Blueberries Mycorrhizal Symbiosis Outside of the Boundaries of Natural Dispersion for Ericaceous Plants in Chile

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Abstract

Blueberry culture in Chile has been expanding its acreage from suitable edaphoclimatic conditions to environments in which some soil characteristics depart from blueberries' requirements, among others, to calcareous soils with pH over 7.5. Under these conditions, specific technological field management is required to keep plants producing fruits; however, little attention has been given to mycorrhizal symbiosis. Several studies have been carried out since 2000 in order to assess the importance of the mycorrhizal symbiosis for cultivated blueberry plants in Mediterranean agroecosystems that do not have native Ericaceous plants. Survey of the mycorrhizal status of commercial blueberry fields and directed inoculation assays with commercial and native mycorrhizal inocula have been conducted. It was observed that under these conditions, field blueberry plants form mycorrhizae with native arbuscular and ectomycorrhizal fungi with a lower infection percentage. In some samples, only arbuscular mycorrhizae were observed. Ericoid mycorrhizae were present to a variable extent, probably as a consequence of contamination at the nursery level. No differences in field plants performance were observed. However, when blueberry plants growing in containers, are inoculated with mycorrhizal fungi at the nursery, the symbiosis performs with different levels of efficiency, from positive to negative when compared to non-inoculated plants, but having native mycorrhizal fungi. In some experiments, ericoid mycorrhiza produced by *Hymenosyphus ericae* had lower plants' biomass (dwt) compared with native mycorrhiza, but in others, they had the highest. The significance of the fungal edaphic adaptation is discussed.

INTRODUCTION

Under some edaphic constraints to higher plants, the mycorrhizal symbiosis can show a coevolutionary pattern between both partners that favors the adaptation of the higher plant to those soil problems or, at least, the symbiosis is able to lower the plant's associated stress level, allowing the plant to keep or increase its competition capacity (Brundrett, 2004). The symbiotic relationship between ericaceous plants and ericoid mycorrhiza is one example of such coevolution (Brundrett, 2002). Ericaceous plants can prosper in soils with low pH, high organic matter and phenolics contents, low NO_3^- and available calcium concentration due to the presence of mycorrhiza (Straker, 1996). Under these soil conditions, ericoid mycorrhizae fungi evolved to help their symbiont partner complete its life cycle and, even, develop as the dominant plant species in a given niche, like heath lands for native *Vaccinium* plants (Read, 1996). As soil is a spatially variable substrate, through the ages, mycorrhizal fungi may have evolved biotypes adapted to

specific soil conditions (Straker, 1996; Brundrett, 2002, 2004). If this is the case, such fungal biotypes may perform less efficiently as the edaphic conditions depart from those in which the symbiosis evolved (Straker, 1996).

Blueberry culture in Chile spreads along almost 875 miles, North to South, with many different agroecosystems, from the arid Mediterranean (30-35°S.L.) to the temperate rainy forest (41°S.L.). In some areas of the Mediterranean zone, the soil and irrigation water is not within the published adaptation range for commercial blueberry cultivation (Eck et al., 1990). Soil pH ranges from 7.5 to 8.4 and contains an elevated calcium content (4-8% of $CaCO_3$), and irrigation water has a pH of 7.8 or higher. Under conventional field management, blueberry plants develop soil-induced nutritional deficiencies and toxicities, and plants may even die if soil stress factors are not compensated. However, through specific cultivation technologies (Ferreyra et al., 2001), it is possible to have a reasonable crop load in Mediterranean zones for many years.

Currently, commercial ericoid and arbuscular mycorrhizal (AM) inocula are available to Chilean growers; however, they have evolved in the Northern Hemisphere under very different ecosystems than the Chilean Mediterranean ecosystems. Under those conditions, such inocula may depart from the label offered performance (Azcón-Bieto and Talón, 2000). In order to assess the native and commercial mycorrhizal inocula performance over blueberry plants at nursery level, two assays were carried out. Also, observations on the mycorrhizal status were made at commercial blueberry fields.

MATERIAL AND METHODS

Experiment 1

One year old Southern Highbush blueberry plants, propagated either by cuttings or tissue culture, and growing in a polyethylene covered greenhouse in 3 L pots containing a substrate consisting in a mix of sawdust (48%), pine acicules (pine needles, 48%) and local soil (4%). These plants were inoculated with one of three mycorrhizal inocula: 1) A commercial inoculum containing *Hymenosyphus ericae* (MYCOSYM TRITON), 2) Arbuscular mycorrhiza inocula given by the Catholic University of Valparaíso, Chile, 3) a naturally occurring mycorrhizal roots from a commercial blueberry field ('O'Neal), located near Valparaíso and 4) control without inoculation. Inoculated plants were arranged in a completely randomized block design with five replications and three subsamples (plants) in each experimental unit. The number and diameter of shoots and root dry weight were determined. Also mycorrhizal status was observed with a light microscope using a combined staining protocol (Vega and Muñoz, 1994).

