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ORIGINAL PAPER

Colonization of Pedunculate oak by the Burgundy truffle fungus is greater with natural than with pelletized lime

Grechen E. Pruett · Johann N. Bruhn · Jeanne D. Mihail

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Abstract European black truffles can be profitable agroforestry crops outside their native ranges. Truffle fungi grow symbiotically as ectomycorrhizae on the roots of host trees, notably hazels and oaks. Conditions in the central USA appear conducive to cultivation of the Burgundy truffle (*Tuber aestivum* Vitt. syn. *T. uncinatum* Chatin), but research is needed to determine effects of management practices on truffle establishment and fruiting. In a greenhouse study we tested the effect of lime type, inoculation technique, and two truffle sources on Pedunculate oak (*Quercus robur* L.) growth and mycorrhizal colonization. We found that the type of lime used to raise potting mix pH can differentially affect the growth rate of root systems inoculated with different selections of Burgundy truffle inoculum. Seedlings inoculated with one selection of the truffle and grown in potting mixes amended with natural crushed dolomitic limestone developed larger root systems with more truffle mycorrhizae compared with potting mix amended with high-calcium pelletized quick-release lime. Seedlings inoculated with a second truffle selection were not affected by lime source and developed root systems as large as those developed with the first truffle source grown with natural lime. Supplemental root dip inoculation did not improve levels of colonization beyond those accomplished by potting mix infestation with truffle ascospores. Use of a hygroscopic polymer to maintain ascospore suspension in the inoculum slurry used to infest the potting mix had no effect on root system development or mycorrhiza formation.

Keywords Cultivation · Mycorrhizae · *Quercus robur* · *Tuber aestivum* · *Tuber uncinatum*

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Introduction

The Périgord and Burgundy black truffles of Europe (*Tuber melanosporum* Vitt., and *T. aestivum* Vitt. syn. *T. uncinatum* Chatin, respectively) are among the most valuable gourmet mushrooms in the world (Hall et al. 2003; Hall and Yun 2001). These fungi function in nature as symbiotic ectomycorrhizal fungi (Smith and Read 1997), aiding their host trees in acquisition of minerals and water from the soil. In Europe, these fungi occur in both extensively and intensively managed truffières (truffle-producing forests and plantations) on suitable sites (Riousset et al. 2001). Even in Europe, the influences of site factors and management practices on successful truffière establishment, maintenance, and productivity are incompletely understood and under vigorous study. Nevertheless, both of these truffle species have been successfully cultivated in their native Europe, and the Périgord black truffle has also been cultivated in New

Zealand (Hall et al. 2001), Australia (James Trappe, pers. comm.), and the USA (Garland 2001). We expect additional successes using these and other truffle species.

Several sets of interdependent decisions need to be made in the process of establishing truffières. First, an appropriate site must be found, based on land use history, landform position, soil properties, and local climate (e.g., (Chevalier et al. 2001; Hall et al. 2001). Local climate will be a determining factor in selecting which truffle and host tree species to consider. Though the soils on many otherwise appropriate sites are too acid to support truffle production, this deficiency can be overcome with large-scale applications of lime to the topsoil prior to planting (Garland 2001; Hall et al. 2001; Hall and Zambonelli 2005). The Burgundy truffle fruits best in the range of pH 7.1–8.0 (Chevalier 1978; Wedén et al. 2004a), whereas soils in the central USA generally range in pH 5.0–6.5 (NRCS on going). Natural dolomitic lime is readily available throughout the central USA, but varies greatly in chemistry, including the Ca/Mg ratio. More highly refined forms of agricultural lime (pelletized, with very high Ca/Mg) are also available from fewer sources, and therefore at greater expense in general due to shipping costs. Our study addresses the effectiveness of these two classes of lime in supporting Burgundy truffle mycorrhiza formation with Pedunculate oak (*Quercus robur* L.) in the seedling production phase.

The second set of decisions in truffière establishment involves production of seedlings well-colonized by the chosen truffle species. Successful truffle mycorrhiza development requires efficient root contact with truffle propagules (ascospores or mycelial fragments) in a potting mix that is conducive to both seedling root growth and truffle mycelial growth. Host seed is often germinated in advance of inoculation, to permit early selection of seedlings based on initial growth performance. Once germlings of the selected host tree species are planted in the infested potting mix, the greenhouse environment must be maintained within limits that favor efficient truffle mycorrhiza formation. In addition to evaluating the effects of lime type on root system growth and Burgundy truffle mycorrhiza formation, our study addressed the effectiveness of supplementary root dip inoculation in addition to potting mix infestation, and the effect of including Stockosorb, a hygroscopic gel, in the inoculum suspension. Our hypothesis was that Stockosorb would enhance mycorrhiza formation by maintaining more uniform levels of propagules in the potting mix.

