

Mycorrhizal infection of wild oats: maternal effects on offspring growth and reproduction

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Received June 25, 1991 / Accepted in revised form December 10, 1991

Summary. The objective of this study was to determine whether infection of *Avena fatua* L. plants by the mycorrhizal fungus *Glomus intraradices* Schenck & Smith could influence the vigor of the offspring generation. Two experiments demonstrated that mycorrhizal infection of the maternal generation had slight but persistent positive effects on offspring leaf expansion in the early stages of growth. In two other experiments, mycorrhizal infection of mother plants had several long lasting effects on their offspring. Offspring produced by mycorrhizal mother plants had greater leaf areas, shoot and root nutrient contents and root:shoot ratios compared to those produced by non-mycorrhizal mother plants. Moreover, mycorrhizal infection of mother plants significantly reduced the weight of individual seeds produced by offspring plants while it increased the *P* concentrations of the seeds and the number of seeds per spikelet produced by offspring plants. The effects of mycorrhizal infections of maternal plants on the vigor and performance of offspring plants were associated with higher seed phosphorus contents but generally lighter seeds. The results suggest that mycorrhizal infection may influence plant fitness by increasing offspring vigor and offspring reproductive success in addition to previously reported increases in maternal fecundity.

Key words: Mycorrhiza – Maternal effects – Offspring vigor – Reproduction – *Avena fatua* L

Mycorrhizal fungi are ubiquitous soil microorganisms that form symbiotic associations with over 80% of terrestrial plant species. In most cases this symbiosis between plant and fungus increases the uptake of phosphorus (*P*) from soils, resulting in significant alterations of the highly integrated nutrient, water and carbon economies of the host plant (Smith 1980; Kucey and Paul 1982; Koide

1985; Allen and Allen 1986). Much of the research on the mycorrhizal symbiosis has concentrated on the effects of the fungus on host vegetative growth and nutrient uptake, or on agronomic yield. Surprisingly little is known about the effects of mycorrhizal infection on plant fitness in natural populations (Law 1988). Two important components of fitness are fecundity and offspring vigor. We have previously shown that mycorrhizal infection can significantly increase seed production (Koide et al. 1988; Bryla and Koide 1990). The purpose of this study was to test whether mycorrhizal infection could influence offspring vigor.

Variation in offspring vigor may determine the demographic patterns of mature vegetation (Harper 1977; Cook 1979). For example, individuals which germinate more rapidly or which grow faster may enjoy greater survivorship because they are larger (Solbrig 1981; Weiner 1988). They may also preempt a greater proportion of the available resources (Ross and Harper 1972; Abul-Fatih and Bazzaz 1979) thereby suppressing less competitive individuals and achieving greater fitness. In some cases, early seedling size may be correlated with eventual reproductive output (Edwards and Emara 1969; Stanton 1984; Roach 1986) although this is not universally true (Lewis and Garcia 1979; Kotecha 1981).

Two of the seed qualities which are of potentially great importance in determining offspring vigor include seed weight (or size) and seed nutrient content. Seed weight has been correlated with the rate of emergence and resultant plant survivorship, size, competitive ability and reproductive output (Lush and Wien 1980; Gross 1984; Schaal 1984; Stanton 1984; Kromer and Gross 1987; Roach 1968; Wulff 1968a, b). In cereals, seed nitrogen (*N*) concentration has been shown to significantly influence the success of seedlings during the establishment phase and subsequent seed production by mature plants, particularly when *N* supply to seedlings is low (Kaufmann and Guitard 1967; Schweizer and Ries 1969; Ries and Everson 1973). Seeds of *Abutilon theophrasti* with higher *N* contents produced more vigorous seedlings than did seeds with lower *N* contents, particularly

when there was competition with other plants (Parrish and Bazzaz 1985).

Similar findings for seed phosphorus (P) have also been reported. For example, Bolland and Paynter (1990) reported that for several legume species increased seed P content resulted in increased vegetative dry matter and shoot P content. Iwata and Eguchi (1958) showed similar effects of seed P on growth of cabbage. Austin (1966) demonstrated that seed yields were higher in those pea plants that were produced by seeds containing greater concentrations of P . These results suggest that infection by mycorrhizal fungi may also influence offspring vigor by influencing seed nutrient content. Our objective was thus to test whether mycorrhizal infection of one generation of plants could influence the growth or reproduction of the subsequent generation.

Materials and methods

Experiment 1. Maternal treatment effects on early growth of offspring; plants grown singly

"Seeds" (caryopses) produced by mycorrhizal (M) and non-mycorrhizal (NM) *Avena fatua* L. (wild oat) plants were obtained from a previous experiment (Koide et al. 1988). In that experiment, M wild oat plants were infected with *Glomus intraradices* Schenck and Smith and M and NM plants were grown in a soil mixture consisting of 2 volumes soil (a low P silt loam from Clearfield County, PA, USA) to 1 volume medium grade sand. Measures of growth and reproduction of this first generation of plants were given in Koide et al. (1988). In this and all subsequent experiments, the individuals of this first generation are referred to as "mother plants" and the "maternal treatment" refers to their mycorrhizal status (M vs. NM). The first generation is referred to as the "maternal" generation although the importance of the paternal contribution to the reported effects is not clear.

