducción de raíces en estacas de *C. avellana* y su micorrización con trufa negra utilizando inóculo de hasta 2 años de almacenamiento, bajo condiciones controladas, permitiendo una rápida producción de plantas para fines comerciales y de reforestación.

Palabras clave: *Tuber melanosporum*, *Corylus avellana*, estacas, rizogénesis, micorrización, tratamientos culturales.

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