The final set of decisions involves the management of truffières once the seedlings are outplanted. While a great deal has been learned about truffière management in recent decades (Chevalier and Frochot 1997; Chevalier et al. 2001; Hall et al. 2001; Ricard 2003; Sourzat 2002), this is the subject of intensive ongoing research. Much of this research is inherently long-term, because truffières generally require four or more years for adequate establishment prior to first fruiting (Hall et al. 2001). Seedling root system size is a determinant of seedling survival (Dey and Parker 1997), and may influence the extent to which root systems colonize truffière sites. Our study reports differential effects of lime type on overall root system development using two strains of the Burgundy truffle.

Materials and methods

Experimental design

This experiment compared the effects on root system development of two types of lime, three root dip inoculation techniques, two soil infestation methods, and two sources of Burgundy truffle inoculum, resulting in 24 treatment combinations. The experiment was arranged in the greenhouse as a randomized complete block experiment with 10 replicates for a total of 240 seedlings.

Potting mix, host plants, and fungal inoculum

The potting mix consisted of a 2:1:1 mixture of air-dried field soil, vermiculite, and perlite (v:v:v). The field soil was loamy silt topsoil excavated at the University of Missouri Horticulture and Agroforestry Research Center, New Franklin, MO, USA from an old alfalfa field that had been cleared of trees for over 100 years. The soil was hand crumbled, and then sufficient lime was incorporated to raise pH to ca. 7.5, well within the range known to support Burgundy truffle fruiting (Wedén et al. 2004a; Wedén et al. 2004b). Half the substrate was amended with MFA Pel-Lime® (Missouri Farmers Association, Columbia

MO, USA); the other half with a natural dolomitic lime with higher magnesium (Mg) content. MFA Pel-Lime® had a $CaCO_3$ equivalent (CCE) of 90.5, an effective neutralizing material (ENM) value of 660, and a Mg content of 3.6% (AOAC 1984). The natural dolomitic lime with substantial magnesium had a CCE of 101.4, an ENM of 639, and a Mg content of 12.6% (AOAC 1984).

We selected *Quercus robur* as our experimental host because it is one of the prominent host species for the Burgundy truffle in Europe. The Burgundy truffle is found as far north as Gotland, Sweden (Wedén et al. 2004b) and south to Spain and Italy (Chevalier et al. 2001). *Quercus robur* acorns from a cold hardy planting in Indiana were acquired from Vallonia State Nursery, Vallonia, IN, USA.

Burgundy truffle ascocarps (truffles) representing one French and one Swedish population of *Tuber aestivum* Vitt. were obtained from Dr. Gérard Chevalier (INRA, Clermont-Ferrand, France) and Dr. Eric Danell (Uppsala University, Sweden), respectively. Both sources were harvested October 2001. The Swedish truffles were shipped fresh by air from Gotland, Sweden, and frozen on arrival. The French truffles were sliced and lightly-dried, and then transported to the U.S.A. in February 2002. Ascospores from lightly-dried truffles are known to function well as inoculum (Chevalier pers. comm.). Ascospore inoculum was produced by removing the outer ''rind'' (peridium) of each truffle, suspending the fragmented fertile interior (gleba) in tap water, and blending the material to produce a suspension of nearly uniform particle size. Inocula produced from the dried French truffles and from the fresh-frozen Swedish truffles are referred to as *Ta*-1 and *Ta*-2, respectively.

Inoculation and seedling production

Acorns were germinated in the greenhouse in Promix (Premier Horticulture Ltd, Quebec, Canada) for approximately 2 months during June-July 2002. In August 2002, the seedlings were removed from their pots, the Promix was washed from their roots, and they were randomly assigned to treatments.

Inoculation involved a potting mix infestation treatment and a root dip treatment with either *Ta*-1 or *Ta*-2, so that each tree had spores from one source incorporated into its potting mix and also placed directly onto its roots. The two potting mix infestation treatments both involved mixing an aqueous slurry of ascospores (W + *Ta)* into the potting mix at the concentration of 0.7×10^6 ascospores L^{-1} potting mix (Fig. 1). In one of the two treatments (W + *Ta* + S*)*, the ascospore slurry was amended with 0.18 mL L^{-1} Stockosorb® (Stockhausen, Greensboro, NC, USA). Stockosorb® is a gel-based compound that when hydrated helps to maintain moisture in potting mixes. We used Stockosorb® to help keep the spores suspended during inoculation treatments.