In this first experiment, 10 seeds were selected for uniformity in weight from each of five mother plants from both of the maternal treatments (for a total of 100 seeds). On 26 August 1988, each seed was planted in the center of a 55 cm³ pot (Cone-tainers, Ray Leach Cone-tainer Nursery, Canby, OR, USA) containing a soil mixture consisting of 2 parts soil (a low P silt loam from Clearfield County, PA, USA) to 1 part medium grade sand. The soil mixture was autoclaved to destroy indigenous mycorrhizal fungi. All offspring were uninoculated (NM). The pots were randomly arranged in a growth chamber held at 50% RH and 25° C/20° C day/night with a 12 h photoperiod. Approximately 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation (400–700 nm) was provided (approximately 30.2 $\text{mol m}^{-2} \text{day}^{-1}$). All pots were watered daily by hand with distilled water and no nutrient amendment was made.

A total of 40 additional seeds (4 seeds randomly selected from each of the 5 mother plants from each maternal treatment) were selected for the measurement of seed nutrient concentrations. Individual seeds were digested and colorimetrically analyzed for total P (molybdophosphate method, Watanabe and Olsen 1965) and total N (Nessler method, Jensen 1962) concentrations. For each maternal treatment, a regression of seed N and P content against seed weight was determined. The equations for these regressions were:

For M maternal treatment:

$$\text{mg } N \text{ seed}^{-1} = 13.6 \times (\text{seed weight, g}) + 0.114 \quad r = 0.87$$

$$\text{mg } P \text{ seed}^{-1} = 2.03 \times (\text{seed weight, g}) + 0.011 \quad r = 0.84$$

For NM maternal treatment:

$$\text{mg } N \text{ seed}^{-1} = 11.1 \times (\text{seed weight, g}) + 0.123 \quad r = 0.77$$

$$\text{mg } P \text{ seed}^{-1} = 1.70 \times (\text{seed weight, g}) + 0.004 \quad r = 0.82$$

These equations were then used to estimate the P and N contents of the seeds planted in this and subsequent experiments.

Total plant leaf areas (LA) were estimated for each plant 8, 10, 12, 14, 17, 19, 25 and 31 days from planting based on measures of leaf length (l) and width (w) using the formula $LA = 0.70 \, l \times w$ (Koide et al. 1988). Approximately 4.5 weeks after sowing, shoots were separated from roots at the soil level. Roots were rinsed free from soil. Both roots and shoots were dried (70° C) and weighed. The following measured and calculated variables were analyzed (see below) for the effect of maternal treatment: weight of individual seeds planted, estimated P and N contents of seeds planted, number of days for seedling emergence, offspring shoot weight, offspring root weight, and offspring root:shoot dry weight ratio.

The primary interest in all experiments was the influence of maternal treatment (M vs. NM) on offspring performance (as assessed in Experiment 1 by leaf area and the several harvest variables mentioned previously). Each mother plant was, therefore, taken to be the experimental unit and the means of the characters of the offspring from each mother were used in the appropriate analyses of variance in each experiment. For the characteristics of the seeds that were sown and for the characteristics of offspring plants determined at the harvest, single factor (maternal treatment) analyses were performed. For leaf area measurements made over time in Experiments 1 and 2, two factor (maternal treatment \times time) analyses were performed. In those analyses, the number of days from sowing to seedling emergence were used as a covariate because independent linear regressions for all offspring combined of this variable against leaf area on the first measurement date in both experiments yielded slopes significantly different from zero (for Experiment 1: d.f. = 89, $T = 18.9$, $p \leq 0.0001$; for Experiment 2: d.f. = 53, $T = 16.7$, $p < 0.0001$). For all experiments, type III sums of squares were calculated in all analyses of variance. Normality of data was assessed by inspection of normal probability plots. Homogeneity of variances was tested using Bartlett's test (STSC, 1991). Untransformed data were sufficient to meet the requirements of the analysis of variance for all characters except leaf areas which were ln-transformed.

According to the results of the analyses of variance the mean weight of individual seeds selected from M and NM mother plants (awns removed for uniformity) and planted in Experiment were not significantly affected by maternal treatment (Table 1). The estimated seed P and N contents were significantly greater for those produced by M mother plants compared to those produced by NM mother plants (Table 1).

Experiment 2. Maternal treatment effects on early growth of offspring; plants grown in the presence of others

The previous experiment utilized seed produced by M and NM mother plants that were selected so as not to differ significantly in weight. In actuality, the seeds produced by M *Avena fatua* (wild oat) plants were slightly lighter than those produced by NM plants (Koide et al. 1988). In Experiment 2, therefore, six seeds of wild oat were randomly selected from each of 5 mother plants in each of the maternal treatments (M vs. NM) from a previous experiment (Koide et al. 1988). In all, 60 pots were used: 2 maternal treatments \times 5 mother plants per maternal treatment \times 6 seeds per mother. Prior to planting, each seed was individually weighed (all awns removed).

