Mycorrhiza (1998) 7:223–235

ORIGINAL PAPER

J. T. Berman · C. S. Bledsoe

Soil transfers from valley oak (*Quercus lobata* Nee) stands increase ectomycorrhizal diversity and alter root and shoot growth on valley oak seedlings

Accepted: 17 August 1997

Abstract Soils from valley oak (*Quercus lobata* Nee) riparian areas of the Cosumnes River Nature Conservancy Preserve near Sacramento, California were added to growth medium of valley oak seedlings grown in a greenhouse or in agricultural fields at Cosumnes which probably once supported valley oak trees and are now replanted with native riparian vegetation or allowed to revegetate naturally. Agricultural field soil from the Cosumnes River Preserve was presumed to be low or lacking in ectomycorrhizal inoculum. The study was designed to (1) determine whether valley oak stand soil transfer could cause mycorrhizal infection on valley oak seedlings in an agricultural field and in a greenhouse, (2) describe ectomycorrhizal morphological types formed on valley oak seedlings, and (3) determine whether seedling growth is enhanced more by transfer of natural valley oak stand soil than agricultural field soil. In the field study, transfer of forest soil increased average ectomycorrhizal diversity (2.4 types) more than transfer of agricultural field soil (1.2 types). Valley oak seedlings were responsive to ectomycorrhizal infection in the field study. With increase in mycorrhizal infection there was an increase in shoot growth at the expense of root growth. In the greenhouse study, both percent mycorrhizal infection and mycorrhizal diversity were increased more by transfer of oak forest and woodland soils than agricultural field soil. Eight morphotypes occurred on seedlings in forest and woodland soils but only three morphotypes in agricultural soil. This result strongly suggests that the agricultural field also harbors ectomycorrhizal propagules but forest and woodland soils support a more abundant and diverse ectomycorrhizal flora.

J. T. Berman · C. S. Bledsoe (✉)
Department of Land, Air and Water Resources,
University of California, Davis, CA 95616-8627, USA
Fax: +1-916-752-1552; e-mail: csbledsoe@ucdavis.edu

Key words *Quercus lobata* · Ectomycorrhizas · Diversity · Soil transfer · Soil sterilization

Introduction

Riparian areas of California's Central Valley were once dominated by both dense and open valley oak (*Quercus lobata* Nee) stands (Thompson 1961). By 1985, an estimated 90% of that vegetation had been removed in the Sacramento Valley and 80% in the San Joaquin Valley (Perryess et al. 1993). Today, there is interest in conserving and re-establishing these natural areas for both aesthetic and conservation reasons (Smith 1977; Muick 1980) and for water-quality enhancement and flood control (Perryess et al. 1993). The use of mycorrhizas in reforestation and their role in ecosystem processes is an area of active research because mycorrhizas are mediators of carbon flow and nutrient cycling in natural ecosystems (Perry et al. 1987; Kropp and Langlois 1990; Vogt et al. 1991). While some information on the physical and chemical soil requirements of valley oak is available, little is known about the microbial component of valley oak establishment and growth (Swiecki and Bernhardt 1991). We found only a single report of mycorrhizas on valley oak from an herbarium specimen of *Pisolithus tinctorius* at California State University, Chico, Calif. (Marx 1977). Knowing the form and function of mycorrhizas in valley oak ecosystems will add to the information base used to manage and re-establish these scarce habitats. This study provides information on valley oak mycorrhizas at the Nature Conservancy Cosumnes River Preserve south of Sacramento, California, where agricultural fields are being revegetated with native riparian vegetation, including valley oaks.

Applying soil collected from undisturbed, natural areas may be a means of supplying mycorrhizal propagules and other soil microbes to degraded sites (Shemakhanova 1967; Amaranthus and Perry 1987; Helm and Carling 1993a) and studying rhizosphere and plant

interactions (Amaranthus and Perry 1989; Colinas et al. 1994). Soil transfers may have a stimulatory effect on plant survival, growth, and mycorrhizal development, especially at environmentally stressed sites (Amaranthus and Perry 1987), acting as a source of mycorrhizal propagules, providing stimulatory effects of non mycorrhizal rhizosphere organisms (Amaranthus and Perry 1989) or protecting from deleterious factors in the receiver soil (Colinas et al. 1994). The dependence on soil source for the stimulation of plant growth and mycorrhizal development points to a role for successional dynamics in plant and fungal response (Amaranthus and Perry 1989; Helm and Carling 1993b).

This study investigated the use of soil from two natural valley oak stands to enhance valley oak seedling growth and mycorrhizal development in a field experiment carried out in an abandoned agricultural field and in a greenhouse experiment. Agricultural field soil was presumed to be lacking in or low in ectomycorrhizal inoculum potential. The study was designed to (1) determine whether valley oak stand soil transfer could cause mycorrhizal infection on valley oak seedlings in an agricultural field and in a greenhouse, (2) describe ectomycorrhizal morphological types formed on valley oak seedlings, and (3) determine whether seedling growth is enhanced more by transfer of natural valley oak stand soils than agricultural field soil. Sterilized and pasteurized soil controls were used to distinguish growth effects of biological mechanisms (i.e. mycorrhizal formation) from those caused by physical and chemical properties of the soil amendments.

Materials and methods

Field site

The Nature Conservancy Cosumnes River Preserve, located near Galt, California, contained valley oak series vegetation (Sawyer and Keeler-Wolf 1995), here called valley oak riparian forest and valley oak riparian woodland. The composition of the forest resembled the plot-based description of Valley Oak Forest by Conard et al. (1977) with an overstory composed primarily of valley oak and Oregon ash *(Fraxinus latifolia* Benth.). The understory included California grape (*Vitis californica* Benth.), Himalayan blackberry (*Rubus discolor* Weihe & Nees), poison oak (*Toxicodendron diversilobum* (Torrey & A. Gray) E. Greene), and other native and non-native shrubs, forbs, and grasses. The woodland overstory was primarily composed of young valley oaks with an understory of *Leymus triticoides* (Buckley) Pilger and *Carex praegracilis* W. Boot. This woodland area had been recently grazed. Soils from these areas were wet sieved and mycorrhizal root tips and *Cenococcum* sp. sclerotia were observed. The field experiment was conducted in an area where agricultural crops (e.g. tomatoes, sugar beets, rice, and pasture grasses) had been grown for at least 60 years, but the area was probably once populated by valley oaks (Dr. Richard Reiner, Nature Conservancy, personal communication).

The valley oak stands and the nearby agricultural field site were located in an unsectionalized area of R5E T5N Bruceville 7.5″ Quadrangle between 38°15′0″ and 38°22′30″ N latitude and between 121°22′30″ and 121°30′0″ W longitude. The "tall forest", where the oak forest soils were collected, extended approximately 1.1 km north from the confluence of Grissley Slough and the Cosumnes River. The valley oak stand where oak woodland soils were collected extended along the west side of an unnamed slough that delineates the western edge of the "tall forest". The experimental field plot was located southeast of the intersection of Bruceville Road and Desmond Road.

Soils

The forest and woodland areas are mapped as Cosumnes silt loam, a fine, mixed, non-acid, thermic Aquic Xerofluvent (Soil Survey, Sacramento County, Calif. 1993). The agricultural field soil is mapped as a Xerarent-San Joaquin complex, 0–1% slopes. Soils in the agricultural field ranged from loam to silt loam to clay loam rather than the sandy loam which dominates the map unit soil description (Table 1). Soils were analyzed for (1) sand, silt and clay content in soil suspension by hydrometer (Gee and Bauder 1979), (2) CEC by barium acetate saturation and calcium replacement (Rible and Quick 1960), (3) total Kjeldahl nitrogen (Isaac and Johnson 1976; Carlson 1978), (4) KCl-extractable phosphate (Olsen et al. 1954), (5) pH (2:1 soil:water), and (6)% organic matter (modified Walkley-Black, Nelson and Sommers 1982). Analyses were made for representative blocks in the field experiment as well as for soil samples collected from the oak forest and woodland. All analyses except pH were carried out by the Division of Agricultural and Natural Resources Laboratory, University of California, Davis.