The three root dip treatments were: a) roots dipped in water with no spores (W); b) roots dipped in an aqueous spore slurry (W + *Ta*); or c) roots dipped in an aqueous spore slurry containing Stockosorb® (W + *Ta* + S). The root dip slurries contained 2×10^7 ascospores L^{-1}, sufficient to provide 10^6 ascospores per plant to the extent that the spores could be distributed equally and completely. In the W + *Ta* + S root dip treatment, 0.18 mL L^{-1} Stockosorb® was added to the ascospore slurry. After root-dipping, seedlings were planted singly in waxed cardboard containers provided with drainage ($10 \times 10 \times 26$ cm deep) and filled to 20 cm with ca. 1.85 L of appropriately limed and inoculated

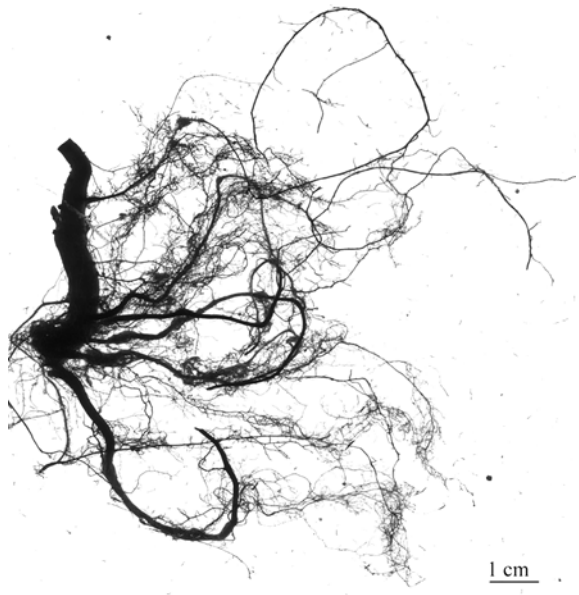

Fig. 1 A typical image produced by scanning a root system with an Epson EU22 flat-bed scanner. Such images were analyzed using WinRhizo software to estimate total root system volume, length, and number of root tips

potting mix. Seedlings were maintained in a greenhouse and watered approximately three times per week.

Data collection

After 11 months in treatment, the seedlings were removed from their pots and their roots were gently washed free of potting mix. Each root system was then spread on a flat surface and scanned with an Epson EU22 scanner (Epson America Inc., Long Beach, CA, USA) (Fig. 1). The scanned image of each root system was analyzed with WinRhizo image analysis software (Régent Instruments, Montreal, Quebec, Canada) to estimate three growth metrics: total root system volume, total root system length, and total number of root tips.

The extent of fungal colonization was also assessed for each root system. Three hundred root tips from each seedling were randomly sampled by spreading the root system onto a numbered grid and harvesting a root segment containing 30 root tips from each of 10 randomly selected locations. These root samples were frozen (−20°C) in 15% glycerol for later examination of fungal colonization. Upon thawing, each root tip was examined by light microscopy (100× and 400×) for the presence of an ectomycorrhizal fungal mantle (Peterson et al. 2004). Ectomycorrhizal root tips were identified microscopically as Burgundy truffle-colonized or ''other'' (Müller et al. 1996; Rauscher et al. 1996). The ectomycorrhizal condition of each seedling was characterized by multiplying the total number of root tips scanned by the percentages of sampled root tips which were ectomycorrhizal or, more specifically, Burgundy truffle-colonized. Broken tips were excluded from analysis. The number of seedlings in each treatment that died during the experiment was also recorded.

Data analysis

Root system metrics (total root system volume, total root system length, and total number of root tips) and fungal colonization metrics (total number of ectomycorrhizal root tips and total number of Burgundy truffle-colonized root tips) for each seedling were analyzed by linear regression (PROC GLM, $\alpha = 0.05$; SAS/STAT System Release 7.1, Inst. Inc., Cary, NC) (Table 1). Least significant means were used to determine differences among treatments.

The effects of the three root dip inoculation techniques and inoculum source on seedling mortality were analyzed separately for the two inoculum sources (*Ta*-1 and *Ta*-2) using contingency table analysis and the χ^2 statistic (Sokal and Rohlf 1995) to compare expected values with observed mortality. All expected values were >3.0, thus providing a robust analysis (Steel and Torrie 1980).