This experiment also differed from Experiment 1 in that other plants were placed in the same pot as the target offspring plant to more realistically simulate growing conditions in the field. On 15 March 1989, each of the "target" seeds was planted in the center of a 150 cm³ pots (Cone-tainers, Ray Leach Cone-tainer Nursery, Canby, Oregon) containing the autoclaved soil mix described above. Again, all offspring were uninoculated (NM). Four randomly selected seeds of *A. fatua* (parentage unknown, Valley Seed Service, Fresno, CA, USA) were also planted in each of the pots

Table 1. Results of type III sums of squares analysis of variance for the dependent variables measured or calculated in Experiments 1 and 2 as influenced by maternal treatment (M vs. NM). In both experiments, d.f. = 1,8

	<i>F</i>	<i>P</i>	Mean (se) of offspring by maternal treatment	
			<i>M</i>	<i>NM</i>
<i>Experiment 1</i>				
Individual weight of seeds planted	3.11	0.12	22.8 (0.4) mg	24.4 (0.8) mg
<i>P</i> content of seeds planted	60.7	≤0.0001	57.4 (0.5) µg	45.1 (1.3) µg
<i>N</i> content of seeds planted	9.50	≤0.05	425 (5) µg	394 (9) µg
Days until seedling emergence	1.67	0.23	6.1 (0.2) d	5.8 (0.1) d
Offspring shoot dry weight	0.054	0.82	109 (2) mg	110 (3) mg
Offspring root dry weight	3.49	0.099	129 (4) mg	122 (2) mg
Root: shoot weight ratio	3.43	0.10	1.20 (0.02)	1.13 (0.03)
<i>Experiment 2</i>				
Individual weight of seeds planted	49.4	≤0.0001	19.3 (0.3) mg	21.7 (0.2) mg
<i>P</i> content of seeds planted	16.5	≤0.0005	50.1 (0.5) µg	40.9 (0.4) µg
<i>N</i> content of seeds planted	7.75	≤0.05	376 (3) µg	364 (3) µg
Days until seedling emergence	0.042	0.84	4.2 (0.3) d	4.1 (0.3) d
Height of other plants in pot	0.132	0.72	5.6 (0.0) cm	5.6 (0.2) cm
Total wt. of other plants in pot	0.098	0.76	356 (9) mg	352 (6) mg
Target offspring shoot dry weight	1.40	0.24	69 (1) mg	62 (3) mg

surrounding the target seed. The pots were randomly arranged on a bench in the greenhouse receiving supplemental illumination. The mean daily total quantum flux density (400–700 nm) was $23.3 \text{ mol m}^{-2} \text{ day}^{-1}$ and the mean daily instantaneous maximum quantum flux density was $1,290 \text{ µmol m}^{-2} \text{ s}^{-1}$. The day of germination for all target plants was recorded. All pots were watered daily by hand. No nutrient amendment was made.

Leaf areas were estimated for each plant from measures of leaf lengths and widths (as above) 8, 11, 14, 18, 21 and 25 days after planting. The effect of time and maternal treatment on ln-transformed leaf areas were assessed as in Experiment 1 using a two factor analysis of variance. The final harvest was performed on 11 April 1989 (approximately 4 weeks after planting) when the shoots of all plants (targets and unselected others) were separated from the roots, rinsed in distilled water, dried in an oven (70°C) and weighed. As in Experiment 1, single factor analyses of variance were used to test the influence of maternal treatment on the following measured and calculated variables (untransformed data): weight of individual seeds planted, estimated *P* and *N* contents of seeds planted, number of days for seedling emergence, offspring shoot weight, mean height of plants from unselected seeds, mean total shoot weight per pot of plants from unselected seeds. According to the analysis of variance, the weights of individual seeds from the *M* mother plants were significantly less than those of seeds from *NM* mother plants and the estimated seed *P* and *N* contents of seeds from *M* mother plants were significantly greater than those of the seeds from *NM* mother plants (Table 1).

Experiment 3. Maternal treatment effects on offspring growth, nutrient uptake and reproductive output; low P supply

In this experiment, wild oat offspring plants were grown from seeds selected from each of 3 mother plants per maternal treatment. As before, the source of the seeds was the set of mother plants grown in a previous experiment (Koide et al. 1988). A total of 12 wild oat plants were grown: 2 maternal treatments (*M* vs. *NM*) × 3 mother plants per maternal treatment × 2 replicate offspring per mother. Seeds were sown on 15 March 1989 into square plastic pots (11.5 cm on a side) containing about 800 g (air-dry) of the autoclaved soil mixture described above. The number of days for emergence to occur was recorded for each plant. The pots were placed in the greenhouse. Each of the offspring plants were twice weekly given

50 mL of a half-strength Hoagland nutrient solution lacking *P* (Machlis and Torrey 1956). Supplemental water was provided by daily drip irrigation. Supplemental lighting was provided by 1000 W high pressure sodium discharge lamps operating 14 h each day. The mean daily total quantum flux density (400–700 nm) under the lamps was $21.8 \text{ mol m}^{-2} \text{ day}^{-1}$ and the mean daily instantaneous maximum quantum flux density was $1,410 \text{ µmol m}^{-2} \text{ s}^{-1}$.