In November 1994, soils were collected from three areas: (1) all 10 blocks in the agricultural field, and along 300-m transects in the (2) oak riparian forest and (3) oak woodland. In the agricul-

Table 1 Characteristics of soils collected from an agricultural field, a valley oak woodland, and a valley oak forest. Values and standard deviations (*SD*) for agricultural field soil are means from single measurements from each of 5 or 10 blocks. Values for woodland and forest soils are means of 3–4 measurements of bulked samples

	Agricultural field soil		Woodland soil		Forest soil	
	Mean	SD	Mean	SD	Mean	SD
pH (2:1, water:soil)	7.3	0.68	5.3	0	5.1	0.071
CEC, meq/100 g soil	28	1.5	37	0.76	36	0.76
Extractable P, ppm	21	4.5	22	0.82	31	0.58
Total Kjeldahl N, %	0.068	0.024	0.24	0.023	0.21	0.027
Organic Matter, %	1.6	0.66	4.9	0.052	4.4	0.14
Texture, %						
sand	23	4.3	11	0.58	7.3	3.2
silt	49	3.3	64	2.1	63	2.9
clay	28	1.6	25	2.6	29	0.58

tural field, randomly selected samples (1.5 l volume) were collected. In the forest and woodland, every 20 m, a 50-cm^2 area of vegetation was clipped, loose surface litter was removed, and soil was collected to a depth of 30 cm from a randomly selected spot within 5 m of the nearest oak tree. The collections of each soil type were bulked and homogenized. For the field experiment, forest soil was steam sterilized for 3 h. For the greenhouse experiment, a different soil sterilization technique was used. Forest soil, woodland soil, and agricultural field soil were oven pasteurized (70 °C) and allowed to stand for 24 h before repeating the process twice. The soil was moistened between heatings to promote germination of microbial propagules. In the greenhouse experiment, the pasteurized soils were dried, broken up, and mixed with a non-sterile commercial potting mix of fir bark, vermiculite, and sphagnum moss.

Acorn collection and preparation

In the fall of 1994, valley oak acorns for the field and greenhouse experiments were collected at the Cosumnes River Preserve from approximately 60 trees. Acorn collections were bulked before random selection of experimental acorns. After recording individual acorn weights and surface sterilizing in 10% bleach for 7 min, acorns were planted in the field or in pots in the greenhouse.

Field study

The field study included three soil treatments: A agricultural field soil, F forest soil, and Fs steam-sterilized forest soil. This study was carried out in the experimental field of a larger study on effects of flooding and weed suppression on revegetation of native shrub and tree species; both studies were established in the same year. The experimental field consisted of 20 contiguous plots, each over 16 m × 22 m. Soil N and P measurements coinciding with visual observation of soil color differences confirmed that heterogeneity existed in the field. The experimental design for this study consisted of 10 non-contiguous 8 m × 11 m plots, chosen according to the blocking and treatment constraints of the larger experiment. Each plot was considered one block so that the experiment was a randomized complete block design with 10 blocks, 3 treatments per plot, and 7 acorns per treatment per block.

Valley oak acorns were planted 2 m apart around the perimeter of each plot and separated by another seedling, 1 m away, chosen randomly from the following California riparian species: valley oak, Fremont cottonwood (*Populus fremontii* S. Watson ssp. *fremontii*), Oregon ash, box elder (*Acer negundo* L. var. *californicum* Torrey and A. Grey), narrow-leaved willow (*Salix exigua* Nutt.), arroyo willow (*Salix laseolepis* Benth.), blue elderberry (*Sambucus mexicanus* C. Presl.), California button willow (*Cephalanthus occidentalis* L. var. *californicus)*, California rose (*Rosa californica* Cham. & Schldl.), Himalayan blackberry, and California blackberry (*Rubus ursinus* Cham & Schldl.). It is unlikely that the willow, cottonwood, ash, box elder, and rose species listed above, which are reported to be ectomycorrhizal (Trappe 1962), were sources of inoculum. The willows and cottonwoods were planted as cuttings and the seedlings were started in fritted clay or commercial potting soil in the same greenhouses used in the greenhouse study reported here. Ectomycorrhizal roots were never observed on plants in these greenhouses. Acorns were planted in the center of a 1-m^2 area where weeds were excluded by woven, black plastic-fiber weed mats. In early December 1994, planting holes (8 cm diameter, 30 cm deep) were dug and filled with equal parts of treatment soil and the soil removed from the hole. The experimental area was flooded naturally from January through March, then irrigated bi-monthly from June until September, when irrigation was discontinued. In October 1995, stems and leaves of all surviving seedlings were harvested at ground level and leaf area, leaf number, stem height, and dry biomass determined. Leaf area was measured with a LI-COR moving belt area meter (LI-COR Inc. Lincoln, Neb.). Leaves and stems were oven dried (80 °C) to constant weight. Statistical analysis of survival was performed on the percentage of surviving seedlings in each treatment per block.

Root systems of 70 seedlings (approximately half of the surviving seedlings) were randomly selected and excavated by removing a cylinder of soil and roots around each seedling. A heavy-gauge PVC cylinder (15 cm diameter, 30 cm long) was inserted into the soil around each seedling, then removed with a shovel. The cylinder, which had been split in half vertically then secured with hose clamps, could be opened and the root system could be removed. Root systems in soil were stored at 4 °C, then processed over a 3-month period.

Greenhouse study

In November 1994, valley oak acorns were planted in a greenhouse in 30-cm-tall, approximately 3-l volume pots. There were six treatments: A agricultural field soil, F forest soil, W woodland soil, Ap, Fp, Wp with and without oven pasteurization, respectively. Each of the treatment soils was mixed (1:1 v:v) with a non-sterile commercial potting mix of fir bark, vermiculite, and sphagnum moss. Acorns were assigned to a randomized complete block design with six treatments and one seedling per treatment in each of 12 blocks. The blocks were arrayed along a moderate temperature gradient in the greenhouse. For each treatment, a fraction of the soil weight at "field capacity" was estimated to allow for adequate drainage and aeration. Pots were weighed 1–2 times per week and water was added as needed to bring them to the estimated fraction of field capacity. Seedlings were watered with a half-strength modified Hoagland's solution (Epstein 1972), which was further altered to reduce micronutrients to 1/100 strength and to supply nitrogen as 63 ppm N (NO_3^-) and 49 ppm N (NH_4^+). Solution pH was adjusted to 5.8–6.2. Greenhouse temperatures were controlled by fans and an evaporative cooler; daytime temperatures were 28–40 °C over the 1-year period of study. In the winter, a heater maintained temperatures above 10 °C.

In January 1996, greenhouse seedlings were harvested using procedures similar to those used in the field study. Shoots were separated from roots above the point of acorn attachment. The roots were stored at 4 °C, then processed over a 4-month period. Leaf area, stem height, leaf number, leaf, stem, and root biomass, root length, and mycorrhizal infection were measured on harvested seedlings.

Root measurements

Roots from the greenhouse study were soaked in tap water for 10–20 min. Roots from the field had to be soaked overnight because of heavy soil clinging to the root mass. Soil was then removed with a gentle stream of tap water. Only roots attached to the oak root system were collected due to the possibility of roots from other potentially ectomycorrhizal seedling species growing into the experimental oak seedling rooting zone. Percent mycorrhizal infection was assessed before roots were oven dried (80 °C) to a constant weight. In the field experiment, roots were separated into four diameter size classes: < 0.5 mm 'very fine' roots, 0.5–2.0 mm 'fine' roots, 2.0–5.0 mm 'small' roots, and > 5.0 mm 'medium' roots. In the greenhouse experiment, roots were divided into only the three size classes 'very fine', 'fine', and 'small', because few roots were greater than 5 mm. In the field experiment, subsamples of 'very fine' roots for each treatment were combusted in a muffle furnace (550 °C overnight) to obtain an average ash content (Sheldick 1984) and ash-free dry weight was calculated. In the greenhouse experiment, all 'very fine' roots were combusted to obtain ash-free dry weights.

Root length of 'very fine' roots was measured with a Comair Root Length Scanner (Hawker De Havilland, Victoria Ltd., Victoria, Australia). For the field seedlings, 'very fine' root length

was measured for all seedlings. In the greenhouse, 'very fine' roots were subsampled and relationships between ash-free dry weight and root length was determined. The regressions were linear for treatments A, Ap, and Fp, logarithmic for Wp, a power function for W, and exponential for F.