Results

Truffle inoculum source

After 11 months in treatment, seedling root systems inoculated with the *Ta*-2 truffle source had grown larger than those inoculated with the *Ta*-1 source (Table 1 and Fig. 2). Inoculation with Ta-2 resulted in 31% greater root volume ($P < 0.001$, Fig. 2a), 29% greater total root length ($P < 0.001$, Fig. 2b), and 25% more total root tips ($P < 0.001$, Fig. 2c) than did inoculation with *Ta*-1. Root systems inoculated with *Ta*-2 also developed 22% more ectomycorrhizal root tips ($P < 0.001$), including more root tips identified as Burgundy truffle-colonized (Table 1 and Figs. 2d, e, respectively). Nevertheless, the numbers of ectomycorrhizal root tips per cm^{-1} of root length and the numbers of truffle-colonized root tips cm^{-1} of root length were very similar for root systems inoculated with either *Ta*-1 or *Ta*-2. Seedlings inoculated with *Ta*-1 and *Ta*-2 developed 2.47 and 2.43 mycorrhizal root tips cm^{-1} of root length and 1.55 and 1.56 truffle-colonized root tips cm^{-1} of root length, respectively. Seedlings inoculated with *Ta*-1 experienced greater mortality than seedlings inoculated with *Ta*-2 (26 vs. 9%, respectively, $\chi^2 = 12.5$, df = 1, $P < 0.001$).

Lime type

Root systems grown in the potting mix amended with natural dolomitic lime developed greater total root length ($P < 0.05$), more total root tips ($P < 0.01$), and more identifiable Burgundy truffle-colonized root tips ($P < 0.05$) than did root systems grown in potting mix amended with Pel-Lime® (Table 1). There was also a clear statistical interaction between inoculum source

Table 1 ANOVA table. Results of analysis of variance to identify effects of lime type, inoculation methods, and truffle inoculum source on *Quercus robur* root system growth and mycorrhization in the greenhouse

Source of variation[a]	Response variables[b]									
	Total root volume		Total root length		Total no. root tips		Total no. mycorr. root tips		Total truffle-colonized root tips	
	df	F	df	F	df	F	df	F	df	F
Model	32	2.07**	32	2.34***	32	2.54***	32	3.01***	32	2.27***
Block	9	1.33	9	1.56	9	2.99**	9	4.74***	9	2.78**
Lime	1	1.91	1	4.73*	1	8.06**	1	3.08	1	6.2*
Root dip	2	10.71***	2	7.57***	2	6.55**	2	10.63***	2	9.02***
Potting mix infest	1	0.37	1	0.17	1	0.10	1	0.41	1	0.38
Source	1	17.53***	1	19.47***	1	15.34***	1	13.38***	1	10.59**
Lime × root dip	2	3.10*	2	3.52*	2	2.10	2	3.69*	2	3.55*
Lime × Potting mix infest	1	0.31	1	1.13	1	0.07	1	1.50	1	0.53
Lime × Source	1	1.77	1	10.93**	1	10.17**	1	6.95**	1	1.81
Root dip × Potting mix infest	2	0.07	2	0.62	2	0.44	2	0.69	2	0.69
Source × Root dip	2	1.41	2	3.70*	2	5.63	2	2.11	2	1.07
Source × Potting mix infest	1	3.34	1	0.81	1	0.82	1	1.17	1	1.89
Lime × Source × Root Dip × Potting mix infest	9	0.48	9	0.74	9	0.82	9	1.38	9	0.91
Within groups	170	–	170	–	170	–	156	–	156	–
Total	202	–	202	–	202	–	188	–	188	–

[a] sources of variation: Block, 10 randomized complete blocks; Lime, Pel-lime® or natural dolomitic lime; Root dip, water (W) or water + *Tuber aestivum* spores (W + *Ta*) or water + *T. aestivum* spores + Stockosorb® (W + *Ta* + S); Potting mix infest., potting mix mixed with water and *T. aestivum spores* (W + *Ta*) or water + *T. aestivum* + Stockosorb® (W + *Ta* + S); Source, *T. aestivum* ascospores from *Ta*-1 or *Ta*-2 inoculum source

[b] df, degrees of freedom

*, **, *** indicate *F*-value significance at $P \leq 0.05$, 0.01, and 0.001, respectively

and the type of lime used (Table 1). Root systems inoculated with *Ta*-1 and grown in potting mix amended with Pel-Lime® were 28% shorter ($P < 0.01$) and produced 30% fewer root tips ($P < 0.01$) of which 22% fewer were ectomycorrhizal ($P < 0.01$), as compared with seedlings grown in potting mix amended with the natural dolomitic lime (Figs. 3b, c, d, respectively). Root systems inoculated with *Ta*-2 performed equally well in potting mix amended with either type of lime (Fig. 3). There was no apparent effect of lime type on mortality of seedlings inoculated with either *Ta*-1 or *Ta*-2.

Potting mix infestation

Addition of Stockosorb® to the aqueous spore suspension used to infest the potting mix with Burgundy truffle ascospores had no effect on root system growth or mycorrhization (Table 1). In addition, no interactions were detected between potting mix infestation treatment and type of lime used, inoculum source, or root dip inoculation treatment (Table 1).