Leaf areas of the offspring plants were estimated 53 days after sowing from measurements of leaf length and width as described above for Experiment 1. Seeds produced by the offspring plants were collected as they matured, then counted and weighed. The spikelets were also counted, and the mean number of seeds per spikelet was calculated from the total number of seeds and the total number of spikelets per plant. These seeds represent the third generation, produced by offspring (second generation) of the original mother plants (first generation). All plants were harvested on 15 September 1989 (approximately 26 weeks after planting). Shoots were separated from roots at the soil level, washed in distilled water, oven dried (70°C) and weighed. Roots were washed free of soil, oven dried and weighed. Shoots and root systems were ground, homogenized and subsamples were acid digested prior to colorimetric determination of total *N* (Nessler method, Jensen 1962) and total *P* (molybdophosphate method, Watanabe and Olsen 1965). All offspring plants were uninoculated and proved to be *NM* at harvest. The following dependent traits (untransformed data) of the offspring were subjected to single factor analyses of variance as in the previous experiments to test for the effect of maternal treatment on offspring performance: planted seed weights, estimated *N* contents of planted seeds, estimated *P* contents of planted seeds, the number of days after sowing until seedling emergence, leaf area, shoot dry weight, shoot *N* concentration, shoot *P* concentration, shoot *N* content, shoot *P* content, root:shoot dry weight ratio, root weight, root *N* concentration, root *P* concentration, root *N* content, root *P* content, number of seeds produced, total seed weight, individual seed weight, number of spikelets, the number of seeds per spikelet, and, for the seed produced by the offspring, seed *N* concentration, seed *P* concentration, seed *N* content and seed *P* content. In both Experiments 3 and 4, the qualities of the seeds (third generation) produced by offspring were assessed by analyzing 3 randomly chosen seeds from a single offspring from each mother plant from each maternal treatment. A single factor (maternal treatment) analysis of variance was performed as previously described using the means of the 3 seeds from each offspring for each seed character.

Seeds from contrasting maternal treatments were selected such that they did not differ significantly (Table 4) in weight (mean 21.5 mg, SE 1.3) or in *N* content (mean 309 $\mu\text{gN seed}^{-1}$, SE 19). Maternal treatment did significantly influence mean (SE) estimated seed *P* content: 54.4 (3.8) $\mu\text{gP seed}^{-1}$ and 40.6 (2.8) $\mu\text{gP seed}^{-1}$ for seeds from M and NM mother plants, respectively (Table 4). All offspring plants were uninoculated and proved to be NM at the harvest.

Experiment 4. Maternal treatment effects on offspring growth, nutrient uptake and reproductive output; high P supply

As in the previous experiment, two wild oat offspring plants were grown from seeds selected from each of 3 mother plants from each maternal treatment. Again, the source of the seeds was the set of mother plants grown in a previous experiment (Koide et al. 1988). According to the analyses of variance (performed as for Experiment 3, see Table 5), seeds from contrasting maternal treatments did not differ significantly in weight (mean 17.5 mg, SE 0.2) or in *N* content (mean 248 $\mu\text{gN seed}^{-1}$, SE 4). Maternal treatment did significantly influence mean (se) estimated seed *P* content: 45.8 (0.8) $\mu\text{gP seed}^{-1}$ and 34.2 (0.3) $\mu\text{gP seed}^{-1}$ for seeds from M and NM mother plants, respectively (Table 5). Seeds were sown on the same day as for those in Experiment 3 and offspring were handled as in Experiment 3. The only difference was that the offspring plants were twice weekly given 50 mL of a half-strength Hoagland nutrient solution containing 333 $\mu\text{M P}$ as KH_2PO_4 (Machlis and Torrey 1956). All offspring plants were uninoculated and proved to be NM at harvest. Statistical analyses of harvest data in Experiment 4 were performed as in Experiment 3.

Results

Experiment 1

According to the analyses of variance, maternal treatment (M vs. NM) did not significantly affect the number of days to emergence, offspring shoot weight, root weight, or root:shoot ratio (Table 1). However, offspring from M mother plants did expand leaves at a significantly greater rate than those from NM mother plants (Table 2). The results from this experiment were essentially the same as for Experiment 2, thus the plot of leaf area vs. time for Experiment 1 is not shown.

Experiment 2

According to the analysis of variance (Table 1), the height and total shoot weight per pot of the four unselected wild oat plants in each pot were not significantly different for the target offspring seedlings produced by M and NM mother plants. Any effects of the unselected plants on the target offspring plants were thus assumed to be the same for those produced by M and NM mother plants. As in the previous experiment, neither the number of days to emergence nor the shoot weight of the target offspring were significantly influenced by maternal treatment (Table 1), but offspring from M mother plants did expand leaves at a significantly greater rate than those

Table 2. Type III sums of squares analysis of variance for ln-transformed leaf areas in Experiment 1

	df	MS	F	P
<i>Covariate</i>				
Days to emergence	1	0.0873	5.39	≤ 0.05
<i>Main effects</i>				
Maternal treatment	1	0.187	11.6	≤ 0.005
Time	7	8.85	547	≤ 0.0001
Maternal treatment \times time	7	0.0207	1.28	0.28
Residual	63	0.0162		
Total (corrected)	79			

Table 3. Type III sums of squares analysis of variance for ln-transformed leaf areas in Experiment 2

	df	MS	F	P
<i>Covariate</i>				
Days to emergence	1	0.284	41.2	≤ 0.0001
<i>Main effects</i>				
Maternal treatment	1	0.348	50.5	≤ 0.001
Time	5	5.32	773	≤ 0.0001
Maternal treatment \times time	5	0.0011	0.163	0.97
Residual	47	0.00688		
Total (corrected)	59			

from NM mother plants (Table 3, Fig. 1). Root:shoot ratios were not calculated.

Experiments 3 and 4

According to the analysis of variance for Experiment 3 (Table 4), maternal treatment (M vs. NM) did not significantly affect the number of days to emergence (mean 3.9 d SE 0.2). The same was true for Experiment 4 (mean 3.8 d, SE 0.1; Table 5).