Assessment of mycorrhizal infection

Two methods were used for assessment of mycorrhizal infection. In the field experiment, it was difficult to separate very fine (< 0.5 mm diameter) roots from soil without some breakage of root tips. Intact live root tips were virtually all mycorrhizal; broken roots showed little evidence of infection; however, it was necessary to estimate a % infection level based on all roots, not just intact root tips. Level of infection was visually estimated as % of roots less than 0.5 mm infected with mycorrhizal fungi, regardless of whether root tips were present or not. Roots were examined with a dissecting microscope (× 10.5–60) in a 6- × 6-cm square petri dish, where categorical percent classes (#1–5), by morphological type (morphotype), were estimated for each 1-cm^2 area: 1 = 0–10%, 2 = 10–25%, 3 = 25–50%, 4 = 50–75%, 5 = 75–100%. The median value of each class, for each 1-cm^2 area, was used to calculate average % mycorrhizal infection for each seedling; this average was used in the statistical analyses.

In the greenhouse experiment, where the soil medium could be removed more easily, it was possible to harvest entire root systems. The 'very fine' roots of each seedling were cut into 1- to 3-cm pieces, then subsampled. A total of 100 segments were observed (dissecting microscope, × 10.5–40). The number of infected tips, by morphotype, was counted for each segment. Only root tips with a complete fungal mantle were scored as mycorrhizal. Percent mycorrhizal infection for each seedling was calculated by averaging the % infection of each root segment examined. Statistical analyses were performed on the total % infection values for each seedling and for each different morphotype.

Morphotypes of different ectomycorrhizas were distinguished by color and gross external surface appearance based on criteria of Agerer (1991). Each morphotype was examined under a light microscope after clearing and staining with trypan blue (Phillips and Hayman 1970; Brundrett 1994) to confirm the presence of a Hartig net and/or mantle. Black and white and color photographs of whole mycorrhizal root tips were made.

Statistical analysis

Treatment effects on oak growth and ectomycorrhizal parameters were analyzed using the SAS general linear model (proc glm) for analysis of variance with a blocking factor included in all models (SAS 1989). Type III hypothesis testing was used and the significance level for all tests was $P \leq 0.05$. All data were first analyzed with analysis of covariance (ANCOVA) with acorn biomass as the covariate. If the covariate term in the model was significant, ANCOVA was used to analyze the data. If the covariate term in the model was not significant, analysis of variance (ANOVA) was used. The absence of covariate by treatment interactions was used to presume homogeneity of slopes in the ANCOVA models. No interactions were found. For the subset of seedlings for which both roots and shoots were harvested, mean acorn biomass was, by chance, significantly greater for seedlings grown in forest soil than for seedlings grown in agricultural field soil. This is a situation in which ANCOVA can be used to guard against mistaking covariate effects for treatment effects. Thus, although a significant treatment by block interaction term for acorn biomass indicated that these differences were not true for all blocks, and although not all measurements were significantly correlated with acorn biomass, ANCOVA was applied to all measurements in this data set.

For the greenhouse data, a two-factor ANOVA was applied to the data with soil type (agricultural field, forest, and woodland) and soil treatment (pasteurized or non-pasteurized) as the two factors. A single-factor ANOVA, combining soil type and soil treatment into six treatments, was also performed on the greenhouse data.

Mean separation techniques depended on the type of analysis. When ANOVA was used, the Ryan-Einot-Gabriel-Welsch Multiple F Test was used to determine significant differences among treatment means. When ANCOVA was used, least square means, corrected for experiment-wise error, were used to determine significant differences among treatment means.

Residuals were examined for consistency with model assumptions and Levene's test ($P \leq 0.05$) was applied to test for similarity in residuals among treatments. With the exception of some mycorrhizal infection measurements and a few measurements in the growth data sets, seedling measurements met the general linear model assumptions of normality and homogeneity of variance. Logarithmic, square root, arcsine, reciprocal, and logit ($x/x+1$) transformations failed to bring the offending data into agreement with model assumptions. For these data, the values were ranked and general linear model procedures and mean separation tests described above were applied to the ranked data (Conover and Ohmann 1981); when ranked and non-ranked, or parametric, results were similar, conclusions based on the analyses performed on the parametric data were considered valid (Zar 1996). In some, cases the results using the non-parametric technique did not agree with the analyses performed on parametric data. In all these cases, analyses performed on parametric data yielded more conservative results that those performed on ranked data. All results shown in tables are for analyses performed on parametric data. Treatment by block interaction terms were also included in the models; any interactions found, as well as any discrepancies between the results from the parametric and non-parametric analyses, are noted in data tables and addressed in the text.

The regression procedure (prog reg) in SAS (1989) was used for regression analyses of relationships between mycorrhizal infection and seedling growth parameters. For the field study data, the stepwise option was used to choose among multiple regression models, using $P \leq 0.05$ as the significance level allowed for entry of independent variables into the model. Increasing mycorrhizal infection was weakly ($P = 0.026$, $r^2 = 0.094$), correlated with increasing acorn biomass. Because acorn biomass was included in the multiple regression models, it was important to avoid collinearity between independent variables. The highest value of the condition index, a measure of collinearity, was 6.7 for the model including acorn biomass and mycorrhizal infection as independent variables. A crude criterion for consideration of the presence of collinearity is a value above 30 (Philippi 1993). Thus we assumed that the relationship between acorn biomass and mycorrhizal infection was weak enough that both could safely be included in the models.

Results

Soil characteristics

The forest and woodland soils were generally more fertile than the agricultural field soil because of higher % organic matter, greater CEC, higher extractable P, and higher total Kjeldahl N (Table 1). The soils had similar amounts of clay but the forest and woodland soils had higher silt contents than the agricultural field soil.

Descriptions of mycorrhizal morphological types

Eight different ectomycorrhizal morphotypes were observed on valley oak seedling roots; pictures of six of

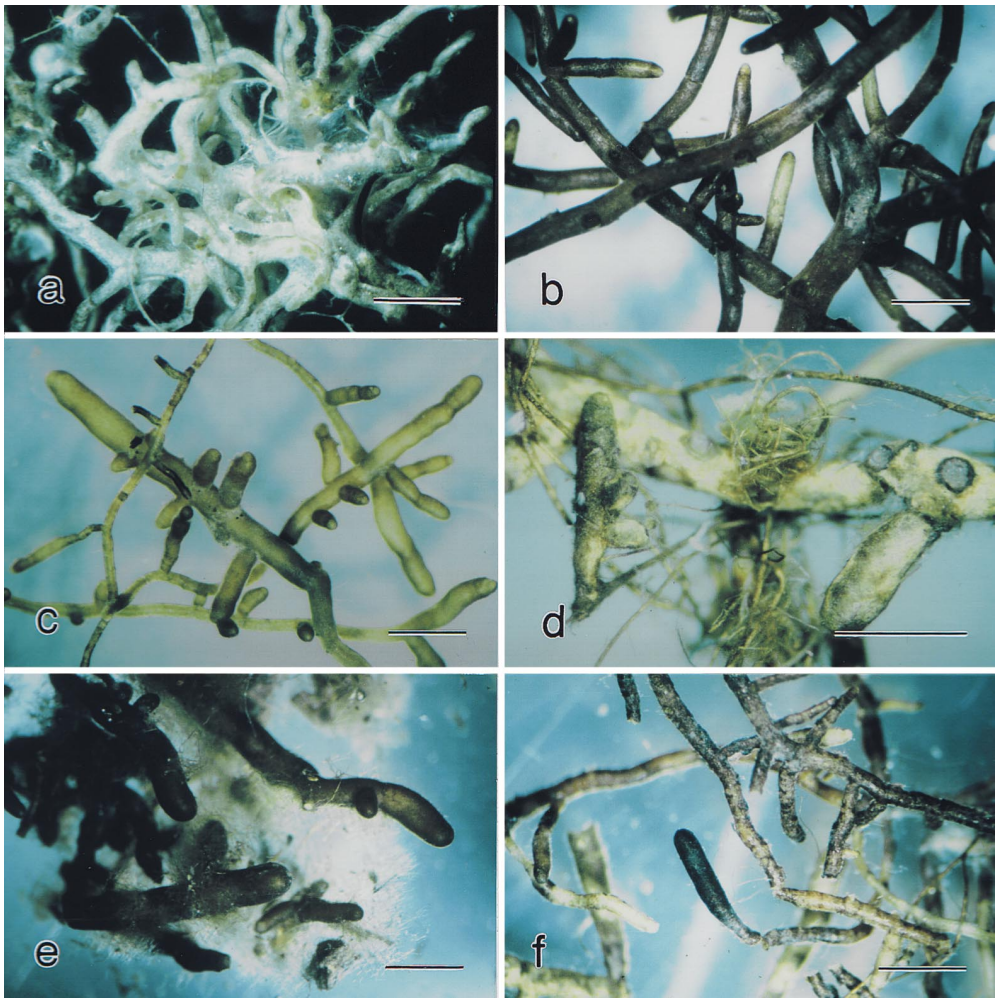

Fig.1a–f Photomicrographs of selected mycorrhizal roots of valley oak seedlings. See results section for descriptions. **a** roots of a field-grown seedling. **b–f** roots of greenhouse-grown seedlings. **a** white, **b** dark brown, **c** light tan, **d** golden-brown, **e** woolly brown, **f** brown-black; *bars* 1 mm