Root dip inoculations

Neither of the root dip inoculation treatments increased root system size or ectomycorrhizal development beyond the effect of potting mix infestation (Fig. 4). Adding Stockosorb® to the aqueous spore suspension (W + *Ta* + S) resulted in smaller and shorter root systems containing fewer ectomycorrhizal root tips ($P < 0.001$) and fewer truffle-colonized root tips ($P < 0.001$) as compared to root systems dipped in an aqueous spore suspension (W + *Ta*) or the water-only root dip (W) (Table 1). The effect of Stockosorb® was greater in the Pel-Lime®

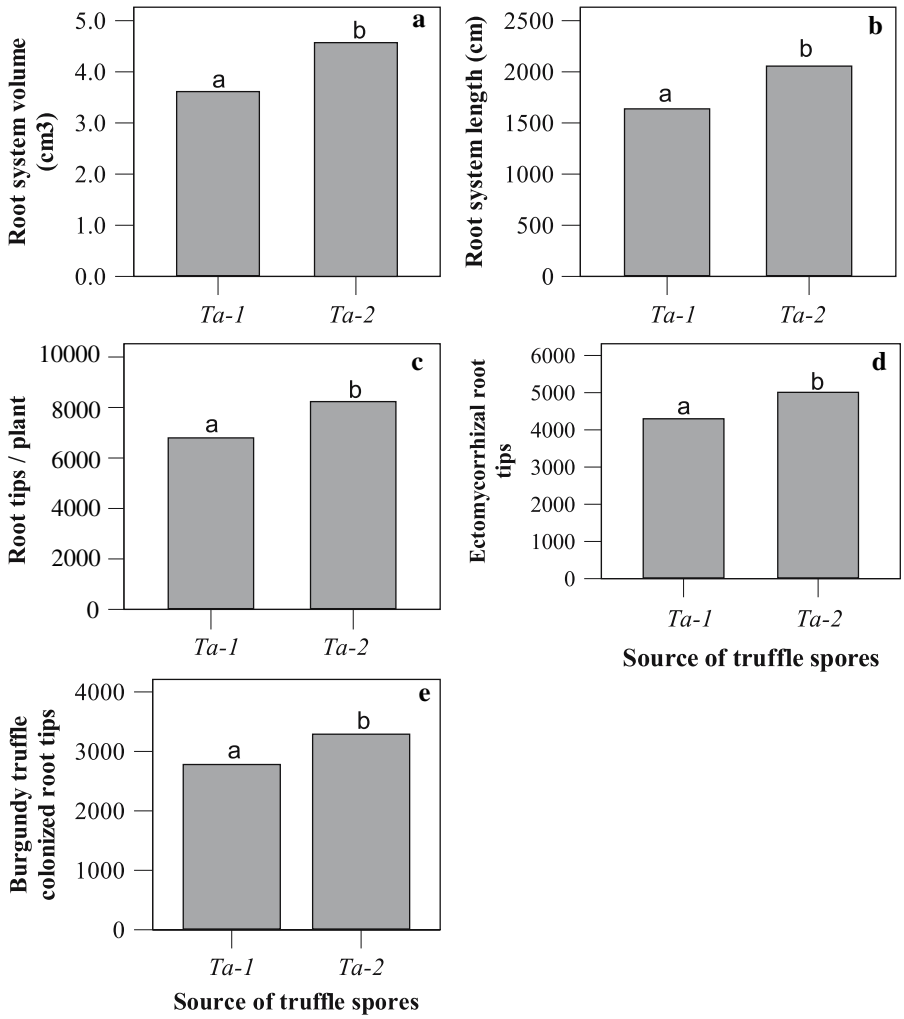

Fig. 2 Characteristics of root systems inoculated with *Ta*-1 or *Ta*-2 sources of Burgundy truffle ascospores (**a**) Root system volume (**b**) Root system length (**c**) Root tips per plant (**d**) Number of ectomycorrhizal root tips (**e**) Number of ectomycorrhizal tips colonized by Burgundy truffle. Within each panel, histogram bars with different letters represent significantly different means ($\alpha = 0.05$)

treatments than in the natural dolomitic lime treatments (Fig. 4). Seedlings dipped in the Stockosorb® amended spore slurry (W + *Ta* + S) experienced 31% mortality compared to 14% mortality for the aqueous spore suspension (W + *Ta*), and 9% for the water-only root dip (W) ($\chi^2 = 15.2$, df = 2, $P < 0.001$).

A significant block effect developed near the end of the study (Table 1), because it took eight weeks to process the seedlings. Blocks were processed sequentially, to protect treatment differences. However, it was apparent that mycorrhizal development continued during processing.