Maternal treatment, however, did have significant effects on offspring vigor that were largely independent of effects of offspring *P* treatment (Figs. 2–5). For $-P$ offspring (Experiment 3), several growth and nutrient uptake variables were greater for those offspring produced by M mother plants compared to those produced by NM mother plants (Figs. 2, 3, Table 4). These included leaf area, shoot *P* concentration, shoot *P* content, root *N* concentration, root *N* content and root *P* content. For $+P$ offspring (Experiment 4), again, several growth and nutrient uptake variables were greater for those offspring produced by M mother plants (Figs. 2, 3, Table 5). These included leaf area, shoot *N* concentration, shoot *N* content, root *N* content and root:shoot dry weight ratio.

The reproductive characters of the offspring generation were also significantly affected by maternal treatment. In Experiments 3 and 4, the number of seeds per spikelet was significantly greater for those offspring produced by M mother plants (Fig. 4, Tables 4, 5). In

Table 4. Results of type III sums of squares analysis of variance for the dependent variables measured or calculated in Experiment 3 as influenced by maternal treatment (M vs. NM). d.f. = 1,4

	<i>F</i>	<i>P</i>
Individual weight of seeds planted	0.005	0.95
<i>N</i> content of seeds planted	0.371	0.58
<i>P</i> content of seeds planted	8.41	≤0.05
Days until seedling emergence	0.211	0.67
Offspring leaf area day 53	60.8	≤0.005
Offspring shoot dry weight	2.58	0.18
Offspring shoot <i>N</i> concentration	0.035	0.86
Offspring shoot <i>P</i> concentration	27.2	≤0.01
Offspring shoot <i>N</i> content	0.927	0.40
Offspring shoot <i>P</i> content	15.2	≤0.05
Offspring root dry weight	5.18	0.09
Offspring root <i>N</i> concentration	310	≤0.0001
Offspring root <i>P</i> concentration	5.55	0.08
Offspring root <i>N</i> content	16.8	≤0.05
Offspring root <i>P</i> content	7.29	≤0.05
Offspring root: shoot	5.79	0.07
No. of seeds produced by offspring	5.59	0.08
Total weight of seeds produced	3.63	0.13
Weight of individual seeds produced	16.3	≤0.05
No. of spikelets produced	1.59	0.28
No. of seeds per spikelet	32.4	≤0.005
<i>P</i> concentration of seeds produced	10.1	≤0.05
<i>N</i> concentration of seeds produced	6.63	0.06
<i>P</i> content of seeds produced	1.24	0.33
<i>N</i> content of seeds produced	0.132	0.74

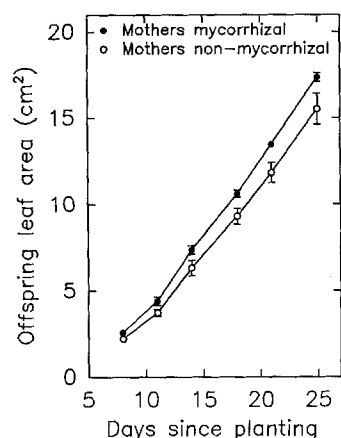


Fig. 1. Leaf area vs. time for offspring *Avena fatua* plants (all non-mycorrhizal) produced by either mycorrhizal or non-mycorrhizal mother plants (Experiment 2). Vertical error bars are ± 1 SE. See Table 3 for the analyses of variance results

contrast, in both Experiments 3 and 4, the weight of individual seeds produced by offspring from M mother plants was significantly lower for those seeds produced by offspring from NM mother plants (Fig. 4, Tables 4, 5). The *P* concentration (Experiments 3 and 4) and the *P* content (Experiment 4) of seeds produced by offspring from M mother plants were significantly higher than for those produced by NM mother plants (Fig. 5, Tables 4, 5), suggesting that the effects of mycorrhizal infection of the first generation plants might still be evident in mature third generation individuals.