these types are shown in Figure 1. Two types, light brown and black, were not photographed. All eight types are briefly described below; the descriptions follow Agerer's (1991) protocol, although include far fewer parameters. Because only a few parameters were used for descriptions, color is the only difference between the descriptions of the light tan, dark brown, and light brown types and further work needs to be done to confirm the differences between these types (see Discussion section). Additionally, because observations were made using a tungsten light source, color names may not be comparable to those made by Agerer , who recommended daylight quality light. All eight types were found on greenhouse-grown valley oak roots, while only four of the eight types (white, light tan, dark brown and black) were found on field-grown seedlings.

(a) *White* Irregularly pinnate mycorrhizal systems with bent to tortuous unramified ends with mantle surface visible; mantle surface silvery and wooly; rhizomorphs abundant, scarcely ramified, with visible margins, occurring at bases of mycorrhizal systems with distinct connections to the mantle; roots from the field study.

(b) *Dark brown* Monopodial pinnate mycorrhizal systems with straight to slightly bent unramified ends with mantle surface visible; mantle surface smooth with no rhizomorphs or emanating hyphae; roots from the greenhouse study.

(c) *Light tan* Monopodial pinnate mycorrhizal systems with straight to slightly bent unramified ends with mantle surface visible; mantle surface smooth with no rhizomorphs or emanating hyphae; roots from the greenhouse study.

(d) *Golden brown* Monopodial pinnate mycorrhizal systems with straight to slightly bent unramified ends with mantle surface visible; shiny, densely grainy mantle surface; rhizomorphs abundant, occurring at bases of mycorrhizal systems, with a distinct connection to the mantle, with distinct margins, and with visible ramifications; roots from the greenhouse study.

(e) *Wooly brown* Monopodial pinnate mycorrhizal systems with bent unramified ends; mantle surface visible; very densely grainy mantle surface; emanating hyphae occurring in dense clumps; roots from the greenhouse study.

(f) *Brown-black* Simple mycorrhizal systems with straight unramified ends and with mantle surface

visible; densely grainy, with no rhizomorphs but with few, single, dark brown emanating hyphae; roots from the greenhouse study.

(g) *Light brown* Monopodial pinnate mycorrhizal systems with straight to slightly bent unramified ends with mantle surface visible; mantle surface smooth with no rhizomorphs or emanating hyphae; roots from the greenhouse study.

(h) *Black* Simple mycorrhizal systems with straight unramified ends and with mantle surface visible; densely grainy, with no rhizomorphs; roots from the greenhouse study.

Field study: mycorrhizal infection

In the field study, total average % infection appeared higher in the forest soil treatments (F = 18%, Fs = 20%) than in the agricultural field soil treatment (A = 10%) (Table 2), but differences were not statistically significant, possibly because of the high variability in percent infection among seedlings (Fig. 2a–d). When % infection was categorized by mycorrhizal morphotype, the data in Table 2 show that the dark brown type did not occur on the A or Fs seedling roots and the black type did not occur on Fs seedlings. Because infection levels were very low (<1%) for the dark brown and black types, it is difficult to know if the presence or absence of these two types was significant. For both the light brown and dark brown types, there were no significant differences among the treatments, although the result from the non-parametric analysis was that F seedlings were more infected with light brown and dark brown types than the A and Fs seedlings. There were no differences among treatments for the white type, which was generally the most abundant. A and Fs treatment seedlings had kinds and numbers of types similar to the F treatment seedlings, indicating that sterilization was probably effective in reducing ectomycorrhizal propagules in the forest soil, and that the source of ectomycorrhizal infection in the Fs seedlings was primarily from the agricultural field soil. There was higher average number of mycorrhizal morphological types found on F seedlings (2.4 types) than on Fs or A seedlings (1.2 and 1.3 types, respectively).

Field study: survival and shoot growth

The data in Table 3 show that survival was higher for the seedlings treated with agricultural soil (71%) and sterilized forest soil (69%) than non-sterilized forest soil (53%). Shoot growth was significantly higher after addition of non-sterilized forest soil to planting holes than agricultural field soil. Seedlings grown in forest soil had greater leaf area, stem biomass, and leaf biomass than seedlings grown in agricultural soil, while seedlings grown in sterilized forest soil had intermediate values. Differences among treatment groups for shoot data for the subset of seedlings whose roots were harvested were not significant.

Field study: root growth

Most of the valley oak roots were in the large-diameter size classes, 2–10 mm, in all treatments (Table 4). Most differences in root biomass among treatments were not significant. One exception was that Fs seedlings allocated more biomass to 'medium' roots than A seedlings. However, the treatment by block interaction for both 'medium' root biomass and 'medium' root biomass as % of total root biomass indicated that in two blocks, A seedlings were larger for these parameters. With the interactions taken into account, we concluded that both A and Fs seedlings allocated more resources to root growth. The root-to-shoot ratio (R/S) of A seedlings was higher than of F seedlings, although root and shoot biomass as % of total seedling biomass were not significantly different across treatments.

Field study: seedling growth and mycorrhizal infection

Although absolute increases in seedling biomass, shown for shoot and root biomass, were more strongly related to acorn biomass than mycorrhizal infection, allocation of biomass was most strongly related to mycorrhizal infection (Table 5). Seedling R/S (all treatments combined) decreased with increasing mycorrhizal infection (Fig. 2d). Figures 2a–c show the relationship between R/S and mycorrhizal infection for each

Table 2 Field study: effect of three soil treatments on development of mycorrhizal morphotypes on roots of valley oak seedlings. Percent infection data are means of observations on 17–22 seedlings per treatment. Values with different letters within a row are significantly different, $P \leq 0.05$. Statistical analyses are described in the Methods section

Soil treatment	Agricultural field soil	Sterilized forest soil	Non-sterilized forest soil
Mycorrhizal type, % infection			
White	8.2a	18a	10a
Light brown[a]	2.0a	2.0a	5.9a
Dark brown[a]	0a	0a	0.97a
Black	0.024ab	0a	0.50b
Total	10a	20a	18a
Average number of types	1.3a	1.2a	2.4b
Total number of types	3	2	4

[a] Data distribution violated ANOVA assumptions of normality and homogeneity of variance, and results of analysis performed on rank-transformed data differed from those performed on parametric data

