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The Effect of Arbuscular Mycorrhizal Fungi on *Bouteloua curtipendula* (Michx.) Torrey in New York

by

Sandra Hecht

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirement for the degree of Doctor of Philosophy, The City University of New York.

2003

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This manuscript has been read and accepted for the Graduate Faculty in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Abstract

The Effect of Arbuscular Mycorrhizal Fungi on *Bouteloua curtipendula* (Michx.) Torrey in New York

by

Sandra Hecht

Advisor- Dwight Kincaid, PhD

Arbuscular mycorrhizal fungi (AMF) are mutualistic with most land plants. The hyphae reaching beyond the rhizosphere transport minerals, particularly phosphorus, to the plant. The host plant supplies the fungi with photosynthate. Field experiments, common garden experiments, and greenhouse experiments were performed to investigate the role of AM fungi in the growth and fitness of *Bouteloua curtipendula* (Michx.) Torr., a grass species rare in New York State.

Nellie Hill, the study site in Dutchess County, yielded two gene pools of *B. curtipendula;* and commercial seed (Prairie Nursery) was obtained from native populations in Nebraska. In long-term factorial experiments, I manipulated AMF colonization using the fungicide benomyl, controlled plant water relations, and varied soil phosphorus availability. I measured various aspects of

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plant growth, size, reproductive output and survivorship as response variables.

The gene pools responded differently to AMF colonization. All had increased growth when fully colonized with AMF and not droughted. When droughted, the Nellie Hill 1 gene pool did not increase growth when fully colonized with AMF, but did respond to additional phosphorous with better survival regardless of mycorrhizal state. It was concluded that Nellie Hill 1 plants benefited from hyphal uptake of phosphorous but may have responded to drought with reduced photosynthesis, and so had no increase of biomass. When droughted and with abundant AMF, the photosynthate drain to the AMF lessened survival so that the plants performed no better than with reduced AMF.

The Nellie Hill 2 gene pool had increased growth and survival with abundant AMF regardless of water availability. When plants had reduced AMF, added phosphorous increased survival. Plants from Prairie Nursery also had increased growth and survival when fully infected, regardless of water availability; however, with reduced AMF, added phosphorous did not increase survival. It was concluded that Prairie Nursery plants benefited from AMF, but not by hyphal uptake of phosphorous. This study

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documents a high degree of poorly understood, intraspecific variability in the dynamics of the plant root-AMF symbiosis.

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ACKNOWLEDGEMENTS

I thank my advisor, Dr. Dwight Kincaid, for his enduring patience and advice, and particularly for his confidence in my research.

I thank The Lower Hudson Chapter of The Nature Conservancy for allowing me to do my field experiment at Nellie Hill in Dover Plains, NY. I thank them for excavating the soil used in my experiments. This project could not have succeeded without their cooperation and assistance.

I thank Dr. Basile for allowing me to use his laboratory, equipment and supplies until other space was available.

I thank Dr. Joseph Morton of INVAM, the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi, for his suggestions on staining the *Bouteloua* roots and for providing me with AM fungal inocula so that I would know what I was looking for.

I am indebted to my cohorts in the Ecology Laboratory at Lehman College for their assistance in carrying gallons of water up the steep climb to Nellie Hill so that I could carry out my field experiment.

I thank Michael Baxter for his superb troubleshooting of whatever problem I came to him with.

I thank David Cain for his assistance and his efficient care of my plants during the greenhouse experiments.

I thank my husband, Michael, and my daughters, Susan and Julia for cheerfully enduring the difficulties my years of study and research have caused them. I also thank them for their unswerving support of my goal. Michael trudged with me to Nellie Hill many times to treat my plots. I particularly thank Susan for giving freely of her time in helping me with the difficult task of harvesting my plants.

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CHAPTER 1

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi form mutualistic associations with most land plants. The external hyphae of the fungi transport minerals, particularly phosphorous, to the plant from beyond the rhizosphere. The AM fungi are also believed to alleviate water stress (Newsham and Watkinson, 1998; Koide, 1993) and to afford protection from root pathogens (Linderman, 2000; Lingua et al, 2002; Abdel-Fattah and Shabana, 2002). The plant, in turn, supplies the fungi with photosynthate as their only source of organic carbon.

The fungal symbiont

A mycorrhiza is an association between a fungus and a plant root, usually providing mutual benefit for both organisms. There are seven known types of mycorrhizae which fit into two broad categories, the ectomycorrhizae and the endomycorrhizae. These two groups differ in numerous ways but the defining one is the extent of hyphal penetration into the plant root (Jeffries, 1987; Morton, 1996). The hyphae of ectomycorrhizae grow between the cells of the outer layer of the cortex. Hyphae of

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endomycorrhizae actually penetrate the walls of the cortical cells.

There is one type of ectomycorrhizae and five types of endomycorrhizae: the arbuscular, ectendo, ericoid, arbutoid, monotropoid and orchidaceous (table 1.1)(Smith and Read, 1997). The ectomycorrhizae and the arbuscular mycorrhizae are the most common mycorrhizal types considering the number of species involved. The ectomycorrhizae are estimated to have ca. 5000 fungal species and ca. 2000 host plants, almost all woody and perennial angiosperms or gymnosperms; arbuscular mycorrhizae - ca. 152 fungal species (Walker and Trappe, 1993) and ca. 300,000 host plants (Jeffries, 1987; Morton, 1996) representing all phyla (Harley and Smith, 1983). The fungi involved in this research form arbuscular mycorrhizae.

Morton and Benny (1990) placed the AM fungi in the class Zygomycetes and the order Glomales. There are 2 suborders, 5 families and 7 genera (Table 1.2)(Morton and Redecker, 2001). Species are described by manner of spore formation and characteristics of layers of the spore wall. Unlike the other mycorrhizal fungi AM fungi appear to have low specificity. They have a wide host range; ca. 152 fungal species are estimated to colonize 80 to 90% of all

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Table 1.1. Types of mycorrhizae. Abbreviations of fungal taxa: Basidiomycetes, Ascomycetes, Zygomycetes; of plant taxa: Bryophyta, Pteridophyta, Gymnospermae and Angiospermae.

	Ecto-mycorrhizae	**Endomycorrhizae**					
	Ecto	Arbuscular (AM)	Ectendo	Arbutoid	Ericoid	Mono-tropoid	Orchid-aceous
Hyphae							
septate	+	-	+	+	+	+	+
aseptate	-	+	-	-	-	-	-
Fungal taxa	Basidio Asco Zygo	Zygo	Basidio Asco	Basidio	Asco	Basidio	Basidio
Fungal species #	Ca. 5,000	Ca. 165					
Plant taxa	Gymno Angio	Bryo Pterido Gymno Angio	Gymno Angio	Ericales	Ericales Bryo	Mono-tropaceae	Orchid-aceae
Plant species #	Ca. 2,000	Ca. 300,000					Ca.17,500

Table 1.2. Classification of arbuscular mycorrhizal fungi

Class Zygomycetes
lack of motile cells, aseptate hyphae (usually), chitinous cell walls

Order Glomales
intraradical hyphae, arbuscules within host cortical cells, obligately asexual spores, unique spore wall structural components

Suborder Glomineae
form vesicles and arbuscules

Family Glomaceae

Genus *Glomus*
spores formed apically from fertile hyphae

Family Acaulosporaceae

Genus *Acaulospora*
spores formed laterally on neck of sporiferous saccule

Genus *Entrophospora*
spores formed within neck of sporiferous saccule

Family Archaeosporaceae

Genus *Archaeospora*
spores with mulitlayered wall formed laterally on neck of sporiferous saccule

Family Paraglomaceae

Genus *Paraglomus*
unique DNA primer sequences

Suborder Gigasporineae
form arbuscules but no vesicles, spores often > 200µ formed on bulbous sporogenous cell

Family Gigasporaceae

Genus *Gigaspora*
inner flexible wall group absent

Genus *Scutellospora*
inner flexible wall group present in spore, germination shield present

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terrestrial plant species (Jeffries, 1987; Trappe, 1987).

The AM fungi are characterized by aseptate hyphae and intracellular arbuscules. Species in the suborder Glomineae also form inter- and intracellular vesicles which are thin-walled, lipid-containing structures believed to function as storage. Until recently AM fungi were known as the vesicular-arbuscular mycorrhizal (VAM) fungi (Sylvia et al, 1997). However, due to the absence of vesicles in the Gigasporaceae, the one family in the suborder Gigasporineae, the fungi are now commonly known as arbuscular mycorrhizal (AM) fungi.

The plant symbiont

The fossil record indicates that colonization of plants by AM fungi is of ancient origin dating back 353-462 Myr (Remy et al., 1994). Rhizoids of *Rhynia* and *Asteroxylon* from the early Devonian were colonized by structures similar to the arbuscules, vesicles and intercellular hyphae of AM fungi. Numerous scientists suggest that ancestral AM fungi were instrumental in the colonization of land by ancient plants (Malloch et al., 1980; Simon et al., 1993; Remy et al., 1994). The fossil record, the origin of the AM fungi and land plants at about the same time (Smith and Read, 1997), and the presence of

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arbuscular mycorrhizae worldwide and their beneficial effect on plant growth and survival help support this hypothesis (Simon et al., 1993).

Although only a small percentage of plant species have been examined for the presence of arbuscular mycorrhizae (Trappe, 1987) ca. 300,000 plant species are believed to form mutualistic associations with the ca. 152 known species of AM fungi. The plants include most families of angiosperms, gymnosperms and pteridophytes (Smith and Read, 1997). In addition, AM fungi colonize the rhizoids of some bryophytes (Pocock and Duckett, 1985; Brundrett, 2002), Psilotales (Bierhorst, 1954; Peterson et al., 1981) and lycopod gametophytes (Gifford and Foster, 1988, Smith and Read, 1997) and sporophytes (Brundrett, 2002).

Certain plant families are considered characteristically 'non-mycorrhizal' as many of their members do not form mycorrhizae, or do so weakly. They include the Brassicaceae, Commelinaceae, Juncaceae, Proteaceae; some members of Capparaceae, Cyperaceae, Polygonaceae, Resedaceae, Urticaceae; and herbaceous members of the Caryophyllales (Amaranthaceae, Caryophyllaceae, Chenopodiaceae, Portulaceae) (Jeffries, 1987).

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The fungal/plant symbiosis

Roots can be colonized from three inoculum sources in soil: spores, previously colonized root fragments and hyphal fragments. Germination from these sources produce limited hyphal growth, approximately 20-30 mm, until successful colonization of a plant root. If this is not attained within 15-20 days hyphal growth from the germinating propagule will stop. Hyphal contact with a root may not be a completely random event as experiments have shown that host plant root exudates stimulate growth and branching of AM hyphae (Giovannetti et al., 1993; Smith and Read, 1997; Nagahashi, 2000).

Upon contact with the root of a host plant, the main hypha (diameter 20-30 µm) produces narrower lateral branches (diameter 2-7 µm) on the root surface. The narrow branches penetrate the root between epidermal cells or through a root hair. The hyphae then grow rapidly from cell to cell in the apoplast of the root cortex. Short hyphal side branches penetrate the cortical cell walls and undergo repeated bifurcation to produce the arbuscules. The plasma membrane of the cortical cell is not penetrated but grows so that it envelops the arbuscule which remains within "an apoplastic compartment" (Smith and Read, 1997). It is believed that the interface between the plasma membrane of

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the fungal arbuscule and the plasma membrane of the plant root cortical cell is the main site for symbiotic nutrient exchange (Allen, 1991; Smith and Read, 1997; Saito, 2000).

After colonization hyphae grow from the root into the soil forming an extensive extraradical mycelium. The external hyphae branch and become progressively narrower until only 2 μm in diameter (Friese and Allen, 1991). These very fine hyphae are able to probe soil pores for mineral absorption. In many species the nutritional gain from AM colonization produces increased growth, reduced root:shoot ratio and increased tissue P concentration. There is also evidence of increased resistance to pathogens and tolerance to water stress, although the mechanisms for these are still in question.

The association between the plant and the colonizing AM fungus is usually a mutualistic one with both parties receiving benefit. There is bidirectional nutrient exchange; the plant receives minerals from the fungus and the fungus receives photosynthate from the plant. However, the association can become parasitic for the plant with above optimum P concentration in the soil or in low sunlight (Smith and Gianinazzi-Pearson, 1988).

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***Bouteloua curtipendula* (Michx.) Torr.**

Bouteloua curtipendula, side-oats grama, is a C_4, warm-season perennial grass that is mycorrhizal dependent in prairie soils, requiring AM fungal colonization to grow to reproductive maturity (Hetrick et al, 1994). It often grows on dry slopes and steep banks in arid or semi-arid places. It outcompetes other prairie grasses in these habitats when droughted or heavily grazed (Weaver, 1954). It is found throughout the United States east of the Rocky Mountains (Hitchcock, 1935) and is common in the West and Southwest where it is an important forage grass. However, in New York State, the northeast edge of its range (Mitchell and Sheviak, 1981), it is rare, known at only five sites (Young, 20003) (fig. 1.1).

Goal of study

This study investigates the effect of arbuscular mycorrhizal fungi on the growth and fitness of *B. curtipendula* as it occurs in New York. Mycorrhizal dependent in the phosphorous deficient prairie soils of the West and Southwest U.S.A., in New York it occurs at sites with adequate phosphorous for plant growth. Since there is a cost to a plant that is colonized by AM fungi the questions are:

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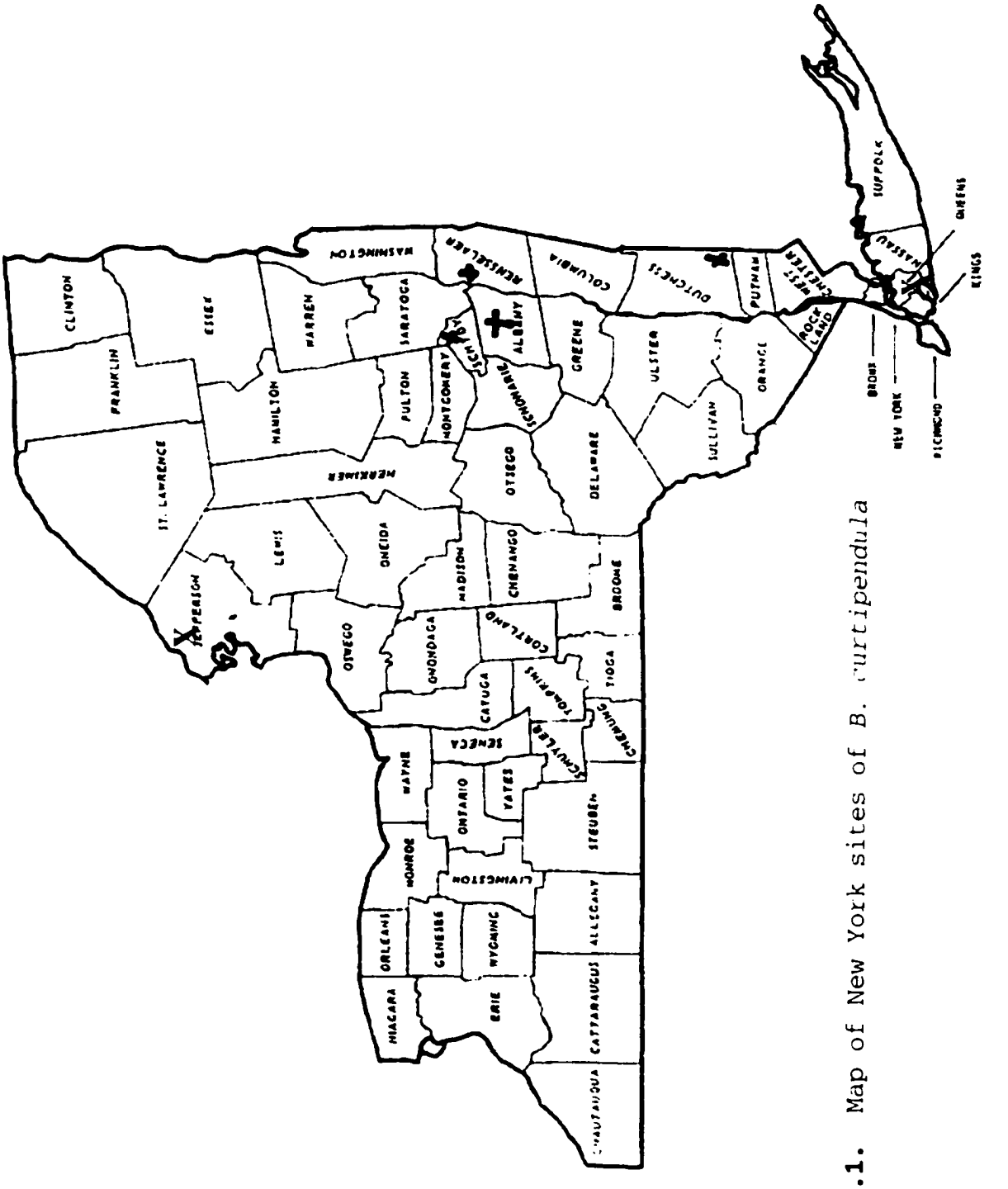

Fig. 1.1. Map of New York sites of *B. curtipendula*

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- Are these fungi beneficial to *B. curtipendula* growing under New York conditions?
- Does the *B. curtipendula* naturally occurring in New York differ in its AM fungal relationships from *B. curtipendula* originating elsewhere?
- Do different gene pools among NY *B. curtipendula* respond differently to AM fungal colonization?

This study seeks to answer these questions.

OVERVIEW OF METHODS USED THROUGHOUT

AM fungal inoculum

The AM fungal propagules that were in the Nellie Hill soil served as the inoculum for the experiments in chapters 2, 3 and 4. The roots and soil from trap plants of *Sorghum bicolor* [L.] Moench augmented the Nellie Hill inoculum in the water deprivation experiment of chapter 5.

Mycorrhizal state

The fungicide, benomyl, ((1-butylcarbamoyl)-2-(benzimidazole) carbamic acid, methyl ester) has been found to be effective in reducing AM fungal colonization without having any direct physiological effects on plant performance (Paul et al, 1989; West et al, 1993;

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Merryweather and Fitter, 1995). To reduce AM fungal colonization of the *B. curtipendula* roots a soil drench of benomyl was applied every two weeks in a concentration of 100 mg of formulated product/kg of soil. Treated plants are designated as "myc(-)." An equal amount of water was applied as a control to the "myc(+)" plants.

Gene pools/Seed source

Seed from three different seed sources of *B. curtipendula* was used. The seed sources are referred to as "gene pools" in this study. *B. curtipendula* is the dominant plant in a section of the Nellie Hill pasture in Dover Plains, NY, one of the five sites of the species' occurrence in NY. Seed was collected from *B. curtipendula* plants approximately 192 m apart at the high (530 ft above sea level) and low (490 feet) ends of that section of the pasture and designated as Nellie Hill 1 gene pool and Nellie Hill 2 gene pool, respectively (fig. 2.1).

The third gene pool was a commercial seed obtained from Prairie Nursery (Westfield, WI 53964) and is called Prairie Nursery seed in this study. This seed originated from native stands in northeastern Nebraska.

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Nellie Hill soil

Nellie Hill soil is classified as FcC - Farmington Galway complex (1991 soil survey of Dutchess County, National Resource Conservation Service [NRCS]). It is described as rolling, very rocky, consisting of "shallow, well and somewhat excessively drained Farmington soils and moderately deep, well and moderately well drained Galway soils. It is on hilltops, narrow ridges, and side slopes that are underlain by folded limestone bedrock."

Two samples of Nellie Hill soil taken 10 m apart from areas of *B. curtipendula* were mixed and submitted for analysis (Rutgers Soil Testing Laboratory). The soil had a pH of 7.8; phosphorous - 20.8 mg/kg; potassium - 36.0 mg/kg; magnesium - 420.0 mg/kg; calcium - 939.3 mg/kg; copper - 1.3 ppm; zinc -5.6 ppm; and manganese - 147.4 ppm. The soil was classified as "sandy loam"; 67% sand, 26% silt and 7% clay.

Analysis of three soil samples taken from each of the Nellie Hill 1 area and Nellie Hill 2 areas are reported in table 1.3. The plant available P ranged from 16.7 - 54.5 mg/kg soil over the three Nellie Hill 1 samples, and from 27.0 - 37.7 mg/kg soil in the samples from Nellie Hill 2 (Rutgers Soil Testing Laboratory).

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Table 1.3. Soil analysis of areas of Nellie Hill 1 (NH 1) and Nellie Hill 2 (NH 2). Mehlich 3 extractable nutrients (mg/kg soil).

sample	texture	Organic matter	pH	P	K	Mg	Ca	Cu	Mn	Zn	B
NH 1.A	Loamy sand	5.88%	7.3	54.5	70.8	680.6	2284.1	2.2	272.1	15.5	2.3
NH 1.B	Sandy loam	6.69%	7.3	53.2	92.1	662.3	2136.0	2.0	240.2	10.8	2.6
NH 1.C	Loam	5.12%	7.1	16.7	63.6	448.4	1499.3	1.0	94.2	2.8	1.2
NH 2.A	Loamy sand	4.04%	7.2	37.7	61.0	398.8	1192.9	1.3	130.2	12.8	1.4
NH 2.B	Sandy loam	6.12%	7.3	34.8	77.1	511.5	1789.0	1.8	217.5	10.0	2.4
NH 2.C	loam	5.77%	7.3	27.0	76.5	537.4	1709.0	1.7	197.3	6.7	2.0

Rutgers Soil Testing Laboratory. New Jersey Agricultural Experiment Station. New Brunswick, NJ 08901

Backup plants

Backup plants in the experiments of chapters 3, 4 and 5 were grown in 0.7 kg (dry weight) of Nellie Hill soil in "deepot" cells, 2.5 cm diameter, 25 cm depth (Stuewe & Sons, Inc.). They were used to replace plants that died and to provide roots for assessment of percentage root colonization. All backup plants received the same treatment as the plants they were backups for. Data were kept on all replacements.

Phosphorous

Soil was treated with additional P from superphosphate (NaH_2PO_4) fertilizer, 0-44-0 (Carolina Biological Supply) in experiments reported in chapters 3 and 4.

Harvest

At harvest, soil and debris were washed from roots. Plants were dried at 65° C for 48 hours. The roots of backup plants were stored in 50% ethanol for future staining and microscopic assessment of percentage root colonization.

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Root colonization assessment

Root segments were cleared with 10% (w/v) KOH and stained with 0.05% trypan blue (w/v) in lactoglycerol as described by Rajapakse and Miller (1992) and examined microscopically for AM fungal structures. The percentage of colonized root segments was assessed using the grid line intersect method (Kormanik and McGraw, 1982) with the modification that root segments were counted as "myc(+)" only when a fungal structure was present at the point of intersection with the grid line.

Statistical analyses

Statistical analyses were performed on Macintosh computers using StatView (ver. 5.0; SAS Inst. Inc.) and JMP (ver 3.2.1; SAS Inst. Inc.).

Analysis of variance (ANOVA) was performed using as the response variable a measure of growth and as treatments the mycorrhizal state and level of phosphorous (chap. 3, 4) or the mycorrhizal state and water availability (chap. 5).

Whenever standard deviations tested as not being equal (Bartlett's test) Welch Anovas were performed along with ANOVA. In every case, the Welch Anova result confirmed that of ANOVA; therefore, ANOVA results were reported.

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Analysis of covariance (ANCOVA) using the sum of the length of green leaves at zero days as covariate was performed in chaps. 3, 4 and 5 to see if plant size variability at time zero had significant effects on the experiment. The ANCOVAs did not change the results of any ANOVAs; therefore, ANOVA results were reported.

The G-test of independence (log-likelihood ratio test) was used to test whether mycorrhizal state, survival and phosphorous level (chapters 3, 4) or water availability (chapter 5) were independent of each other.

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CHAPTER 2

FIELD STUDY

INTRODUCTION

When grown in the impoverished soils of the West and Southwest U.S.A. *B. curtipendula* requires colonization by arbuscular mycorrhizal fungi (AMF) to reach reproductive maturity (Hetrick et al, 1994; Wilson and Harnett, 1999). At a New York site where *B. curtipendula* is the dominant species, the soil has adequate nutrients for plant growth. Since there is a cost to the plant in sustaining AMF colonization I question the presence of AMF in these plants; and if present, what benefit does the plant gain from the association? I hypothesize that if *B. curtipendula* in New York soils is colonized by AMF then the association for the plant is facultative: plants are able to survive without AM fungal colonization but gain an advantage from the mycorrhizal relationship. This hypothesis was tested by comparing the growth and fitness of mycorrhizal and "non-mycorrhizal" *B. curtipendula* at one of its New York sites.

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METHODS

Experimental Design

Description of field site

The Nellie Hill site (Fig. 2.1) in Dover Plains, Dutchess County, NY is a sloping pasture of approximately 50 acres which is no longer grazed. It is part of a 144 acre land parcel that is owned and maintained by The Nature Conservancy. I chose Nellie Hill as my primary investigation site as it has the largest NY occurrence of *B. curtipendula*, and is the most accessible of the five New York sites. The soil is sandy with a pH of 7.8 and a phosphorous content of 21 µg/g (Rutgers Soil Testing Laboratory, New Jersey Agricultural Experiment Station).

1996

Four 5 x 5 meter plots were established in the Nellie Hill pasture (Fig.2.2). Each plot was subdivided into 25 one meter square quadrants. In September, data were collected from alternate quadrants on the number of *B. curtipendula* flowering stems and the heights of those stems as measurements of growth and fitness.

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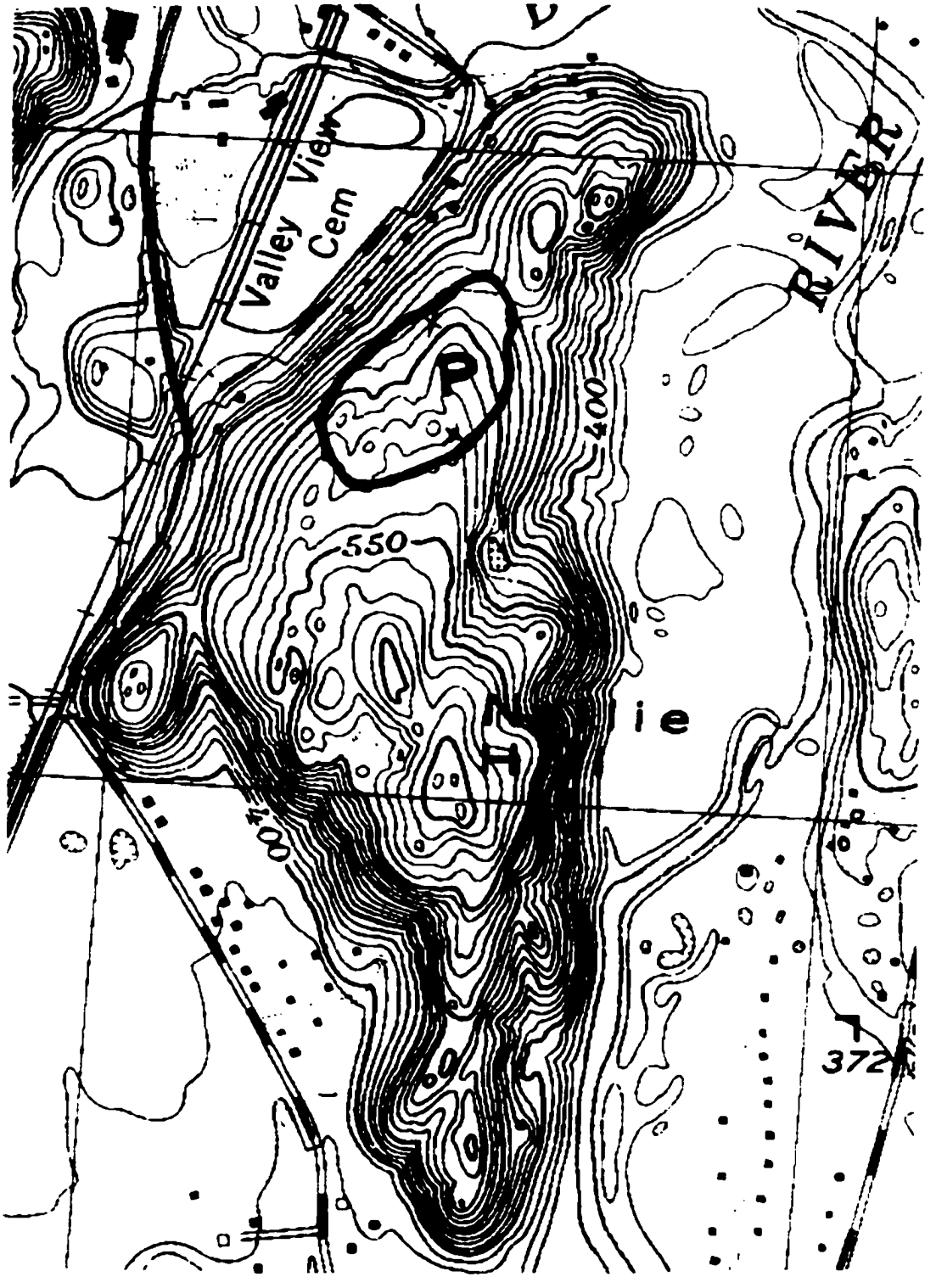

Fig. 2.1. Nellie Hill, Dover Plains, NY.
"P" denotes pasture, area of investigation.