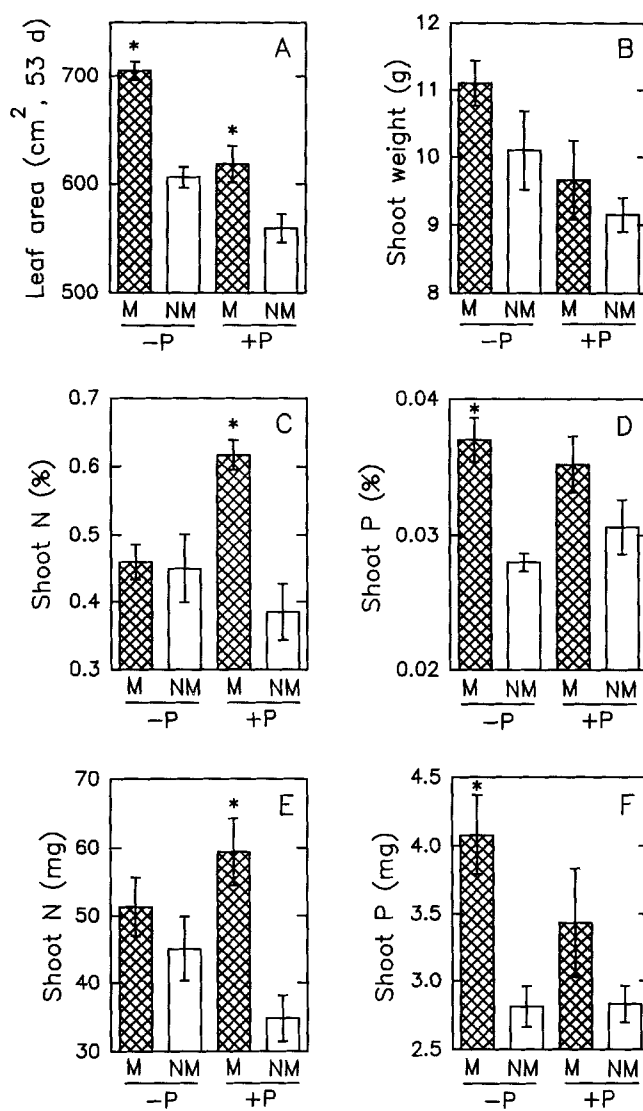


Fig. 2A-F. Measures of shoot growth and nutrient content for offspring *Avena fatua* plants produced by either mycorrhizal (M) or non-mycorrhizal (NM) mother plants and given two levels of *P* amendment ($-P$ and $+P$) in Experiments 3 and 4, respectively. Vertical error bars are ± 1 SE. Asterisks indicate that offspring from M mother plants differed significantly ($p < 0.05$) from offspring from NM mother plants within an experiment. Tables 4 and 5 give complete results from the analyses of variance which tested for the effect of maternal treatment (see Materials and Methods for the details of the analyses)

Discussion

The results of these four experiments show that mycorrhizal infection of *Avena fatua* (wild oat) plants did have significant effects on the growth and reproduction of offspring plants that were associated with increases in seed *P* content. These effects were not consequences of different rates of seedling emergence as there were never significant effects of maternal treatment on emergence. The effects, therefore, were due to differential growth and allocation to reproduction after emergence.

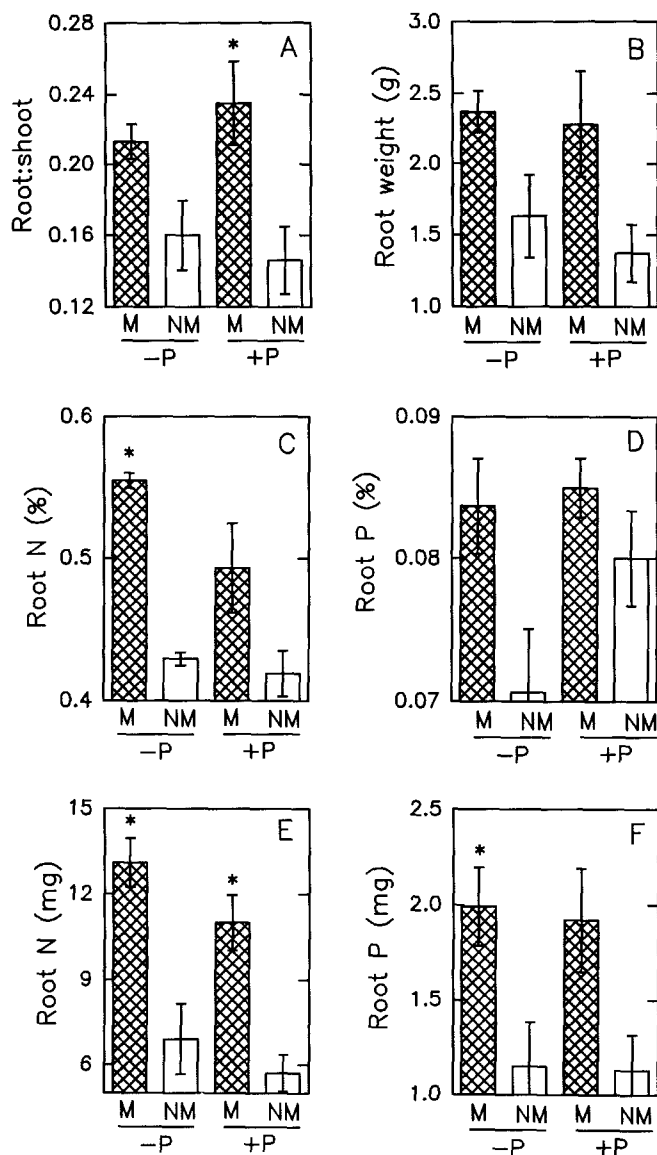


Fig. 3A-F. Measures of root growth and nutrient content for offspring *Avena fatua* plants produced by either mycorrhizal (M) or non-mycorrhizal (NM) mother plants and given two levels of P amendment (-P and +P) in Experiments 3 and 4, respectively. Error bars and asterisks are the same as for Fig. 2. Tables 4 and 5 give complete results from the analyses of variance which tested for the effect of maternal treatment (see Materials and Methods for the details of the analyses)

Even when the seeds from M mother plants were significantly lighter than those from NM mother plants and when offspring were in close proximity to several unselected plants (Experiment 2), the positive effect of mycorrhizal infection of mother plants on leaf expansion was evident. Since the results from Experiment 2 were essentially the same as those from Experiment 1, the results from Experiment 1 were probably not simply artifacts due to the nonrandom selection of seeds of similar weight from M and NM mother plants.