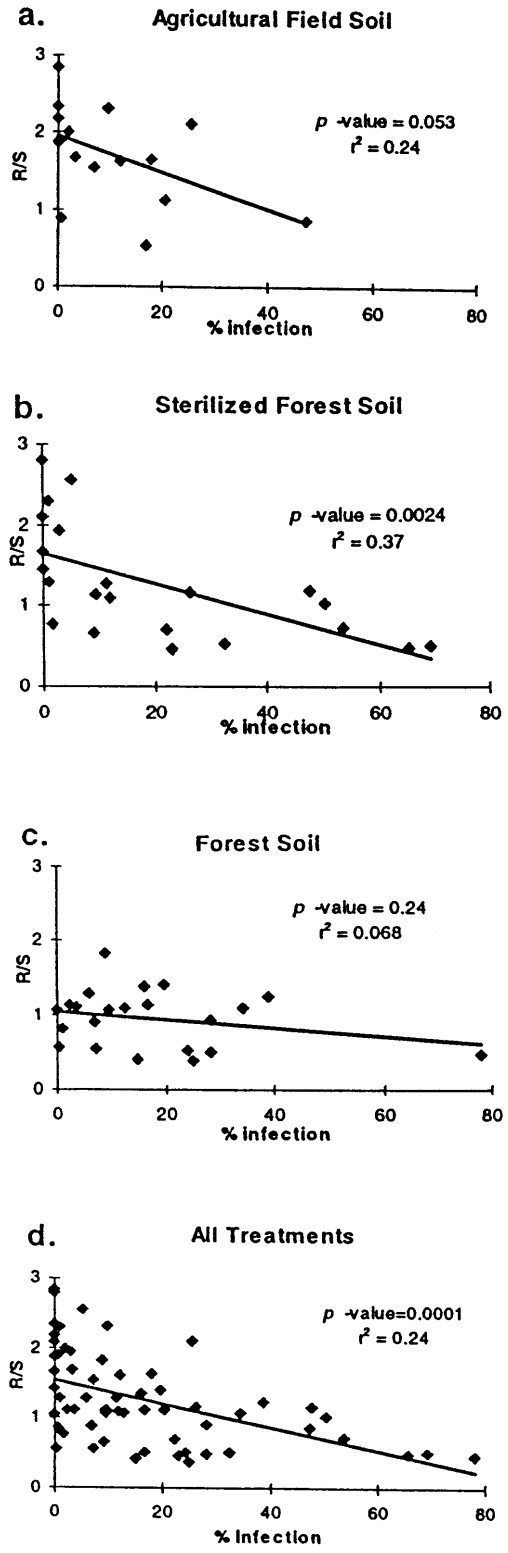

Fig. 2a–d Regressions of root-to-shoot ratio and mycorrhizal infection for seedlings grown in **a** agricultural field soil, **b** sterilized forest soil, **c** non-sterilized forest soil, **d** all data combined. r^2 values are not adjusted

treatment. The relationship was highly significant for seedlings grown in sterilized forest soil, marginal for seedlings grown in agricultural soil, and not significant for seedlings grown in non-sterilized forest soil. The mycorrhizal infection data explained 37% of the variation in the root-to-shoot ratio of the Fs seedlings (Fig. 2b).

Greenhouse study: mycorrhizal infection

Transfers of forest and woodland soils to the growth medium dramatically increased percent mycorrhizal infection on valley oak seedlings and also increased the number of mycorrhizal morphotypes from three to eight (Table 6), compared with transfers of agricultural field soil. Pasteurization reduced both mycorrhizal abundance and diversity to very low levels in woodland and forest soil treatments. The four dominant morphotypes for both forest and woodland soils were white (6.9%), light brown (7.5%), light tan (7.7%), and dark brown (8.8%). Two types, golden-brown (11%) and wooly brown (11%), were dominant only in forest soil. The only morphotype present in all treatments was the white type. There were no differences in white type infection among treatments, although the result of the analysis performed on ranked data was that W seedlings were more infected with the white type than Wp and Ap seedlings. The white type probably was not an airborne or potting soil contaminant in the greenhouse because it was not found on other valley oak seedlings in the same greenhouse, growing in potting soil only. The analysis performed on ranked data for the light tan and light brown types also yielded a less conservative result than for the parametric data (Table 6), that W and F seedlings were more infected with these types than seedlings in all other treatments. The average number of mycorrhizal types per seedling was greater for seedlings in the W and F treatments than both the A treatment and all pasteurized treatments.

Greenhouse study: shoot and root growth

A two-factor analysis of greenhouse data showed that growth was higher for seedlings grown with pasteurized soils than non-pasteurized soils. Further, seedlings grown in woodland soil and/or forest soil were larger than those grown in agricultural field soil and/or forest soil. (Table 7). There were two major exceptions to the results of the two-factor analysis. First, root length and specific root length ('very fine' size class) of seedlings grown in agricultural field soil were higher than for those grown in woodland and forest soil. Secondly, % 'very fine' root biomass was higher for non-pasteurized than pasteurized treatments. The two-factor analysis also showed that the root-to-shoot ratios of agricultural soil-grown seedlings were higher than for woodland soil-grown seedlings.

Table 3 Field study: survival and shoot parameters for 135 valley oak seedlings; 70 acorns were planted for each treatment. Values with different letters within a row are significantly different, $P \leq 0.05$. Statistical analyses are described in the Methods section

	Agricultural field soil	Sterilized forest soil	Non-sterilized forest soil
Number of seedlings	50	48	37
Survival, %	71a	69a	53b
Acorn biomass, g	5.3a	6.0a	6.0a
Stem height, cm	21a	26b	34b
Leaf number	25a	36a	43a
Leaf area, cm^2	107a	168ab	208b
Shoot biomass, g/seedling			
Stem	1.1a	1.9ab	2.3b
Leaf	1.1a	1.7ab	2.1b
Total shoot	2.2a	3.6ab	4.4b

Table 4 Field study: root parameters for small data set of 61 valley oak seedlings. Values with different letters within a row are significantly different, $P \leq 0.05$. *Asterisks* signify significant treatment by block interactions. Statistical analyses are described in the Methods section

	Agricultural field soil	Sterilized forest soil	Non-sterilized forest soil
Number of seedlings	17	22	22
Acorn biomass*, g	4.7a	5.9ab	6.4b
Root biomass by root diameter class, g/seedling			
Very fine, 0–0.5 mm	0.13a	0.16a	0.20a
Fine, 0.5–2.0 mm	0.20a	0.24a	0.28a
Small, 2.0–5.0 mm	1.7a	1.3a	1.5a
Medium*, 5.0–10.0 mm	0.91a	3.7b	2.6ab
Total Root	2.9a	5.4a	4.6a
Root biomass by root diameter class, % of total root			
Very fine	4.7a	3.5a	4.6a
Fine	7.8a	6.0a	7.5a
Small	64a	29b	42ab
Medium*	24a	61b	46ab
Root biomass, % of total seedling	60a	52a	47a
Root-to-shoot ratio	1.7a	1.3ab	0.94b
Very 'fine' root length, m	8.5a	9.0a	10a
Specific 'very fine' root length, m/g	71a	58a	53a

Table 5 Field study: multiple regression statistics for models of seedling growth response as functions of mycorrhizal infection and acorn weight; *na* signifies variable not allowed into model. Statistical analyses are described in the Methods section

Dependent variable	Independent variable entered into model	Order of entry into model	[a]Model r^2	[b]*P*-value	Relationship
Shoot biomass	Acorn weight	1	0.23	0.0001	positive
	Mycorrhizal Infection	2	0.32	0.0066	positive
Root biomass	Acorn weight	1	0.29	0.0001	positive
	Mycorrhizal Infection	2	na	–	–
Root-to-shoot ratio	Mycorrhizal Infection	1	0.24	0.0001	negative
	Acorn weight	2	0.27	0.13	negative
Shoot biomass, % of total	Mycorrhizal Infection	1	0.26	0.0001	positive
	Acorn weight	2	0.31	0.047	positive
Root biomass, % of total	Mycorrhizal Infection	1	0.26	0.0001	negative
	Acorn weight	2	0.31	0.047	negative
Leaf biomass, % of total	Mycorrhizal Infection	1	0.30	0.0001	positive
	Acorn weight	2	na	–	–

[a] The model r^2 for the second independent variable entered describes the fit of the two-variable model

[b] The *P*-value for the second independent variable entered indicates whether its addition explains significantly more variation in the model than without it

Table 6 Greenhouse study: development of mycorrhizal morphotypes on roots of valley oak seedlings grown in three soil mixes without (−) or with (+) pasteurization. Means are of 9–12 seedlings. Values with different letters within a row are significantly different, $P \leq 0.05$. Statistical analyses are described in the Methods section

Pasteurization	Agricultural soil		Forest soil		Woodland soil	
	−	+	−	+	−	+
Mycorrhizal type, % infection						
White[a]	4.6a	1.4a	5.0a	7.9a	7.8a	0.61a
Dark brown	0b	0b	7.5a	0b	10a	0b
Light tan[a]	3.0ab	0b	9.3a	0.22b	6.0ab	2.2ab
Golden brown	0b	0b	11a	0b	2.8b	1.2b
Woolly brown	0b	0b	11a	0b	0.43b	0b
Brown-black	0b	0b	2.8a	0b	2.6a	0b
Light brown[a]	0.44b	0b	5.2ab	0b	9.8a	0b
Black	0a	0a	0.21a	0a	0.28a	0a
Total	8.1b	1.4b	52a	8.1b	40a	4.0b
Average number of types	0.50b	0.11b	4.7a	0.20b	4.0a	0.56b
Total number of types	3	1	8	2	8	2