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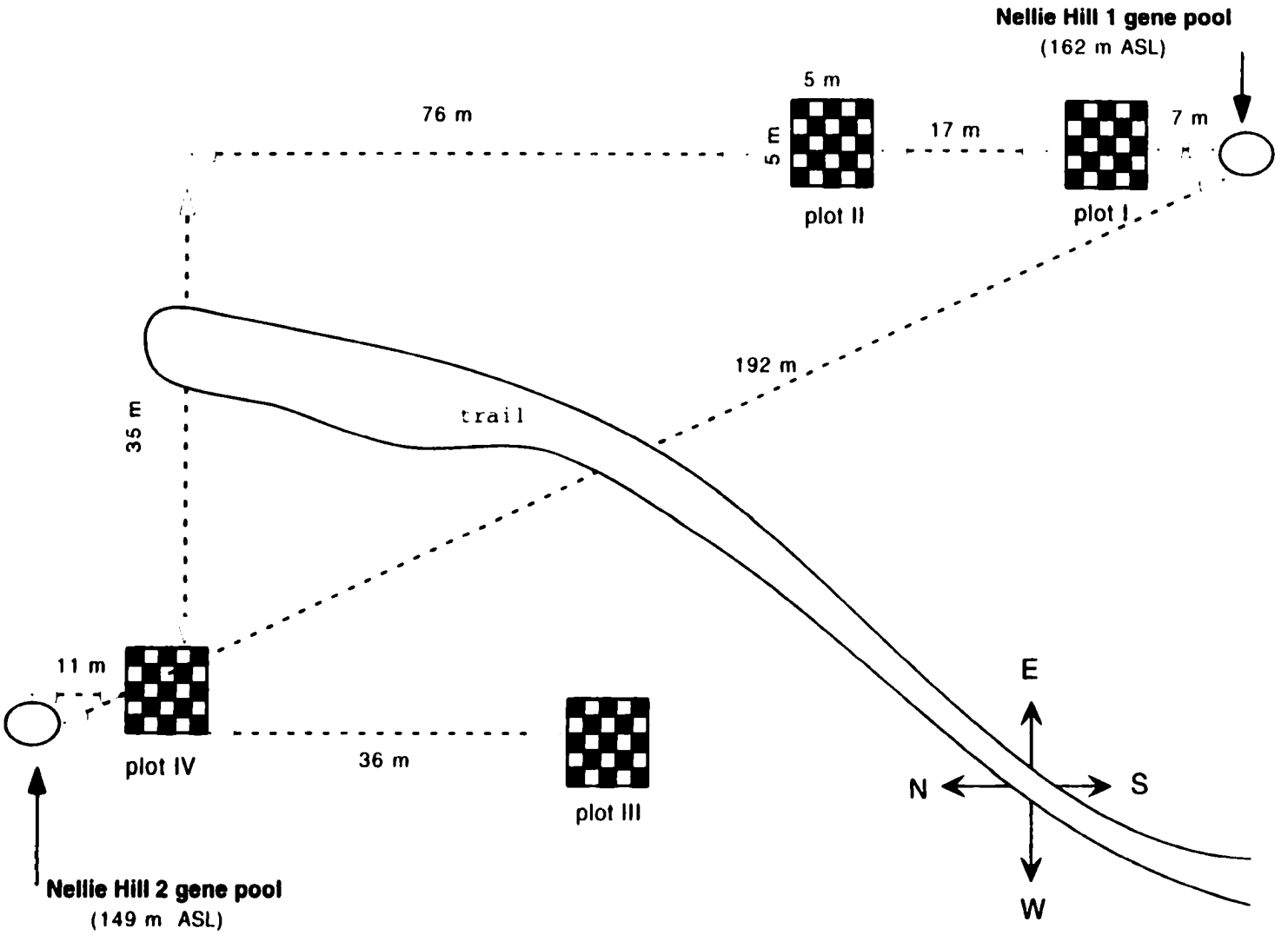

Fig. 2.2. Nellie Hill pasture plots. Data taken from shaded quadrats. Distances approximate and not drawn to scale. ASL = above sea level.

Analysis of the number and heights of flowering stems from the four plots in 1996 showed that plots III and IV were statistically very similar. These two plots became the treatment plots for the 1997 field experiment.

1997

Benomyl (2.5g of formulated product) was delivered in one liter of water to each square meter within plot III and 0.25 meters beyond the plot perimeter approximately every two weeks from 5/12/97 to 8/29/97 for a total of eight treatments. Water was similarly delivered to plot IV as a control. In September, data were collected on the number of *B. curtipendula* flowering stems and their heights from alternate quadrants in all four plots. Samples of *B. curtipendula* roots were harvested from all four plots for assessment of percentage of AMF colonization (Methods, Chapter 1).

Data Analysis

1996

ANOVAs tested for differences in the four plots regarding the numbers of flowering culms and their heights. The Bonferonni procedure for pairwise comparisons revealed the two plots that were most alike in the numbers of

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flowering culms. These plots then served as the benomyl-treated and water control plots in the 1997 experiment.

1997

A one way analysis of variance tested for difference among the means of the numbers and the heights of the flowering stems of the benomyl-treated Plot III and the water control Plot IV.

Following ANOVA, multiple comparisons were used to explore differences in the percentage of colonized roots among the plots.

A one-way analysis of variance tested for difference among the mean percentages of colonized roots from the benomyl-treated Plot III and the water control Plot IV.

1996 vs. 1997

A one-way analysis of variance tested for difference between the 1996 number of flowering stems and the 1997 for each plot. Due to large standard deviations, the Mann-Whitney U test, which uses ranks, was performed. As these results did not differ significantly from ANOVA, only ANOVA results are reported.

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RESULTS

1996

The number and heights of *B. curtipendula* flowering culms in the four plots were compared to determine the two plots most similar.

Plot I

The 13 quadrants of Plot I had a total of 557 *B. curtipendula* flowering culms with a median of 28, mean of 42.8 and a standard deviation of 49.34 (2.1). Distribution of the culms ranged from a low of zero culms in three quadrants to a high of 153 in one quadrant. Flowering culms were present in 10 quadrants.

The heights of the flowering culms of Plot I had a mean of 52.65 cm and a standard deviation of 15.86 (2.4). The minimum height was 15, maximum 89.

Plot II

The 13 quadrants of Plot II had a total of 69 *B. curtipendula* flowering culms with a median of 0, mean of

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Table 2.1. Descriptive statistics of flowering culm numbers, 1996. Median = 0 in Plot II as more than half of the quadrats had a value of 0. N = 13 quadrats per plot.

Plot	Median	Mean	SD	95% CI	Min	Max	Count
I	28	42.8	49.34	12.6-72.3	0	153	557
II	0	5.30	8.55	0.14-10.5	0	25	69
III	29	25.3	16.3	15.5-35.2	3	61	329
IV	26	25.9	18.27	14.9-37.0	0	71	337

Table 2.2. ANOVA of numbers of flowering culms, 1996

Source of variation	df	SS	F	P	Power
Plots I -IV	3	9193	3.9	0.01	0.81
Unexplained	48	777			

Table 2.3. Pairwise plot comparisons of flowering culm numbers, 1996. N = 13 quadrats per plot.

	Mean difference	P-value (Bonferroni)
Plots I, II	37.538	0.0012
Plots I, III	17.538	0.1152
Plots I, IV	16.923	0.1282
Plots II, III	-20.000	0.0735
Plots II, IV	-20.615	0.0654
Plots III, IV	-0.615	0.9553

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Table 2.4. Descriptive statistics of flowering culm heights (cm), 1996

	Mean	SD	Count	Minimum	Maximum
Plot I	52.65	15.86	557	15	89
Plot II	50.73	18.32	69	18	91
Plot III	51.09	18.84	329	10	97
Plot IV	53.52	16.28	337	13	99

Table 2.5. ANOVA of heights of flowering culms, 1996

Source of variation	df	SS	F	P	Power
Plots I -IV	3	1218	1.4	0.23	0.37
Unexplained	1288	368058			

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5.3 and a standard deviation of 8.55 (Table 2.1). Distribution of the culms ranged from a low of zero in seven quadrants to a high of 25 in one quadrant. Flowering culms were present in six quadrants.

The heights of the flowering culms of Plot II had a mean of 50.73 cm, standard deviation of 18.32, minimum height of 18 and maximum of 91 (Table 2.4).

Plot III

B. curtipendula flowering culms totaled 329 in the 13 quadrants of plot III with a median of 29, mean of 25.3 and a standard deviation 16.30 (Table 2.1). The counts ranged from a low of three in one quadrant to a high of 61 in one quadrant. Flowering culms were present in all 13 quadrants.

The heights of the flowering culms of Plot III had a mean of 51.09 cm, standard deviation of 18.84, minimum height of 10 and maximum of 97 (Table 2.4).

Plot IV

The 13 quadrants of Plot IV had a total of 337 *B. curtipendula* flowering stems (Table 2.1). The median was 26, mean 25.9 and the standard deviation 18.27. The counts

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ranged from 0 in one quadrant to 71 in one quadrant. Flowering culms were present in 12 quadrants.

The heights of the flowering culms of Plot IV had a mean of 53.52 cm, standard deviation of 16.28, minimum height of 13 and maximum of 99 (Table 2.4).

Plots I - IV

ANOVA of the numbers of flowering culms in the four plots showed a significant difference ($F = 3.9$, $P = 0.01$, $df = 3,48$, power = 0.81; (Table 2.2)).

Bonferroni pairwise comparison tests were used to investigate, in this case, where similarities existed between the plots regarding the number of flowering culms (Table 2.3). Plots III and IV had the smallest mean difference (-0.615) and the largest P-value (0.9553), and thus were considered most similar. The similarity between plots III and IV and dissimilarity of the other plots can be seen graphically in Fig. 2.3.

ANOVA of the heights of the flowering culms in the four plots showed no significant difference (Table 2.5) ($F = 1.4$, $P = 0.23$, $df = 3,1288$, power = 0.37).

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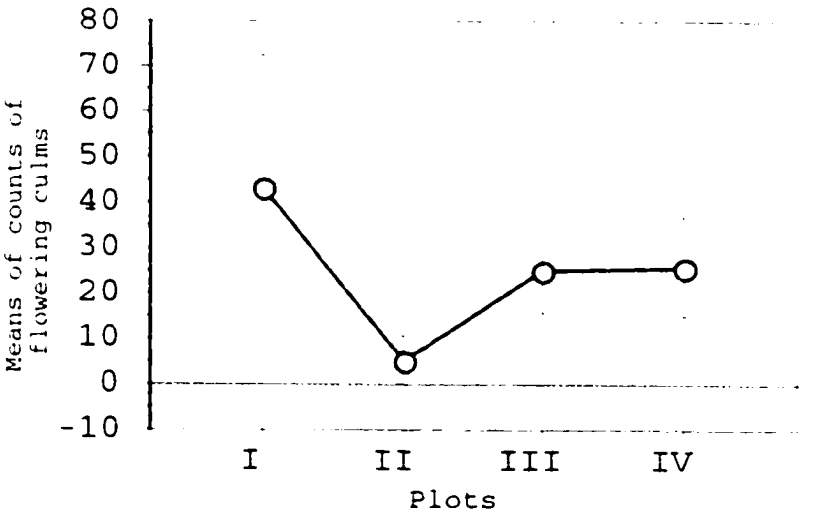

Fig. 2.3. Flowering culm numbers, 1996. Bars are 95% confidence intervals.

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1997

Plot I

The 13 quadrants of Plot I had a total of 281 *B. curtipendula* flowering culms with a median of 14, mean of 21.6 and a standard deviation of 22.16 (Table 2.6). Distribution of the culms ranged from a low of zero culms in three quadrants to a high of 74 in one quadrant.

The mean of the flowering culm heights for the 10 quadrants in Plot I that grew flowering stems was 50.22 cm (Table 2.7). The standard deviation was 15.57, minimum height 15.5 cm and maximum height 94.5.

Plot II

The 13 quadrants of Plot II had a total of 28 *B. curtipendula* flowering culms with a median of zero, mean of 2.15 and a standard deviation of 4.58 (Table 2.6). Distribution of the culms ranged from a low of zero in eight quadrants to a high of 15 in one quadrant.
The mean of the flowering culm heights for the five quadrants in Plot II that grew flowering stems was 52.34 cm (Table 2.7). The standard deviation was 17.58, minimum height 14.5 cm and maximum height 88.0.

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Table 2.6. Descriptive statistics of flowering culm numbers, 1997. Median = 0 in plot II as more than half the quadrats have a value of 0. N = 13 quadrats in each plot.

	Median	Mean	SD	95% CI of mean	Min	Max	Sum
Plot I (untreated)	14	21.6	22.16	8.2-35.0	0	74	281
Plot II (untreated)	0	2.15	4.58	0.61-4.9	0	15	28
Plot III (benomyl)	12	12.2	7.10	7.9-16.4	3	25	158
Plot IV (water control)	24	24.8	14.52	16.0-33.5	4	55	322

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Table 2.7. Descriptive statisics of flowering culm heights, 1997

	Mean	SD	Count	Minimum	Maximum
Plot I (untreated)	50.22	15.57	281	15.5	94.5
Plot II (untreated)	52.34	17.58	28	14.5	88.0
Plot III (benomyl)	55.65	18.72	158	17.0	95.5
Plot IV (water control)	56.69	17.06	322	16.0	106.0

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Plot III

B. curtipendula flowering culms totaled 158 in the 13 quadrants of benomyl-treated plot III, with a median of 12, mean of 12.2 and a standard deviation of 7.10 (Table 2.6). The counts ranged from a low of three in two quadrants to a high of 25 in one quadrant.

The mean of the flowering culm heights for the 13 quadrants in Plot III was 55.65 cm (Table 2.7). The standard deviation was 18.72, the minimum height 17.0 cm and the maximum height 95.5.

Plot IV

Plot IV had 322 *B. curtipendula* flowering stems in the 13 quadrants (Table 2.6). The median was 24, mean 24.8 and standard deviation 14.52. The counts ranged from four in one quadrant to 55 in one quadrant.

The mean of the flowering culm heights for the 13 quadrants in Plot IV was 56.69 cm (Table 2.7). The standard deviation was 17.06, minimum height 16.0 cm and maximum height 106.0.

Plot III vs. Plot IV

The number of flowering culms in the benomyl-treated Plot III was significantly less than those in the water

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control Plot IV (F = 7.92, P = 0.01, df = 1,24, Power = 0.78; Table 2.8). There was no significant difference in the heights of flowering culms in the benomyl-treated plot compared to the water control plot (F = 1.09, P = 0.31, df = 1.24, Power = 0.16; Table 2.9).

Percentage root colonization

The mean percentages of root sections colonized with AM fungi in samples taken from the plots were: Plot I - 68.50%; Plot II - 70.30%; Plot III - 47.93%; and Plot IV - 68.58% (Table 2.10). ANOVA found significant difference between plots I - IV (F = 13.02, P = 0.0002, df = 3,14, power = 1.0).

Bonferroni/Dunn multiple comparison tests show significant differences between Plots I and III, II and III, and III and IV (Table 2.11).

ANOVA showed that the mean percentage of colonized root in the water control Plot IV (68.58%) was statistically greater than in the benomyl-treated Plot III (47.93%) (F = 32.3, P = 0.0005, R-sq. = 80.2%, df = 1,8, power = 0.99; Table 2.12).

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Table 2.8. ANOVA of culm numbers in plots III and IV in 1997

Source of variation	DF	SS	F	P	R-sq.	$Power_{0.05}$
Plots III vs. IV	1	1034	7.92	0.01	24.8%	0.78
Unexplained	24	3134				
Total	25	4168				

	N	Mean	SD
Plot III	13	12.2	7.1
Plot IV	13	24.8	14.5

Plot III - benomyl treatment

Plot IV - water control

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Table 2.9. ANOVA of means of culm heights in plots III and IV, 1997

Source of variation	DF	SS	F	P	R-sq.	Power$_{0.05}$
Plots III vs. IV	1	54	1.09	0.31	4.3%	0.16
Unexplained	24	1198				
Total	25	1252				

	N	Mean	SD
Plot III	13	53.91	8.78
Plot IV	13	56.80	4.77

Plot III - benomyl treatment

Plot IV - water control

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Table 2.10. Percentage of root sections colonized with AM fungi

	N	Mean ± SD
Plot I (untreated)	3	68.50 ± 9.1
Plot II (untreated)	5	70.30 ± 7.11
Plot III (benomyl)	6	47.93 ± 3.31
Plot IV (water control)	4	68.58 ± 8.13

Table 2.11. Bonferroni pairwise comparisons of percentage of colonized root sections in all plots. Significant ANOVA ($F = 13.02$, $P = 0.0002$, $df = 3,14$, Power = 1.0). *Bonferroni procedure significant at 0.05 when $P \leq 0.0083$.

	P-value
Plot I(untreated) vs. II(untreated)	0.7242
Plot I(untreated) vs. III(benomyl)	0.0008*
Plot I(untreated) vs. IV(water control)	0.9888
Plot II(untreated) vs. III(benomyl)	<0.0001*
Plot II(untreated) vs. IV(water control)	0.7128
Plot III(benomyl) vs. IV(water control)	0.0004*

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Table 2.12. ANOVA of percentage of colonized root sections in benomyl-treated and water control plots

Source of Variation	Df	SS	F	P	R-sq.	Power (5%
Plots III vs. IV	1	1022.6	32.35	0.0005	80.2%	0.99
Unexplained	8	252.9				
Total	9	1275.5				

	N	Mean	SD
Plot III (benomyl)	6	47.93	3.31
Plot IV (water control)	4	68.58	8.13

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1996 vs. 1997

Compared to 1996 there was a decrease in 1997 of the numbers of flowering culms in all plots (Fig. 2.4) although the decrease was statistically significant only in the benomyl-treated Plot III (Table 2.13). The decrease in Plot I was 49%; Plot II, 60%; Plot III, 52% and Plot IV, 5%.

DISCUSSION

Plots III and IV were selected as the test plots for the 1997 experiment. This was based on the similarity of their statistics; the mean, median, standard deviation, minimum, maximum and count of the number of flowering culms in those plots in 1996 (Table 2.1; Fig. 2.3). Since the probability that there is no difference between groups increases as the P-value approaches 1.0, the P-value, 0.96, obtained in the Bonferroni pairwise comparison tests (Table 2.3) for the number of flowering culms in plots III and IV supports that these plots were very similar. As there were no differences among the plots in the means of the culm heights (Table 2.5) they did not have to be considered. After the benomyl treatments of 1997 the number of culms in plot III was significantly less than the control Plot IV.

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Table 2.13. *B. curtipendula* culm numbers, 1996 vs. 1997 with means and coefficient of variation (CV). F, P-values and R-square (R-sq.) generated by one-way ANOVA. * denotes statistical significance.

Plots	1996 means	1996 CV	1997 means	1997 CV	Apparent % decrease	F	P	R-sq.
I	42.5	1.16	21.6	1.02	49%	1.9	0.18	7.4%.
II	5.3	1.61	2.1	2.13	60%	1.38	0.25	5.4%
III	25.3	0.644	12.2	0.585	52%	7.11 *	0.01 *	22.9%
IV	26.0	0.705	24.8	0.586	5%	0.03	0.86	0.13%

Plot I: '96 - untreated
'97 - untreated

Plot II: '96 - untreated
'97 - untreated

Plot III: '96 - untreated
'97 - benomyl

Plot IV: '96 - untreated
'97 - water control

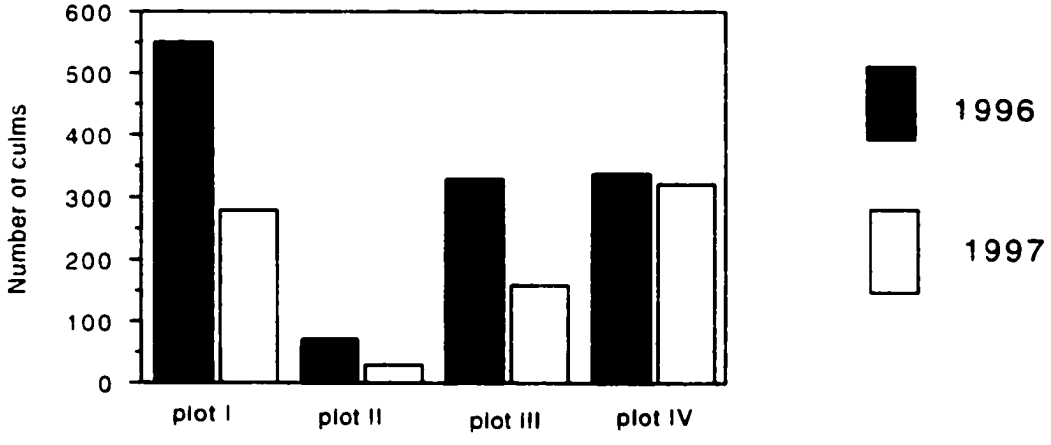

Fig. 2.4. *B. curtipendula* culm numbers, 1996 vs.1997

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Since the *B. curtipendula* roots from the benomyl-treated plot III showed a significantly smaller percentage of AM fungal colonization compared to all the untreated plots (Table 2.11) it is likely that the decreased presence of these fungi reduced the amount of phosphorous obtained by the plants, causing a reduction in their growth and fitness which was expressed as a decrease in the number of flowering culms. Although Nellie Hill soil has "sufficient" P it is probable that the P concentration is not at an optimum and that AM fungi normally benefit the Nellie Hill *B. curtipendula* by providing additional P from the rhizosphere.

There was a reduction in flowering culm numbers in all plots in 1997 as compared to 1996 (Table 2.13; Fig. 2.4). This may be explained by the decrease in precipitation during the growing season of 1997 (Table 2.14 ; Fig. 2.5). The untreated plots, I and II, had 49 and 60% reductions respectively. However, the water-control plot, plot IV, which received one liter water/ sq. meter every two weeks from mid May through August had only a 5% reduction in culm number. Plot III, the benomyl-treated plot, had a 52% reduction. This plot received as much water as plot IV and number. Plot III, the benomyl-treated plot, had a 52%

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Table 2.14. Precipitation (mm), Dutchess County, NY. Data as graphed in Fig. 2.5.

	1996	1997
April	180.0	52.8
May	74.3	83.7
June	92.2	38.0
July	188.3	106.2
August	45.1	104.7
September	150.0	46.6
Total	729.9	432.0

Source: Institute of Ecosystem Studies Environmental Monitoring Program.

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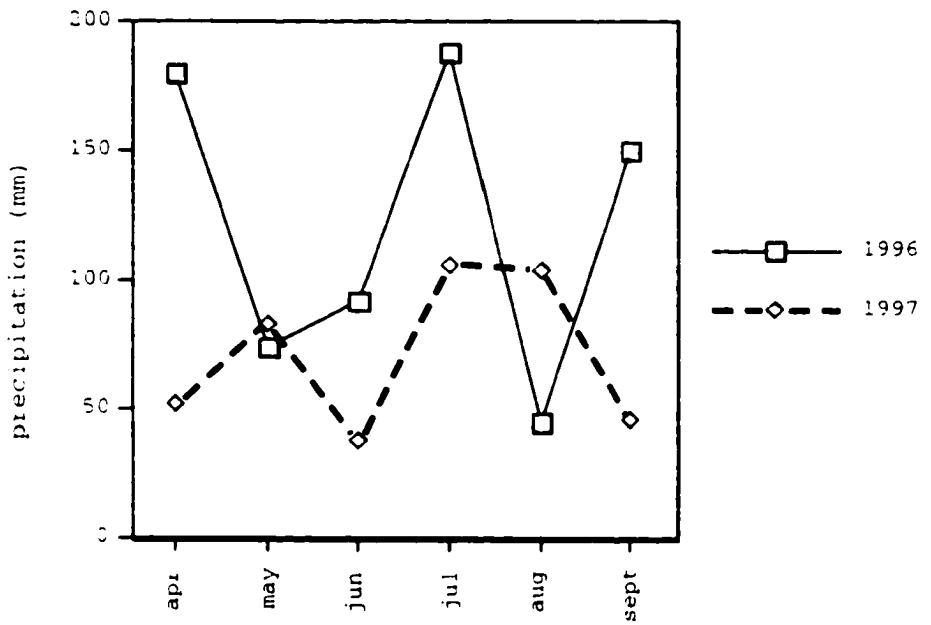

Fig. 2.5. Precipitation, Dutchess County, NY
See Table 2.14.

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reduction. This plot received as much water as plot IV and might be expected on that basis to also have had a very small difference in *Bouteloua* flowering culm numbers. However, Plot III having also received the fungicide, benomyl, with every water application, consequently had significantly less mycorrhizae (Tables 2.10, 2.11). I believe that the reduced mycorrhizae caused a reduction in mineral uptake which reduced the number of flowering stems of these plants. The benefit of the water application alleviating drought (Table 2.14) conditions, as in Plot IV, was offset by the reduction in mycorrhizal fungi.

The number of *B. curtipendula* flowering stems increased with addition of water during a drought season and decreased with reduced mycorrhizal colonization. The heights of the flowering stems did not change with increased water or decreased AMF. I, therefore, believe that the number of flowering stems can be considered a measure of growth and fitness but the heights of the stems cannot.

In conclusion, *B. curtipendula*, occurring at Nellie Hill, Dover Plains, NY, is colonized by AMF, and benefits from that colonization with increased growth and fitness. Although the mycorrhizal association may be facultative,

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that cannot be concluded from this study as the "myc(-)" group was never completely devoid of all AMF colonization.

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CHAPTER 3

COMMON GARDEN EXPERIMENT

INTRODUCTION

Since there is an energy cost to a plant colonized by AM fungi there are questions to be answered:

- What benefit, if any, do New York grown *B. curtipendula* plants receive from the mycorrhizal association?
- Is this benefit related to soil phosphorous concentration?
- Do Nellie Hill *B. curtipendula* differ in their interaction with AM fungi compared to that of *B. curtipendula* from elsewhere?
- Do different gene pools among NY *B. curtipendula* respond differently to AM fungal colonization?

The experiment reported in this chapter was designed to answer the above questions. Plants from three gene pools of *B. curtipendula* plants were grown in Nellie Hill soil with three different concentrations of P. Half of the plants were grown with a normal complement of arbuscular mycorrhizal (AM) fungi and half with a reduced complement.

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The experiment spanned two growing seasons, July 1998 through August 1999.

METHODS

Experimental design

Sixty *B. curtipendula* seedlings from each of three gene pools were transplanted into Nellie Hill soil in two outdoor raised beds (Fig. 3.1) in Pine Plains, NY. Each gene pool was given three levels of P. One raised bed was treated every two weeks with the fungicide benomyl to create a reduced mycorrhizal state "myc(-)." The control bed was given an amount of water equal to that of the fungicide, and was designated "myc(+)". Each treatment had 10 replicates.

Raised beds

Rigid polyfoam was laid at the bottom of each raised bed. Holes were punched for drainage. The polyfoam was covered with landscape cloth that extended up the sides of the raised bed to prevent contact of the roots with the underlying substrate. Cedar planks were used to construct 18 bottomless boxes in two rows of nine each for each

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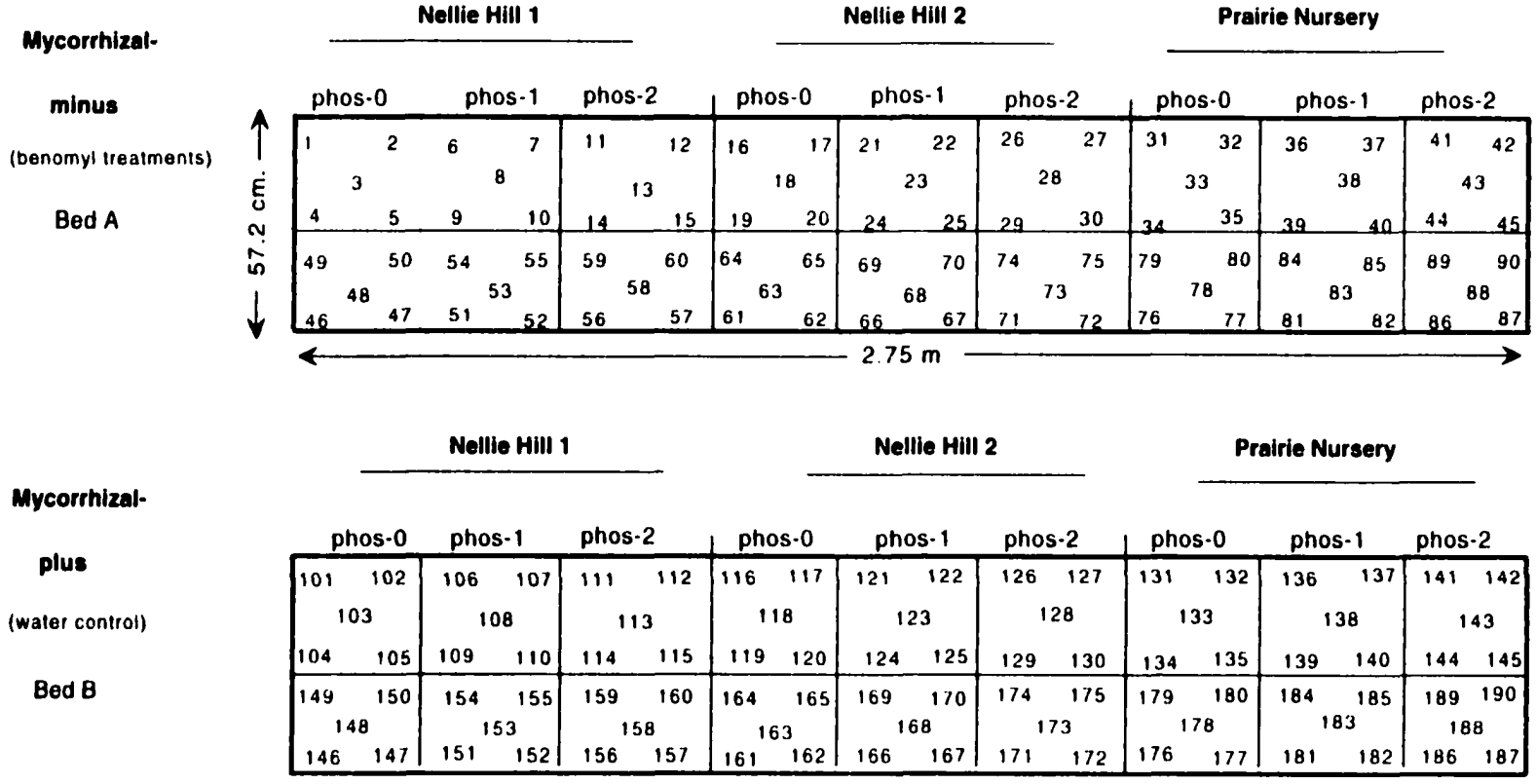

Figure 3.1. Design of common garden experiment. Phos-1 (50 ppm) and phos 2 (100 ppm) achieved with one treatment of P at beginning of experiment. "Mycorrhizal-minus" condition maintained with regular applications of benomyl. "Mycorrhizal-plus" achieved with fungal propagules naturally occurring in Nellie Hill soil. Each of of the two large rectangles represents a raised bed filled with Nellie Hill soil. Each of the 36 component boxes measured 28.6 cm x 30.5 cm x 25.4 cm deep.

raised bed. The sides of each box measured 28.6 x 30.5 cm x 25.4 cm deep (11.25 x 12 x 10 inches). The open bottom of the box rested on the landscape cloth-covered polyfoam.