Discussion

Seedlings inoculated with *Ta*-1, the French truffle source, produced smaller root systems in potting mix amended with Pel-Lime® than did seedlings inoculated with *Ta*-2, the truffle source from Sweden. However, the number of root tips and of *Ta*-colonized root tips specifically cm^{-1} root length were very similar for both truffle sources regardless of the form of lime used. This result indicates a differential effect of our two forms of lime on structural root growth as mediated by the two Burgundy truffle sources used as mycorrhizal inoculum.

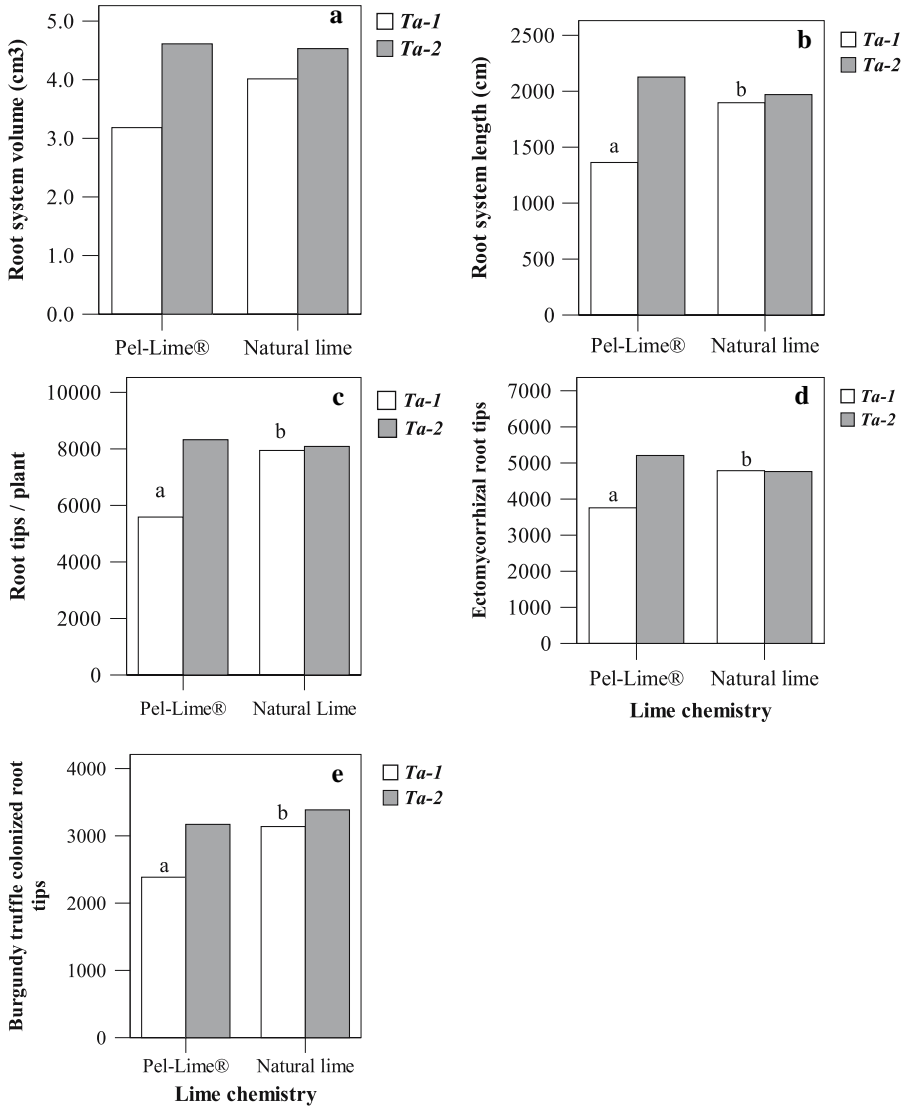

Fig. 3 Characteristics of root systems grown in potting mix amended with Pel-Lime® or natural dolomitic lime and inoculated with *Ta*-1 or *Ta*-2 sources of Burgundy truffle ascospores (**a**) Root system volume (**b**) Root system length (**c**) Root tips per plant. (**d**) Number of ectomycorrhizal root tips (**e**) Number of ectomycorrhizal tips identified as Burgundy truffle. Mean response values were compared separately for each inoculum source. Within each panel, histogram bars with different letters represent significantly different means ($\alpha = 0.05$). There were no significant differences in any response variable with respect to lime source using the *Ta*-2 inoculum source