[a] Data distribution violated ANOVA assumptions of normality and homogeneity of variance, and results of analysis performed on rank-transformed data differed from those performed on parametric data

Table 7 Greenhouse study: effect of six soil treatments on selected measurements of valley oak seedling growth. For treatment means, single-factor ANOVA results are shown and values with different letters within a row are significantly different, $P \leq 0.05$. Direction of significance, $P \leq 0.05$, is shown for two-factor ANOVA analysis of soil type (*a* agricultural soil, *f* forest soil, *w* woodland soil) and soil treatment (*n* non-pasteurized, *p* pasteurized); *ns* signifies analysis was not significant. Statistical analyses are described in the Methods section

Pasteurization	Treatment means						Two-factor ANOVA analysis	
	Agricultural soil		Forest soil		Woodland soil		Type a, f, w	Treatment n, p
	−	+	−	+	−	+		
No. of seedlings	12	9	9	10	12	9		
Acorn biomass, g	6.3a	7.0a	6.9a	5.5a	4.8a	5.8a	ns	ns
Leaf number[a]	9.1c	13bc	9.4c	18a	15abc	22a	w>a	p>n
Leaf area, cm^2	27b	43ab	74ab	50ab	89a	84a	w>a	ns
Biomass, g/seedling								
Leaf[a]	0.23b	0.36ab	0.64ab	0.56ab	0.73a	0.83a	w, f>a	ns
Stem[a]	0.61b	0.91b	0.65b	1.4b	1.1b	2.8a	w>a, f	p>n
Total root	5.4c	9.4ab	4.6c	11ab	6.9bc	11a	ns	p>n
Total seedling	6.2c	11abc	6.6c	13ab	8.7bc	14a	w>a	p>n
Root biomass, g/seedling								
Very fine	0.8a	1.1a	1.0a	1.0a	1.4a	1.2a	w>a	ns
Fine[a]	0.2c	0.5bc	0.1c	1.0a	0.3bc	0.7ab	ns	p>n
Small	4.4c	7.7ab	3.5c	8.8a	5.1c	8.8a	ns	p>n
%, Very fine	20abc	12bc	21a	9.6c	21ab	11bc	ns	n>p
%, Total roots	83a	87a	79a	86a	79a	77a	ns	ns
Root length, m	86b	141a	57b	66b	65b	72b	a>w, f	p>n
Sp. root length, m/g	113b	128a	61c	66c	47d	69c	a>w, f	p>n
Root-to-shoot ratio	8.3a	7.8ab	4.1bc	7.2abc	4.1c	4.6abc	a>w	ns

[a] Data distribution violated ANOVA assumptions of normality and homogeneity of variance, and results of analysis performed on rank-transformed data differed from those performed on parametric data.

Treatment means and results of single-factor ANOVA on seedling growth data are also shown (Table 7). For the parameters leaf number, leaf and stem biomass, and 'fine' root biomass, where parametric and non-parametric analysis differed, neither analysis led to an overall conclusion different from the two-factor ANOVA results. Linear regression relationships of total seedling growth parameters against mycorrhizal infection were not significant.

Discussion

Mycorrhizal infection

Soil transfers in the greenhouse were a good source of ectomycorrhizal inoculum, producing higher infection levels and numbers of mycorrhizal morphotypes on seedlings inoculated with non-pasteurized forest and

woodland soils than those inoculated with the other soils. Because numbers and types of mycorrhizae on A seedlings in the greenhouse were similar to those on A seedlings in the field, the greenhouse results approximately represented field conditions for the agricultural soil. Although it is very possible that mycorrhizal types growing on W and F seedlings in the greenhouse did not reflect the total number of ectomycorrhizal types potentially living in these soils, greenhouse soil transfer results strongly suggest that mycorrhizal diversity and abundance is higher in the valley oak forest and woodland than in the agricultural field.

For the F seedlings in the field study, it is unclear to what extent the non-sterilized forest soil acted as a source of inoculum. Because the white and light brown types were abundantly present on the roots treated with forest soil in the greenhouse, it is equally possible that the propagules came from the forest soil inoculum or from the agricultural field. One soil transfer study reported the appearance of an ectomycorrhizal morphotype on seedlings grown in transfer soil versus site soil (Amaranthus and Perry 1989) but it is not clear that soil transfers act in this way in general (Colinas et al. 1994). This is an example of the difficulty of inoculating fields with non-indigenous ectomycorrhizal fungi and, furthermore, distinguishing the difference between indigenous and introduced fungi on the roots (Bledsoe 1992).

We assume that the types in the greenhouse and field studies with the same color characterizations are similar. However, this determination was based only on color differences and visual differences in gross morphology. Color characterizations of fresh material under daylight and quantification of gross morphological characteristics such as size, examinations of mantle preparations, chemical tests, and DNA characterization are required to be more certain of differences among ectomycorrhizal morphotypes (Ingleby et al. 1990; Agerer 1991; Goodman et al. 1996).

Field study: survival and seedling growth

Although there might have been some stimulation of seedling growth by the addition of non-sterilized forest soil, survival was greatest for seedlings in the A and Fs treatments (Table 3). This suggests that there were pathogens present in the forest soil which inhibited germination or killed the very young seedlings. Once the seedlings survived these early-colonizing pathogens and established a root system, the growth-promoting factors in the forest soil could become established.

R/S ratios were higher for the A seedlings than for F seedlings and there was a slight trend towards increased allocation of root biomass in the A and Fs seedlings (Table 4). For the larger set of shoot data only, an increase in shoot biomass and leaf area occurred with the addition of forest soil (Table 3). It is possible that the same root-to-shoot adjustments occurring among treatments for the smaller, whole seedling data set were occurring for this larger set of seedlings. An increase in shoot growth at the expense of root growth is a classic response to increased fertility and improved nutrient acquisition (Marschner 1995). Because differences in these parameters were not detected between F and Fs seedlings or between A and Fs seedlings, it is possible that these increases were due solely to the higher fertility and other shared factors of the F and Fs soils (Table 1); the stem height data support this conclusion (Table 3). However, because differences in these measurements were generally only detected between A and F seedlings, it is also possible that there was a biological factor present in the non-sterilized forest soil promoting nutrient acquisition for the F seedlings. One such biological factor could be the increased average number of mycorrhizal types on F seedling roots compared with A and Fs seedlings. Perhaps the combination of fungal types, with different functional capacities, led to improved plant response. The general class of plant growth-promoting rhizobacteria, the presence of free-living nitrogen fixers, or nutrient enrichment from microbial cycling are other candidates for this biological factor (Azcon-Aguilar and Barea 1992).

Valley oak seedlings appeared to be responsive to the effects of mycorrhizal infection at these nutrient levels. Figures 2a–c demonstrate that the R/S ratio decreased with increasing mycorrhizal infection, although the relationship was not significant for F seedlings (Fig. 2c). Although average infection levels were not significantly different among the treatments, the highest percent infection values did occur on Fs seedlings (Fig. 2b) and the relationship was probably detected most clearly in this set of seedlings. In situations where nutrients are limiting for non-mycorrhizal plants, mycorrhizal infection can act to increase nutrient acquisition (Marschner 1995). At the same time, the fungal infection can be an increased sink for photosynthates, possibly due to increased fungal respiration rates (Rygiewicz and Andersen 1994). This fungal demand can stimulate photosynthetic rates (Rosseau and Reid 1990; Conjeaud et al. 1996), but can also exert a carbon drain on the plant, reducing carbon to roots (Dosskey et al. 1990; Coalpert et al. 1996). In this study, it appears that mycorrhizal infection neither substantially enhanced nor depressed overall valley oak seedling growth, but acted to increase carbon allocation to shoots (Tables 3, 5). It is possible that photosynthesis was stimulated either by increased mineral uptake or by increased sink demand, thereby increasing photosynthate available for shoot growth. However, because there was no increased allocation of photosynthate to roots such that root growth was not substantially increased, R/S decreased with increasing mycorrhizal infection.