Gene pools/ Seed source

Seed of the three gene pools, Nellie Hill 1, Nellie Hill 2 and Prairie Nursery (see Methods, Chapter 1), was sown on the surface of vermiculite in plastic boxes, moistened with a water spray, covered with plastic film and placed under fluorescent lights. Seeds germinated in four to six days and were transplanted into vermiculite in "cone-tainer" cells, 3.8 cm diameter by 21 cm deep (Stuewe & Sons, Inc.). Since the seed was aggregated into spikes, care was taken that only one seedling per cone-tainer be allowed to develop.

At approximately two weeks age the seedlings were placed in a protected area outdoors to harden off. At three weeks the seedlings were transplanted into sections in the raised beds filled with Nellie Hill soil. Backup plants for each treatment were transplanted into "deepot" cells, 6.4 cm diameter by 25 cm deep (Stuewe & Sons, Inc.), filled with Nellie Hill soil and placed next to their respective sections. Leaf and stem lengths were measured at the time of transplant into the raised beds and deepots.

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Soil

Soil was excavated at the Nellie Hill pasture, from an area of approximately 1.8 m x 1.8 m x 0.3 m deep. The soil was transported to Pine Plains where upper plant debris and rocks were removed. Roots were retained and mixed with the soil as a source of AM fungal propagules. Approximately 23 kg dry weight of soil was distributed into each of the 36 cedar sections in the two raised beds.

Phosphorous

The plants were grown in 3 different levels of soil P. Phos-zero was the control and had no P added. Phos-one (P-1) had 50 ppm P added to the soil before transplant; phos-two (P-2) had 100 ppm P added. To determine the plant available P four soil samples of each P treatment were analyzed.

Mycorrhizal state

The soil in raised bed A was drenched with benomyl before transplant and approximately every two weeks during both growing seasons of the experiment. These plants were referred to as "myc(-)". The soil in raised bed B was treated similarly with water as a control to produce "myc(+)" plants.

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Root samples were taken at harvest (end of second season), stained and assessed for the percentage of root that was colonized by AM fungi (see Methods, Chapter 1.).

Backup plants

A total of thirty-seven backup plants was eventually used to replace plants as they died during the first growing season.

Data collection

Plant growth during the first growing season was measured by length and number of green leaf blades and of vegetative stems. At the end of the second growing season dry weight of stems and leaves was measured. Leaf lengths and vegetative stems were measured throughout the first season on plants. However, the P-1 and P-2 plants grew too large for leaves to be measured in a timely fashion; only vegetative stems were measured after 34 days.

As an assessment of fitness, at the end of the second growing season, dry weight of seed was measured. The data measurements and time periods were as follows: lengths of green leaf blades at transplant into the raised beds, and again at 19 days; leaf blade lengths and vegetative stem lengths and number at 34 days; leaf blade lengths and

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vegetative stem lengths and number for plants at 56 days; leaf blade lengths, vegetative stem lengths and number for plants at 63 and 81 days (end of the first growing season); vegetative stem lengths and number for P-1 and P-2 plants at 63 and 81 days; dry weight of stems and leaves and dry weight of seed at the end of the second growing season for all P levels.

The numbers of surviving vs. non-surviving plants were recorded at 19 days, 27 days, 34 days, 56 days, 63 days, 81 days and at the end of the second growing season.

Statistical analysis

In four cases, as described in Methods, Chapter 4, approximate randomization tests were performed as cells of the contingency tables had a frequency less than five; G-test may be suspect. In all cases, there were no changes in the P-value. Therefore, I did not proceed with the intense computational process required to analyze each of the contingency tables in this chapter beyond the log-likelihood ratio test (G-test).

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RESULTS

Soil P

Analysis of plant available P in the raised beds showed a mean of 24.5 mg/kg soil for P-zero; 34.3 mg/kg soil for P-1 (50ppm); and 64.6 mg/kg soil for P-2 (100 ppm) (Table 3.1). The optimum concentration of plant available P is 41-96 mg/kg. A "low" concentration is 14-25 mg/kg; "medium" is 26-40 mg/kg (Table 3.2).

Growth

Zero days

At time of transplant, zero days, there were no significant differences between the plants grouped by mycorrhizal state and soil P levels, as measured by the sum of the lengths of the green leaf blades of each plant.

At P-zero an ANOVA of the means of the sum of the lengths of the green leaf blades of the myc(+) and myc(-) plants showed no significant difference in any of the three gene pools: Nellie Hill 1, $F = 0.858$, $P = 0.37$, $df = 1,18$;

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Table 3.1. Analysis of plant available P (mg/kg soil) in raised beds

Added P	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Mean**
Zero (phos-zero)	23.6	27.5	22.5	24.2	24.5
50 ppm (phos-one)	30.9	31.5	37.1	37.6	34.3
100 ppm (phos-two)	59.0	70.2	70.8	58.4	64.6

Table 3.2. Optimum levels of plant available P (mg/kg soil)

	Below optimum			optimum	
	Very low	**Low**	**Medium**	**High**	**Very high**
Plant available P (mg/kg soil)	0-13	14-25	26-40	41-96	>96

Source: Rutgers Soil Testing Laboratory

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Nellie Hill 2, F = 0.003, P = 0.96, df = 1,18; Prairie Nursery, F = 2.771, P = 0.11, df = 1,18. Similarly, at P-1 there were no significant differences in any of the three gene pools: Nellie Hill 1, F = 1.893, P = 0.19, df = 1,17; Nellie Hill 2, F = 0.326, P = 0.57, df = 1,18; Prairie Nursery, F = 5.635E-5, P = 0.99, df = 1,18. At P-2, again there were no significant differences in any of the three gene pools: Nellie Hill 1, F = 0.639, P = 0.43, df = 1,18; Nellie Hill 2, F = 0.823, P = 0.38, df = 1,18; Prairie Nursery, F = 0.587, P = 0.45, df = 1,18.

The sums of the lengths of the green leaf blades of each plant at zero days were used as the covariate in performing ANCOVAs.

19 days

(Table 3.3)

Nineteen days after the *B. curtipendula* seedlings were transplanted into the raised beds, using the sum of lengths of green leaf blades as the response variable, the Nellie Hill 1 gene pool showed no significant differences between the myc(+) and myc(-) plants at any P level.

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Table 3.3. Green leaf lengths (mm) at 19 days. Degrees of freedom (df) = 1,18. Significant results in bold type. LSN is least significant number when alpha = 0.05. N_{pow} is prospective N when power = 0.8.

Added Phosphorous

	0 ppm (phos-zero)				**50 ppm** (phos-one)				**100 ppm** (phos-two)			
SEED SOURCE	**Myc (+)** Mean (SD)	**Myc (-)** Mean (SD)	ANOVA % R-sq. (POWER)	LSN (N_{LSN})	**Myc (+)** Mean (SD)	**Myc (-)** Mean (SD)	ANOVA % R sq. (POWER)	LSN (N_{LSN})	**Myc (+)** Mean (SD)	**Myc (-)** Mean (SD)	ANOVA % R-sq. (POWER)	LSN (N_{LSN})
Nellie Hill one	79.1 (29.65)	87.0 (24.77)	F=0.418 P= 0.53 2.3% (0.09)	186 (378)	186.0 (42.98)	146.8 (71.55)	F=2.206 P=0.16 10.9% (0.28)	37 (74)	153.4 (60.26)	115.1 (83.65)	F=1.380 P=0.26 7.1% (0.19)	58 (116)
Nellie Hill two	102.2 (37.18)	61.4 (18.74)	**F=9.601 P=0.006** 35% (0.85)	11	180.4 (63.31)	183.4 (65.11)	F=0.011 P=0.92 0.06% (0.05)	7043 (14386)	187.1 (74.07)	150.0 (101.12)	F=0.876 P=0.36 4.6% (0.14)	90 (182)
Prairie Nursery	204.7 (60.14)	114.9 (45.85)	**F=14.019 P=0.0015** 44% (0.96)	8	314.4 (60.73)	294.0 (115.56)	F=0.244 P=0.63 1.3% (0.08)	317 (646)	381.7 (86.38)	217.1 (136.38)	**F=10.397 P=0.005** 37% (0.86)	10

At P-zero both Nellie Hill 2 and Prairie Nursery gene pools had significantly higher means for myc(+) plants over myc(-) ($F = 9.601$, $P = 0.006$, R-sq. = 35%, df = 1,18, Power = 0.85; $F = 14.019$, $P = 0.0015$, R-sq. = 44%, df = 1,18, Power = 0.96, respectively).

At P-1, means for myc(+) and myc(-) were not significantly different for either Nellie Hill 2 or Prairie Nursery gene pools.

At P-2 means for myc(+) and myc(-) were not significantly different for Nellie Hill 2, but myc(+) was significantly higher than myc(-) for Prairie Nursery plants ($F = 10.397$, $P = 0.005$, R-sq. = 37%, df = 1,18, Power = 0.86).

34 days

(Table 3.4)

At thirty four days, using the sum of lengths of green leaf blades as the measurement variable, an ANOVA of Nellie Hill 1 gene pool showed no significant differences at any P level.

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Table 3.4. Green leaf lengths (mm) at 34 days. Degrees of freedom (df) = 1,18. Significant results in bold type. LSN is least significant number when alpha = 0.05. N_{pow} - prospective N when power = 0.8.

	Added Phosphorous											
	0 ppm (phos-zero)				**50 ppm** (phos-one)				**100 ppm** (phos-two)			
SEED SOURCE	**Myc (+)** Mean (SD)	**Myc (-)** Mean (SD)	ANOVA % R-sq. (POWER)	LSN (N_{pow})	**Myc (+)** Mean (SD)	**Myc (-)** Mean (SD)	ANOVA % R-sq. (POWER)	LSN (N_{pow})	**Myc (+)** Mean (SD)	**Myc (-)** Mean (SD)	ANOVA % R-sq. (POWER)	LSN (N_{pow})
Nellie Hill one	104.3 (61.44)	97.4 (86.38)	F = 0.04 P = 0.83 0.23% (0.05)	1815 (3710)	454.5 > (93.42)	402 3 (154.92)	F= 0.81 P= 0.38 0.05% (0.14)	93 (187)	356.9 (117.12)	358.9 (189.86)	F=0.001 P=0.98 0.005% (0.05)	95580
Nellie Hill two	181.8 (80.85)	67.8 (19.75)	**F = 18.5 P =0.0004** 51% (0.98)	7	388.1 < (106.34)	559 6 (211.66)	**F= 5.2 P= 0.03** 22.6% (0.58)	17 (32)	471.1 < (181.98)	526.7 (252.58)	F= 0.32 P= 0.58 1.7% (0.08)	243 (494)
Prairie Nursery	325.1 (87.26)	123.3 (39.31)	**F = 44.5 P=<0.0001** 71.2% (1.00)	5	664.2 < (147.78)	794 1 (180.41)	F = 3.1 P = 0.1 14.7% (0.39)	27 (53)	808.0 > (188.71)	723.6 (246.58)	F =0.74 P =0.4 3.9% (0.13)	106 (216)

At P-zero, both Nellie Hill 2 and Prairie Nursery gene pools had significantly higher means for myc(+) plants over myc(-) (F= 18.5, P = 0.0004, R-sq. = 51%, df = 1,18, Power = 0.98; and F = 44.5, P = <0.0001, R-sq. = 71%, df = 1,18, Power = 1.00 respectively).

However, at the P-1 level the Nellie Hill 2 gene pool plants had a myc(-) result significantly higher than myc(+) (F = 5.2, P = 0.03, R-sq. = 22.6%, df = 1,18, Power = 0.58). Prospective Power analysis showed that an N of 32 would achieve Power of 0.8.

There was no significant difference at the P-2 concentration. The least significant number (LSN), the smallest N required to show significance between these means, was 243. At P-1 and P-2 the Prairie Nursery plants showed no significant differences.

56 days

(Table 3.5)

(Fig. 3.2)

At fifty six days measurements were taken on the lengths of green leaf blades on only the plants. ANOVA showed no differences in the myc(+) and myc(-) plants for Nellie Hill 1 gene pool.

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Table 3.5. Green leaf lengths (mm) at 56 Days. Degrees of freedom (df) = 1,18. Significant results in bold type. LSN is least significant number when alpha = 0.05. $N_{pow\ .8}$ is prospective N when power = 0.8.

SEED SOURCE	**Phos-zero**			
	Myc(+)	**Myc(-)**	ANOVA	LSN
	Mean (SD)	Mean (SD)	% R-sq. (Power)	($N_{pow\ .8}$)
Nellie Hill one	266.7 (179.45)	175.3 (212.67)	F = 1.079 P = 0.31 5.7% (0.17)	74 (149)
Nellie Hill two	528.2 (329.1)	98.8 (67.9)	**F = 16.3** **P = 0.0008** 47.6% (0.97)	8
Prairie Nursery	925.2 (176.0)	269.1 (127.5)	**F = 91.134** **P = <0.0001** 83.5% (1.00)	4

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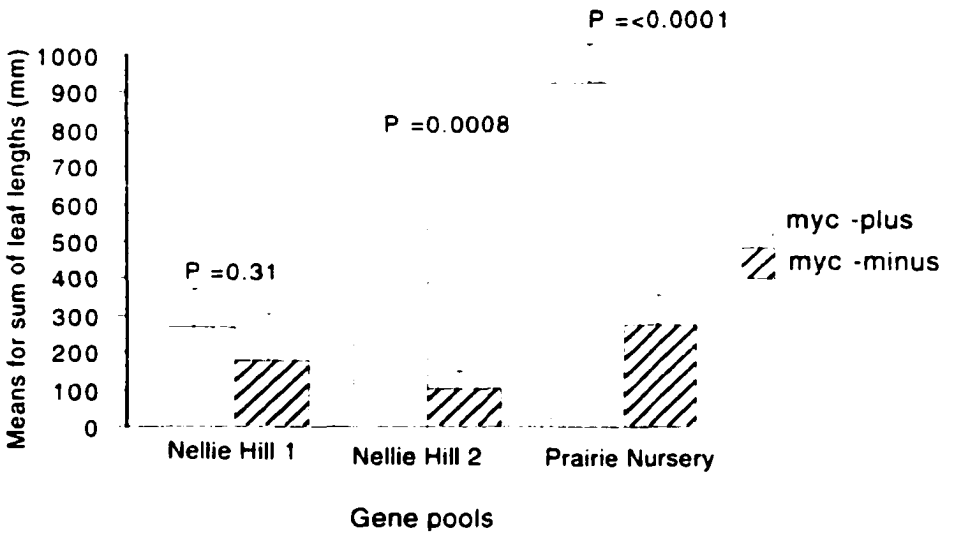

Fig. 3.2. Growth at 56 days without added P. Error bars show 95% confidence intervals. P-value from ANOVA testing for significant difference between the means of myc-plus and myc-minus plants.

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For the Nellie Hill 2 and Prairie Nursery gene pools the sum of the lengths of green leaf blades of the myc(+) plants was significantly higher than the myc(-) plants (Nellie Hill 2: F = 16.33, P = 0.0008, R-sq. = 47.6%, df = 1,18, Power = 0.97); (Prairie Nursery: F = 91.134, P = <0.0001, R-sq. = 83.5%, df = 1,18, Power = 1.00).

63 days

(Table 3.6)

(Fig. 3.3)

At 63 days, an ANOVA of vegetative stem lengths showed no significant differences in the Nellie Hill 1 gene pool plants at any P level.

For the Nellie Hill 2 and Prairie Nursery gene pools, at P-zero, the means for myc(+) were significantly higher than that for myc(-) (F = 16.3, P = 0.0008, R-sq. = 47.5%, df = 1,18, Power = 0.96; and F = 34.79, P = <0.0001, R-sq. = 65.9%, df = 1,18, Power = 1.00 respectively).

At P-1 the results were reversed in that the means for myc(-) were significantly higher than for myc(+) (Fig. 3.3). An ANOVA of the sum of vegetative stem lengths for Nellie Hill 2 generated an F = 8.797, P = 0.008, df = 1,18, R-sq. = 32.8%, Power = 0.8. The Prairie Nursery results

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Table 3.6. Sum of vegetative stem lengths (mm) at 63 days. Degrees of freedom (df) = 1,18. Significant results in bold type. LSN is least significant number when alpha = 0.05. $N_{pow\ 8}$ is prospective N when power = 0.8.

Added Phosphorous

	0 ppm (phos-zero)				**50 ppm** (phos-one)				**100 ppm** (phos-two)			
SEED SOURCE	**Myc(+)** Mean (SD)	**Myc(-)** Mean (SD)	ANOVA % R-sq. (POWER)	LSN ($N_{pow\ 8}$)	**Myc (+)** Mean (SD)	**Myc (-)** Mean (SD)	ANOVA % R-sq. (POWER)	LSN ($N_{pow\ 8}$)	**Myc (+)** Mean (SD)	**Myc (-)** Mean (SD)	ANOVA % R-sq. (POWER)	LSN ($N_{pow\ 8}$)
Nellie Hill one	21.4 (14.30)	18.9 (38.57)	F=0.037 P=0.85 0.2% (0.05)	2082 (4251)	508.1 (129.99)	492.7 (214.83)	F=0.487 P=0.50 2.6% (0.10)	160 (325)	441.9 (252.06)	637 (368.27)	F=1.911 P=0.18 9.5% (0.26)	43 (85)
Nellie Hill two	61.2 > (45.89)	2.4 (3.75)	**F=16.30 P=0.0008** 47.5% (0.96)	8	279.8 < (155.04)	558.1 (263.0)	**F=8.797 P=0.008** 32.8% (0.8)	11	430.5 < (234.85)	629.3 (453.01)	F=1.518 P=0.23 7.8% (0.21)	53 (106)
Prairie Nursery	83.5 > (34.19)	17.7 (8.71)	**F=34.79 P=<0.0001** 65.9% (1.00)	5	689 < (244.57)	1423.7 (886.30)	**F=13.30 P=0.002** 42.5% (0.93)	9	1020.6 (401.86)	1058.3 (606.82)	F=0.027 P=0.87 0.15% (0.05)	2866 (5860)

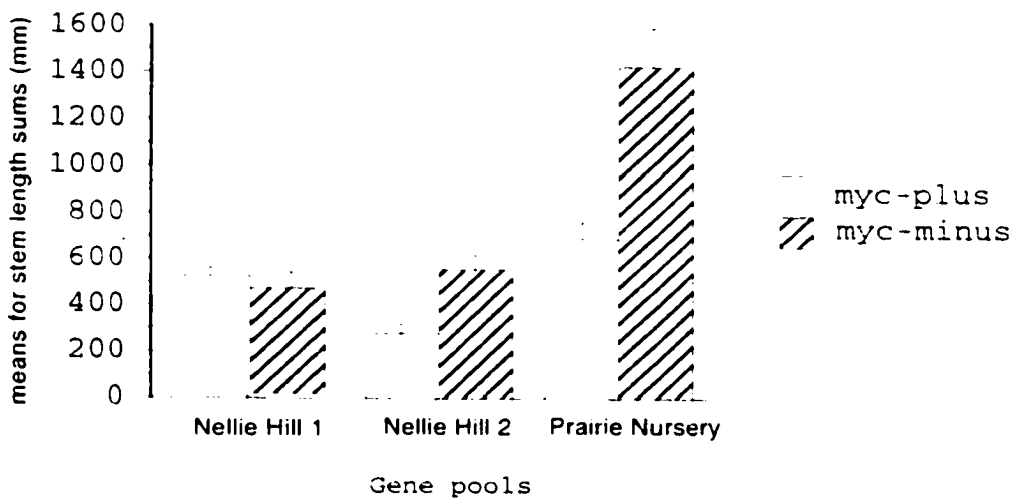

Fig. 3.3. Growth at 63 days with 50 ppm added P. Bars are SEM. Note parasitic effect of mycorrhizal colonization in two gene pools.

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were F = 13.298, P = 0.002, df = 1,18, R-sq. = 42.5%, Power = 0.93.

At P-2 there were no significant differences in vegetative stem lengths for either Nellie Hill 2 or Prairie Nursery gene pools.

81 days; end of 1st season

The final measurements taken in the 1st growing season were at 81 days.

Sum of green leaf lengths; P-zero

(Table 3.7)

Nellie Hill 1 gene pool. Using the sum of the green leaf lengths as the measurement variable, an ANOVA showed no difference between myc(+) and myc(-) *B. curtipendula* at P-zero in the Nellie Hill 1 gene pool plants (F = 1.06, P = 0.32, df = 1,18).

Nellie Hill 2 gene pool. The mean of the sum of the green leaf lengths for Nellie Hill 2, P-zero, was 880.4 mm for myc(+) and 136.5 mm for myc(-). An ANOVA of these means showed a highly significant difference (F = 12.04, P = 0.0027, R-sq.= 40%, df = 1,18, Power = 0.92).

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Table 3.7. Sums of green leaf lengths (mm) at 81 days for P-zero. Significant results in bold type.

Nellie Hill 1

Source of variation	df	SS	F	P	Power
Mycorrhizal state	1	75399	1.06	0.317	15.7%
Unexplained	18	1281067			
Total	19	1356466			

	N	Mean	SD
Myc-plus	10	343.4	243.2
Myc-minus	10	220.6	288.4

Nellie Hill 2

Source of variation	df	SS	F	P	R-sq	Power
Mycorrhizal state	1	2766936	**12.04**	**0.0027**	40%	92.2%
Unexplained	18	4135183				
Total	19	6902119				

	N	Mean	SD
Myc-plus	10	880.4	669.9
Myc-minus	10	136.5	103.2

Prairie Nursery

Source of variation	df	SS	F	P	R-sq	Power
Mycorrhizal state	1	5150110	**73.09**	**<0.0001**	80%	100%
Unexplained	18	1268409				
Total	19	6418519				

	N	Mean	SD
Myc-plus	10	1413.6	323.3
Myc-minus	10	398.7	190.8

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Prairie Nursery gene pool. The mean of the sum of the green leaf lengths for Prairie Nursery, P-zero was 1413.6 mm for myc(+) and 398.7 mm for myc(-). An ANOVA of these means showed a very highly significant difference (F = 73.09, P = <0.0001, R-sq. = 80%, df = 1,18, Power = 1.00).

Sum of main stem lengths

(Table 3.8)

Nellie Hill 1 gene pool. Using the sum of main stem lengths as the measurement variable, ANOVAs of Nellie Hill 1 gene pool at P-ZERO, P-1 and P-2 showed no significant differences between the mycorrhizal and non-mycorrhizal states.

Nellie Hill 2 gene pool. The mean of the sum of the main stem lengths for Nellie Hill 2, P-zero was 113.7 mm for myc(+) and 4.1 mm for myc(-). An ANOVA of these means showed a significant difference (F = 8.096, P = 0.01, R-sq. = 31%, df = 1,18, Power = 0.77). An increase of N to only 22 would give a Power of 0.8.

At P-1, the myc(-) mean (1087.9 mm) was now higher than the myc(+) mean (525 mm). An ANOVA of these means showed a highly significant difference (F = 9.014, P = 0.007, R-sq. = 34%, df = 1,18, Power = 0.81).

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Table 3.8. Sum of vegetative stem lengths (mm) at 81 days. Degrees of freedom (df) = 1,18. Significant results in bold type. LSN is least significant number when alpha = 0.05. $N_{i..w.}$ is prospective N when power = 0.8.

Added Phosphorous

	0 ppm (phos-zero)				**50 ppm** (phos-one)				**100 ppm** (phos-two)			
SEED SOURCE	**Myc (+)** Mean (SD)	**Myc (-)** Mean (SD)	ANOVA % R-sq. (POWER)	LSN ($N_{i..}$)	**Myc (+)** Mean (SD)	**Myc (-)** Mean (SD)	ANOVA % R-sq. (POWER)	LSN ($N_{i..}$)	**Myc (+)** Mean (SD)	**Myc (-)** Mean (SD)	ANOVA % R-sq. (POWER)	LSN ($N_{i..}$)
Nellie Hill one	34.9 (26.25)	26.5 (60.79)	F=0.161 P=0.69 0.9% (0.07)	480 (978)	719.1 (235.86)	736 (364.79)	F=0.015 P=0.90 0.08% (0.05)	5078	645.5 < (325.49)	798.5 (337.67)	F=1.064 P=0.32 5.5% (0.16)	75 (150)
Nellie Hill two	113.7 > (121.7)	4.1 (6.26)	**F = 8.096 P = 0.01** 31% (0.77)	12 (22)	525 < (279.92)	1087.9 (519.32)	**F=9.014 P=0.007** 34% (0.81)	11	653.2 < (367.23)	1327.9 (921.62)	**F=4.625 P=0.05** 20% (0.53)	19 (36)
Prairie Nursery	139.7 > (59.17)	22.3 (14.57)	**F= 37.115 P=<0.0001** 67% (1.00)	5	835.6 < (338.69)	1796.8 (646.01)	**F=17.362 P=0.0006** 49% (0.98)	7	1234.1< (474.32)	1482.6 (729.73)	F=0.815 P=0.38 4.3% (0.14)	97 (195)

At P-2, the myc(-) mean was barely significantly higher than the myc(+) (F = 4.625, P = 0.05, R-sq. = 20%, df = 1,18, Power = 0.53). An increase of N to 36 would give a Power of 0.8.

Prairie Nursery gene pool. The mean of the sum of the main stem lengths for Prairie Nursery at P-zero was 139.7 for myc(+) and 22.3 for myc(-). An ANOVA of these means showed a very highly significant difference (F = 37.115, P = <0.0001, R-sq. = 67%, df = 1,18, Power = 1.00).

At P-1, the myc(-) mean (1796.8 mm) was now higher than the myc(+) (835.6 mm). An ANOVA of these means showed a very highly significant difference (F = 17.362, P = 0.0006, R-sq. = 49%, df = 1,18, Power = 0.98).

At P-2, the myc(+) means (1234.1 mm) and the myc(-) means (1482.6 mm) were not significantly different. The LSN was 97; an N of 195 would give Power of 0.8.

End of second season

Dry weight of upper plant parts

(Table 3.9)

Nellie Hill 1 gene pool. The upper plant parts (leaves and stems; seeds excluded) of the seven surviving

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Table 3.9. Dry weight (gms) of upper plant at end of 2nd season. Significant results in bold type.

SEED SOURCE	Phos-zero			Phos-one			Phos-two		
	Myc(+)	Myc(-)	ANOVA	Myc(+)	Myc(-)	ANOVA	Myc(+)	Myc(-)	ANOVA
	Mean (SD) N	Mean (SD) N		Mean (SD) N	Mean (SD) N	%R-sq. Power$_{05}$	Mean (SD) N	Mean (SD) N	%R-sq. Power$_{05}$
Nellie Hill one	4.97 > (4.43) N = 7	4.42 (4.52) N = 5	F=0.044 P=0.84	9.0 < (2.97) N = 10	11.78 (4.39) N = 10	F=2.74 P=0.114	6.86 < (3.74) N = 10	13.51 (8.54) N = 10	**F=5.10 P=0.04** 22% 0.56
Nellie Hill two	3.02 < (1.81) N = 9	3.66 (2.59) N = 4	F=0.27 P=0.61	4.92 < (4.28) N = 9	10.48 (7.02) N = 10	F=4.23 P=0.06	7.04 < (5.07) N = 7	14.79 (8.73) N =10	**F=4.42 P=0.05** 23% 0.49
Prairie Nursery	3.34 > (2.42) N = 9	2.37 (1.61) N = 7	F=0.84 P=0.37	6.72 < (3.06) N = 10	14.34 (6.83) N = 10	**F=10.37 P=0.005** 37% 0.88	8.19 < (2.95) N = 10	14.7 (9.21) N = 10	**F=4.53 P=0.05** 20% 0.51

myc(+), P-zero plants of the Nellie Hill 1 gene pool had a mean dry weight of 4.97 gms. The mean dry weight of the five surviving myc(-) was 4.42 gms. An ANOVA of these means showed no significant difference ($F = 0.044$, $P = 0.84$, $df = 1,10$).

At P-1, the 10 surviving myc(+) plants had a mean dry weight of 9.00 gms. The mean dry weight of the 10 myc(-) plants was 11.78 gms. An ANOVA of these means showed no significant difference ($F = 0.27$, $P = 0.12$, $df = 1,18$).

At P-2, the 10 myc(+) plants had a mean dry weight of 6.86 gms. The mean dry weight of the 10 myc(-) plants was 13.51 gms. An ANOVA of these means showed a significant difference ($F = 5.10$, $P = 0.04$, $df = 1,18$). The R-sq. was 22% and Power at alpha = 0.05 was 0.56.

Nellie Hill 2 gene pool. The upper plant parts (leaves and stems; seeds excluded) of the nine surviving myc(+), P-zero plants of the Nellie Hill 2 gene pool had a mean dry weight of 3.02 gms. The four surviving myc(-) plants had a mean dry weight of 3.66 gms. An ANOVA of these means showed no significant difference ($F = 0.27$, $P = 0.61$, $df = 1,11$).

The nine surviving myc(+), P-1 plants had a mean dry weight of 4.92 gms. The mean dry weight of the 10 myc(-)

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plants was 10.48 gms. An ANOVA of these means showed no significant difference (F = 4.23, P = 0.06, df = 1,17).

The seven surviving myc(+), P-2 plants had a mean dry weight of 7.04 gms. The mean dry weight of the 10 myc(-) plants was 14.8 gms. An ANOVA of these means showed a significant difference (F = 4.42, P = 0.05, df = 1,15). The R-sq. was 23% and Power at alpha = 0.05 was 0.49.

Prairie Nursery gene pool. The upper plant parts (leaves and stems; seeds excluded) of the nine surviving myc(+), P-zero plants of the Prairie Nursery gene pool had a mean dry weight of 3.34 gms. The seven surviving myc(-) plants had a mean dry weight of 2.37 gms. An ANOVA of these means showed no significant difference (F = 0.84, P = 0.37, df = 1,14).

The 10 myc(+), P-1 plants had a mean dry weight of 6.72 gms. The mean dry weight of the 10 myc(-) plants was 14.34 gms. An ANOVA of these means showed a significant difference (F = 10.37, P = 0.005, df = 1,18). The R-sq. was 37% and Power at alpha = 0.05 was high at 0.88.

The 10 myc(+), P-2, plants had a mean dry weight of 8.19 gms. The mean dry weight of the 10 myc(-) plants was 14.7 gms. An ANOVA of these means showed a significant

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difference (F= 4.53, P = 0.05, df = 1,18). The R-sq. was 20% and Power at alpha = 0.05 was 0.51.

Estimated Fitness

Dry weight of seed

(Table 3.10)

Nellie Hill 1 gene pool. There were no significant differences between the mean dry weight of the myc(+) and myc(-) Nellie Hill 1 seed at any P level, although the direction of the difference changed with added P.