The basis for the sensitivity of the *Ta*-1 biotype to the pelletized, quick-release Pel-Lime® is uncertain and merits further study. High levels of calcium in European soils (commonly associated with soil pH > 7.0) are widely thought to be important for the growth and/or fruiting of many *Tuber* species (Chevalier et al. 2001; Hall et al. 2001; Riousset et al. 2001; Sourzat 2002). García-Montero et al. (2005) observed greater truffle production in Portuguese soils with higher active carbonate content and suggested that high soil carbonate activity specifically is more closely related to truffle fruiting than is the total quantity of calcium in the soil. Although we did not measure the carbonate activity of the differently limed potting mixes in our study, the CCE (calcium carbonate equivalent) of the dolomitic lime was 11% higher than the CCE of the Pel-Lime®. Nevertheless, the calcite Pel-lime® contained negligible magnesium compared to our dolomitic lime source. Because calcium and magnesium compete for cation exchange sites on soil particles, we suspect that our *Ta*-1 truffle source is more susceptible to the exceptionally high Ca:Mg ratio established using calcite Pel-lime® compared with dolomitic lime. We certainly can not draw any geographic conclusions from our two *T. aestivum* sources. Wedén et al. (2004b) pointed out that productive truffle sites in both France and Sweden vary widely in their soil

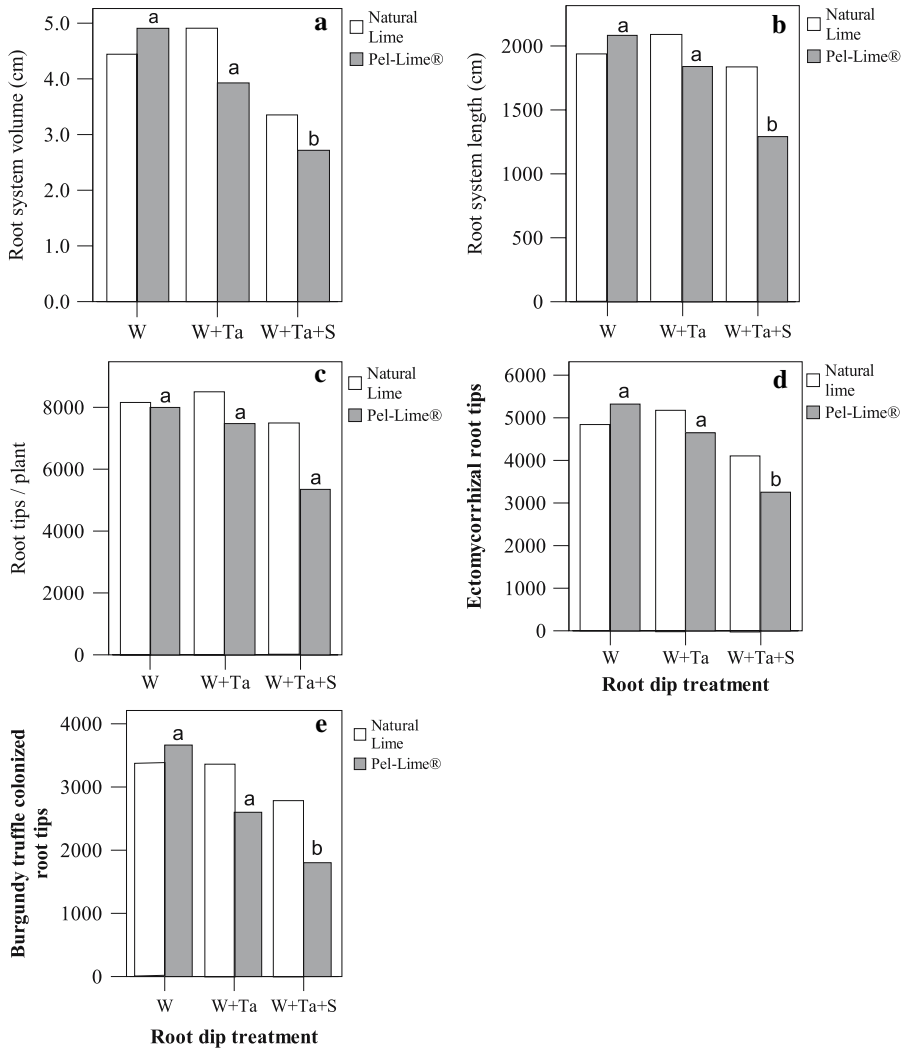

Fig. 4 Characteristics of root systems which experienced different root dip techniques and grown in potting mix amended with Pel-Lime® or natural dolomitic lime (**a**) Root system volume (**b**) Root system length (**c**) Root tips per plant (**d**) Number of ectomycorrhizal root tips (**e**) Number of ectomycorrhizal tips identified as Burgundy truffle. Root dip treatments consisted of: water (W); and aqueous suspension of *Ta*-1 or *Ta*-2 ascospores (W + *Ta*); an aqueous suspension of *Ta*-1 or *Ta*-2 ascospores and Stockosorb® (W + *Ta* + S). Mean response values were compared separately for each lime type. Within each panel, histogram bars with different letters represent significantly different means ($\alpha = 0.05$). There were no significant differences in response variables among root dip treatments where natural dolomitic lime was used