Greenhouse study: seedling growth

Seedlings grown in pasteurized woodland and forest soils were larger than in other treatments (Table 7, two-way ANOVA results). Sterilization of soil for greenhouse use is a routine practice for the elimination of soil-borne plant pathogens (Lawrence 1956). Although pathogenic infection of roots was not observed, growth suppression can be caused by "minor pathogens" which infect and parasitize surface cells (Katan 1996), or by microorganisms which produce toxins and inhibit growth (Bolton et al. 1989). Soil sterilization can also affect soil physical and chemical properties. Both autoclaving and applying dry heat to soil has been shown to increase plant-available N and P and to produce growth increases in barley (Jakobsen and Andersen 1982). However, N and P measurements were made on a sample of each of the pasteurized and non-pasteurized forest and agricultural soils, and variations were minimal between pasteurized and non-pasteurized soils; it is unlikely that pasteurization increased nutrient availability in this study. The process of wetting, drying, and breaking up the pasteurized soils may have created a more aggregated structure than in the non-pasteurized soils. The less-aggregated non-pasteurized soil could have caused oxygen deficiency, so that seedling leaf and root growth in the non-pasteurized treatments was suppressed (Marschner 1995).

The secondary result of the two-way ANOVA, that woodland and forest soil treatments increased growth of valley oak seedlings more than the agricultural field soil treatments, was probably due to the beneficial physical and chemical characteristics of the soils (Table 1). The exceptions to the increased growth of seedlings in forest and woodland soils in the greenhouse were that total root length of 'very fine' roots and specific root length were higher for seedlings grown in agricultural soil than for those grown in either forest or woodland soil (Table 7). These results are a further expression of the less-fertile conditions in the agricultural field because increases in root surface area can be a response to low fertility, especially to P deficiency (Marschner 1995).

Because shoot and root biomass allocation was influenced by mycorrhizal infection in the field study, and because it has been shown that ectomycorrhizal infection can negatively affect shoot and root growth (Coalpert et al. 1996), it is possible that the overall effect of reduced growth of seedlings in the non-pasteurized treatments was due to carbon allocation to mycorrhizas. However, because mycorrhizal infection was uniformly low for seedlings grown in agricultural soil, and Ap seedlings were still generally larger than A seedlings, it seems unlikely that decreased growth in the non-pasteurized treatments was primarily caused by mycorrhizal infection. Separating effects of mycorrhizal fungi from those of other microbes is a fundamental problem in field- and field soil-based mycorrhizal research (Fitter and Garbaye 1994). Because this study did not separate these effects, it is impossible to attribute a negative effect on growth, observed between the pasteurized and non-pasteurized soils, to mycorrhizal infection.

Under greenhouse and other controlled conditions, increased growth of mycorrhizal seedlings over non-mycorrhizal seedlings has been reported for several oak and oak-related species (Daughtridge et al. 1986; Newton 1991; Tam and Griffiths 1994). It is less clear whether mycorrhizal inoculum enhances growth of oaks inoculated with mycorrhizas in the nursery and then outplanted in the field (Parker et al. 1986), or of mycorrhizal oak seedlings in natural systems (Newton and Pigott 1991). However, it has been noted that the adaptive advantage of the symbiosis to the plant may not always be expressed through growth parameters such as biomass and height (Harley 1989). Measures such as establishment, survival, and reproductive success may be more valuable indices of adaptive advantage (Francis and Read 1995; Newsham et al. 1994).

Mycorrhizal diversity

Other findings not directly related to the originally stated objectives concern the mycorrhizal diversity data. In both studies, the white type was a dominant morphotype; infection level of this type was not significantly different across treatments. It was the dominant type in the agricultural soil treatments and it also appeared to be resistant to pasteurization in the greenhouse experiment. Although average infection levels were low, the agricultural field soil was capable of infection, mostly from the white type, in valley oak seedlings in both the field (10%) and greenhouse (8%) experiments, despite having been in cultivation for at least 60 years. The white type may produce abundant propagules, enabling it to colonize the agricultural field. Alternatively, or in addition, it may produce very resistant propagules, enabling it to survive cultivation (and pasteurization). The colonization ability of this fungus is probably also enhanced by its production of abundant rhizomorphs (Fig. 1a). These results suggest that this fungus may play a unique role in the ecology of valley oak riparian systems, for instance as an early-colonizer after disturbance.

There were few differences between the types found on the W and F seedlings in the greenhouse experiment. Although the oak woodland was younger than the oak forest, and recently grazed, it had apparently maintained ectomycorrhizal diversity compared with the less-disturbed, adjacent oak forest. One exception was that the F seedlings were more infected with the woolly (11%), and golden-brown (11%) types than the W seedlings (2.8%, 0.43%, respectively). Perhaps these types are somehow adapted to the more complex soil environment of the valley oak forest, with its more diverse plant community.

In the field study, roots of seedlings in the F treatment had, on the average, higher numbers of mycorrhizal types than seedlings in A and Fs treatments. While this could be attributed to the presence of additional inoculum in the root zone of these seedlings, another explanation might be the stimulating effects of other microorganisms in the non-sterilized forest soil, such as mycorrhization helper bacteria (Fitter and Garbaye 1994).

Ectomycorrhizal morphological diversity is much higher than for the arbuscular mycorrhizae, although it is still uncertain whether the morphological diversity reflects functional diversity (Bruns 1995). Ectomycorrhizal functional diversity within a single plant root system has been suggested as a means by which plants are buffered from changing environmental conditions (Perry et al. 1987). If this functional diversity exists, fungal populations may play a role in regulating plant community dynamics (Allen et al. 1995). Our results suggest that intact riparian valley oak stands, such as those in the Cosumnes River Preserve, act as reservoirs of soil microbial diversity. These reservoirs could be a source of propagules, which, through natural dispersal mechanisms, could assist the efforts to rehabilitate and expand riparian valley oak ecosystems.

Acknowledgements This study was funded by a grant from The Nature Conservancy to C. Bledsoe. The authors wish to thank the following persons: Darlene Chirman, Cathy Millikin, Brendan Ishikawa, Tony Hartshorn, Jennifer Katcher, Sue Mahoney, Jeff Maurer, Wendy Stevens, Kevin Fort, Betsey Beeman, Robert Pickering, Manuel Ortega, and the Nature Conservancy staff at Cosumnes for field assistance, Helen Solanum, Beverley Derish, Nancy Pergam, Robin Miller, Jena Ferrarese, and Kris Lepine for lab. help. Other assistance was provided by Machelle Wilson. JH Richards and KJ Rice provided valuable assistance throughout the project and reviewed the manuscript. The authors are also grateful to David Janos and the anonymous reviewers for their comments on the manuscript.

References

Agerer R (1991) Characterization of ectomycorrhizas. In: Norris JR, Read DJ, Varma AK (eds) Techniques for mycorrhizal research. Academic, San Diego, Calif., pp 26–71

Allen EB, Allen MF, Hlem DJ, Trappe J, Molina R, Rincon E (1995) Patterns and regulation of mycorrhizal plant and fungal diversity. Plant Soil 170:47–62

Amaranthus MP, Perry DA (1987) Effect of soil transfer on ectomycorrhiza formation and the survival and growth of conifer seedlings on old, non-reforested clear-cuts. Can J For Res 17:944–950

Amaranthus MP, Perry DA (1989) Interaction effects of vegetation type and Pacific madrone soil inocula on survival, growth, and mycorrhiza formation of Douglas-fir. Can J For Res 19:550–556

Azcon-Aguilar C, Barea JM (1992) Interactions between mycorrhizal fungi and other rhizosphere organisms. In: Allen MF (ed) Mycorrhizal functioning, an integrative plant-fungal process. Chapman & Hall, New York, pp 163–198

Bolton H Jr, Elliott LF, Gurusiddaiah S, Fredrickson JK (1989) Characterization of a toxin produced by a rhizobacterial *Pseudomonas* sp. that inhibits wheat growth. Plant Soil 114:279–287

Bledsoe CS (1992) Physiological ecology of ectomycorrhizae: implications for field application. In: Allen MF (ed) Mycorrhizal functioning, an integrative plant-fungal process. Chapman & Hall, New York, pp 249–300

Brundrett M (1994) Clearing and staining mycorrhizal roots. In: Brundrett M, Melville L, Peterson L (eds) Practical methods in mycorrhizal research. Mycologue, University of Guelph, Guelph, Ontario, pp 42–46