The mean dry weight of the seed at P-zero was larger for myc(+) (0.807 gms) than myc(-) (0.422 gms). An ANOVA showed no significant difference (F = 0.825, P = 0.39, df = 1,10).

At P-1 the mean myc(-) seed weight (1.387) was now larger than the myc(+) (1.107 gms). An ANOVA showed no significant difference (F = 1.526, P = 0.23, df = 1,18).

At P-2 the mean myc(-) seed weight (1.446) was also larger than the myc(+) (0.749 gms). An ANOVA again showed no significant difference (F = 2.89, P = 0.11, df = 1,18).

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Table 3.10. Dry weight (gms) of seed. Significant results in bold type. Degrees of freedom = df.

SEED SOURCE	Phos-zero			Phos-one			Phos-two		
	Myc(+)	Myc(-)	ANOVA	Myc(+)	Myc(-)	ANOVA df	Myc(+)	Myc(-)	ANOVA df
	Mean (SD)	Mean (SD)	df	Mean (SD)	Mean (SD)	%R-sq. (Power$_{.05}$)	Mean (SD)	Mean (SD)	%R-sq. (Power$_{.05}$)
Nellie Hill one	0.807 > (0.832)	0.422 (0.523)	F= 0.825 P= 0.39 df=1,10	1.107 < (0.549)	1.387 (0.460)	F= 1.526 P= 0.23 df = 1,18	0.749 < (0.307)	1.446 (1.26)	F=2.89 P= 0.11 df = 1,18
Nellie Hill two	0.346 > (0.300)	0.237 (0.242)	F= 0.397 P= 0.54 df= 1,11	0.788 < (0.774)	1.407 (1.241)	F= 1.655 P= 0.22 df = 1,17	0.754 < (0.612)	1.825 (1.30)	**F=5.106 P= 0.04** df=1,15 23% 0.56
Prairie Nursery	0.281 > (0.225)	0.191 (0.235)	F= 0.603 P= 0.45 df= 1,14	0.585 < (0.304)	1.527 (0.761)	**F= 13.229 P= 0.0019** df = 1,18 42% 0.95	0.565 < (0.343)	1.620 (1.078)	**F=8.7 P=0.009** df=1,18 33% 0.81

Nellie Hill 2 gene pool. The differences between the mean dry weight of the myc(+) and myc(-) Nellie Hill 2 seed for P-zero and P-1 were not statistically significant, but again they changed direction with added P (P-1, P-2). There was a statistical difference at P-2.

The mean dry weight of the seed of Nellie Hill 2 gene pool plants at P-zero was 0.346 gms for myc(+) and 0.237 for myc(-). An ANOVA showed no significant difference ($F = 0.397$, $P = 0.54$, $df = 1,11$).

At P-1 the mean myc(-) seed weight (1.407) was larger than myc(+) (0.788 gms). An ANOVA showed no significant difference ($F = 1.655$, $P = 0.22$, $df = 1,17$).

The mean dry weight of the seed at P-2 was 0.754 gms for myc(+) and 1.825 for myc(-), a significant parasitic difference ($F = 5.106$, $P = 0.04$, $df = 1,15$). The R-sq. was 23% and Power at alpha = 0.05 was 0.56.

Prairie Nursery gene pool. The mean dry weight of the seed of the Prairie Nursery gene pool plants for myc(+) and myc(-) was not significantly different at P-ZERO but had a significant parasitic difference at P-1 and P-2.

At P-zero the myc(+) mean was 0.281 and myc(-) was 0.191. An ANOVA showed no significant difference ($F = 0.603$, $P = 0.45$, $df = 1,14$).

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At P-1 the mean for myc(+) was 0.585 gms and 1.527 gms for myc(-). An ANOVA showed a significant difference (F = 13.229, P = 0.0019, df = 1,18). The R-sq. was 42%, and Power at alpha = 0.05 was 0.95.

The mean dry weight of the seed at P-2 was 0.565 gms for myc(+) and 1.620 for myc(-). An ANOVA showed a significant difference (F = 8.7, P = 0.009, df = 1,18). The R-sq. was 33%, and Power at alpha = 0.05 was 0.81.

Survival

19 days

(Table 3.11)

At 19 days, seven of the 180 plants had died. Of these seven, one (14.3%) was myc(+) and six (85.7%) were myc(-). A G-test of independence showed a significant association between the mycorrhizal state and survival (G = 4.107, P = 0.04).

Nellie Hill 1 gene pool. Of the 60 Nellie Hill 1 gene pool plants, one myc(+) and one myc(-) plant did not survive. A G-test of independence showed no significant

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Table 3.11. G-test of independence for survival vs. mycorrhizal state at 19 days. Column percent in parentheses after count.

	Survived		
All gene pools G = 4.107 P = 0.04	No	Yes	Total
Myc (+)	1 (14.3%)	89 (51.4%)	90
Myc (-)	6 (85.7%)	84 (48.6%)	90
Total	7	173	180 = N
Nellie Hill 1 gene pool G = 0 P = 1.0			
Myc (+)	1 (50.0%)	29 (50.0%)	30
Myc (-)	1 (50.0%)	29 (50.0%)	30
Total	2	58	60 = N
Nellie Hill 2 gene pool G = 4.317 P = 0.04			
Myc (+)	0 (0.0%)	30 (52.6%)	30
Myc (-)	3 (100%	27 (47.4%)	30
Total	3	57	60 = N
Prairie Nursery gene pool G = 2.842 P = 0.09			
Myc (+)	0 (0%)	30 (51.7%)	30
Myc (-)	2 (100%)	28 (48.3%)	30
Total	2	58	60 = N

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association between the mycorrhizal state and survival (G = 0, P = 1.0).

Nellie Hill 2 gene pool. Of the 60 Nellie Hill 2 gene pool plants, three myc(-) did not survive. A G-test of independence showed a significant association between the mycorrhizal state and survival (G = 4.317, P = 0.04).

Prairie Nursery gene pool. Of the 60 Prairie Nursery gene pool plants two did not survive, both myc(-). A G-test of independence for the association between the mycorrhizal state and survival generated a P-value of 0.09, slightly above the accepted 0.05 level of significance.

27 days

(Table 3.12)

By 27 days, a total of nine of the 180 plants had died. Of these nine, two (22.2%) were myc(+) and seven (77.8%) were myc(-). A G-test of independence for the association between the mycorrhizal state and survival now

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Table 3.12. G-test of independence for survival vs. mycorrhizal state at 27 days. Column percent in parentheses after count.

	Survived		
All gene pools G = 3.09 P = 0.08	No	Yes	Total
Myc (+)	2 (22.2%)	88 (51.5%)	90
Myc (-)	7 (77.8%)	83 (45.8%)	90
Total	9	171	180 = N
Nellie Hill 1 gene pool G = 0.357 P = 0.55			
Myc (+)	2 (66.7%)	28 (49.1%)	30
Myc (-)	1 (33.3%)	29 (50.9%)	30
Total	3	57	60 = N
Nellie Hill 2 gene pool G = 5.831 P = 0.02			
Myc (+)	0 (0.0%)	30 (53.6%)	30
Myc (-)	4 (100%)	26 (46.4%)	30
Total	4	56	60 = N
Prairie Nursery gene pool G = 2.842 P = 0.09			
Myc (+)	0 (0%)	30 (51.7%)	30
Myc (-)	2 (100%)	28 (48.3%)	30
Total	2	58	60 = N

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generated a P-value of 0.08, slightly above the accepted 0.05 level of significance.

Nellie Hill 1 gene pool. Three of the dead plants were from the Nellie Hill 1 gene pool. Two were myc(+) and one was myc(-). A G-test of independence showed no significant association between the mycorrhizal state and survival (G = 0.357, P = 0.55).

Nellie Hill 2 gene pool. Four of the dead plants were from the Nellie Hill 2 gene pool. All four, (100%) were myc(-). A G-test of independence showed a significant association between the mycorrhizal state and survival (G = 5.831, P = 0.02).

Prairie Nursery gene pool. Two of the dead plants were from the Prairie Nursery gene pool. Both (100%) of the dead plants were myc(-). A G-test of independence for the association between the mycorrhizal state and survival generated a P-value of 0.09, slightly above the accepted 0.05 level of significance.

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34 days

(Table 3.13)

By 34 days two additional plants had died, all myc(-). This gave a total of 11 deaths out of 180 plants. Of these deaths 18.2% (two plants) were myc(+); 81.8% (nine plants) were myc(-). A G-test of independence showed a significant association between the mycorrhizal state and survival (G = 5.108, P = 0.02).

Nellie Hill 1 gene pool. No additional Nellie Hill 1 gene pool plants had died by 34 days.

Nellie Hill 2 gene pool. No additional Nellie Hill 2 gene pool plants had died by 34 days.

Prairie Nursery gene pool. The two additional dead plants at 34 days were both myc(-) plants from the Prairie Nursery gene pool. A G-test of independence showed a significant association between the mycorrhizal state and survival (G = 5.831, P = 0.02).

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Table 3.13. G-test of independence for survival vs. mycorrhizal state at 34, 56, and 63 days. Column percent in parentheses after count.

	Survived		
All gene pools G = 5.10 P = 0.02	No	Yes	Total
Myc (+)	2 (18.2%)	88 (52.1%)	90
Myc (-)	9 (81.8)	81 (47.9%)	90
Total	11	169	180 = N
Nellie Hill 1 gene pool G = 0.357 P = 0.55			
Myc (+)	2 (66.7%)	28 (49.1%)	30
Myc (-)	1 (33.3%)	29 (50.9%)	30
Total	3	57	60 = N
Nellie Hill 2 gene pool G = 5.831 P = 0.02			
Myc (+)	0 (0.0%)	30 (53.6%)	30
Myc (-)	4 (100%)	26 (46.4%)	30
Total	4	56	60 = N
Prairie Nursery gene pool G = 5.831 P = 0.02			
Myc (+)	0 (0.0%)	30 (53.6%)	30
Myc (-)	4 (100%)	26 (46.4%)	30
Total	4	56	60 = N

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56 days

(Table 3.13)

At 56 days there were no additional plant deaths since observation at 34 days.

63 days

(Table 3.13)

At 63 days, survival continued to remain unchanged from 34 days.

81 days; end of first season

By 81 days, the end of the first growing season, a total of 14 plants had died and been replaced by backups. In one instance, a backup used to replace a previously expired plant also died, and was replaced by another backup, giving a total N of 181. Of the 14 dead plants three (21.4%) were myc(+) and 11 (78.6%) were myc(-) (Table 3.14). A G-test of independence showed a significant association between the mycorrhizal state and survival ($G = 5.340$, $P = 0.02$).

Seven of the 14 dead plants were P-zero (no added P), two were P-1 and five were P-2. A G-test of independence showed no significant association between P level and survival ($G = 3.12$, $P = 0.21$).

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Table 3.14. G-test of independence for survival vs. mycorrhizal state at 81 days. Row and column percents in parentheses after count (row%, column%).

	Survived		
All gene pools G = 5.34 P = 0.02	No	Yes	Total
Myc (+)	3 (3.3%, 21.4%)	88 (96.7%, 52.7%)	91
Myc (-)	11 (12.2%, 78.6%)	79 (87.8%, 47.3%)	90
Total	14	167	181
Nellie Hill 1 gene pool G = 0.185 P = 0.67			
Myc (+)	3 (9.7%, 60%)	28 (90.3%, 50%)	31
Myc (-)	2 (6.7%, 40%)	28 (90.3%, 50%)	30
Total	5	56	61
Nellie Hill 2 gene pool G = 7.387 P = 0.007			
Myc (+)	0 (0.0%, 0.0%)	30 (100%, 53.6%)	30
Myc (-)	5 (16.7%, 100%)	25 (83.3%, 46.4%)	30
Total	5	55	60
Prairie Nursery gene pool G = 5.831 P = 0.02			
Myc (+)	0 (0.0%, 0.0%)	30 (100%, 53.6%)	30
Myc (-)	4 (13.3%, 100%)	26 (86.7%, 46.4%)	30
Total	4	56	60

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When the association between the P level and survival was looked at for each mycorrhizal state the myc(+) state showed significance (G = 6.661, P = 0.04); the myc(-) state did not (G = 1.549, P = 0.46)(Table 3.15).

The above results were further explored by considering each gene pool independently.

Nellie Hill 1 gene pool. Of the five Nellie Hill 1 gene pool plants that died by 81 days three (60%) were myc(+) and two (40%) were myc(-). A G-test of independence showed no association between survival and the mycorrhizal state (G = 0.185, P = 0.67) (Table 3.14). However, when looking at the P level, all five of the non-surviving plants are found to be P-ZERO (no added P) (Table 3.16). A G-test between survival and the P level showed a highly significant association (G = 11.54, P = 0.003).

Looking at the association between survival and the P level for each mycorrhizal state (Table 3.17) slight significance is found at the myc(+) state (G = 6.821, P = 0.03) and non-significance at the myc(-) state (G = 4.688, P = 0.09).

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Table 3.15. G-tests of independence for survival vs. phosphorous level for each mycorrhizal state at 81 days. Gene pools combined; row and column percents in parentheses below count (row%, column%).

mycorrhizal-plus

G = 6.661 P = 0.04

Phosphorous Level	Survival: No	Survival: Yes	Total
P zero	3 (9.7%, 100%)	28 (90.3%, 31.8%)	31
P one	0	30 (100, 34.1%)	30
P two	0	10 (100, 34.1%)	30
total	3	88	91

mycorrhizal-minus

G = 1.549 P = 0.46

Phosphorous Level	Survival: No	Survival: Yes	Total
P zero	4 (13.3%, 36.4%)	26 (86.7%, 32.9%)	30
P one	2 (6.7%, 18.2%)	28 (93.3%, 35.4%)	30
P two	5	25 (83.3%, 31.6%)	30
total	11	79	90

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Table 3.16. G-test of independence for survival vs. phosphorous level for Nellie Hill 1 gene pool at 81 days. Column percent in parentheses after count.

G = 11.54 P = 0.003

	Survival		
Phosphorous level	No	Yes	Total
P zero	5 (100%)	16 (28.6%)	21
P one	0	20 (35.7%)	20
P one	0	20 (35.7%)	20
Total	5	56	61

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Table 3.17. G-tests of independence for survival vs. phosphorous level for each mycorrhizal state in Nellie Hill 1 gene pool at 81 days. Row and column percents in parentheses below count (row%, column%).

Nellie Hill 1 mycorrhizal-plus

G = 6.821 P = 0.03

Phosphorous Level	Survival: No	Survival: Yes	Total
P zero	3 (27.3%, 100%)	8 (72.7%, 28.6%)	11
P one	0	10 (100, 35.7%)	10
P two	0	10 (100, 35.7%)	10
total	3	28	31

Nellie Hill 1 mycorrhizal-minus

G = 4.688 P = 0.09

Phosphorous Level	Survival: No	Survival: Yes	Total
P zero	2 (20%, 100%)	8 (80%, 28.6%)	10
P one	0	10 (100%, 35.7%)	10
P two	0	10 (100%, 35.7%)	10
total	2	28	30

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Nellie Hill 2 gene pool. All five of the non-surviving Nellie Hill 2 gene pool plants were myc(-). A G-test of independence showed a highly significant association between survival and the mycorrhizal state (G = 7.387, P = 0.007) (Table 3.14). Two of the dead plants were P-zero and three were P-2. A G-test of independence between survival and the P level showed no significant association (G = 4.509, P = 0.10). Another G-test of independence showed no association between survival and P level at the myc(+) state (Table 3.18). The G-statistic is zero as there were no plant deaths in the mycorrhizal-plus state. There was also no significant association between survival and P level at the myc(-) state (G = 4.8, P = 0.09)(Table 3.18).

Prairie Nursery gene pool. All four of the non-surviving Prairie Nursery gene pool plants were myc(-). A G-test of independence showed a significant association between the mycorrhizal state and survival (G = 5.831, P = 0.02)(Table 3.14). A G-test of independence between survival and the P level showed no significant association (G = 3.385, P = 0.18). Another G-test of independence showed no association between survival and P level at the

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Table 3.18. G-tests of independence for survival vs. phosphorous level for each mycorrhizal state in Nellie Hill 2 gene pool at 81 days. Row and column percent in parenthesis below count (row%, column%).

Nellie Hill 2 mycorrhizal-plus

G = 0.0 P = 0.0

Phosphorous Level	Survival: No	Survival: Yes	Total
P zero	0	10 (100%, 33.3%)	10
P one	0	10 (100%, 33.3%)	10
P two	0	10 (100%, 33.3%)	10
total	0	30	30

Nellie Hill 2 mycorrhizal-minus

G = 4.8 P = 0.09

Phosphorous Level	Survival: No	Survival: Yes	Total
P zero	2 (20%, 40%)	8 (80%, 32%)	10
P one	0	10 (100%, 40%)	10
P two	3 (30%, 60%)	7 (70%, 28%)	10
total	5	25	30

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myc(+) state (Table 3.19). The G-statistic is zero as there were no plant deaths in the myc(+) state. There was also no significant association between survival and P level at the myc(-) state (G = 3.544, P = 0.17).

End of second season

By the end of the second growing season a total of 37 plants had died; 12 myc(+) and 25 myc(-) (Table 3.20). A G-test of independence showed a significant association between the mycorrhizal state and survival (G = 5.458, P = 0.02) with the myc(-) group having 67.6% of the plant losses compared to 32.4% in the myc(+) group.

Another G-test of independence showed a very highly significant association between the P level and survival (G = 26.148, P = <0.0001) (Table 3.21). The P-zero group had 70.27% (26 plants) of the plant losses, P-1 8% (three plants) and P-2, 21.62% (eight plants).

Exploring these results further, a G-test was performed on survival and the P level for each mycorrhizal state (Table 3.22). This showed a significant association for survival and the myc(+) condition (G = 6.984, P = 0.03) and a very highly significant association for survival and the myc(-) condition (G = 20.143, P = <0.0001).

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Table 3.19. G-tests of independence for survival vs. phosphorous level for each mycorrhizal state in Prairie Nursery gene pool at 81 days. Row and column percents in parentheses below count (row%, column%).

Prairie Nursery mycorrhizal-plus

G = 0.0 P = 0.0

	Survival		
Phosphorous Level	No	Yes	Total
P zero	0	10 (100%, 33.3%)	10
P one	0	10 (100%, 33.3%)	10
P two	0	10 (100%, 33.3%)	10
total	0	30	30

Prairie Nursery mycorrhizal-minus

G = 3.544 P = 0.17

	Survival		
Phosphorous Level	No	Yes	Total
P zero	0 (0%, 0%)	10 (100%, 38.5%)	10
P one	2 (20%, 50%)	8 (80%, 30.8%)	10
P two	2 (20%, 50%)	8 (80%, 30.8%)	10
total	4	26	30

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Table 3.20. G-test of independence for survival vs. mycorrhizal state at end of 2nd season. Row and column percents in parentheses after count (row%, column%).

	Survived		
All gene pools G = 5.458, P = 0.02	No	Yes	Total
Myc (+)	12 (13.0%, 32.4%)	80 (87.0%, 53.7%)	92
Myc (-)	25 (26.6%, 67.6%)	69 (73.4%, 46.3%)	94
Total	37	149	186
Nellie Hill 1 gene pool G = 0.097 P = 0.76			
Myc (+)	6 (18.8%, 46.2%)	26 (81.3%, 51.0%)	31
Myc (-)	7 (21.9%, 53.9%)	25 (78.1%, 49.0%)	30
Total	13	51	61
Nellie Hill 2 gene pool G = 2.59 P = 0.11			
Myc (+)	5 (16.7%, 31.3%)	25 (83.3%, 54.4%)	30
Myc (-)	11 (34.4%, 68.8%)	21 (65.6%, 45.7%)	31
Total	16	56	61
Prairie Nursery gene pool G = 5.76 P = 0.02			
Myc (+)	1 (3.3%, 12.5%)	29 (96.7%, 55.8%)	30
Myc (-)	7 (23.3%, 87.5%)	23 (76.7%, 44.2%)	30
Total	8	52	60

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Table 3.21. G-test of independence for survival vs. phosphorous level at end of 2nd season. All gene pools combined; column percent in parentheses after count.

G = 26.148,
P = <0.0001

	Survival		
Phosphorous level	No	Yes	Total
Phos-zero	26 (70.27%)	40 (26.85%)	66
Phos-one	3 (8%)	57 (38.26%)	60
Phos-two	8 (21.62%)	52 (34.9%)	60
Total	37	149	186

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Table 3.22. G-test of independence for survival vs. phosphorous level for each mycorrhizal state at end of 2nd season. All gene pools combined; column percent in parentheses after count.

Mycorrhizal-plus

G = 6.984
P = 0.03

Phosphorous level	Survival: No	Survival: Yes	Total
Phos-zero	8 (66.67%)	24 (30.00%)	32
Phos-one	1 (8.33%)	29 (36.25%)	30
Phos-two	3 (25.00%)	27 (33.75%)	30
Total	12	80	92

Mycorrhizal-minus

G = 20.143
P = <0.0001

Phosphorous level	Survival: No	Survival: Yes	Total
Phos-zero	18 (72.00%)	16 (23.19%)	34
Phos-one	2 (8.00%)	28 (40.58%)	30
Phos-two	5 (20.00%)	25 (36.23%)	30
Total	25	69	94

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Of the 12 myc(+) plants that did not survive eight (66.7%) were P-zero, one (8.3%) was P-1, and three (25%) were P-2. Of the 25 myc(-) plants that did not survive 18 (72%) were P-zero, two (8%) were P-1, and five (20%) were P-2.

These results were further explored by considering each gene pool independently.

Nellie Hill 1 gene pool. Thirteen Nellie Hill 1 gene pool plants had died by the end of the second season; six myc(+) and seven myc(-). A G-test of independence showed no association between survival and the mycorrhizal state (G = 0.097, P = 0.76)(Table 3.20).

All of the 13 non-survivors were P-zero, no added P. A G-test between survival and the level of P showed a very highly significant association (G = 31.498, P = <0.0001) (Table 3.23). Looking at the association between survival and the P level for each mycorrhizal state a highly significant association is found in both myc(+) (G = 14.25, P = 0.0008) and myc(-) (G = 17.32, P = 0.0002) conditions (Table 3.24).

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Table 3.23. G-test of independence for survival vs. phosphorous level in Nellie Hill 1 gene pool at end of 2nd season. Row and column percents in parentheses below count (row%, column%).

G = 31.498
P = <0.0001

Phosphorous level	Survival: No	Survival: Yes	Total
Phos-zero	13 (54.2%, 100%)	11 (45.8%, 21.6%)	24
Phos-one	0 (0%, 0%)	20 (100%, 39.2%)	20
Phos-two	0 (0%, 0%)	20 (100%, 39.2%)	20
total	13	51	64

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Table 3.24. G-tests of independence for survival vs. phosphorous level for each mycorrhizal state in Nellie Hill 1 gene pool at end of 2nd season. Row and column percents in parentheses below count (row%, column%).

Nellie Hill 1 mycorrhizal-plus

G = 14.25 P = 0.0008

	Survival		
Phosphorous Level	No	Yes	Total
Phos-zero	6 (50%, 100%)	6 (50%, 23.1%)	12
Phos-one	0	10 (100, 38.5%)	10
Phos-two	0	10 (100, 38.5%)	10
total	6	26	32

Nellie Hill 1 mycorrhizal-minus

G = 17.32 P = 0.0002

	Survival		
Phosphorous Level	No	Yes	Total
Phos-zero	7 (58.3%, 100%)	5 (41.7%, 20%)	12
Phos-one	0	10 (100%, 40%)	10
Phos-two	0	10 (100%, 40%)	10
total	7	25	32

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Nellie Hill 2 gene pool. Sixteen Nellie Hill 2 gene pool plants had died by the end of the second season. Five (31.3%) were myc(+) and 11 (68.8%) were myc(-). A G-test of independence showed no association between survival and the mycorrhizal state (G = 2.59, P = 0.11) (Table 3.20).

A G-test between survival and P showed a significant association (G = 8.66, P = 0.01)(Table 3.25). When the association between survival and P was looked at for each mycorrhizal state, a G-test showed no association in the myc(+) state (G = 1.81, P = 0.40 (Table 3.26). However, a highly significant association was found between survival and P in the myc(-) state (G = 13.69, P = 0.001). Of the 11 myc(-) plants that did not survive eight (72.7%) were P-zero, none were P-1 and three (27.3%) were P-2.

Additionally, when a G-test was run on the association between survival and mycorrhizal state at each P level a highly significant result was found at P-zero (G = 7.99, P = 0.005 (Table 3.27) but no significance was found at P-1 (G = 1.44, P = 0.23) or P-2 (G = 0, P = 0). Of the nine Nellie Hill 2 plants at P-zero that did not survive, eight (88.9%) were myc(+) and 1 (11.1%) was myc(-). At P-1 there was only one non-surviving plant; it was myc(+).

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Table 3.25. G-test of independence for survival vs. phosphorous level in Nellie Hill 2 gene pool at end of 2nd season. Row and column percents in parentheses below count (row%, column%).

G = 8.664
P = 0.01

	Survival		
Phosphorous level	No	Yes	Total
Phos-zero	9 (40.9%, 56.3%)	13 (59.1%, 28.3%)	22
Phos-one	1 (5.0%, 6.3%)	19 (95.0%, 41.3%)	20
Phos-two	6 (30.0%, 37.5%)	14 (70.0%, 30.4%)	20
total	16	46	62

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Table 3.26. G-tests of independence for survival vs. phosphorous level for each mycorrhizal state in Nellie Hill 2 gene pool at end of 2nd season. Row and column percents in parentheses below count (row%, column%).

Nellie Hill 2 mycorrhizal-plus

G = 1.81 P = 0.40

Phosphorous Level	Survival: No	Survival: Yes	Total
Phos-zero	1 (10%, 20%)	9 (90%, 36%)	10
Phos-one	1 (10%, 20%)	9 (90%, 36%)	10
Phos-two	3 (30%, 60%)	7 (70%, 28%)	10
total	5	25	30

Nellie Hill 2 mycorrhizal-minus

G = 13.69 P = 0.001

Phosphorous Level	Survival: No	Survival: Yes	Total
Phos-zero	8 (66.7%, 72.7%)	4 (33.3%, 19.1%)	12
Phos-one	0	10 (100%, 47.6%)	10
Phos-two	3 30%, 27.3%)	7 (70%, 33.3%)	10
total	11	21	32

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Table 3.27. G-tests of independence for survival vs. mycorrhizal state at each phosphorous level for Nellie Hill 2 gene pool at end of 2nd season. Row and column percents in parentheses below count (row%, column%).

Phosphorous-zero
G = 7.99 P = 0.005

	Survival		
Mycorrhizal state	No	Yes	Total
myc-plus	1 (10%, 11.11%)	9 (90%, 69.23%)	10
myc-minus	8 (66.67%, 88.89%)	4 (33.33%, 30.77%)	12
Total	9	13	22

Phosphorous-one
G = 1.44 P = 0.23

	Survival		
Mycorrhizal state	No	Yes	Total
myc-plus	1 (10%, 100%)	9 (90%, 47.4%)	10
myc-minus	0 (0%, 0%)	10 (100%,52.6%)	10
Total	1	19	20

Phosphorous-two
G = 0.0 P = 0.0

	Survival		
Mycorrhizal state	No	Yes	Total
myc-plus	3 (30%, 50%)	7 (70%, 50%)	10
myc-minus	3 (30%, 50%)	7 (70%, 50%)	10
Total	6	14	20

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At P-2 there were six non-survivors; three (50%) were myc(+), and three (50%) were myc(-).

Prairie Nursery gene pool. Eight Prairie Nursery gene pool plants had died by the end of the second season; one (12.5%) was myc(+) and seven (87.5%) were myc(-). A G-test of independence showed a significant association between survival and the mycorrhizal state (G = 5.76, P = 0.02) (Table 3.20). G-tests of independence showed no association between survival and P at any level (G = 1.1, P = 0.58)(Table 3.28) or at either mycorrhizal state (Table 3.29).

Percent root colonization

There was a significantly larger percentage of root length colonized by AM fungi in the myc(+) plants (mean of 61.6%) compared to the myc(-) (mean of 41.9%); (F = 57.50, P = <0.0001, df = 1,24, R-sq. = 0.71, Power = 1.00). There were no significant differences in percent colonization between the different P levels.

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Table 3.28. G-tests of independence for survival vs. phosphorous level in Prairie Nursery gene pool at end of 2nd season. Row and column percents in parentheses; column% above count, row% below count.

G = 1.10
P = 0.58

	Survival		
Phosphorous Level	No	Yes	Total
Phos-zero	(50%) 4 (20%)	(30.8%) 16 (80%)	20
Phos-one	(25%) 2 (10%)	(34.6%) 18 (90%)	20
Phos-two	(25%) 2 (10%)	(34.6%) 18 (90%)	20
Total	8	52	60

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Table 3.29. G-tests of independence for survival vs. phosphorous level for each mycorrhizal state in Prairie Nursery gene pool at end of 2nd season. Row and column percents in parentheses; column % above count; row % below count.

Prairie Nursery mycorrhizal-plus

G = 2.27 P = 0.32

	Survival		
Phosphorous	No	Yes	Total
Phos-zero	1 (100%) (10%)	9 (31.0%) (90%)	10
Phos-one	0 (100%)	10 (34.5%)	10
Phos-two	0 (100%)	10 (34.5%)	10
Total	1	29	30

Prairie Nursery mycorrhizal-minus

G = 0.36 P = 0.83

	Survival		
Phosphorous	No	Yes	Total
Phos-zero	3 (42.9%) (30.0%)	7 (30.4%) (70.0%)	10
Phos-one	2 (28.6%) (20.0%)	8 (34.8%) (80.0%)	10
Phos-two	2 (28.6%) (20.0%)	8 (34.8%) (80.0%)	10
Total	7	23	30

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DISCUSSION

Plants of both the Nellie Hill 2 and Prairie Nursery gene pools benefited in growth and survival from AM fungal colonization when grown outdoors in Nellie Hill soil without added P. However, neither the growth nor survival of the Nellie Hill 1 gene pool plants was significantly affected by AM fungal colonization. Sexual reproductive fitness in each of the three gene pools, evaluated by the dry weight of seed at the end of the second season, was not apparently affected by AM fungal colonization.