Experiment 2

Commercially propagated 'O'Neal' blueberry plants growing in 1 L pots under a shade net, (photosynthetic photon flux density at noon = 1000 µmol m^{-2} s^{-1}), were inoculated with three different mycorrhizal inoculum. There was also a non-inoculated control. Roots with established native Chilean mycorrhizal population were used as inocula. These were collected in three different locations: 1) From a commercial 'O'Neal' blueberry field, located at Lampa (33º 10' S.L.; calcareous loam soil, pH 7.8) 2) From a commercial 'O'Neal' blueberry field located at Peralillo (34º 30' S.L.; granitic, clay-loam soil, pH 5.8), and 3) Native *Pernettya* sp. (Ericaceae) roots from a soil derived from Andean volcanic ash (38º 30' S.L.; pH 5.8). In all cases only mycorrhizal washed roots, without rhizospheric soil were used. For each inoculation treatment, three irrigation tap water pH levels were tested: a) 2.0, b) 5.0 and, c) 7.8. In the first two pH levels, H_2SO_4 was added to lower the pH to the desired level. In all cases, the watering was done at least 24 hours after storing the water in a 100 L plastic container in order to eliminate chlorine. Plants were arranged in a completely randomized blocks design with a 4x3 factorial structure (4 inocula and 3 irrigation water pH), with six replications of a single plant. Data recorded included the shoot and root dry weight and mycorrhizal status (as in Experiment 1) after 8 months.

In both experiments, analysis of variance (ANOVA) was performed and means were separated using Tukey's multiple range test if significant differences ($P \leq 0.05$) were detected.

RESULTS AND DISCUSSION

Experiment 1

1. Shoot Length. Shoot length data was the sum of the length of all shoots on the plant. No differences in shoot length were observed as a consequence of native mycorrhizal inoculation treatments. However, plants inoculated with *Hymenosyphus ericae* (commercial inoculum), differed in size depending on propagation technique. Plants propagated by cuttings showed higher shoot length than tissue cultured plants (Fig. 1A). These results agree with the work of Starret (2003), who did not find differences between ericoid fungus inoculated and non-inoculated 'Bluecrop' blueberry plants. Starret (2003) indicated that if nutrients are at an adequate concentration, the symbiosis benefits may not be evident. The higher shoot growth of cutting-propagated plants may be due to the photosynthate reserves, which could be less in vitro propagated plants (not determined). However, the inoculum doses is also a factor in eventual responses to the symbiosis, as observed in 'Dixi' and 'Stanley' blueberry plants inoculated with 50 mg/plant, which showed less shoot growth than plants that received 1/10 of that doses (Powell and Bagyaraj, 1984).

2. Shoot Diameter. After five months of growth, the total shoot diameter per experimental unit (three plants) showed some effect of inoculum source. With field-grown blueberry roots as inoculum, the smaller diameter was observed, being different from *H. ericae* inoculated in micropropagated plants, but not for the same inoculum when applied to cutting-propagated plants (Fig. 1B). The differential effect of the *H. ericae* inoculum according to the propagation system may be related to the transplant process, which occurred before inoculation. Cutting plants can receive some damage to the roots and divert some photosynthates to roots regeneration, may diminish those allocated to sustain the initial mycorrhizal fungus colonization. If carbon availability diminishes, the nitrogen source utilization may be less efficient, affecting the fitness of the symbiosis (Gwen-Aëlle et al., 2005), and finally the plant growth. The same may be valid for treatments inoculated with mycorrhizal roots of field grown blueberry plants (which had ericoid mycorrhizal fungi) (Fig. 1B). No differences were observed with the other treatments.

3. Root Dry Weight. Plants inoculated with ericoid mycorrhizal fungi and propagated in vitro showed the highest root dry weight (Fig. 2A), compared to plants propagated by cuttings and inoculated either by roots or non-inoculated. Local soil inoculum applied to cutting plants is less effective than ericoid mycorrhizal fungi inoculated to tissue cultured plants (Fig. 2A). This may be because the substrate used in this experiment is very different than the local soil from where the root used as inoculum was harvested, probably because such inocula are different strains of ericoid mycorrhizal fungi, with different soil or substrate specializations, like calcicole or calcifuge, which may respond very differently to the substrate and, as consequence, its performance as symbiont (Straker, 1996).