The P content of seeds is often correlated with the dry weight, P content and seed production of the resultant plants (Austin 1966; Demircakmak and Kaufmann

Table 5. Results of type III sums of squares analysis of variance for the dependent variables measured or calculated in Experiment 4 as influenced by maternal treatment (M vs. NM). d.f. = 1,4

	F	P
Individual weight of seeds planted	2.41	0.20
N content of seeds planted	5.97	0.07
P content of seeds planted	197	≤0.0001
Days until seedling emergence	0	1.0
Offspring leaf area day 53	7.67	≤0.05
Offspring shoot dry weight	0.647	0.47
Offspring shoot N concentration	24.3	≤0.01
Offspring shoot P concentration	2.46	0.19
Offspring shoot N content	17.0	≤0.05
Offspring shoot P content	2.02	0.23
Offspring root dry weight	4.61	0.10
Offspring root N concentration	4.41	0.10
Offspring root P concentration	1.46	0.29
Offspring root N content	20.6	≤0.05
Offspring root P content	5.74	0.07
Offspring root: shoot	8.87	≤0.05
No. of seeds produced by offspring	3.77	0.12
Total weight of seeds produced	1.31	0.32
Weight of individual seeds produced	22.5	≤0.01
No. of spikelets produced	0.297	0.62
No. of seeds per spikelet	17.6	≤0.05
P concentration of seeds produced	57.5	≤0.005
N concentration of seeds produced	2.45	0.19
P content of seeds produced	11.0	≤0.05
N content of seeds produced	0.535	0.51

1963). Thus, differential maternal provisioning of seeds with P as seen here and more fully described elsewhere (Lu and Koide 1991) may have been involved in the effects seen in these studies. Others have reported that maternal effects on seed quality may have long-lasting effects on offspring (Demircakmak and Kaufmann 1963; Harper and Obeid 1967; Kaufmann and Guitard 1967; Austenson and Walton 1970; Ahmed and Zuberi 1973; Kromer and Gross 1987), although this is not universally true (Burris Edje and Wahab 1973).

Although in Experiments 1 and 2 the N and P contents of the planted seeds differed according to maternal treatment, we suspect that the effects on offspring performance had more to do with seed P content than seed N content. In Experiments 3 and 4 the N contents of the planted seeds were not significantly affected by maternal treatment and yet persistent maternal treatment differences were apparent in the offspring.

The findings of Austin (1966) suggest that if greater vigor of offspring from M mother plants was simply due to increased seed P provisioning, additions of P to offspring should have reduced the differences in growth and reproduction between offspring produced by M and NM mother plants. In fact, addition of P to offspring plants in Experiment 4 did not eliminate all the differences caused by maternal treatment seen in the -P offspring plants of Experiment 3. This result was similar to that reported in Lewis and Koide (1990) for *Abutilon theophrasti* Medic., but the present study documented significant maternal effects much later in the growth cycle of offspring plants, including those affecting reproduction.

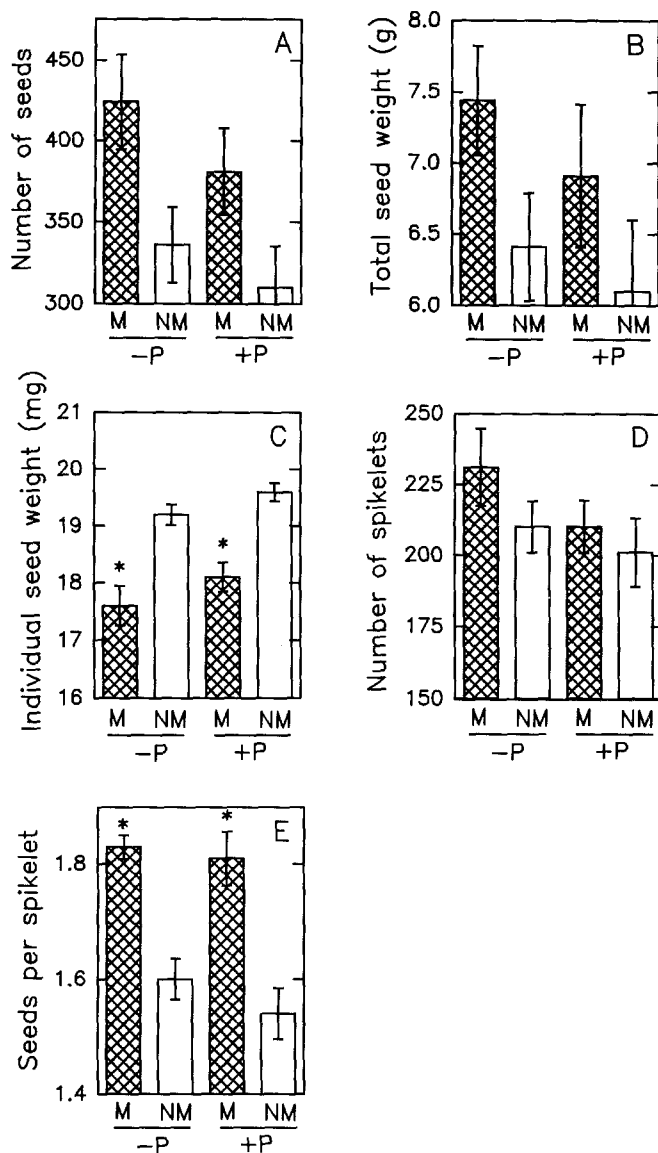


Fig. 4A-E. Reproductive variables for offspring *Avena fatua* plants produced by either mycorrhizal (M) or non-mycorrhizal (NM) mother plants and given two levels of P amendment (-P and +P) in Experiments 3 and 4, respectively. Error bars and asterisks are the same as for Fig. 2. Tables 4 and 5 give complete results from the analyses of variance which tested for the effect of maternal treatment (see Materials and Methods for the details of the analyses).