Survival

Nineteen days after the seedlings were exposed to AMF propagules (spores, hyphae, colonized root fragments) naturally occurring in the soil, the myc(+) plants of the Nellie Hill 2 and Prairie Nursery gene pools had a significantly better chance of surviving than their myc(-) plants. This advantage of better survival for the myc(+) plants increased considerably at 34 days, and remained unchanged until 63 days after exposure (Fig. 3.4). Survival of the Nellie Hill 1 gene pool plants was not

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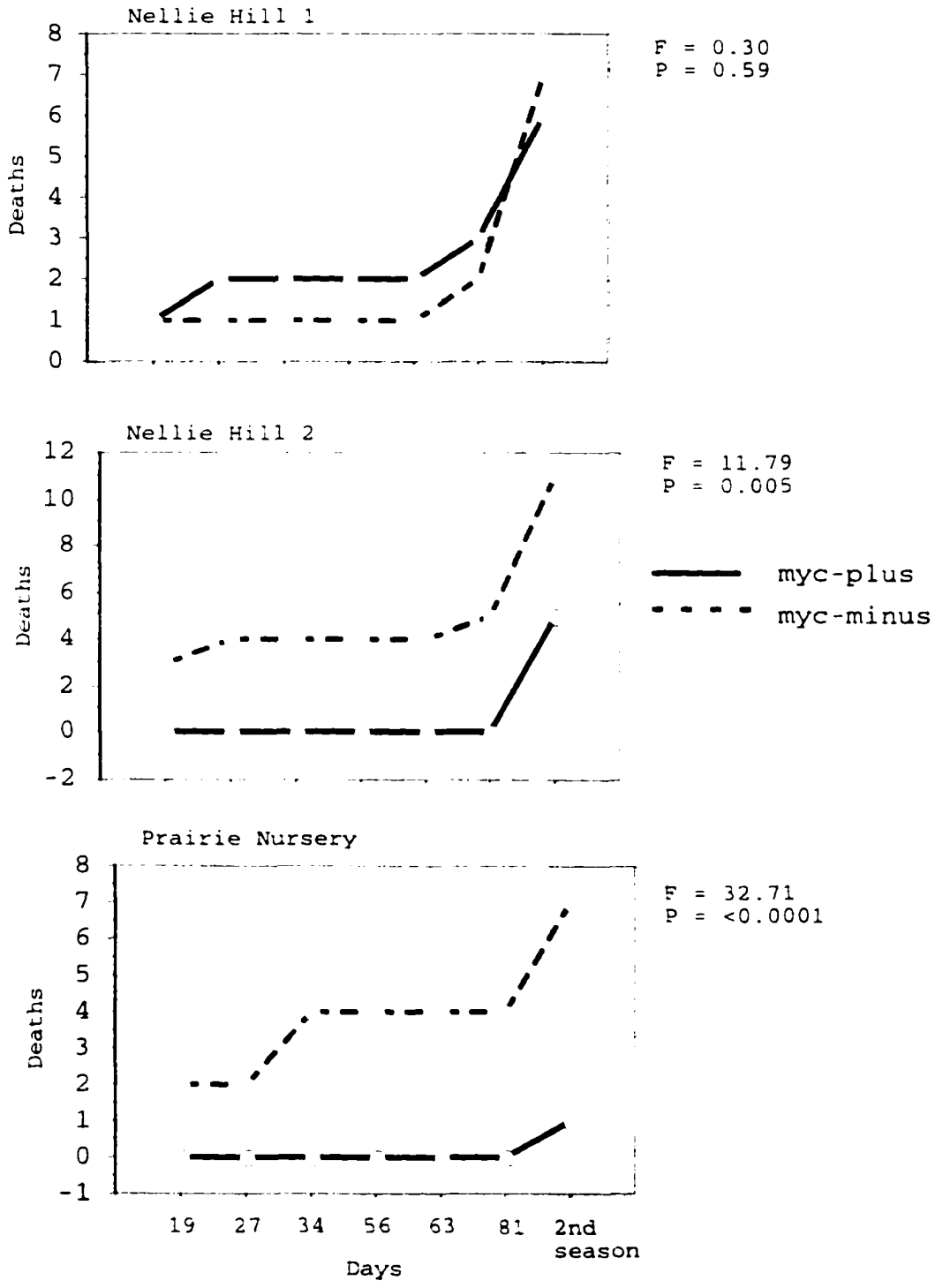

Fig. 3.4. The mycorrhizal state of non-survivors over time. Days after exposure to AMF propagules by transplanting into Nellie Hill soil. F and P-values generated by one-way ANOVA of number of non-survivors and mycorrhizal state at end of 2nd season.

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related to the presence or absence of AM fungi, but was strongly affected by the P level.

Nellie Hill 1 gene pool. Survival of the Nellie Hill 1 gene pool plants did not appear to be affected by the presence or absence of AM fungal colonization. At all seven observation intervals non-surviving Nellie Hill 1 plants were almost evenly distributed between the myc(+) and myc(-) states (Tables 3.11, 3.12, 3.13, 3,14). However, the P level appeared to be very important in the survival of the Nellie Hill 1 plants. The non-survivors were always from the P-zero group (no added P). At 81 days, the end of the 1st growing season, there was a statistically highly significant association between survival and P level (G = 11.54, P = 0.003) (Table 3.16). By the end of the second growing season there was a very highly significant association (G = 31.498, P = <0.0001) (Table 3.23).

Since all Nellie Hill 1 gene pool plant losses occurred in soil with no added P, and the losses in the myc(+) and myc(-) states were almost equal, I conclude that increased soil P is very important to Nellie Hill 1 survival, but arbuscular mycorrhizal fungi did not seem to help under the conditions of this experiment.

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Nellie Hill 2 gene pool. Survival of Nellie Hill 2 plants was independent of the P level when the plants were heavily colonized with AM fungi (myc(+))(Table 3.26). However, when AM fungal colonization was reduced (myc(-)) P became important to plant survival. With no P added to the Nellie Hill soil (P-zero) 66.7% (eight out of 12) of the myc(-) plants died (Table 3.26; Table 3.30). With added P only 15% (three out of 20) did not survive (Table 3.30). In contrast, the myc(+) Nellie Hill 2 plants had only a 10% loss in their P-zero group and 20% with added P.

Apparently, Nellie Hill 2 gene pool plants relied on the AM fungi to bring in the additional P they require. This additional P enhanced the survival of plants from this gene pool.

Prairie Nursery gene pool. Prairie Nursery plants respond to AMF but do not respond to P levels in general(Table 3.28) or specifically at either mycorrhizal state (Table 3.29). Table 3.20 shows a significant association between survival and mycorrhizal state for Prairie Nursery. Of the 8 non-survivors 87.5% (seven plants) were myc(-) and 12.5% (one plant) was myc(+). For the myc(+) plants 96.7% (29 plants) survived compared to

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Table 3.30. G-tests of independence for survival vs. mycorrhizal state with no added phosphorous (Phos-zero) and with added phosphorous (Phos-one + Phos-two) for Nellie Hill 2 gene pool at end of 2nd season. Row and column percents in parentheses below count (row%, column%).

No added phosphorous (Phos-zero)

G = 7.99 P = 0.005

	Survival		
Mycorrhizal state	No	Yes	Total
myc-plus	1 (10%, 11.1%)	9 (90%, 69.2%)	10
myc-minus	8 (66.7%, 88.9%)	4 (33.3%, 30.8%)	12
Total	9	13	22

Added Phosphorous (Phos-one + Phos-two)

G = 0.174 P = 0.68

	Survival		
Mycorrhizal state	No	Yes	Total
myc-plus	4 (20%, 57.1%)	16 (80%, 48.5%)	20
myc-minus	3 (15%, 42.9%)	17 (85%,51.5%)	20
Total	7	33	40

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76.7% (23 plants) of the myc(-) plants. This indicates that the myc(+) condition is advantageous to the survival of Prairie Nursery plants. However, the seven plant losses in the myc(-) group are distributed almost evenly among the three P levels (Table 3.29). If the AM fungi were enhancing survival by bringing in needed P the myc(-)/P-zero group should have significantly more losses than the P-1 and P-2 groups. Since this is not the case the AM fungi may be helping these plants in a different way, one unrelated to increased P uptake.

Although increased P uptake is considered the most important contribution of AM fungal colonization, the fungi are reportedly beneficial to the host plant in other ways, including the uptake and transport of zinc, copper and nitrogen (George, 2000); increased resistance to drought (Augé, 2000) and promoting increased tolerance to disease (Linderman, 2000; Dumas-Gaudot et al, 2000). Newsham et al (1994, 1995) found that AM fungi benefited the annual grass *Vulpia ciliata* ssp. *ambigua* by protecting it against root pathogens, not by enhancing P uptake. With AM fungi present there was a significant reduction in the number of pathogenic fungal hyphae in the roots of *V. ciliata* and an increase in the root length and shoot biomass. The AM

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fungi did not increase the biomass without the presence of the pathogen indicating that the AM fungi benefited the plant by its effect on the pathogen. Also, P enhancement was not a factor as AM fungi did not increase leaf P concentration. The mechanisms that explain how the AM fungi confer protection against root pathogens are not yet known. Some hypotheses are: development of a mechanical barrier (i.e. increased lignification of cell walls) (Newsham et al, 1995); production of antibiotic (anti-fungal) compounds (Sylvia, 1997); and induction of plant defense response (Dumas-Gaudot, 2000).

In conclusion, regarding survival, I have looked at three different gene pools of *B.curtipendula* and have found three different responses. One group (Nellie Hill 1) appears to be strongly dependent on increased soil P for survival but unable to get the P under these conditions from the AM fungi. Another group (Nellie Hill 2) also appears strongly dependent on increased soil P, but these plants are able to benefit from the AM fungi. The third group (Prairie Nursery), although apparently not responsive to increased P, may be helped in some other manner by the AM fungi.

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Growth

With no added P myc(+) Nellie Hill 2 and Prairie Nursery plants grew significantly more than the myc(-) plants throughout the 1st growing season (Tables 3.3, 3.4, 3.5, 3.6, 3.7, 3.8; fig 3.2). However, when 50 ppm P was added to the Nellie Hill soil the myc(-) plants grew significantly more than the myc(+) plants (Tables 3.6, 3.8; Fig. 3.3). This indicates that with increased soil P the benefits to the plant from AM fungal colonization were more than offset by the disadvantage of providing the fungi with photosynthate as their carbon source. Normally the fungi provide the plant with needed P, but with the increased soil P the plant apparently no longer required additional P for optimal growth. The relationship, mutualistic when the soil P level was lower, now was parasitic with the plant as the host and the fungus as the parasite.

Nellie Hill 1 gene pool plants showed no significant differences in growth between the myc(+) and myc(-) states at any P level. This may indicate that any benefit from the fungi was offset by the disadvantage of providing the fungi with their carbon source. If the fungi had provided

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no benefit at all then I would expect the colonized plants to have exhibited a parasitic effect from the AM fungi inhabiting their roots.

Second season: fitness and growth

Fitness was evaluated using the dry weight of the seed harvested at the end of the second season of growth. Since the robustness of a plant might be expected to affect the seed production it was not surprising that the results of the seed weights paralleled those of growth. Regarding both the growth at the end of the second season and the seed weight, Nellie Hill 2 and Prairie Nursery showed no significant differences between myc(+) and myc(-) states at P-zero but showed some parasitic effects (myc(-) significantly higher values than myc(+)) with added P (Table 3.9, Table 3.10). In the field experiment (Chapter 2) benomyl treatments significantly reduced the number of flowering culms of *B. curtipendula* growing naturally at Nellie Hill. This contradicts the seed weight results reported here, as flowering culm numbers and total seed weight would be positively correlated. A likely explanation is that by the end of the second year, crowding of the external hyphae in the confined space of the growth

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boxes may very well have decreased their efficiency in obtaining nutrients for the host (Parke and Kaeppler, 2000). As for the parasitic effect, when grown in P-enriched soil, benefit from the AM fungi was negated by the now plentiful P, and the colonizing fungi would confer only a drain to the plant.

The validity of all the results from the end of the second season may need to be questioned. In the raised beds, when the sides of the boxes that held and partitioned the plants into different P levels were removed, it was found that plant roots had crept along the sides and through the joinings of the partitions, so that roots from one P level grew into the box holding the next P level. Therefore, the P levels were no longer entirely distinct in the second season. In addition, roots were crowded in the boxes in some instances. Consequently, second season results may not be completely reliable.

In conclusion, *B. curtipendula*, naturally occurring in Dover Plains, NY, is found to be colonized by arbuscular mycorrhizal fungi, and often benefits from this association in both growth and survival. The benefit is most likely due to additional P that the fungi make available to the plant. However, *B. curtipendula* from two different parts

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of the field at Nellie Hill in Dover Plains, NY show a different response to AM fungal colonization, one group clearly benefiting from the association and the other having no net benefit under these conditions. The reasons for this are not known. Further investigation is required. AM fungi also benefit a commercial strain of *B. curtipendula* originating in northeastern Nebraska, possibly in some way other than providing additional P.

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CHAPTER 4

GREENHOUSE EXPERIMENT I

INTRODUCTION

Previous work (see Chapter 3 - common garden experiment) has shown that *B. curtipendula* plants grown from seed gathered from different areas of the Nellie Hill pasture are affected differently by indigenous arbuscular mycorrhizal (AM) fungi. The Nellie Hill 1 gene pool plants grew at the highest point of the pasture, approximately 162 meters above sea level (Fig 2.1). The Nellie Hill 2 plants grew 13 meters down the slope, 149 meters ASL, and approximately 192 meters southwest of Nellie Hill 1 (Fig. 2.2). When grown in Nellie Hill soil in raised beds, seed collected from the Nellie Hill 1 area produced plants that were not significantly affected in their growth or survival by AM fungi regardless of the soil P level. Similarly grown seed from the Nellie Hill 2 area produced plants that benefited from root colonization with AM fungi.

This experiment was designed to further investigate the response to AM fungi of *B. curtipendula* plants grown from Nellie Hill 1 and Nellie Hill 2 seed by growing them under greenhouse conditions.

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METHODS

B. curtipendula seed from the Nellie Hill 1 and Nellie Hill 2 locations were sown 3/8/99 in flats in vermiculite in the research greenhouse of Lehman College, Bronx, NY. As the seeds germinated they were transplanted to vermiculite in "cone-tainer" cells, 3.8 cm diameter by 21 cm deep (Stuewe & Sons, Inc). On 4/13/99, sixty Nellie Hill 1 and sixty Nellie Hill 2 seedlings were transplanted to "deepot" cells, 6.4 cm diameter by 25 cm deep (Stuewe & Sons, Inc.) which contained 706 gms of Nellie Hill soil.

Inoculum

As with the raised beds experiment (chapter 3) the arbuscular mycorrhizal fungi naturally occurring in Nellie Hill soil was the inoculum.

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Mycorrhizal state

Half of the "deepots" were treated with a benomyl suspension (see Chapter 1, methods) before transplanting and, thereafter, every two to three weeks to produce myc(-) plants; the other half received an equal amount of water and were designated as myc(+) (Fig.4.1).

Phosphorous

The plants were grown in three different levels of soil P. Phos-A had 25 ppm P added before planting; phos-B had 50 ppm added. P-zero was the control, and that soil was pre-treated only with water.

Data collection

As an assessment of plant growth, data were collected on the lengths of the green leaf blades and the vegetative stems, and the dry weight of the upper and lower plant parts. The data measurements and time periods were as follows: lengths of green leaf blades and stems at

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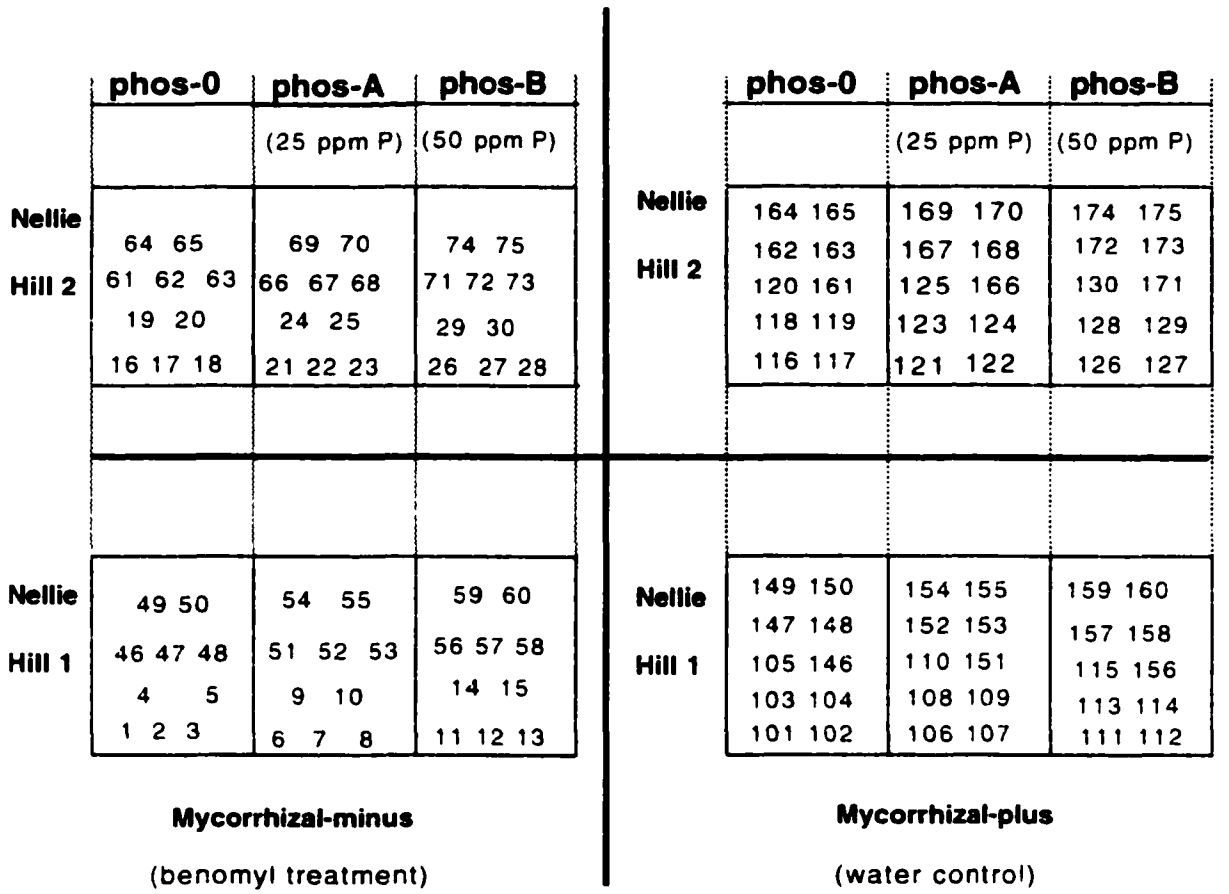

Fig. 4.1. Design for investigation of response of *B.curtipendula* to mycorrhizae and P under greenhouse conditions. 'Mycorrhizal-plus' achieved with AM fungal propagules naturally occurring in Nellie Hill soil. 'Mycorrhizal-minus' maintained with regular applications of benomyl.

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transplant, and again at 17 days, 30 days, and 50 days; dry weight of plant parts at 70 days.

Statistical analysis

See methods, Chapter 1 for details on statistical analysis.

In four analyses regarding survival, when testing for association in row by column contingency tables, several cells had frequencies less than 5; G-test may be suspect. Therefore, approximate randomization tests were designed by Dr. Dwight T. Kincaid (2003) to test the null hypotheses in question. The attained P-values after 99,999 randomizations (preserving row and column tables) were in agreement with the G-test in all four situations.

Mycorrhizal dependency

Mycorrhizal dependency was calculated as [(dry mass myc(+) plant) - (dry mass myc(-) plant) / dry mass myc(+) plant] x 100 (Plenchette et al, 1982).

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RESULTS

Survival

17 days

At 17 days, two out of 120 plants had died. They were both myc(+), phos-B. One was from Nellie Hill 1 and one from Nellie Hill 2. There were no significant differences in survival between the mycorrhizal state and/or the P concentration in either of the two gene pools.

30 days

By 30 days, four out of 120 plants had died. All were myc(+), three were Nellie Hill 2, one was Nellie Hill 1; two were P-zero and two were phos-B. There were no significant differences in survival between the mycorrhizal state and/or the P concentration in either of the two gene pools.

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50 days

By 50 days three additional plants died, making a total of seven deaths out of 120 plants. There were no significant differences in survival between the mycorrhizal state and/or the P concentration in either of the two gene pools.

70 days

By the conclusion of the experiment, 70 days, 14 plants had died (Table 4.1). Eight were myc(+), six were myc(-). Of the myc(+), three were Nellie Hill 1, one being P-zero and two being phos-B. The other five myc(+) deaths were Nellie Hill 2, three being P-zero, one phos-A and one phos-B. Of the myc(-) deaths, two were Nellie Hill 1 and two were Nellie Hill 2. Of the Nellie Hill 1, one was P-zero and one was phos-B. Of the Nellie Hill 2 three were phos-A and one was phos-B.

A log-likelihood ratio test (G-test) for association between survival and seed source for the mycorrhizal-plus state showed no significant difference ($G = 0.508$, $df = 1$, $P = 0.48$)(Table 4.2). Approximate randomization

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Table 4.1. Survival at 70 days

Added phosphorous	Mycorrhizal-plus				Mycorrhizal-minus			
	Nellie Hill 1		Nellie Hill 2		Nellie Hill 1		Nellie Hill 2	
	SURVIVAL				SURVIVAL			
	Yes	No	Yes	No	Yes	No	Yes	No
0 (phos-zero)	9	1	8	3	9	1	10	0
25 ppm (phos-A)	10	0	9	1	10	0	7	3
50 ppm (phos-B)	8	2	9	1	9	1	9	1
Total	27	3	26	5	28	2	26	4

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Table 4.2. Survival at 70 days for mycorrhizal-plus

Log-likelihood ratio test for association between survival and seed source

	Survival		
	yes	no	total
Nellie Hill 1	27	3	30
Nellie Hill 2	26	5	31
total	53	8	61

G = 0.508,
df = 1
P = 0.48

Log-likelihood ratio test for association between survival and phosphorous treatment for Nellie Hill 1 and 2 combined.

	Survival		
	yes	no	total
0ppm P	17(81%)	4	21
25ppm P	19(95%)	1	20
50ppm P	17(85%)	3	20
total	53	8	61

G = 2.1
df = 2
P = 0.35

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analysis, to test the null hypothesis that there was no association between survival and seed source, confirmed the results of the G-test that there was no significant difference. Therefore, I merged the two seed sources and performed a log-likelihood ratio test for association between survival and P treatment for the mycorrhizal-plus state (Yable 4.2). There were no significant differences ($G = 2.1$, $df = 2$, $P = 0.35$). Again approximate randomization testing confirmed the results of the G-test.

A log-likelihood ratio test for association between survival and seed source for the mycorrhizal-minus state showed no significant difference ($G = 0.75$, $df = 1$, $P = 0.39$) (Table 4.3). Again, the non-significance allowed me to merge the two seed sources to run the log-likelihood ratio test for association between survival and P treatment for the mycorrhizal-minus state Table 4.3). There were no significant differences ($G = 1.158$, $df = 2$, $P = 0.56$). Both these test results were confirmed with approximate randomization testing.

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Table 4.3. Survival at 70 days for mycorrhizal-minus

Log-likelihood ratio test for association between survival and seed source

	Survival		
	yes	no	total
Nellie Hill 1	28	2	30
Nellie Hill 2	26	4	30
total	54	6	60

G = 0.75
df = 1
P = 0.39

Log-likelihood ratio test for association between survival and phosphorous treatment

	Survival		
	yes	no	total
0ppm P	19(95%)	1	20
25ppm P	17(85%)	3	20
50ppm P	18(90%)	2	20
total	54	6	60

G = 1.158
df = 2
P = 0.56

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Growth

Zero days

At time of transplant (zero days), there were no significant differences between the plants grouped by mycorrhizal state and soil P levels, as measured by the sum of the lengths of the green leaf blades of each plant.

At P-zero an ANOVA of the means of the sum of the lengths of the green leaf blades of the myc(+) and myc(-) plants showed no significant difference in either of the two gene pools: Nellie Hill 1, $F = 0.012$, $P = 0.91$, $df = 1,18$; Nellie Hill 2, $F = 1.204$, $P = 0.29$, $df = 1,18$. Similarly, at Phos-A there were no significant differences: Nellie Hill 1, $F = 3.560$, $P = 0.08$, $df = 1,18$; Nellie Hill 2, $F = 0.124$, $P = 0.73$, $df = 1,18$. There were also no significant differences at Phos-B: Nellie Hill 1, $F = 0.755$, $P = 0.40$, $df = 1,18$; Nellie Hill 2, $F = 3.274$, $P = 0.09$, $df = 1,18$.

17 days

Seventeen days after the *B. curtipendula* seedlings were transplanted into the deepots, using the sum of

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lengths of green leaf blades as the response variable, there were no significant differences at either mycorrhizal state and/or at any P level for either Nellie Hill 1 or 2.

30 days

At 30 days there were again no significant differences at any P level or either mycorrhizal state for either Nellie Hill 1 or 2.

50 days

(Table 4.4)

At 50 days, using the sum of lengths of green leaf blades as the measurement variable, an ANOVA of the Nellie Hill 1 gene pool showed a significantly higher means for myc(+) plants over myc(-) at P-zero ($F = 10.608$, $P = 0.004$, R-sq.= 37%, df = 1,18, power = 0.87). There were no significant differences at phos-A or B for Nellie Hill 1.

There were no significant differences at any P level for Nellie Hill 2.

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Table 4.4. Green leaf lengths (mm) at 50 days. Degrees of freedom (df) = 1,18. Significant results in bold type. LSN is least significant number when alpha = 0.05. $N_{power\ 8}$ is prospective N when power = 0.8.

Added Phosphorous

	0 ppm (phos-zero)				**25 ppm** (phos-A)				**50 ppm** (phos-B)			
SEED SOURCE	**Myc(+)** Mean (SD)	**Myc(-)** Mean (SD)	ANOVA % R-sq. (POWER)	LSN ($N_{power\ 8}$)	**Myc(+)** Mean (SD)	**Myc(-)** Mean (SD)	ANOVA % R-sq. (POWER)	LSN ($N_{power\ 8}$)	**Myc(+)** Mean (SD)	**Myc(-)** Mean (SD)	ANOVA % R-sq. (POWER)	LSN ($N_{power\ 8}$)
Nellie Hill one	249.8 > (121.9)	117.5 (40.5)	**F=10.608** **P=0.004** 37% (0.87)	10 (17)	508.8 > (172.4)	381.1 (131.8)	F=3.461 P=0.08 16% (0.42)	25 (48)	804.3 < (174.7)	1048.2 (416.4)	F=2.917 P=0.10 0.14% (0.37)	29 (56)
Nellie Hill two	137.8 > (76.8)	102.1 (18.0)	F=2.047 P=0.17 10% (0.27)	40 (79)	344.9 < (229.1)	389.7 (235.0)	F=0.186 P=0.67 0.04% (0.07)	415 (845)	579.7 < (247.0)	596.5 (319.0)	F=0.017 P=0.90 0.0009% (0.05%)	4434 (9056)

70 days (harvest)

Dry weight of upper plant parts

(Table 4.5)

Nellie Hill 1 genepool. The upper plant parts (leaves and stems) of the myc(+), P-zero plants of Nellie Hill 1 gene pool had a mean dry weight of 0.079 gms. The mean dry weight of the myc(-) plants was 0.027 gms. An ANOVA of these means showed a significant difference ($F = 7.752$, $P = 0.01$, df = 1,17, R-sq.= 31.3%, power = 0.75). Prospective power analysis shows that an N of 22 would give power of 0.8.

At phos-A there was no significant difference between the mean dry weight of the myc(+) (0.177 gms) and the myc(-) plants (0.163 gms).

At phos-B an ANOVA showed the mean dry weight of the myc(-) plants (0.457 gms) was now significantly higher than that of the myc(+) plants (0.268 gms); ($F = 4.597$, $P = 0.05$, df = 1,17, R-sq.= 21.3%, power = 0.5). A prospective N of 34 would give power of 0.8.

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Table 4.5. Dry weight (gms) of upper plant at harvest (70 days). Significant results in bold type. LSN is least significant number when alpha = 0.05. $N_{pow\ 8}$ is prospective N when power = 0.8.

	Added Phosphorous											
	0 ppm (phos-zero)				**25 ppm** (phos-A)				**50 ppm** (phos-B)			
SEED SOURCE	**Myc(+)** Mean (SD) N	**Myc(-)** Mean (SD) N	ANOVA % R-sq. (POWER)	LSN ($N_{pow\ 8}$)	**Myc(+)** Mean (SD) N	**Myc(-)** Mean (SD) N	ANOVA % R-sq. (POWER)	LSN ($N_{pow\ 8}$)	**Myc(+)** Mean (SD) N	**Myc(-)** Mean (SD) N	ANOVA % R-sq. (POWER)	LSN ($N_{pow\ 8}$)
Nellie Hill one	0.079 > (0.053) 10	0.027 (0.018) 9	**F=7.75 P=0.01** 31.3% (0.75)	12 (22)	0.177 > (0.054) 10	0.163 (0.065) 10	F=0.26 P=0.62 0.01% (0.08)	299 (610)	0.268 < (0.214) 10	0.457 (0.163) 9	**F=4.60 P=0.05** 21.3% (0.5)	19 (34)
Nellie Hill two	0.031 > (0.026) 9	0.018 (0.10) 10	F=2.15 P=0.16 0.11% (0.28)	37 (72)	0.105 < (0.076) 9	0.139 (0.118) 9	F=0.55 P=0.47 0.03% (0.10)	128 (259)	0.162 < (0.073) 9	0.238 (0.141) 9	F=2.30 P=0.15 0.11% (0.30)	36 (71)

Nellie Hill 2 gene pool. There were no significant differences between the upper plant mean dry weights of the Nellie Hill 2 gene pool myc(+) and myc(-) plants at any P level.

Dry weight of roots

(Table 4.6)

Nellie Hill 1 gene pool. At P-zero an ANOVA showed the myc(+) Nellie Hill 1 plant roots had a mean dry weight (0.074 gms) significantly higher than that of the myc(-) (0.017 gms) ($F = 11.930$, $P = 0.003$, $df = 1,17$, R-sq.= 42.2%, power = 0.9).

At phos-A an ANOVA showed that the mean dry weight of the roots of the myc(-) plants (0.126 gms) was now significantly higher than that of the myc(+) (0.029 gms) ($F = 7.595$, $P = 0.01$, $df = 1,18$, R-sq.= 29.9%, power = 0.74). A prospective N of 23 would give power of 0.8.

At phos-B there was no significant difference between the mean dry weight of the myc(+) roots (0.284 gms) and the myc(-) (0.245 gms)($F = 0.834$, $P = 0.37$, $df = 1,17$).