4. Mycorrhizal Status. After five months of experimental time, the infection level observed was higher than those reported in the literature for blueberries (Eynard, and Czesnik, 1989; Scagel, 2005). Even non-inoculated plants (Fig. 2B), growing in a substrate disinfested three times with fluent vapor at 100°C, showed high infection levels, except the latter that differ from findings made by Scagel et al. (2005) on blueberry nursery plants. Plants inoculated with an arbuscular mycorrhizal fungus (local isolate) were those with higher infection level, however, since these plants had lower biomass production, symbiont fitness may have been lower than the other inocula. Some reports have indicated that ericoid mycorrhizal fungi may have vesicular-arbuscular mycorrhizal

fungi as ancestors and that Ericaceous plants, in absence on ericoid mycorrhizal inoculum, can associate to VAM fungi (Brundrett, 2002).

Experiment 2

At the beginning of this experiment, all plants used in this study showed a low intensity mycorrhizal infection by vesicular-arbuscular (2.1%) and ericoid (5.3%) fungi. This contamination could have occurred at the nursery before the experiment began.

1. Plant Dry Weight and Mycorrhizal Status. There were no factorial treatment interactions and no differences among treatments in plant dry weight (Fig. 3) or mycorrhizal infection (Fig. 4). The data set had a high coefficient of variation that indicated high variability in the raw data. It is possible that the number of replications in the experiment was insufficient, or that the experimental time shorter than ideal. Caution must be used in interpreting the data, because there are reports in which this variation (CV about 20-200%), is associated with the infection dynamics itself (St. John and Hunt, 1983). Mycorrhizal infection usually does not follow a linear pattern of development in roots tissues under experimental conditions, and could be fitted by a negative binomial distribution. This means that the infection starts in one or more points in the root system, and from those points, it disperses in a radial fashion to the other roots. This gives an infection pattern where there are simultaneously points with high infection intensity, as well as, other with a mild or no mycorrhizal infection (St. John and Hunt, 1983). Therefore, if the mycorrhizal infection has not reached a dynamic equilibrium in the host plant root system, and with the other fungal endophytes competing for the same niche, an efficient mycorrhizal infection assessment will be dependent on the sampling procedures (Reich and Barnard, 1984).

CONCLUSIONS

The mycorrhizal infection percentage varies widely, even among replications in an assay; however, a pattern was observed where vesicular-arbuscular micorrhizae were able to infect blueberry plants in the field and in an experimental set up under greenhouse conditions. In the latter, VAM mycorrhiza reached even to 80% of infection, however, with lower benefits for the host plant than with the ericoid mycorrhizal symbiosis.

Ericoid mycorrhizal fungi may have biotypes that have evolved to specific edaphic conditions, even for calcareous soils. If so, it should be possible to isolate fungal strains that are more efficient as a symbiont for blueberry plants growing out of their natural range of soil conditions.

More research is needed to evaluate the mycorrhizal performance at the field, with emphasis in ericoid fungi.

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Figures

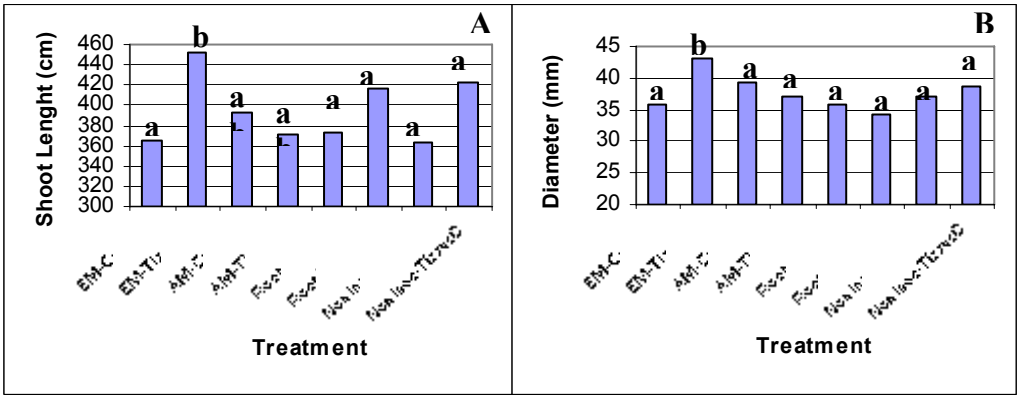

Fig. 1. Growth response of greenhouse grown 'O'Neil' Blueberry plants to different mycorrhizal inocula. A. Shoots length per experimental unit (cm). B. Shoots diameter per experimental unit (mm). EM, ericoid mycorrhizal inoculum (*Hymenosyphus ericae*); AM, arbuscular mycorrhizal inoculum; TC, Tissue culture propagated; Root, Mycorrhizal roots from commercial blueberry field used as inoculum (mainly ericoid mycorrhiza); Non Inoc, Non inoculated plants. Same letters above columns indicate no significant differences between treatments ($P \leq 0.05$).