Ca:Mg ratios. Rather, our results demonstrate that sensitivity to exceptionally high Ca:Mg ratios may be common among strains of *T. aestivum.*

All seedlings were planted into potting mix infested with ascospores of one or the other truffle source. The spore suspension used to infest half of the potting mix with each truffle source contained Stockosorb®. Addition of Stockosorb® to the inoculum suspension used to infest the potting mix had no influence on root system size or infection.

We anticipated that a supplemental root-dip inoculation with an aqueous spore suspension would increase at least root infection level beyond that achieved by potting mix infestation, but this was not the case. In fact, addition of Stockosorb® to the root dip suspension significantly reduced root system size and *Ta* infection level in potting mix amended with Pel-Lime®. Other studies have indicated that gel-based polymers enhance, suppress, or act neutrally towards plant growth depending on the system (Austin and Bondari 1992; Volkmar and Chang 1995). In our case, however, addition of Stockosorb®-amended root dip treatment was counter productive in potting mix amended with Pel-Lime®. The Pel-Lime® component of this effect may again be explained by the exceptionally high Ca:Mg ratio of calcitic Pel-Lime®. Additionally, the Stockosorb® particles may have physically separated some spores from fine roots and/or maintained a moisture content unfavorable for *Ta* infection. Because spores were more uniformly distributed throughout Stockosorb® suspensions compared to simple aqueous suspension,

spores may have become largely distributed along structural root sections (where infection is less likely) instead of flowing to more susceptible root tips as they settled out of the aqueous suspension. The basis for the interaction between Stockosorb® root-dip inoculation and Pel-Lime® is uncertain.

While our source of *Q. robur* was selected for it's cold-hardiness, it was also uniformly susceptible to powdery mildew. Powdery mildew infection levels were uniform across all treatments throughout the experiment, resembling levels observed in Sweden (Johansson 2001). We did not attempt to treat the powdery mildew, as the potential effects of fungicides on truffle ecology are uncertain. We suspect that the greater mortality experienced among seedlings inoculated with *Ta*-1 was associated with the smaller root systems of these seedlings in general. A host more resistant to powdery mildew might have fared better.

Our seedlings experienced low levels of colonization by other ectomycorrhizal fungi. No effort was made to identify these species. Most likely these competitors were introduced both during the greenhouse growth period and in the field live soil which formed the basis for our potting mix. Although the soil excavation site has lacked ectomycorrhizal hosts for perhaps 100 years, various mycophagous mammals (e.g. deer and burrowing rodents) have undoubtedly deposited fecal pellets rich in ectomycorrhizal inoculum over this period (Ashkannejhad and Horton 2006). We intentionally used live field soil from our eventual outplanting site in order not to exclude the effects of indigenous microbiota, including competing ectomycorrhizal fungi, from consideration.

Conclusions

This study was conceived as a direct response to concerns expressed by nurserymen eager both to grow planting stock well-infected by Burgundy truffle and to know the importance of cation balance for truffle infection and ultimately fruiting in limed soils. The critical issue is the cost of transporting the large volumes of lime needed to raise soil pH to favorable levels at plantation sites. Many potential growers are closer to sources of natural dolomitic lime than they are to manufacturers of more highly refined lime products with the highest Ca-levels (e.g., calcite Pel-Lime®). We conclude from this study that calcite Pel-Lime® is not superior to our test source of natural dolomitic lime in supporting Burgundy truffle mycorrhiza formation with Pedunculate oak. In fact, Pel-Lime® appears capable of suppressing structural root development in partnership with some sources of Burgundy truffle. We can not recommend that growers undertake extra expense in order to use a refined high-Ca liming product.

Neither Stockosorb® nor root-dip inoculation improved Burgundy truffle infection levels beyond those accomplished by potting mix infestation using an aqueous suspension of truffle ascospores. Jimenez-Aguilar and Uceda-Marfil (2001) had similar findings in Spain.

We have shown that different sources of Burgundy truffle responded differently to calcite Pel-Lime® in partnership with our source of Pedunculate oak. No difference was detected between truffle sources in response to our source of dolomitic lime. From this we also conclude that the gentle drying technique used to prepare our *Ta*-1 source for transportation did not adversely affect inoculum viability. Researchers use various ways to prepare inoculum (Hall et al. 2003). Our findings indicate that there is not a significant difference between freezing and drying.

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