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Table 4.6. Dry weight (gms) of roots at harvest (70 days). Significant results in bold type. LSN is least significant number when alpha = 0.05. $N_{pow\ 8}$ is prospective N when power = 0.8.

	Added Phosphorous											
	0 ppm (phos-zero)				**25 ppm** (phos-A)				**50 ppm** (phos-B)			
SEED SOURCE	**Myc(+)** Mean (SD) N	**Myc(-)** Mean (SD) N	ANOVA % R-sq. (POWER)	LSN ($N_{pow\ 8}$)	**Myc(+)** Mean (SD) N	**Myc(-)** Mean (SD) N	ANOVA % R-sq. (POWER)	LSN ($N_{pow\ 8}$)	**Myc(+)** Mean (SD) N	**Myc(-)** Mean (SD) N	ANOVA % R-sq. (POWER)	LSN ($N_{pow\ 8}$)
Nellie Hill one	0.074 > (0.049) 10	0.017 (0.009) 9	**F=11.930** **P=0.003** 41.2% (0.9)	9 (15)	0.029 < (0.080) 10	0.126 (0.05) 10	**F=7.595** **P=0.01** 29.9% (0.74)	13 (23)	0.284> (0.087) 10	0.245 (0.101) 9	F=0.834 P=0.37 0.05% (0.14)	90 (181)
Nellie Hill two	0.025 > (0.023) 9	0.012 (0.007) 9	F=2.691 P=0.12 0.14% (0.34)	28 (56)	0.101 (0.082) 9	0.106 (0.08) 9	F=0.02 P=0.9 .00097% (0.05)	4433	0.149 < (0.064) 10	0.160 (0.082) 10	F=0.114 P=0.74 .006% (0.06)	676 (1380)

Nellie Hill 2 gene pool. There were no significant differences between the root mean dry weights of the Nellie Hill 2 myc(+) and myc(-) plants at any P level.

Roots and shoots

(Table 4.7)

Root:shoot ratio. In both Nellie Hill 1 and 2, at each P level the root:shoot ratio was higher for the myc(+) plants compared to the myc(-).

Mycorrhizal dependence. The percentage mycorrhizal dependence of the Nellie Hill 1 plants with no added P (P-zero) was 71.5%. This greatly decreased as the P concentration increased. The Nellie Hill 2 plants at P-zero had a 45.7% mycorrhizal dependence. As with Nellie Hill 1, this also decreased considerably with increased P concentration.

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Table 4.7. Effects of AM fungi and P nutrition on growth (gms) of roots and shoots under greenhouse conditions (70 days). Root and shoot weights are means of 10(9) replicates. Percentage mycorrhizal dependence = [(dry wt mycorhizal-plus plant) - (dry wt mycorrhizal-minus plant) / (dry wt mycorrhizal-plus plant)] X 100

Gene pool	Mycorrhizal state	Added P (ppm)	Dry weight of tissue			Root: Shoot ratio	(%) mycorrhizal dependence
			Root (N)	Shoot (N)	Total		
	minus	0	0.0167 (9)	0.0267 (9)	0.0434	0.625	
	plus	0	0.0741 (10)	0.0786 (10)	0.1527	0.943	71.5
Nellie	minus	25	0.1256 (10)	0.1630 (10)	0.2886	0.771	
	plus	25	0.2088 (10)	0.1766 (10)	0.3854	1.182	25.1
Hill 1							
	minus	50	0.2449 (9)	0.4571 (9)	0.7020	0.536	
	plus	50	0.2843 (10)	0.2685 (10)	0.5528	1.059	-21.3
	minus	0	0.0122 (9)	0.0185 (10)	0.0307	0.659	
	plus	0	0.0254 (9)	0.0311 (9)	0.0565	0.817	45.7
Nellie	minus	25	0.1062 (9)	0.1394 (9)	0.2456	0.762	
	plus	25	0.1013 (9)	0.1047 (9)	0.2060	0.968	-19.2
Hill 2							
	minus	50	0.1603 (10)	0.2382 (10)	0.3985	0.673	
	plus	50	0.1492 (10)	0.1620 (10)	0 3112	0.920	-28

DISCUSSION

Nellie Hill 1

By 50 days after transplant, the Nellie Hill 1 gene pool plants appeared to benefit from mycorrhizal colonization, as the untreated group (myc(+)) grew significantly larger than the benomyl-treated (myc(-)) (Table 4.4). It would seem that the benefit was due to improved P nutrition since the significant growth difference between the treated and non-treated groups disappeared when 25 ppm P was added to the soil (Fig. 4.2). Hetrick et al (1986) attributed mycorrhizal benefit in tall grass prairie plants to increased P availability when, after providing additional P, the mycorrhizal benefit disappeared.

When 50 ppm P was added to the soil the benomyl-treated plants now grew larger than the untreated. At 50 days this difference was not significant, but the least significant number (LSN) at alpha = 0.05 would be an increase to only 29 (Table 4.4). At 70 days this difference was statistically significant (Table 4.5). The increased growth of the myc(-) plants compared to the

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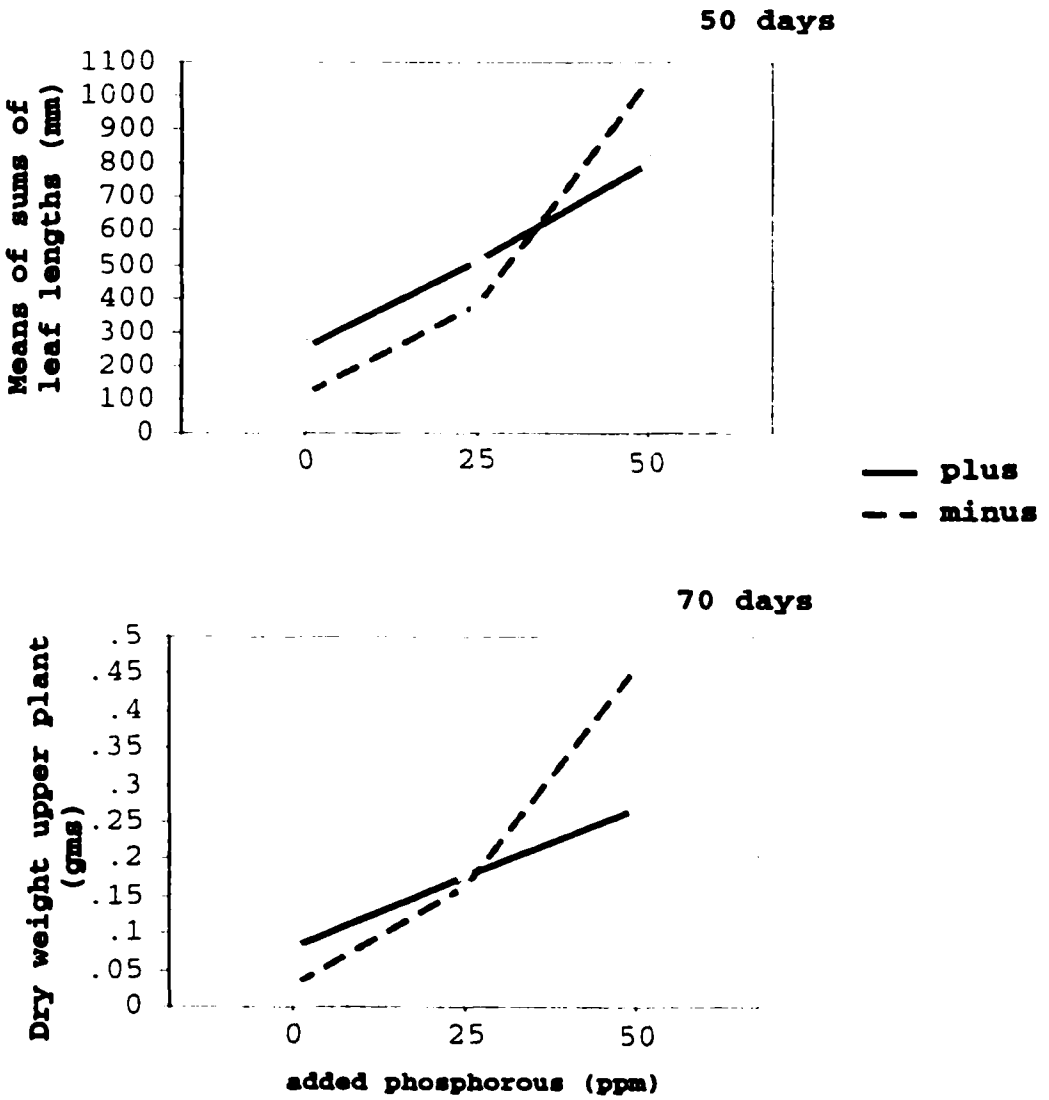

Fig. 4.2. Growth vs. P nutrition vs. mycorrhizal state in Nellie Hill 1

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myc(+) could be attributed to a parasitic effect of the AM fungi. The fungi normally provide the host plant with additional soil P. However, since the soil was now P-rich the plant could obtain optimum P on its own. The presence of the fungi then became an energy drain to the plant, a liability. Plants with reduced fungal colonization from the benomyl treatment now grew larger than the untreated. This change in growth as the P concentration increased is reflected in the percentage mycorrhizal dependence of each of the groups (Table 4.7). As the P concentration increased the percentage mycorrhizal dependence decreased. The growth change with increased P can be seen graphically in Fig. 4.2.

Nellie Hill 2

As for the Nellie Hill 2 gene pool plants, although the difference in growth between the benomyl-treated and the untreated was not statistically significant, the percentage mycorrhizal dependence (Table 4.7) showed the same pattern as that of the Nellie Hill 1 plants, a decrease as the P concentration increased. This would lead

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me to believe that AM fungi were also a benefit to this gene pool when the P concentration was low.

Roots and shoots

When plants benefit from mycorrhizal colonization their roots have increased growth as well as their shoots. The root:shoot index, however, will typically be reduced (Smith and Read, 1997;, Doud et al, 2000). It is explained that the mycorrhizal root, being more efficient, can support a larger mass of shoot than the non-mycorrhizal root. I have no explanation as to why the root:shoot index (Table 4.7) of the myc(+) plants in this experiment was not reduced compared to the myc(-).

In conclusion, it became apparent that when grown under greenhouse conditions the Nellie Hill 1 and Nellie Hill 2 gene pools responded to arbuscular mycorrhizal fungi differently than when grown outdoors in raised beds. Outdoors, the Nellie Hill 1 *B. curtipendula* had no significant response to AM fungi (Chapter 3). However, under greenhouse conditions, at P-zero the myc(+) Nellie

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Hill 1 plants grew significantly larger than the myc(-), although survival was not affected by either mycorrhizal state or P level. Similarly, the Nellie Hill 2 *B. curtipendula* showed very significant differences in both growth and survival between myc(+) and myc(-) treatments when grown outdoors, but no statistically significant differences with these treatments at any P level in the greenhouse. Perhaps the different conditions in these two environments affected the interaction of the AM fungi with the plants of the two gene pools, Nellie Hill 1 and Nellie Hill 2. Light intensity, temperature and water availability would be among the factors that differ in the two settings. Since the seed from the two gene pools came from different elevations in the sloping pasture, water availability may have been a determinant in shaping the different gene pools and thus would be important in the response of the plants. This requires further investigation.

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CHAPTER 5

GREENHOUSE EXPERIMENT II

INTRODUCTION

In previous experiments it was found that

- Plants of *B. curtipendula* from the Nellie Hill 1 gene pool, with AM fungi (myc(+)), when grown in raised beds outdoor, showed no significant differences in growth from benomyl-treated (myc(-)) plants of the same gene pool. However, when grown in the greenhouse Nellie Hill 1 myc(+) plants grew significantly larger than Nellie Hill 1 myc(-) plants.

- Plants of *B. curtipendula* from the Nellie Hill 2 gene pool, with AM fungi (myc(+)), when grown in raised beds outdoors, grew significantly larger than the benomyl-treated (myc(-)) Nellie Hill 2 plants. However, when grown in the greenhouse there were no significant growth differences between the myc(+) and myc(-) plants of this gene pool.

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- Plants of *B. curtipendula* from the Prairie Nursery gene pool, with AM fungi (myc(+)), when grown both in raised beds outdoors and in the greenhouse grew significantly larger than the benomyl-treated (myc(-)) plants.

I reasoned that water availability may differ between the outdoor and greenhouse environments, and might account for the differences in growth responses in the presence or absence of AM fungi. This experiment was designed to investigate the effects of water availability and AM fungal colonization on the three different gene pools of *B. curtipendula* under greenhouse conditions (Fig. 5.1).

METHODS

Nellie Hill 1 gene pool, Nellie Hill 2 gene pool and Prairie Nursery *B. curtipendula* seeds were sown into flats of vermiculite in the Lehman College research greenhouse. As the seeds germinated they were transplanted into vermiculite in "cone-tainer" cells, 3.8 cm diameter by 21 cm deep (Stuewe & Sons, Inc.). When 40 seeds of each gene

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gene pools	water (+) [water-abundant]	water (-) [water-deprived]
Nellie Hill 1	1 2 3 4 5 6 7 8 9 10	11 12 13 14 15 16 17 18 19 20
Nellie Hill 2	21 21 23 24 25 26 27 28 29 30	31 32 33 34 35 36 37 38 39 40
Prairie Nursery	41 42 43 44 45 46 47 48 49 50	51 52 53 54 55 56 57 58 59 60

Mycorrhizal-minus
(benomyl treatment)

gene pools	water (+) [water-abundant]	water (-) [water-deprived]
Nellie Hill 1	101 102 103 104 105 106 107 108 109 110	111 112 113 114 115 116 117 118 119 120
Nellie Hill 2	121 122 123 124 125 126 127 128 129 130	131 132 133 134 135 136 137 138 139 140
Prairie Nursery	141 142 143 144 145 146 147 148 149 150	151 152 153 154 155 156 157 158 159 160

Mycorrhizal-plus
(water control)

Fig. 5.1. Design of water deprivation experiment. Numbers refer to serial numbers of *B. curtipendula* plants.

pool had germinated the 120 seedlings were transplanted into Nellie Hill soil in "deepot" cells, 6.4 cm diameter by 25 cm deep (Stuewe & Sons, Inc.). Due to poor and spotty germination Nellie Hill 1 and Nellie Hill 2 seedlings were from seven to 30 days old at time of transplant. The Prairie Nursery seedling were seven to eight days old. The lengths of the leaves were measured at the time of this final transplant.

Inoculum

As with the raised beds experiment (Chapter 3) and the greenhouse/phosphorous experiment (Chapter 4) the arbuscular mycorrhizal fungi naturally occurring in Nellie Hill soil was the inoculum. However, because there may have been a reduction in the number of active AMF propagules due to the soil having been stored for 20 months I added the cut-up roots and the soil from ten 15 cm pots of trap plants, pot cultures of Sudan grass (*Sorghum bicolor* [L.] Moench) grown in Nellie Hill soil.

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Mycorrhizal state

Half of the deepots were treated with the fungicide benomyl to create a reduced mycorrhizal state "myc(-)". The dates of the benomyl treatments were 3/14/00, 3/25/00, 4/11/00, 4/26/00 and 5/10/00. The plants in the control half "myc(+)" were given an amount of water equal to that of the benomyl suspension.

Water treatment

Half of the myc(+) and half of the myc(-) plants were designated as "water-deprived." This group received water only at the times of the benomyl treatments except for a period of particularly hot weather when an extra watering was given. Water-deprived plants that were also myc(-) received only the water that made up the benomyl suspension. The water-deprived plants that were myc(+) received an amount of water equal to that of the benomyl suspension. The watering dates of the water-deprived group were 3/14/00, 3/25/00, 4/11/00, 4/26/00, 5/4/00 (extra watering), 5/10/00 and 5/17/00. The plants that were in the "water-abundant" group received water as needed, daily.

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Leaf water potential

At harvest two leaves were cut from five water-deprived and five abundantly-watered *B. curtipendula* plants. Their midday water potential was measured with a Scholander pressure bomb. The cut leaves were saved and included for assessment of dry mass.

Data collection

As an assessment of plant growth, data were collected on lengths of green leaf blades and vegetative stems, and dry weight of upper and lower plant parts. These measurements and time periods were as follows: lengths of green leaf blades and stems at transplant into Nellie Hill soil (zero days), and again at 28 days, 44 days, and 57 days; dry weight of plant parts at 79 days.

Statistical analysis

Analysis of covariance (ANCOVA), using the sum of the length of green leaves at zero days as covariate, was performed with all green leaf length data from measurements

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at 28, 44 and 57 days to help see if plant size variability at time zero had significant effects on the experiment. The ANCOVAs did not change the results of any ANOVAs; therefore, ANOVA results were reported.

One-way analysis of variance (ANOVA) was performed using growth (sum of green leaf lengths or dry weight) as the measurement variable, and mycorrhizal state and water availability as the treatments.

At the conclusion of the experiment (79 days) a three-way ANOVA was performed using seed source, mycorrhizal state and water availability as the main effects.

In four cases, as described in Chapter 4, approximate randomization tests were performed because some cells of the contingency tables were less than five which makes G-tests suspect. In all cases, there were no meaningful changes in P-value. Therefore, I did not proceed with the intense computational process required to analyze each of the contingency tables in this chapter beyond the conventional log-likelihood ratio test (G-test).

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Mycorrhizal dependency

Percentage mycorrhizal dependency is calculated as [(dry mass myc(+) plant) - (dry mass myc(-) plant) / dry mass myc(+) plant] x 100 (Plenchette et al, 1982).

RESULTS

Growth

Zero days

Despite minor variability at the start of the experiment, this did not contribute significantly to the outcome of the experiment (ANCOVA), as previously noted.

28 days

Mycorrhizal-plus

(Table 5.1)

Nellie Hill 1 gene pool. At 28 days, using the mean of the sum of the lengths of the green leaf blades as the measurement of growth, an ANOVA of myc(+) Nellie Hill 1

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Table 5.1. Green leaf lengths (mm) at 28 days re: mycorrhizal state. Degrees of freedom (df) = 1,18. Significant results in bold type. LSN - least significant number when alpha = 0.05. $N_{pow\ .8}$- prospective N when power = 0.8.

GENE POOL	Mycorrhizal-plus				Mycorrhizal-minus			
	Water-abundant Mean (SD)	**Water-deprived** Mean (SD)	ANOVA R-sq. (Power)	LSN ($N_{pow\ .8}$)	**Water-abundant** Mean (SD)	**Water-deprived** Mean (SD)	ANOVA R-sq. (Power)	LSN ($N_{pow\ .8}$)
Nellie Hill One	172.7 > (53.93)	93.4 (42.75)	**F = 13.28 P = 0.002** 42.5% (0.93)	7	85.4 < (30.62)	89.1 (31.90)	F = 0.07 P = 0.79 0.38% (0.06)	1100 (2244)
Nellie Hill two	246.6 > (61.75)	178.8 (67.65)	**F = 5.48 P = 0.03** 23.3% (0.60)	17 (31)	80.4 < (37.46)	110.6 (39.66)	F = 3.06 P = 0.10 14.5% (0.38)	28 (53)
Prairie Nursery	326.9 > (70.93)	222.1 (102.84)	**F = 7.04 P = 0.02** 28.1% (0.71)	14 (24)	133.7 < (51.80)	157.8 (42.53)	F = 1.30 P = 0.27 6.7% (0.19)	62 (125)

gene pool plants showed a highly significantly greater mean for water-abundant plants compared to water-deprived ($F = 13.28$, $P = 0.002$, $df = 1,18$, R-sq. = 42.5%, Power = 0.93).

Nellie Hill 2 gene pool. The myc(+) Nellie Hill 2 gene pool had a significantly greater mean for its water-abundant plants than its water-deprived ($F = 5.48$, $P = 0.03$, $df = 1,18$, R-sq. = 23.3%, Power = 0.60). Prospective power analyses showed that an increase of N to only 31 would attain statistical power of 0.8.

Prairie Nursery. The myc(+) Prairie Nursery plants also had a significantly greater mean for its water-abundant plants than its water-deprived ($F = 7.04$, $P = 0.02$, $df = 1,18$, R-sq. = 28.1%, Power = 0.71). Prospective power analyses showed that an increase of N to only 24 would attain statistical power of 0.8.

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Mycorrhizal-minus

(Table 5.1)

Nellie Hill 1 gene pool. At 28 days, using the mean of the sum of the lengths of the green leaf blades as the measurement of growth, an ANOVA of myc(-) Nellie Hill 1 gene pool plants showed no significant difference between the water-abundant and water-deprived ($F = 0.07$, $P = 0.79$ $df = 1,18$, Power = 0.06). The least significant number (LSN), the sample size for realizing a significant effect at $P = 0.05$, was 1100. Prospective power analyses showed that an N of 2244 was required in order to have statistical power of 0.8.

Nellie Hill 2 gene pool. As with Nellie Hill 1 the mean growth of myc(-) Nellie Hill 2 plants showed no significant difference between the water-abundant and water-deprived ($F = 3.06$, $P = 0.10$ $df = 1,18$, Power = 0.38). The LSN was 28; the N for prospective power of 0.8 was 53.

Prairie Nursery. The mean growth of the plants of the third gene pool, Prairie Nursery, also showed no significant difference between the water-abundant and water-deprived ($F = 1.30$, $P = 0.27$ $df = 1,18$, Power =

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0.19). The LSN was 62; the N for prospective power of 0.8 was 125.

Water-abundant

(Table 5.2)

Nellie Hill 1 gene pool. At 28 days, using the mean of the sum of the lengths of the green leaf blades as the measurement of growth, an ANOVA of water-abundant Nellie Hill 1 gene pool plants showed a very highly significantly greater means for myc(+) compared to myc(-) ($F = 19.82$, $P = 0.0003$, df = 1,18, R-sq. = 52.4%, Power = 0.99).

Nellie Hill 2 gene pool. As with Nellie Hill 1, the mean for growth of the water-abundant Nellie Hill 2 gene pool plants was very highly significantly greater for myc(+) compared to myc(-) ($F = 52.96$, $P = <0.0001$, df = 1,18, R-sq. = 74.6%, Power = 1.00).

Prairie Nursery. The third gene pool, Prairie Nursery, also had a very highly significantly greater mean for its myc(+) plants compared to myc(-) when water

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Table 5.2. Green leaf lengths (mm) at 28 days re: water availabilty. Degrees of freedom (df) = 1,18. Significant results in bold type. LSN - least significant number when alpha = 0.05. $N_{pow.8}$- prospective N when power = 0.8. Data rearranged from table 5.1 in order to test different hypotheses.

GENE POOL	Water - abundant				Water - deprived			
	Myc(+) Mean (SD)	**Myc(-)** Mean (SD)	ANOVA R-sq. (Power)	LSN ($N_{pow.8}$)	**Myc(+)** Mean (SD)	**Myc(-)** Mean (SD)	ANOVA R-sq. (Power)	LSN ($N_{pow.8}$)
Nellie Hill one	172.7 > (53.93)	85.4 (30.62)	**F=19.82 P=0.0003** 52.4% (0.99)	7	93.4 > (42.75)	89.1 (31.90)	F = 0.07 P = 0.80 0.4% (0.06)	1185 (2420)
Nellie Hill two	246.6 > (61.75)	80.4 (37.46)	**F=52.96 P=<0.0001** 74.6% (1.00)	5	178.8 > (67.65)	110.6 (39.66)	**F = 7.56 P = 0.01** 29.6% (0.74)	13 (24)
Prairie Nursery	326.9 > (70.93)	133.7 (51.80)	**F=48.39 P=<0.0001** 72.8% (1.00)	5	222.1 > (102.84)	157.8 (42.53)	F = 3.34 P = 0.08 15.0% (0.41)	26 (50)

was abundant (F = 48.39, P = <0.0001, df = 1,18, R-sq. = 72.8%, Power = 1.00).

Water-deprived

(Table 5.2)

Nellie Hill 1 gene pool. When water-deprived there was no significant difference in growth between the myc(+) and the myc(-) Nellie Hill 1 plants (F = 0.07, P = 0.80 df = 1,18. Power = 0.06). The LSN was 1185; the N for prospective power of 0.8 was 2420.

Nellie Hill 2 gene pool. When water-deprived the mean of the sum of the lengths of the green leaf blades of the myc(+) plants was significantly greater that that of the myc(-) for Nellie Hill 2 (F = 7.56, P = 0.01, df = 1,18, R-sq. = 29.6%, Power = 0.74). The N for prospective power of 0.8 was 24.

Prairie Nursery. When water-deprived there was no significant difference in growth between the myc(+) and the myc(-) Prairie Nursery plants (F = 3.34, P = 0.08 df = 1,18, Power = 0.41). The LSN was 26; the N for prospective power of 0.8 was 50.

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44 days

Mycorrhizal-plus

(Table 5.3)

Nellie Hill 1 gene pool. At 44 days, using the mean of the sum of the lengths of the green leaf blades as the measurement of growth, an ANOVA of myc(+) Nellie Hill 1 gene pool plants showed a highly significantly greater mean for water-abundant plants compared to water-deprived ($F = 12.76$, $P = 0.002$, $df = 1,18$, R-sq. = 41.5%, Power = 0.90).

Nellie Hill 2 gene pool. As with Nellie Hill 1, the myc(+) Nellie Hill 2 gene pool had a highly significantly greater mean for growth for water-abundant plants compared to water-deprived ($F = 15.55$, $P = 0.001$, $df = 1,18$, R-sq. = 46.3%, Power = 0.96).

Prairie Nursery. The mean for growth for myc(+) Prairie Nursery plants was very highly significantly greater for its water-abundant plants compared to water-deprived ($F = 21.12$, $P = 0.0002$, $df = 1,18$, R-sq. = 54%, Power = 0.99).

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Table 5.3. Green leaf lengths (mm) at 44 days re: mycorrhizal state. Degrees of freedom (df) = 1,18. Significant results in bold type. LSN - least significant number when alpha = 0.05. $N_{pow\ 8}$- prospective N when power = 0.8.

GENE POOL	Mycorrhizal-plus				Mycorrhizal-minus			
	Water-abundant Mean (SD)	**Water-deprived** Mean (SD)	ANOVA R-sq. (Power)	LSN ($N_{pow\ 8}$)	**Water-abundant** Mean (SD)	**Water-deprived** Mean (SD)	ANOVA R-sq. (Power)	LSN ($N_{pow\ 8}$)
Nellie Hill One	338.90 > (131.40)	138.80 (118.80)	**F = 12.76 P = 0.002** 41.5% (0.90)	9	109.4 > (74.59)	97.2 (72.14)	F = 0.14 P = 0.71 0.7% (0.06)	558 (1138)
Nellie Hill two	499.10 > (88.65)	298.30 (134.41)	**F = 15.55 P = 0.001** 46.3% (0.96)	8	86.8 < (41.88)	114.5 (67.37)	F = 1.22 P = 0.28 6.3% (0.18)	65 (131)
Prairie Nursery	614.7 > (102.68)	338.9 (159.60)	**F = 21.12 P = 0.0002** 54% (0.99)	6	197.4 > (106.46)	138.2 (44.60)	F = 2.63 P = 0.28 12.7% (0.34)	32 (62)

Mycorrhizal-minus

(Table 5.3)

Nellie Hill 1 gene pool. At 44 days, using the mean of the sum of the lengths of the green leaf blades as the measurement of growth, an ANOVA of myc(-) Nellie Hill 1 gene pool plants showed no significant difference between the water-abundant and water-deprived ($F = 0.14$, $P = 0.71$ $df = 1,18$, Power = 0.06). The LSN was 558; N at prospective power of 0.8 was 1138.

Nellie Hill 2 gene pool. As with Nellie Hill 1, the mean growth of myc(-) Nellie Hill 2 plants showed no significant difference between the water-abundant and water-deprived ($F = 1.22$, $P = 0.28$ $df = 1,18$, Power = 0.18). The LSN was 65; N at prospective power of 0.8 was 131.

Prairie Nursery. The mean growth of the plants of the third gene pool, Prairie Nursery, also showed no significant difference between the water-abundant

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and water-deprived (F = 2.63, P = 0.28 df = 1,18, Power = 0.34). The LSN was 32; N for prospective power of 0.8 was 62.

Water-abundant

(Table 5.4)

(Fig.5.2)

Nellie Hill 1 gene pool. At 44 days, using the mean of the sum of the lengths of the green leaf blades as the measurement of growth, an ANOVA of water-abundant Nellie Hill 1 gene pool plants showed a very highly significantly greater means for myc(+) compared to myc(-) (F = 23.08, P = 0.0001, df = 1,18, R-sq. = 56%, Power = 1.00).

Nellie Hill 2 gene pool. As with Nellie Hill 1, the mean for growth of the water-abundant Nellie Hill 2 gene pool plants was very highly significantly greater for myc(+) compared to myc(-) (F = 176.84, P = <0.0001, df = 1,18, R-sq. = 91%, Power = 1.00).

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Table 5.4. Green leaf lengths (mm) at 44 days re: water availabilty. Degrees of freedom (df) = 1,18. Significant results in bold type. LSN - least significant number when alpha = 0.05. $N_{pow.8}$- prospective N when power = 0.8.