Perhaps this indicates an inability to alter a growth trajectory established in the very earliest phases of growth. Lewis and Koide (1990) and Lu and Koide (1991) reported that the amount of phytic-acid P (a seed storage form of P of particular importance to seedlings) was higher in seeds produced by M mothers. It is possible then, that the effects of phytate P in seeds could not be mimicked by phosphate supplied exogenously to the seedlings.

That the maternal effects on offspring performance were not entirely due to differences in seed P provisioning is suggested by the fact that mycorrhizal infection of the maternal generation of plants had such long lasting effects on the offspring generation. For example, offspring

from M mother plants produced seeds of higher nutrient content and lower weight than offspring from NM mother plants. This maternal effect on the quality of seeds produced by offspring is similar to the direct effect of mycorrhizal infection on the weight and P content of individual seeds produced by the infected generation (Koide et al. 1988). This suggests the possibility of inheritance of an acquired characteristic (Durrant 1962; Hill 1965) but we have no direct evidence for this.

The reduced individual seed weight produced by offspring plants which were themselves the progeny of the original M mother plants was associated with a significant increase in the number of seeds produced per spikelet. This indicates that there may be a resource-mediated tradeoff between seed weight and the number of seeds per spikelet. Since P content was actually higher in those seeds produced by offspring from M mother plants, the limiting resource would not appear to be P. A greater P availability as a consequence of greater root development and root phosphatase activity could have resulted in more seeds per spikelet but lighter individual seeds as a consequence of a limitation of carbon or some other resource.

We note that in Experiments 3 and 4 higher root N concentrations and contents were observed in the off-

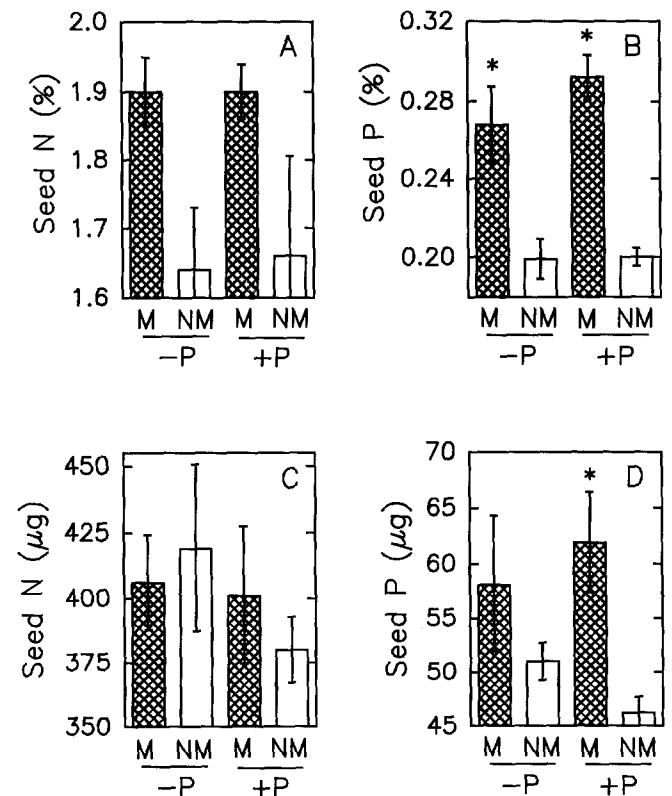


Fig. 5A-D. Characteristics of *Avena fatua* seeds (third generation) produced by offspring *Avena fatua* plants having either mycorrhizal (M) or non-mycorrhizal (NM) parentage and given two levels of P amendment (-P and +P) in Experiments 3 and 4, respectively. Error bars and asterisks are the same as for Fig. 2. Tables 4 and 5 give complete results from the analyses of variance which tested for the effect of maternal treatment (see Materials and methods for the details of the analyses).

spring from M mother plants. This is consistent with higher levels of phosphatase and phytase activities in such offspring previously reported (Lu and Koide 1991). Thus there may be a connection between root *N* status and plant *P* economy. While offspring from M mother plants are better provisioned with *P* in the seed, their ability to subsequently acquire *P* from the soil may also be enhanced via greater root phosphatase activity. Greater root phosphatase activity could result in long lasting and significant consequences for the overall nutrient economies of the offspring.

Results of these experiments show that mycorrhizal infection of *Avena fatua* plants had significant, long-lasting positive effects on offspring growth and reproduction that were associated with, but not necessarily caused by, increases in seed *P* content. As always, these experiments must be cautiously interpreted. Spatial heterogeneity of the field environment could, possibly, diminish the importance of any variability in offspring vigor introduced by maternal mycorrhizal infection. These experiments do suggest, however, that field work should be conducted to determine the importance of the maternal effects observed in these experiments. We suggest that, in addition to influencing fecundity (Koide et al. 1988; Bryla and Koide 1990), mycorrhizal infection may influence plant fitness, population dynamics and community composition via its effects on offspring vigor.

Acknowledgements. We thank the A.W. Mellon Foundation and the National Science Foundation (DMB-8657186) for financial support of this project. We also thank Mingguang Li, Carla Picardo, Robert Robichaux, Ian Sanders, Roger Schreiner, Margot Stanley and two anonymous reviewers for comments on earlier versions of the manuscript. Drs. Durland Shumway, Victoria Sork and Marvin Risius contributed to substantial improvements in the statistical analysis and presentation of data.

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