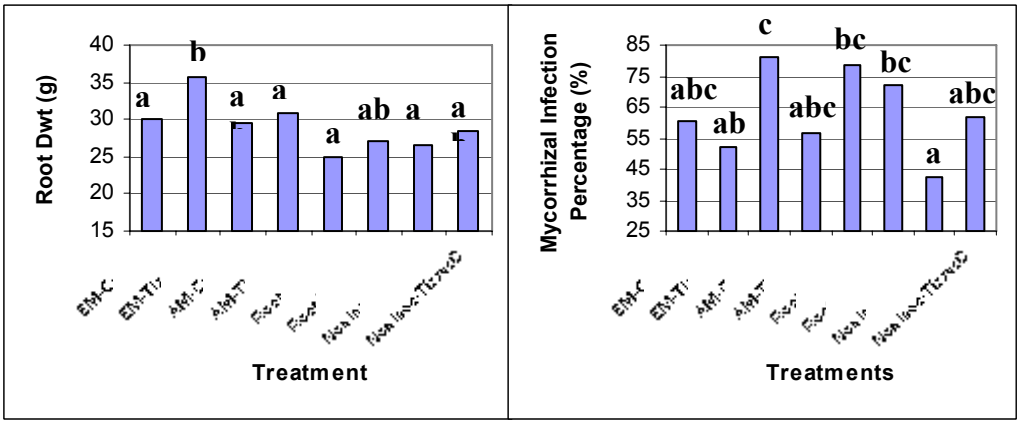

Fig. 2. Root dry weight (A) and mycorrhizal infection (B) in 'O'Neil blueberry plants inoculated with different mycorrhizal inocula and growing under greenhouse conditions. EM, ericoid mycorrhizal inoculum (*Hymenosyphus ericae*); AM, arbuscular mycorrhizal inoculum; Root, Mycorrhizal roots from commercial blueberry field used as inoculum. Same letters above columns indicate no significant differences between treatments (P≤0.05).

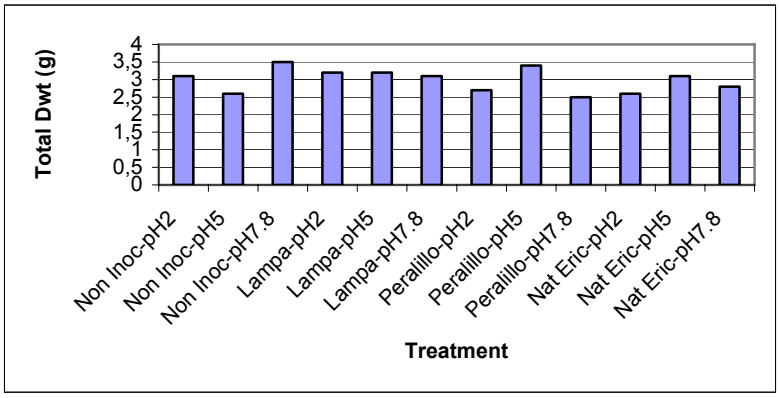

Fig. 3. Total dry weight of O'Neal blueberry plants growing under a shade net and inoculated with different native mycorrhizal inocula and watered with different pH water. There were no interactions between factors and no treatment differences. Non-Inoc, Non-inoculated; Lampa and Peralillo are different locations where commercial blueberries are growing. Nat Eric, Native ericoid mycorrhizal inoculum.

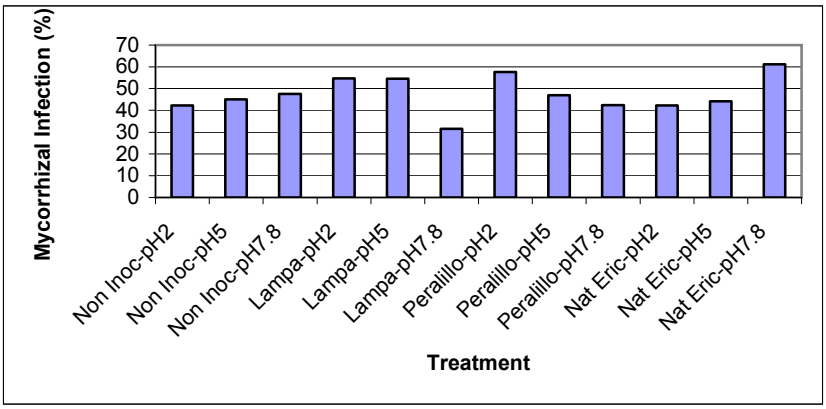

Fig. 4. Mycorrhizal status of O'Neal blueberry plants growing under a shade net and inoculated with different native mycorrhizal inocula and watered with different pH water. There were no interaction between factors and no treatment differences. Non-Inoc, Non-inoculated; Lampa and Peralillo are different locations where commercial blueberries are growing. Native ericoid mycorrhizal inoculum.