GENE POOL	Water - abundant				Water - deprived			
	Myc(+)	**Myc(-)**	ANOVA	LSN	**Myc(+)**	**Myc(-)**	ANOVA	LSN
	Mean (SD)	Mean (SD)	R-sq. (Power)	($N_{pow.8}$)	Mean (SD)	Mean (SD)	R-sq. (Power)	($N_{pow.8}$)
Nellie Hill One	338.90 > (131.40)	109.40 (74.59)	**F = 23.08 P = 0.0001** 56% (1.00)	6	138.80 > (118.80)	97.20 (72.14)	F = 0.896 P = 0.36 4.7% (0.15)	88 (177)
Nellie Hill two	499.1 > (88.65)	86.8 (41.88)	**F = 176.84 P = <0.0001** 91% (1.00)	4	298.3 > (134.41)	114.5 (67.37)	**F = 14.95 P = 0.001** 45% (0.95)	8
Prairie Nursery	614.7 > (102.68)	197.4 (106.46)	**F = 79.60 P = <0.0001** 82% (1.00)	4	338.9 > (159.60)	138.2 (44.60)	**F = 14.7 P = 0.001** 45% (0.95)	8

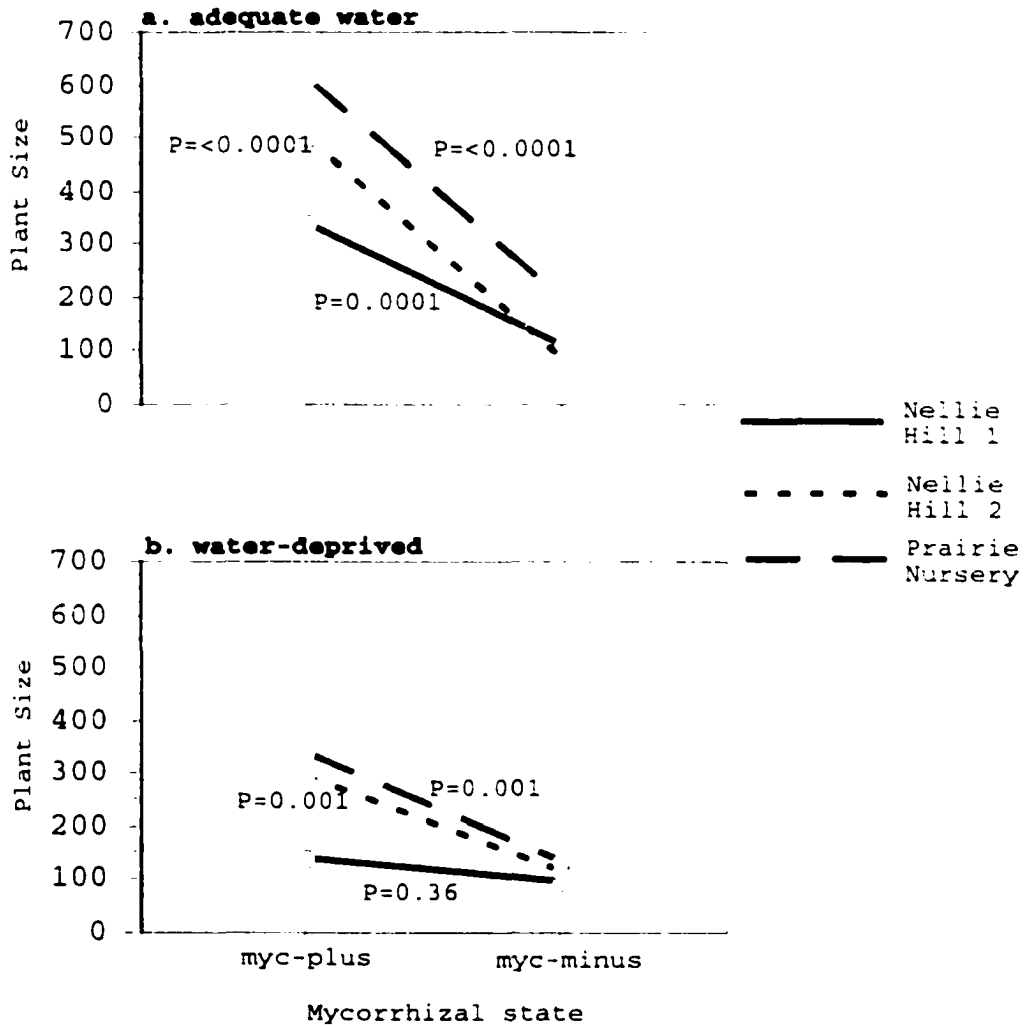

Fig. 5.2. Effect of mycorrhizal state on growth at 44 days when watered (a) and droughted (b). Plant size is mean sum of leaf lengths (mm). P-values from ANOVA of plant size (response variable) and mycorrhizal state (independent variable).

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Prairie Nursery gene pool. The third gene pool, Prairie Nursery, also had a very highly significantly greater mean for its myc(+) plants compared to myc(-) when water was abundant ($F = 79.60$, $P = <0.0001$, $df = 1,18$, $df = 1,18$, R-sq. = 82%, Power = 1.00).

Water-deprived

(Table 5.4)

(Fig. 5.2)

Nellie Hill 1 gene pool. When water-deprived there was no significant difference in growth between the myc(+) and the myc(-) Nellie Hill 1 plants ($F = 0.896$, $P = 0.36$ $df = 1,18$, Power = 0.15). The LSN was 88; N for prospective power of 0.8 was 177.

Nellie Hill 2 gene pool. When water-deprived the mean of the sum of the lengths of the green leaf blades of the myc(+) plants was highly significantly greater than that of the myc(-) for Nellie Hill 2 ($F = 14.95$, $P = 0.001$, $df = 1,18$, R-sq. = 45%, Power = 0.95).

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Prairie Nursery. As with Nellie Hill 2, water-deprived Prairie Nursery myc(+) plants had a highly significantly greater mean for the sum of the lengths of the green leaf blades than that of myc(-) ($F = 14.70$, $P = 0.001$, $df = 1,18$, R-sq. = 45%, Power = 0.95).

57 days

Mycorrhizal-plus

(Table 5.5)

Nellie Hill 1 gene pool. At 57 days, using the mean of the sum of the lengths of the green leaf blades as the measurement of growth, an ANOVA of myc(+) Nellie Hill 1 gene pool plants showed very highly significantly greater mean for water-abundant plants compared to water-deprived ($F = 17.63$, $P = 0.0005$, $df = 1,18$, R-sq. = 49.5%, Power = 0.98).

Nellie Hill 2 gene pool. The myc(+) Nellie Hill 2 gene pool had a highly significantly greater mean for

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Table 5.5. Green leaf lengths (mm) at 57 days re: mycorrhizal state. Degrees of freedom (df) = 1,18. Significant results in bold type. LSN - least significant number when alpha = 0.05. $N_{pow\ .8}$- prospective N when power = 0.8.

GENE POOL	Mycorrhizal-plus				Mycorrhizal-minus			
	Water-abundant Mean (SD)	**Water-deprived** Mean (SD)	ANOVA R-sq. (Power)	LSN ($N_{pow\ .8}$)	**Water-abundant** Mean (SD)	**Water-deprived** Mean (SD)	ANOVA R-sq. (Power)	LSN ($N_{pow\ .8}$)
Nellie Hill One	504.3 > (127.27)	213.4 (178.31)	**F = 17.63 P = 0.0005** 49.5% (0.98)	7	171.2 > (157.71)	120.3 (92.04)	F = 0.795 P = 0.38 4.2% (0.14)	99 (200)
Nellie Hill two	779.9 > (118.60)	515.2 (238.0)	**F = 9.91 P = 0.006** 35.5% (0.85)	11	149.3 > (115.91)	135.5 (80.68)	F = 0.125 P = 0.73 0.6% (0.06)	616 (1256)
Prairie Nursery	857.6 > (140.60)	476.7 (236.60)	**F = 19.15 P = 0.0004** 51.6% (0.99)	7	346.7 > (211.64)	166.3 (90.24)	**F = 6.148 P = 0.02** 25.5% (0.65)	15 (28)

growth for water-abundant plants compared to water-deprived (F = 9.91, P = 0.006, df = 1,18, R-sq. = 35.5%, Power = 0.85).

Prairie Nursery. The mean for growth for myc(+) Prairie Nursery plants was very highly significantly greater for its water-abundant plants compared to water-deprived (F = 19.15, P = 0.0004, df = 1,18, R-sq. = 51.6%, Power = 0.99).

Mycorrhizal-minus

(Table 5.5)

Nellie Hill 1 gene pool. At 57 days, using the mean of the sum of the lengths of the green leaf blades as the measurement of growth, an ANOVA of myc(-) Nellie Hill 1 gene pool plants showed no significant difference between the water-abundant and water-deprived (F = 0.795. P = 0.38 df = 1,18, Power = 0.14). The LSN was 99; the N for prospective power of 0.8 was 200.

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Nellie Hill 2 gene pool. As with Nellie Hill 1, the mean growth of myc(-) Nellie Hill 2 plants showed no significant difference between the water-abundant and water-deprived (F = 0.125, P = 0.73 df = 1,18, Power = 0.06). The LSN was 616; the N for prospective power of 0.8 was 1256.

Prairie Nursery. The mean growth for myc(-) Prairie Nursery plants was significantly greater for its water-abundant plants compared to water-deprived (F = 6.148, P = 0.02, df = 1,18, R-sq. = 25.5%, Power = 0.65). The N for prospective power at 0.8 was 28.

Water-abundant

(Table 5.6)

Nellie Hill 1 gene pool. At 57 days, using the mean of the sum of the lengths of the green leaf blades as the measurement of growth, an ANOVA of water-abundant Nellie Hill 1 gene pool plants showed a very highly significantly

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Table 5.6. Green leaf lengths (mm) at 57 days re: water availabilty. Degrees of freedom (df) = 1,18. Significant results in bold type. LSN - least significant number when alpha = 0.05. $N_{pow\ 8}$- prospective N when power = 0.8.

GENE POOL	Water - abundant				Water - deprived			
	Myc(+) Mean (SD)	**Myc(-)** Mean (SD)	ANOVA R-sq. (Power)	LSN ($N_{pow\ 8}$)	**Myc(+)** Mean (SD)	**Myc(-)** Mean (SD)	ANOVA R-sq. (Power)	LSN ($N_{pow\ 8}$)
Nellie Hill One	504.3 > (127.27)	171.8 (157.71)	**F = 26.92 P = <0.0001** 59.9% (1.00)	6	213.4 > (178.31)	120.3 (92.04)	F = 2.15 P = 0.16 10.7% (0.28)	38 (75)
Nellie Hill two	779.9 > (118.62)	149.3 (115.90)	**F = 144.58 P = <0.0001** 88.9% (1.00)	4	515.2 > (238.01)	133.5 (80.68)	**F = 23.07 P = 0.0001** 56.2% (1.00)	6
Prairie Nursery	857.6 > (140.61)	346.7 (211.64)	**F = 40.43 P = <0.0001** 69.2% (1.00)	5	476.7 > (236.61)	166.3 (90.24)	**F = 15.03 P = 0.001** 45.5% (0.96)	8

greater means for myc(+) compared to myc(-) (F = 26.92, P = <0.0001, df = 1,18, R-sq. = 59.9%, Power = 1.00).

Nellie Hill 2 gene pool. As with Nellie Hill 1, the mean for growth of the water-abundant Nellie Hill 2 gene pool plants was very highly significantly greater for myc(+) compared to myc(-) (F = 144.58, P = <0.0001, df = 1,18, R-sq. = 88.9%, Power = 1.00).

Prairie Nursery. The third gene pool, Prairie Nursery, also had a very highly significantly greater mean for its myc(+) plants compared to the myc(-) when water was abundant (F = 40.43, P = <0.0001, df = 1,18, R-sq. = 69.2%, Power = 1.00).

Water-deprived

(Table 5.6)

Nellie Hill 1 gene pool. When water-deprived there was no significant difference in growth between the myc(+) and the myc(-) Nellie Hill 1 plants (F = 2.15, P = 0.16, df = 1,18, Power = 0.28). The LSN was 38; the N for prospective power of 0.8 was 75.

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Nellie Hill 2 gene pool. When water-deprived the mean of the sum of the lengths of the green leaf blades of the myc(+) plants was very highly significantly greater than that of the myc(-) for Nellie hill 2 ($F = 23.07$, $P = 0.0001$, $df = 1,18$, R-sq. = 56.2%, Power = 1.00).

Prairie Nursery. When water-deprived the mean growth of the myc(+) Prairie Nursery plants was highly significantly greater than that of the myc(-) ($F = 15.03$, $P = 0.001$, $df = 1,18$, R-sq. = 45.5%, Power = 0.96).

Summary of plant growth for 28, 44 and 57 days

(Fig. 5.3)

Figure 5.3 shows graphically the plant growth response to AMF colonization and water availability for the three gene pools over time. In all three gene pools, with abundant water myc(+) plants grew significantly more than myc(-) plants. This growth difference increased with time. At 44 and 57 days the droughted myc(+) Nellie Hill 2 and

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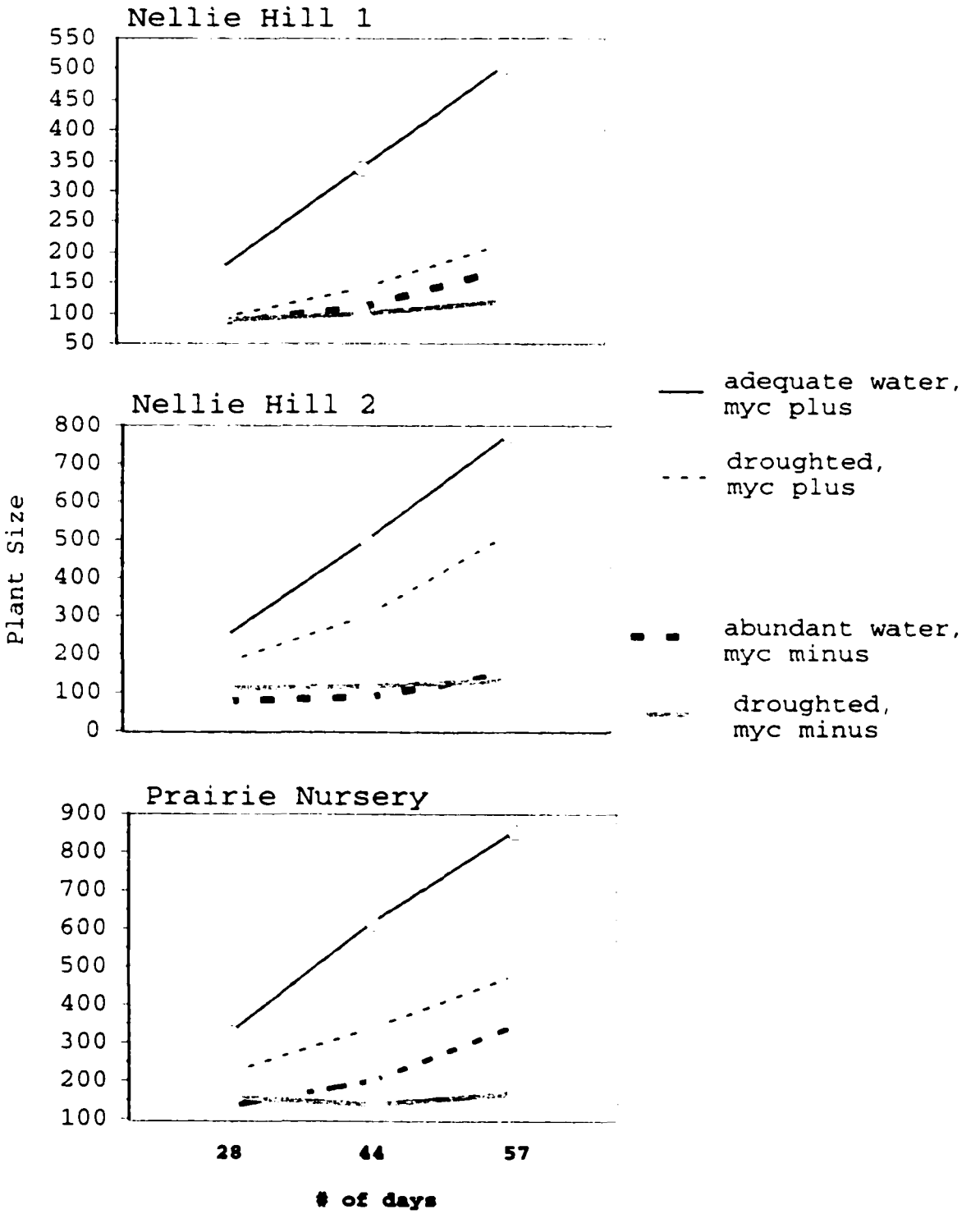

Fig. 5.3. Plant growth response to AMF colonization and water availability. Plant size is mean sum of leaf lengths (mm).

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Prairie Nursery plants also grew significantly more than the myc(-) plants, although with less of a difference. Under droughted conditions the Nellie Hill 1 plants had no significant growth difference between the myc(+) and myc(-) state.

79 days

Mycorrhizal-plus

(shoots - Table 5.7;

roots - Table 5.8)

Plants were harvested at 79 days enabling dry weight (shoots - Table 5.7 ; roots - Table 5.8) to be used as the measurement of growth.

Nellie Hill 1 gene pool. At 79 days, using the mean of the dry weight of the upper plant parts as the measurement of growth, an ANOVA of myc(+) Nellie Hill 1 gene pool plants showed a highly significantly greater mean for water-abundant plants compared to water-deprived ($F = 11.52$, $P = 0.003$, $df = 1,18$, R-sq. = 38.8%, Power = 0.89).

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Table 5.7. Dry weight (gms) of upper plant at 79 days re: mycorrhizal state. Degrees of freedom (df) = 1,18. Significant results in bold type. LSN is least significant number when alpha = 0.05. $N_{pow.8}$ is prospective N when power = 0.8.

GENE POOL	Mycorrhizal-plus				Mycorrhizal-minus			
	Water-abundant Mean (SD)	**Water-deprived** Mean (SD)	ANOVA R-sq. (Power)	LSN ($N_{pow.8}$)	**Water-abundant** Mean (SD)	**Water-deprived** Mean (SD)	ANOVA R-sq. (Power)	LSN ($N_{pow.8}$)
Nellie Hill One	0.113 > (0.055)	0.041 (0.039)	**F = 11.52 P = 0.003** 38.8% (0.89)	9	0.038 > (0.042)	0.017 (0.017)	F = 2.05 P = 0.17 10% (0.27)	40 (80)
Nellie Hill two	0.185 > (0.051)	0.101 (0.067)	**F = 9.90 P = 0.006** 35.4% (0.84)	11	0.021 (0.017)	0.019 (0.013)	F = 0.06 P = 0.82 0.3% (0.06)	1404 2867
Prairie Nursery	0.193 > (0.055)	0.092 (0.054)	**F = 17.40 P = 0.0006** 49% (0.97)	7	0.078 > (0.06)	0.027 (0.018)	**F = 6.63 P = 0.02** 27.1% (0.68)	14 (26)

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Table 5.8. Dry weight (gms) of roots at 79 days re: mycorrhizal state. Significant results in bold type. LSN - least significant number when alpha = 0.05. $N_{pow\ 8}$- prospective N when power = 0.8.

GENE POOL	Mycorrhizal-plus				Mycorrhizal-minus			
	Water-abundant Mean (SD) N	**Water-deprived** Mean (SD) N	ANOVA R-sq. (Power)	LSN ($N_{pow\ 8}$)	**Water-abundant** Mean (SD) N	**Water-deprived** Mean (SD) N	ANOVA R-sq. (Power)	LSN ($N_{pow\ 8}$)
Nellie Hill One	0.109 > (0.063) 8	0.037 (0.047) 7	**F = 6.087** **P = 0.03** 31.9% (0.63)	12 (22)	0.021 > (0.027) 9	0.012 (0.012) 9	F = 0.857 P = 0.37 5.1% (0.14)	83 (168)
Nellie Hill two	0.158 > (0.040) 10	0.105 (0.076) 10	F = 3.782 P = 0.07 17.4% (0.44)	23 (44)	0.012 (0.013) 7	0.013 (0.010) 10	F = 0.051 P = 0.82 0.34% (0.06)	1274 (2600)
Prairie Nursery	0.184 > (0.040) 9	0.169 (0.240) 8	F = 0.034 P = 0.86 0.23% (0.05)	1932 (3944)	0.058 > (0.051) 10	0.020 (0.016) 9	**F = 4.479** **P = 0.05** 20.1% (0.51)	19 (36)

An ANOVA of the myc(+) root dry weight showed a significantly greater mean for water-abundant plants compared to water-deprived (F = 6.087, P = 0.03, R-sq. = 31.9%, df = 1,13, Power = 0.63). An N of 22 would give a prospective power of 0.8.

Nellie Hill 2 gene pool. As with Nellie Hill 1, the myc(+) Nellie Hill 2 gene pool had a highly significantly greater mean for growth for water-abundant upper plant parts compared to water-deprived (F = 9.90, P = 0.006, df = 1,18, R-sq. = 35.4%, Power = 0.84).

An ANOVA of the myc(+) root dry weight showed no significant differences between the water-abundant and water-deprived (F =3.782, P = 0.07, df = 1,18, Power = 0.44). The LSN was 23; an N of 44 would give a prospective power of 0.8.

Prairie Nursery. The mean for growth for myc(+) Prairie Nursery plants was very highly significantly greater for its water-abundant upper plant parts compared to water-deprived (F = 17.40, P = 0.0006, df = 1,18, R-sq. = 49%, Power = 0.97).

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An ANOVA of the myc(+) root dry weight showed no significant differences between the water-abundant and water-deprived (F = 0.034, P = 0.86, df = 1,15, Power = 0.05). The LSN was 1932; an N of 3944 would give a prospective power of 0.8.

Mycorrhizal-minus
(shoots - Table 5.7;
roots - Table 5.8)

Nellie Hill 1 gene pool. At 79 days, using the mean of the dry weight of the upper plant parts as the measurement of growth, an ANOVA of myc(+) Nellie Hill 1 gene pool plants showed no significant difference between the water-abundant and water-deprived (F = 2.05, P = 0.17 df = 1,18, Power = 0.27). The LSN was 40; the N for prospective power of 0.8 was 80.

An ANOVA of the myc(-) root dry weight showed no significant difference between the water-abundant and water-deprived (F = 0.857, P = 0.37, df = 1,16, Power = 0.14). The LSN was 83; the N for prospective power of 0.8 was 168.

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Nellie Hill 2 gene pool. As with Nellie Hill 1 the mean of growth of myc(-) Nellie Hill 2 upper plants showed no significant difference between the water-abundant and water-deprived (F = 0.06, P = 0.82 df = 1,18, Power = 0.06). The LSN was 1404; the N for prospective power of 0.8 was 2867.

An ANOVA of the myc(-) root dry weight showed no significant difference between the water-abundant and water-deprived (F = 0.051, P = 0.82, df = 1,15, Power = 0.06). The LSN was 1274; the N for prospective power of 0.8 was 2600.

Prairie Nursery. The mean for growth for myc(-) Prairie Nursery plants was significantly greater for its water-abundant upper plant parts compared to the water-deprived (F = 6.63, P = 0.02, df = 1,18, R-sq. = 27.1%, Power = 0.68). The N for prospective power of 0.8 was 26.

An ANOVA of the myc(-) root dry weight showed a significantly greater mean for the water-abundant compared to the water-deprived (F = 4.479, P = 0.05, R-sq. = 20.1% df = 1,17, Power = 0.51). The N for prospective power of 0.8 was 36.

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Water-abundant

(shoots - Table 5.9;

roots - Table 5.10)

Nellie Hill 1 gene pool. At 79 days, using the mean of the dry weight of the upper plant parts as the measurement of growth, an ANOVA of water-abundant Nellie Hill 1 gene pool plants showed a highly significantly greater means for myc(+) compared to myc(-) ($F = 11.73$, $P = 0.003$, $df = 1,18$, R-sq. = 39.4%, Power = 0.9).

An ANOVA of the water-abundant roots showed a highly significantly greater means for myc(+) compared to myc(-) ($F = 14.559$, $P = 0.002$, $df = 1,15$, R-sq. = 49%, Power = 0.96).

Nellie Hill 2 gene pool. The mean for growth of the water-abundant Nellie Hill 2 gene pool upper plant parts was very highly significantly greater for myc(+) compared to myc(-) ($F = 92.64$, $P = <0.0001$, $df = 1,18$, R-sq. = 83.3%, Power = 1.00).

An ANOVA of the water-abundant roots showed a very highly significantly greater means for myc(+) compared to

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Table 5.9. Dry weight (gms) of upper plant at 79 days re: water availabilty. Degrees of freedom (df) = 1,18. Significant results in bold type. LSN - least significant number when alpha = 0.05. $N_{pow\ .8}$- prospective N when power = 0.8.

GENE POOL	Water - abundant				Water - deprived			
	Myc(+)	**Myc(-)**	ANOVA	LSN	**Myc(+)**	**Myc(-)**	ANOVA	LSN
	Mean (SD)	Mean (SD)	R-sq. (Power)	($N_{pow\ .8}$)	Mean (SD)	Mean (SD)	R-sq. (Power)	($N_{pow\ .8}$)
Nellie Hill One	0.113 > (0.055)	0.038 (0.042)	**F = 11.73 P = 0.003** 39.4% (0.90)	9	0.041 > (0.039)	0.017 (0.017)	F = 3.01 P = 0.10 14% (0.37)	28 (55)
Nellie Hill two	0.185 > (0.051)	0.021 (0.017)	**F = 92.64 P = <0.0001** 83.8% (1.00)	4	0.101 > (0.067)	0.019 (0.013)	**F = 14.45 P = 0.0013** 44.7% (0.95)	8
Prairie Nursery	0.193 > (0.055)	0.078 (0.060)	**F = 20.09 P = 0.0003** 52.8% (0.99)	7	0.092 > (0.054)	0.027 (0.018)	**F = 12.83 P = 0.002** 42.0% (0.92)	9

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Table 5.10. Dry weight (gms) of roots at 79 days re: water availabilty. Significant results in bold type. LSN - least significant number when alpha = 0.05. $N_{pow\ 8}$- prospective N when power = 0.8.

GENE POOL	Water - abundant				Water - deprived			
	Myc(+)	**Myc(-)**	ANOVA	LSN	**Myc(+)**	**Myc(-)**	ANOVA	LSN
	Mean (SD) N	Mean (SD) N	R-sq. (Power)	($N_{pow\ 8}$)	Mean (SD) N	Mean (SD) N	R-sq. (Power)	($N_{pow\ 8}$)
Nellie Hill One	0.109 > (0.063) 8	0.021 (0.027) 9	**F = 14.559 P = 0.002** 49% (0.96)	17	0.037 > (0.047) 7	0.012 (0.012) 9	F = 2.385 P = 0.14 14.5% (0.29)	28 (55)
Nellie Hill two	0.158 > (0.040) 10	0.012 (0.013) 7	**F = 86.336 P = <0.0001** 85% (1.00)	4	0.105 > (0.076) 10	0.013 (0.010) 10	**F = 14.575 P = 0.001** 44.7% (0.96)	8
Prairie Nursery	0.184 > (0.040) 9	0.058 (0.051) 10	**F = 34.883 P = <0.0001** 67.2% (1.00)	5	0.169 (0.240) 8	0.020 (0.016) 9	F = 3.499 P = 0.08 18.9% (0.41)	21 (39)

myc(-) (F = 86.336, P = <0.0001, df = 1,15, R-sq. = 85%, Power = 1.00).

Prairie Nursery. The third gene pool, Prairie Nursery, also had a very highly significantly greater mean for its myc(+) upper plant parts compared to myc(-) when water was abundant (F = 20.09, P = 0.0003, df = 1,18, R-sq. = 52.8%, Power = 0.99).

The water-abundant roots of Prairie Nursery also had a very highly significantly greater mean for the myc(+) plants compared to the myc(-) (F = 34.883, P = <0.0001, df = 1,17, R-sq. = 67.2%, Power = 1.00).

Water-deprived

(shoots - Table 5.9;

roots - Table 5.10)

Nellie Hill 1 gene pool. When water-deprived there was no significant difference in growth between the myc(+) and myc(-) Nellie Hill 1 upper plant parts (F = 3.01, P = 0.10 df = 1,18, Power = 0.37). The LSN was 28; the N for prospective power of 0.8 was 55.

As with the upper plant parts the water-deprived roots had no significant difference between the myc(+) and the

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myc(-) (F = 2.385, P = 0.14, df = 1,14, Power = 0.29). The LSN was 28; the N for prospective power of 0.8 was 55.

Nellie Hill 2 gene pool. When water-deprived the mean of the dry weight of the myc(+) upper plant parts was highly significantly greater than that of the myc(-) for Nellie Hill 2 (F = 14.45, P = 0.0013, df = 1,18, R-sq. = 44.7%, Power = 0.95).

As with the upper plant parts the water-deprived Nellie Hill 2 roots had a highly significantly greater weight for the myc(+) compared to the myc(-) (F = 14.575, P = 0.001, df = 1,18, R-sq. = 44.7%, Power = 0.96).

Prairie Nursery. The third gene pool, Prairie Nursery, also had a very highly significantly greater mean for its myc(+) upper plant parts compared to the myc(-) when water-deprived (F = 12.83, P = 0.002, df = 1,18, R-sq. = 42.0%, Power = 0.92).

An ANOVA of the water-deprived Prairie Nursery roots showed no significant difference between the myc(+) and myc(-) plants (F = 3.499, P = 0.08, df = 1,15, Power =

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0.41). The LSN was 21; the N for prospective power of 0.8 was 39.

Overall experiment

(Table 5.11)

The overall experiment can also be expressed as a three-way ANOVA with the dry weight of the shoots as the dependent variable and the mycorrhizal state, water availability and seed source as the main effects. The mycorrhizal state (myc(+), myc(-)) was very highly significant (F = 114.360, P = <0.0001, R-sq. = 35.65%, Power = 1.00). Water availability was very highly significant (F = 45.440, P = <0.0001, R-sq. = 14.16%, Power = 1.00). Seed source was also very highly significant (F = 10.504, P = <0.0001, R-sq. = 6.55%, Power = 0.99).

The interaction of water availability and the mycorrhizal state was highly significant (F = 14.206, P = 0.0003, R-sq. = 4.42%, Power = 0.98). The interaction of seed source and mycorrhizal state was significant (F = 6.836, P = 0.0016, R-sq. = 4.26%, Power = 0.93). The interaction of seed source and water availability was not

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Table 5.11. Three-way ANOVA with dry weight of shoots as dependent variable; N = 120.

Source of variation	DF	Sum of squares	F-value	P-value	R-sq.	Power
Mycorrhizal state (myc-plus vs. myc-minus)	1	0.22812	114.360	<0.0001	35.65%	1.00
Water availability (adequate water vs. drought)	1	0.09064	45.440	<0.0001	14.16%	1.00
Seed source (Nellie Hill 1 vs. Nellie Hill 2 vs. Prairie Nursery)	2	0.04190	10.504	<0.0001	6.55%	0.99
Water availability * mycorrhizal state	1	0.02834	14.206	0.0003	4.42%	0.98
Seed source * mycorrhizal state	2	0.02727	6.836	0.0016	4.26%	0.93
Seed source * water availability	2	0.00668	0.0068	0.1923	1.04%	0.33
Seed source * water availability * mycorrhizal state	2	0.00157	0.0016	0.6759	0.25%	0.11
Unexplained	108	0.21543			33.66%	
Total		0.63994				

significant (F = 0.0068, P = 0.1923, R-sq. = 1.04%, Power = 0.33). The interaction of the three effects, seed source * water availability * mycorrhizal state was also not significant (F=0.0016, P=0.68, R-sq.= 0.25%, Power = 0.11).

Root:shoot ratio

(Table 5.12)

In all three gene pools the root:shoot ratio was higher in the myc(+) state compared to the myc(-) when either abundantly watered or droughted.