Bruns T (1995) Thoughts on the processes that maintain local species diversity of ectomycorrhizal fungi. Plant Soil 170:63–73

Carlson RM (1978) Automated separation and conductimetric determination of ammonia and dissolved carbon dioxide. Anal Chem 48:1528–1531

Coalpert JV, Van Laere A, Van Assche JA (1996) Carbon and nitrogen allocation in ectomycorrhizal and non-mycorrhizal *Pinus sylvestris* L. seedlings. Tree Physiol 16:787–793

Colinas C, Molina R, Trappe J, Perry D (1994) Ectomycorrhizas and rhizosphere microorganisms of seedlings of *Pseudotsuga menziesii* (Mirb.) Franco planted on a degraded site and inoculated with forest soils pretreated with selective biocides. New Phytol 127:529–537

Conard SG, MacDonald RL, Holland RF (1977) A short review of the status of riparian forests in California. In: Sands A (ed) Riparian forests in California, their ecology and conservation. Institute of Ecology, University of California, Davis, Calif., 15:47–55

Conjeaud C, Scheromm P, Mousain D (1996) Effects of P and ectomycorrhizas on maritime pine seedlings (*Pinus pinaster*). New Phytol 120:345–351

Conover WJ, Ohmann RJ (1981) Rank transformations as a bridge between parametric and non-parametric statistics. Am Stat 35:124–133

Daughtridge AT, Pallardy SG, Garrett HG, Sander IL (1986) Growth analysis of mycorrhizal and nonmycorrhizal black oak (*Quercus velutina* Lam.) seedlings. New Phytol 103:473–480

Dosskey MG, Linderman RG, Boersma L (1990) Carbon-sink stimulation of photosynthesis in Douglas fir seedlings by some ectomycorrhizas. New Phytol 115:269–274

Epstein E (1972) Mineral nutrition of plants: principles and perspectives. Wiley, New York

Fitter AH, Garbaye J (1994) Interactions between mycorrhizal fungi and other soil organisms. Plant Soil 159:123–132

Francis R, Read DJ (1995) Mutualism and antagonism in the mycorrhizal symbiosis, with special reference to impacts on plant community structure. Can J Bot 73:S1301–S1309

Gee GW, Bauder JW (1979) Particle size analysis by hydrometer: a simplified method for routine textural analysis and a sensitivity test of measurement parameters. Soil Sci Soc Am J 43:1004–1007

Goodman DM, Durall DM, Trofymow JA, Berch SM (1996) A manual of concise descriptions of North American ectomycorrhizas: including microscopic and molecular characterization. Mycologue Publications, Sydney, BC, Canada

Harley JL (1989) The significance of mycorrhizas. Mycol Res 92:129–139

Helm DJ, Carling DE (1993a) Use of soil transfer for reforestation on abandoned mined lands in Alaska. I. Effects of soil and phosphorus on growth and mycorrhizal formation by *Populus balsamifera*. Mycorrhiza 3:97–106

Helm DJ, Carling DE (1993b) Use of soil transfer for reforestation on abandoned mined lands in Alaska. II. Effects of soil transfers from different successional stages on growth and mycorrhizal formation by *Populus balsamifera* and *Alnus crispa*. Mycorrhiza 3:107–114

Ingleby K, Mason PA, Last FT, Fleming LV (1990) Identification of ectomycorrhizas. ITE Research Publication No. 5, Institute of Terrestrial Ecology, HMSO, London, UK

Isaac RA, Johnson WC (1976) Determination of total nitrogen in plant tissue. J Assoc Off Anal Chem 59:98–100

Jakobsen I, Andersen AJ (1982) Vesicular-arbuscular mycorrhiza and growth in barley: effects of irradiation and heating of soil. Soil Biol Biochem 14:171–178
Katan J (1996) Interactions of roots with soil-borne pathogens. In: Waisel Y, Eshel A, Kafkafi U (eds) Plant roots: the hidden half, 2nd edn. Marcel Dekker, New York, pp 811–822
Kropp BR, Langlois CG (1990) Ectomycorrhizas in reforestation. Can J For Res 20:438–451
Lawrence WJC (1956) Soil sterilization. George Allen and Unwin, London
Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic, San Diego, Calif
Marx DH (1977) Tree host range and world distribution of the ectomycorrhizal fungus *Pisolithus tinctorius*. Can J Microbiol 23:217–223
Muick PC (1980) A CA native plant society oak-hardwood policy. Fremontia 18:101–102
Nelson DW, Sommers LE (1982) Total carbon, organic carbon, and organic matter. In: Page AL, Miller RH, Keeney DR (eds) Methods of soil analysis: part 2. Chemical and microbiological methods. Monograph Number 9 (2nd edn). Am Soc Agron, Madison, Wisc, pp 574–577
Newsham KK, Fitter AH, Watkinson AR (1994) Root pathenogenic and arbuscular mycorrhizal fungi determine fecundity of asymptomatic plants in the field. J Ecol 82:805–814
Newton AC (1991) Mineral nutrition and mycorrhizal infection of seedling oak and birch. III. Epidemiological aspects of ectomycorrhizal infection and the relationship to seedling growth. New Phytol 117:53–60
Newton AC, Pigott CD (1991) Mineral nutrition and mycorrhizal infection of seedling oak and birch. I. Nutrient uptake and the development of mycorrhizal infection during seedling establishment. New Phytol 117:37–44
Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Dept Agric Circular No. 939, pp 1–19
Parker WC, Moorhead DJ, Pallardy SG, Garrett HE, Dixon RK (1986) Six-year field performance of container-grown and bare-root black oak seedlings inoculated with *Pisolithus tinctorius* and outplanted on two Ozark clear-cuts. Can J For Res 16:1339–1344
Perry DA, Molina R, Amaranthus MP (1987) Mycorrhizae, mycorrhizospheres, and reforestation: current knowledge and research needs. Can J For Res 17:929–940
Perryess E, Tietje W, Barrett R (1993) Oak woodland riparian habitat: values and management notes. University of California Integrated Hardwood Range Management Program, Berkeley, Calif
Philippi TE (1993) Multiple regression: herbivory. In: Scheiner SM, Gurevitch J (eds) Design and analysis of ecological experiments. Chapman and Hall, New York, pp 183–210
Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–160
Rible JM, Quick J (1960) Tentative methods of analysis for diagnostic purposes. Method S–19:1. University of California Agricultural Experimental Service
Rosseau JVD, Reid CPP (1990) Effects of phosphorous and ectomycorrhizas on the carbon balance of loblolly pine seedlings. For Sci 36:101–112
Rygiewicz PT, Andersen CP (1994) Mycorrhizae alter quality and quantity of carbon allocated below ground. Nature 369:58–60
SAS (1989) Version 6.09. SAS Institute, Cary, NC
Sawyer JO, Keeler-Wolf T (1995) A manual of California vegetation. California Native Plant Society, Sacramento, Calif
Sheldick BH (1984) Analytical methods manual. LRRI Contribution No. 84-30. Research Branch, Agriculture, Canada, p 45/1
Shemakhanova NM (1967) Mycotrophy of woody plants. Institute of Microbiology, Academy of Sciences, Moscow, Russia
Smith F (1977) A short review of the status of riparian forests in California. In: Sands A (ed) Riparian forests in California, their ecology and conservation. Institute of Ecology Pub No. 15, University of California, Davis, pp 1–2
Soil Survey of Sacramento County, California (1993) Soil Conservation Service, US Department of Agriculture, Sacramento, Calif., April 12, 1993
Swiecki TJ, Bernhardt EA (1991) Minimum input techniques for restoring valley oaks on hardwood rangelands. Forest and Rangeland Resources Assessment Program, Dept Forestry and Fire Protection, Sacramento, Calif
Tam PCF, Griffiths DA (1994) Mycorrhizal associations in Hong Kong Fagaceae. Mycorrhiza 4:169–172
Thompson K (1961) Riparian forest of the Sacramento valley, California. Ann Assoc Am Geograph 51:294–315
Trappe JM (1962) Fungus associates of ectotrophic mycorrhizae. Bot Rev 28:538–606
Vogt KA, Publicover DA, Vogt DJ (1991) A critique of the role of ectomycorrhizas in forest ecology. Agric Ecosyst Environ 35:171–190
Zar JH (1996) Biostatistical analysis, 3rd edn. Prentice Hall, Upper Saddle River NJ