Survival

28 days

(Table 5.13)

At 28 days, 11 out of 120 plants had died. Of the non-survivors, seven were myc(+), four were myc(-). Three of the myc(+) had been abundantly watered; four were water-deprived. Of the four myc(-) non-survivors two had been abundantly watered and two were water-deprived. There were no significant differences in survival between the mycorrhizal state and/or the water state in any of the three gene pools.

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Table 5.12. Effects of AM fungi and water deprivation on growth (gms) of roots and shoots. Root and shoot weights are means of the N replicates. Percentage mycorrhizal dependence = [(dry wt mycorhizal plus plant) - (dry wt mycorrhizal minus plant) / (dry wt mycorrhizal plus plant)] X 100.

Gene pool	Mycorrhizal state	Water state	Dry weight of tissue			Root:shoot ratio	(%) mycorrhizal dependence
			Root (N)	Shoot (N)	Total		
	minus	abundant	0.021 (9)	0.040 (9)	0.061	0.525	
Nellie	plus	abundant	0.109 (8)	0.120 (8)	0.229	0.908	73.4%
Hill 1	minus	droughted	0.012 (9)	0.017 (9)	0.029	0.705	
	plus	droughted	0.037 (7)	0.045 (7)	0.082	0.822	64.6%
Nellie	minus	abundant	0.012 (7)	0.020 (8)	0.032	0.600	
	plus	abundant	0.158 (10)	0.185 (10)	0.343	0.854	90.6%
Hill 2							
	minus	droughted	0.013 (10)	0.019 (10)	0.032	0.684	
	plus	droughted	0.105 (10)	0.101 (10)	0.206	1.040	84.5%
Prairie	minus	abundant	0.058 (10)	0.078 (10)	0.136	0.743	
	plus	abundant	0.184 (9)	0.199 (9)	0.383	0.924	64.5%
Nursery							
	minus	droughted	0.020 (9)	0.029 (9)	0.049	0.690	
	plus	droughted	0.169 (8)	0.097 (8)	0.266	1.742	81.6%

Table 5.13. Survival at 28 days

GENE POOL	Mycorrhizal-plus				Mycorrhizal-minus			
	Water-abundant		Water-deprived		Water-abundant		Water-deprived	
	SURVIVAL				SURVIVAL			
	Yes	No	Yes	No	Yes	No	Yes	No
Nellie Hill one	8	2	8	2	9	1	9	1
Nellie Hill two	10	0	10	0	9	1	10	0
Prairie Nursery	9	1	8	2	10	0	9	1
Total	27	3	26	4	28	2	28	2

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44 days

At 44 days, 12 out of 120 plants had died. Of the non-survivors, seven were myc(+), five were myc(-). Four of the myc(+) had been abundantly watered; four had been water-deprived. Of the four myc(-) non-survivors two had been abundantly watered and two were water-deprived. There were no significant differences in survival between the mycorrhizal state and/or the water state in any of the three gene pools.

57 days

At 57 days, there were no additional plant deaths.

79 days

By the conclusion of the experiment, 79 days, 13 out of 120 plants had died (Table 5.14). Of the non-survivors, eight were myc(+) and 5 were myc(-); six had had abundant water and seven were water-deprived.

A log-likelihood ratio test for association between survival and the water state for the myc(+) showed no significant difference ($G = 0.582$, $df = 1$, $P = 0.45$)

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Table 5.14. Survival at 79 days

GENE POOL	Mycorrhizal-plus				Mycorrhizal-minus			
	Water-abundant		Water-deprived		Water-abundant		Water-deprived	
	SURVIVAL				SURVIVAL			
	Yes	No	Yes	No	Yes	No	Yes	No
Nellie Hill one	8	2	7	3	9	1	9	1
Nellie Hill two	10	0	10	0	8	2	10	0
Prairie Nursery	9	1	8	2	10	0	9	1
Total plants	27	3	25	5	27	3	28	2

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(Table 5.15a). Therefore, I merged the two water states and performed a log-likelihood ratio test for association between survival and seed source (Table 5.15b). A significant association was found (G = 7.719. df = 2, P = 0.02).

A log-likelihood ratio test for association between survival and the water state for the myc(-) showed no significant difference (G = 0.22, df = 1, P = 0.64) (Table 5.16a). Again, the non-significance allowed me to merge the two water states before testing for association between survival and seed source (Table 5.16b). There were no significant differences (G = 0.47, df = 2, P = 0.79).

Leaf water potential

The water potential values of plants growing in the temperate zone with ample water usually range from -0.1 to -0.5 MegaPascals (MPa) (Nelsen, 1987). Mildly water-stressed leaf water potentials range from -0.5 to -1.5 MPa and severely stressed from -1.5 to -3.0 MPa.

Here, the mean leaf water potential of the abundantly watered plants ranged from a high of -0.35 MPa to a low of

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Table 5.15 (a,b). Survival at 79 days for mycorrhizal-plus

a. Log-likelihood ratio test for association between survival and water state

	Survival: yes	Survival: no	
abundant-water	27	3	30
water-deprived	25	5	30
	52	5	60

G = 0.582
df = 1
P = 0.45

b. Log-likelihood ratio test for association between survival and seed source

	Survival: yes	Survival: no	
Nellie Hill 1	15	5	20
Nellie Hill 2	20	0	20
Prairie Nursery	17	3	20
	52	8	60

G = 7.719
df = 2
P = 0.02

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Table 5.16 (a,b). Survival at 79 days for mycorrhizal-minus

a. Log-likelihood ratio test for association between survival and water state

	Survival: yes	Survival: no	
abundant-water	27	3	30
water-deprived	28	2	30
	55	5	60

G = 0.22
df = 1
P = 0.64

b. Log-likelihood ratio test for association between survival and seed source

	Survival: yes	Survival: no	
Nellie Hill 1	18	2	20
Nellie Hill 2	18	2	20
Prairie Nursery	19	1	20
	55	5	60

G = 0.47
df = 2
P = 0.79

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-0.5 with a group mean of -0.45 (SEM 0.028)(Table 5.17). The droughted plants had a high of -0.9 MPa and a low of -2.9 with a group mean of -1.87 (SEM 0.287). An analysis of variance (ANOVA) comparing the leaf water potentials of the abundantly watered with the droughted showed a highly significant difference (F = 24.431, P = 0.0006, R-sq. = 0.71, df - 1,10).

When considering only the myc(+) plants there was no significant difference in the water potential of the abundantly watered compared to the droughted (F = 7.089, P = 0.06, R-sq. = 0.64, N = 6). However, prospective power analysis showed that an increase of N to 9 would exhibit significance (alpha = 0.05) at a power of 0.8.

When considering only the myc(-) plants the droughted had a very significantly lower water potential (mean -1.78 ± 0.26 than the abundantly watered (mean -0.48 ± 0.02)(F = 24.986, P = 0.0075, R-sq. = 0.86, Power = 0.958).

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Table 5.17. ANOVA results for leaf water potential. Measurements made with Scholander pressure bomb. Droughted plants received no water for 15 days. Abundantly watered received daily water. Power calculated at alpha = 0.05.

Source of variation	DF	SS	F	P	R-sq.	Power	N	Mean (MPa)	SEM
Water state	1	6.092	24.431	0.0006	0.71	0.996	12		
Abundant water							6	-0.446	0.028
Droughted							6	-1.871	0.287
Mycorrhizal - plus									
Water state	1	3.604	7.089	0.0562	0.64	0.523	6		
Abundant water							3	-0.408	0.046
Droughted							3	-1.958	0.580
Mycorrhizal - minus									
Water state	1	2.535	24.986	0.0075	0.86	0.958	6		
Abundant water							3	-0.483	0.021
Droughted							3	-1.783	0.259

DISCUSSION

Although it is widely accepted that colonization with AM fungi can affect the water relations of plants, the mechanisms are still unclear. At present, most researchers believe that under drought conditions mycorrhizal plants may do better than the non-mycorrhizal because of better nutritional status (Nelsen, 1987; Fitter, 1988; Gupta, 1991; Smith and Read, 1997; Augé, 2000). However, there is growing support for the view that non-nutritional effects of AM fungi may also be involved (Augé, 2001). Nonetheless, the uptake of minerals, primarily P, continues to be accepted as the most important, albeit not the only contribution of AMF to the water relations of colonized plants.

Phosphate ions, plant available P, are in low concentration in the soil solution and diffuse slowly to the plant roots which absorb them readily. As a result, zones of depletion form around the absorbing roots. The low rate of diffusion of the phosphate ions from the outer rhizosphere to the depletion zones causes the zones to widen so that even less P is available to the plant root. In AMF colonized roots the extraradical hyphae are able to

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extend beyond the zones of depletion and mine phosphate ions from areas in the rhizosphere unavailable to the plant roots. Also, the diameter of the hyphae, which can be as narrow as 2 μm, (Friese and Allen, 1991) compared to that of the root hair, 10-20 μm (Sanchez-Diaz and Honrubia, 1994). enable them to penetrate soil pores that are not accessible to the roots. In addition, the hyphae are able to absorb the phosphate ions more rapidly than the roots because the distance of diffusion of the P ion to a hyphae will most always be shorter than to a root (George, 2000). Thus, under normal conditions, mycorrhizal plants would be expected to have a nutritional advantage over the non-mycorrhizal.

During periods of water stress hyphal uptake of nutrients may become even more important to the plant (Graham and Syvertsen, 1987; Nelsen, 1987). As the soil dries, the rate of diffusion decreases so that phosphate ions become even less available to the plant root (Smith and Gianinazzi-Pearson, 1988). Nelsen and Safir (1982) found that with water-stress non-mycorrhizal onion plants had reduced growth and deficient tissue P even when soil P levels were high. In the same experiment water-stressed mycorrhizal onions were significantly larger, and were not P deficient even when soil P was low. The researchers

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speculated that the droughted soil made the P less mobile and thus unavailable to the non-mycorrhizal plants, causing P deficiency and consequent stunting. In contrast, the mycorrhizal onions were not affected by the rate of diffusion of the P ions, as they obtained P via the AM fungal hyphae. Being adequately nourished the mycorrhizal onions were significantly larger.

Although P is considered the most significant nutrient moved into plants by AM mycorrhizae there is strong evidence that at least two relatively immobile micronutrients, zinc and copper, are also absorbed and translocated by the fungi (Stribley, 1987; George, 2000; Liu et al., 2000). The hyphal uptake of these minerals may become more important during drought as well.

There is also good evidence for the uptake and transport of inorganic nitrogen by AM fungi, but the nutritional importance to the host plant is not certain (Smith and Read, 1997; George, 2000). Plant available nitrogen is in the form of ammonium and nitrate ions. Ammonium ions predominate in cold or acidic soils; nitrate ions in warm moist soils. Ames et al (1983), Johansen et al (1992) and Frey and Schüepp (1993), in separate experiments, found AM hyphal uptake and transport of labeled ammonium ions with $(^{15}NH_4)_2SO_4$ as the N source,

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although there was no increase in plant growth. Tobar et al (1994a) found that with unlimited water lettuce (*Latuca sativa* L), colonized by the AM fungal species *Glomus mossae,* responded to $^{15}NH_4^+$ with uptake and increased size; when colonized with *Glomus fasciculatum* plant size did not increase although there was evidence of uptake and transport. However, when water-stressed, the lettuce responded to $^{15}NH_4^+$ with increased size when colonized with either fungal species indicating that differing plant/AM fungal species associations and environmental conditions may interact to produce different results. The ammonium ion is relatively non-mobile and so may not readily diffuse to plant roots, particularly in droughted soil. Transport by mycorrhizal hyphae would theoretically be of importance in climax communities where the ammonium ion is often the primary source of plant available N.

In agricultural soils the primary N source is the nitrate ion (NO_3^-) which is mobile and diffuses readily under normal conditions. Using $^{15}NO_3^-$ as the labeled N source Tobar et al (1994b) found no difference in ^{15}N content in the plant tissues of mycorrhizal and non-mycorrhizal lettuce when adequately watered. However, with water stress the mycorrhizal lettuce plants had four times the ^{15}N content of

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the non-mycorrhizal and were very significantly larger than the non-mycorrhizal. Using $K^{15}NO_3$ with maize plants (*Zea mays cv.* 'Tuxpeño sequia' selection cycle C0) Subramanian and Charest (1999) found a significantly increased ^{15}N content for mycorrhizal plants compared to non-mycorrhizal under moderate drought conditions. It was concluded that the nitrate ion was much less mobile in dry soil, and that mycorrhizal uptake and transport of nitrate was important under these conditions (Tobar et al. 1994b; Smith and Read, 1997; Subramanian and Charest, 1999).

The purpose of this study was to investigate the effects of water availability and arbuscular mycorrhizal colonization on three gene pools of *B. curtipendula*. The results show that the response to water availability was strongly influenced by the mycorrhizal state of the plants and their gene pool (Fig. 5.4). When looking at the response to water availability in each mycorrhizal state it was found that abundant water was beneficial to the Nellie Hill 1 and 2 plants only when myc(+)(Tables 5.1, 5.3, 5.5, 5.7). Similarly, the myc(+) Prairie Nursery plants had a much stronger significant benefit from abundant water

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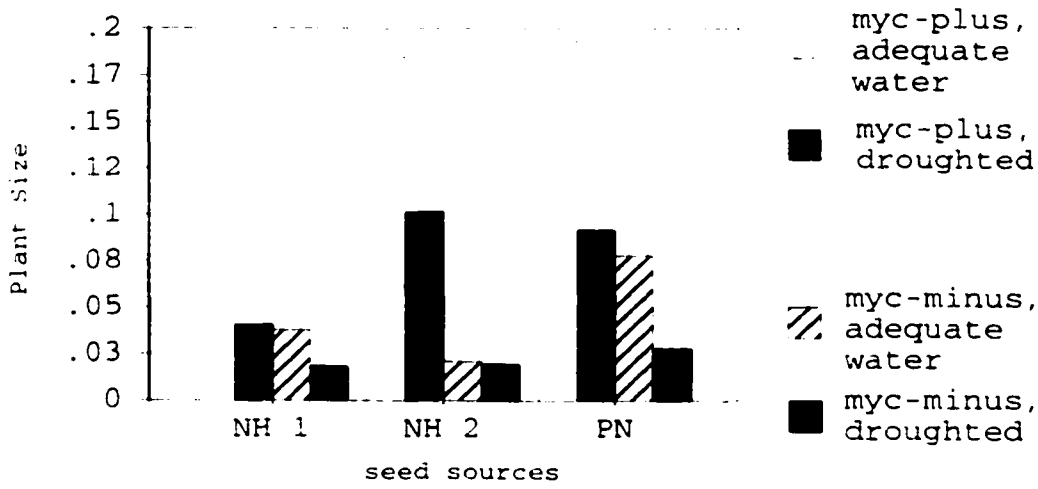

Fig. 5.4. Effect of mycorrhizal state and water availability on dry weight (gms) of upper plant. NH 1 - Nellie Hill 1;NH 2 - Nellie Hill 2; PN - Prairie Nursery.

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than the myc(-) (Tables 5.5, 5.7). Since plant available P is usually a limiting growth factor the myc(-) plants, not having the additional P supplied by the AM fungi, may have been growth limited and unable to make use of the additional water.

When looking at the response to the mycorrhizal state with respect to water availability it was found that when abundantly watered, the myc(+) plants grew very significantly larger than the myc(-) (Tables 5.2, 5.4, 5.6, 5.9) (Fig. 5.3) in all gene pools. Under drought conditions the Nellie Hill 2 and Prairie Nursery myc(+) also grew better than the myc(-) (Tables 5.4, 5.6, 5.9), although without as large a difference. However, when droughted, the myc(+) Nellie Hill 1 plants did not grow better than the myc(-). Thus, colonization with AM fungi appeared to benefit the Nellie Hill 2 and Prairie Nursery plants under both abundantly-watered and droughted conditions, but the Nellie Hill 1 plants benefited only when abundantly-watered.

I can only speculate as to why droughted Nellie Hill 1 plants appeared not to benefit from AMF colonization. It is known that AM fungi take photosynthate from the plants they colonize. If the droughted Nellie Hill 1 plants were

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not benefiting from the colonization; if they were providing photosynthate to the fungi without receiving any return, a parasitic effect should be evident. The myc(+) plants would have had reduced size compared to the myc(-). This is, in fact, what happened when plants were treated with enough additional P to offset the advantage of AM colonization in Chapter 4 (Table 4.5; Fig. 4.2). However, that is not the case here. The growth (leaf length or dry weight) of the droughted Nellie Hill 1 myc(+) plants was still higher than the droughted myc(-) although the difference was not statistically significant (Table 5.4, 5.6, 5.9). Calculation of the percentage mycorrhizal dependency (Plenchette et al, 1983) in Table 5.12 of the droughted Nellie Hill 1 plants shows a definite benefit from AMF colonization. That the droughted myc(+) plants did not grow significantly larger than the myc(-) might be because the limiting factor was
not nutritional, but something unrelated to mycorrhizal involvement.

When under water stress, plants respond with strategies that help conserve water. Among them is the production of abscisic acid (ABA) which causes stomatal closing which decreases transpiration and photosynthesis. Seed of the Nellie Hill 1 gene pool was taken from the

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highest elevation in the sloping Nellie Hill pasture. If these plants have adapted to living in this drier location they may respond to drought more rapidly and/or more intensely with water conserving measures such as increased ABA production. This could then ultimately lead to reduced plant growth. Support for this premise may be found in results of the raised bed experiment reported in Chapter 3. The two growing seasons during which that experiment took place (summers of 1998 and 1999) had markedly reduced precipitation (Fig. 5.5); 161 mm in 1998 and 180 mm in 1999; the normal average is approximately 316 mm (Institute of Ecosystem Studies). Under those conditions, the Nellie Hill 1 plants again had no statistical difference in growth between the myc(+) and myc(-) states. If the Nellie Hill 1 plants responded to the reduced precipitation with a water conserving strategy such as described above, this could account for there being no growth difference between the myc(+) and (-) conditions. A future study to detect increased production of ABA in water-stressed Nellie Hill 1 plants would provide evidence in support of this hypothesis.

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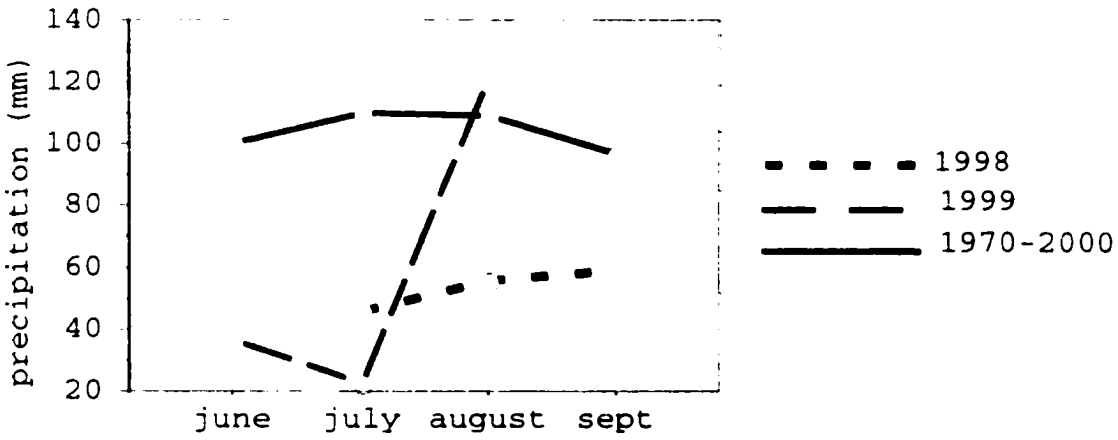

Fig. 5.5. Precipitation of summers of 1998 snd 1999 compared to 30 year average, Dutchess County, NY.

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However, it needs to be noted and addressed that although in the raised beds experiment the Nellie Hill 1 plants did not have a statistical growth response to the mycorrhizal colonization at any of the three P levels, the level of P seemed important for survival. By the conclusion of that experiment (late August, 1999) 20% of the Nellie Hill 1 plants had died; all in the phos-zero group, comprising 54% of that group (Table 3.23). The deaths were almost evenly divided among the myc(+) and myc(-) plants (Table 3.24). Since there were no deaths in the groups with added P it strongly suggests that P was important to survival, although the AM fungi did not appear to aid in this need. I believe that this may partly again be a response to drought. If, as I suggested above, that the Nellie Hill 1 plants respond to drought by reducing photosynthesis then a colonized plant might be very stressed by losing a considerable percent of its photosynthate to the fungi, even though the plant would be gaining P from the colonization. As a result, in the phos-zero group, when colonized, the plants might die from the added drain of colonization, but when not colonized they may die from not having the needed P. This would account

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for a large percentage of the phos-zero group dying, half of them myc(+) and half myc(-).

The droughted plants in this experiment were clearly under water stress as evidenced by their significantly lower (more negative) leaf water potential compared to the abundantly watered plants (Table 5.17; Fig. 5.6). This water stress continues to be evident when looking at just the myc(-) plants ($F = 24.986$, $P = 0.0075$, R-sq. = 0.86, Power = 0.958). However, when considering the myc(+) plants the water potentials of the droughted and abundantly watered are not clearly significantly different ($F = 7.089$, $P = 0.0562$, R-sq. = 0.64, Power = 0.523). Although an increase of only three myc(+) plants would show significance at a power of 0.8; and although the difference between the means of the myc(+) droughted and well-watered, and the difference between the means of the myc(-) droughted and well-watered is almost of the same order, the ANOVA results suggest that further investigation may be warranted. It is possible that the AM fungal colonization could be affording the host plant a resistance to water stress that is evidenced by a higher (less negative) leaf water potential when droughted. Subramanian et al, (1995) found that both drought-sensitive and drought-resistant tropical maize cultivars had higher leaf

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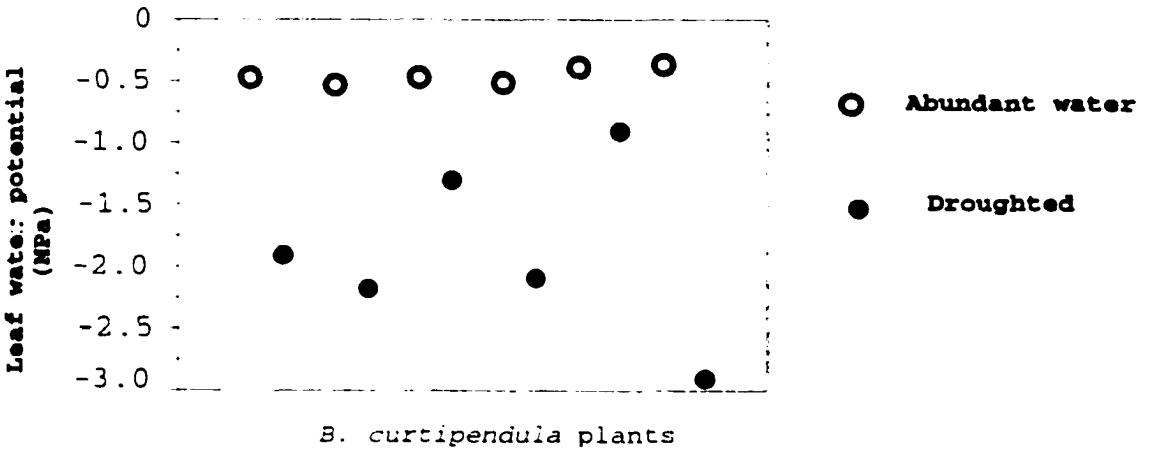

Fig. 5.6. Midday leaf water potential of 12 plants. The droughted plants had received no water for 15 days. The abundantly watered had received daily watering.

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water potentials when myc(+) compared to myc(-). In an early study Safir et al (1971) found that myc(+) soybean plants recovered from water stress more rapidly than myc(-) but that with fertilization (Safir et al, 1972) this difference was eliminated.

The root:shoot ratio was higher in the myc(+) state compared to the myc(-) regardless of water availability (Table 5.12). As explained in Chapter 4 (Discussion) AMF colonization usually causes a decrease in the root:shoot ratio as the colonized root is more efficient and can support a larger shoot mass. Why in both greenhouse experiments (Chapter 4, 5) the root:shoot ratio did not decrease with colonization is not known. However, Allen (1991) reports AM fungi caused increased root:shoot ratio in half the papers he examined and the opposite response in the other half. He suggests that this is caused by the interaction of a particular species or cultivar and the environment.

Another experimental outcome that needs to be addressed is the mixed response of Nellie Hill 2 plants to AM fungal colonization among three experiments. In both the raised beds experiment (Chapter 3) and the water deprivation experiment (Chapter 5) Nellie Hill 2 gene pool plants showed significant benefit from being colonized.

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However, in the greenhouse/phosphorous experiment (Chapter 4) there were no significant growth differences between the myc(+) and myc(-) conditions. Several conditions might possibly have contributed to this discrepancy. The test plants used in these three experiments were grown from *B. curtipendula* seed gathered from two separate areas of the Nellie Hill pasture and seed from a commercial seed company. I consider Nellie Hill 1, Nellie Hill 2 and Prairie Nursery as three different gene pools of *B. curtipendula*. There is much genetic variability possible among the seeds within each gene pool; a situation very different than from one using clones. This variability plus the small N of 20 (10 myc(+) and 10 myc(-)) might account for inconsistency in the Nellie Hill 2 gene pool response to colonization in the three experiments.

Another variable among the three experiments was the composition of the AM fungal species in the soil used for each experiment. Soil used in the raised beds experiment in June 1998 had been freshly excavated from the Nellie Hill pasture. The remaining soil was stored in covered plastic pails in the Lehman College research greenhouse. It was used in April, 1999 for the greenhouse/phosphorous

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experiment and again in April, 2000 for the water deprivation experiment. The long storage time caused concern regarding the viability of the AM fungal propagules. Consequently, cut root segments of 10 trap plants were added to the soil for the water deprivation experiment. The storage times of the Nellie Hill soil and the use of trap plants may have caused variations in the fungal flora in the three experiments.

Two of the three experiments were done in a greenhouse where temperatures reached 110°F. Since the experiments were done in different years they were subjected to differing temperatures, sometimes extreme.

The interaction of these uncontrolled conditions, that of genetic variability, inocula of differing AM fungal flora populations, and varying climactic conditions may conceivably have contributed to inconsistencies among these three experiments. To minimize these elements in future studies, I would use freshly collected soil as the inoculum; use the seeds of clones, attempt better control over greenhouse temperatures, greatly increase severity of imposed drought and increase N.

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In summary, in response to water availability the three *B. curtipendula* gene pools responded similarly to abundant water and differently to drought. When mycorrhizal, all gene pools had increased growth when well-watered. With reduced mycorrhizae, the well-watered Nellie Hill 1 and 2 gene pools had no statistical increase in biomass, and the Prairie Nursery gene pool had only a small statistical increase (Table 5.7). Thus the mycorrhizae appear beneficial to all the gene pools when not water-stressed.

When droughted, mycorrhizal Nellie Hill 2 and Prairie Nursery still exhibited increased growth but Nellie Hill 1 did not. With water stress Nellie Hill 1 plants may reduce photosynthesis. With reduced photosynthesis AMF may cause a photosynthate drain that offsets the benefits of hyphal P uptake.

In conclusion, *B. curtipendula*, mycorrhizal-dependent in low P soils, was found to benefit from AMF colonization in soils of less than optimal P but suffer parasitic effects when soil P is high. AMF colonization may improve resistance to water stress by maintaining a higher leaf water potential. When water-stressed a drought-sensitive *B. curtipendula* variant may lose its net benefit from AMF colonization.

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CONCLUSIONS

The investigations described in this research were conducted to study the effects of arbuscular mycorrhizal fungi (AMF) on *B. curtipendula*, a grass species rare in New York State. Nellie Hill, the study site in Dutchess County, yielded two gene pools of *B. curtipendula*; commercial seed (Prairie Nursery) was obtained from native populations in Nebraska. In long-term factorial experiments, I manipulated AMF colonization using the fungicide benomyl, controlled plant water relations, and varied soil phosphorus availability. I measured various aspects of plant growth, size and reproductive output as well as survivorship as response variables. The conclusions of these experiments are set forth in the following paragraphs.

Field Study (chapter 2)

Natural stands of *B. curtipendula* are colonized by AMF at Nellie Hill. The plants benefit from the mycorrhizal association with increased growth and fitness. This was determined by a decrease in the number

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of flowering culms when the percentage of AMF colonization was reduced. It was also determined that the heights of the flowering culms are not affected by AMF.

Common Garden Experiment (chapter 3)

Variants of *B. curtipendula* can respond differently to AMF colonization. The Nellie Hill 1 gene pool showed increased survival when phosphorous was increased and AMF colonization reduced; with abundant AMF and no added phosphorous there was no increase in survival. The Nellie Hill 2 gene pool showed increased survival with either an increase in phosphorous or with abundant AMF colonization. Prairie Nursery showed increased survival with abundant AMF but not with increased phosphorous.

Greenhouse Experiment I (chapter 4)

Variants of *B. curtipendula* can change their response to AMF colonization under different environmental conditions. This was concluded as Nellie

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Hill 1 plants did not respond to AMF in a common garden experiment but did respond under greenhouse conditions. Nellie Hill 2 plants responded to AMF in the common garden experiment but did not respond in the greenhouse.

B. curtipendula, mycorrhizal-dependent in low phosphorous soils, benefits from AMF colonization in soils of less than optimal phosphorous, but suffers parasitic effects when soil phosphorous is high. This was determined considering that the Nellie Hill 1 plants showed increased growth from added phosphorous when colonization was lessened, but with abundant colonization and added phosphorous growth decreased.

Greenhouse Experiment II (chapter 5)

AMF colonization may improve resistance to water stress as leaf water potential was higher (less negative) in plants with abundant colonization compared to those with reduced colonization.

When water-stressed a drought-sensitive *B. curtipendula* variant may lose its net benefit from AMF colonization. The abundantly colonized Nellie Hill 1

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plants showed increased growth only when adequately watered. When droughted, the Nellie Hill 1 plants did not increase growth with abundant AMF, but did respond to additional phosphorous with better survival regardless of mycorrhizal state. It was concluded that Nellie Hill 1 plants benefited from hyphal uptake of phosphorous but may have responded to drought with reduced photosynthesis, and so had no increase of biomass. When droughted and with abundant AMF, the photosynthate drain to the AMF lessened survival so that the plants performed no better than with reduced AMF.

This study documents a high degree of poorly understood, intraspecific variability in the dynamics of the plant root-AMF symbiosis.

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