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Plant life span and response to inoculation with vesicular-arbuscular mycorrhizal fungi

II. Species from weakly mycotrophic genera

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Summary. To determine if annual and perennial species from weakly mycotrophic families exhibit the same differential patterns of responsiveness to vesicular-arbuscular mycorrhizae (VAM) as do members of the strongly mycotrophic grass family (Poaceae), this study examined the responses of congeneric, sympatric annual and perennials species from the Caryophyllaceae and Solanaceae to inoculation with the VAM fungus *Glomus intraradices*. The perennial *Solanum carolinense* responded as positively to *G. intraradices* as did perennial grasses. The other perennials examined, *Solanum dulcamara* and *Silene nivea*, were both relatively unresponsive to VAM inoculation and had mean responsiveness values below the typical level for annuals from strongly mycotrophic families. The annual *Solanum nigrum* exhibited a small positive response to inoculation, and was as responsive as many annuals from more mycotrophic families, especially at low P supply rates. The annual *Silene noctiflora* was strongly inhibited by inoculation. Mortality over 8 weeks was fivefold higher in VAM-inoculated *Silene noctiflora* than in uninoculated plants. Among the four herbaceous species examined, the species which persists longest in old-field succession in eastern North America was also the most responsive to VAM inoculation. Mechanisms are proposed for successional persistence and replacement based on VAM responsiveness and soil nutrient pool sizes.

Key words: Vesicular-arbuscular mycorrhizae - Caryophyllaceae - Solanaceae - Responsiveness - Succession

Introduction

The temporal gradient of secondary succession in a humid, mesic environment has many similarities to spatial gradients of increasing physiological stress (Pickett 1976). Light intensity at the soil surface, moisture, and nutrient availability are all typically lower in later successional sites than immediately following disturbance or abandonment. Thus, species of later successional communities must share many stress-tolerating physiological adaptations with species of inherently stressful sites (Grime 1979). These adaptations include long leaf and root life span, high root:shoot ratio, and "the presence of mechanisms which allow the intake of resources during temporarily favourable conditions" (Grime 1979). Among these mechanisms is the mycorrhizal habit (Grime 1979).

Janos (1980) developed a model for the impact of vesicular-arbuscular mycorrhizal (VAM) fungi on succession in humid environments. This model predicts that non-mycotrophic species should dominate successions for long periods only when VAM propagule density is low. Long-lived mycorrhizal species should eventually dominate any succession, regardless of fertility level, as long as VAM propagule density is sufficient.

This leads to the question of whether non-mycotrophic or weakly mycotrophic species are displaced during succession simply because they cannot form the mycorrhizal associations necessary to acquire mineral nutrients from later successional, less fertile soils. Or, alternatively, can the inability of weakly mycotrophic species to respond to mycorrhizal infections when they form be at least partially responsible for their lack of persistence in late successional communities? In the first paper of this series (Boerner 1992), I presented data demonstrating that later successional, perennial species of two genera of grasses were more responsive to and dependent on infection by *Glomus intraradices* than were congeneric, sympatric annuals. In this study, we extend these experiments to congeneric, sympatric species from genera considered to be weakly mycotrophic: *Silene* (Caryophyllaceae) and *Solanum* (Solanaceae).

Of the species in the Caryophyllaceae or Caryophyllales examined to date, 50% and 62% have been judged to be non-mycorrhizal, respectively (Newman and Riddell 1987; Trappe 1987). In the Solanales, overall 45% have been judged to be non-mycorrhizal, though the percentage among short-lived species is probably greater (Trappe 1987). Our observations of *Solanum* species from the field indicate that $<60\%$ carry VAM infections and that the percentage of root length colonized in the VAM plants is <15%. In contrast, Newman and Riddell (1987) list 91% of species in the Poaceae, the strongly mycotrophic family used in the earlier study, as facultatively or obligately mycorrhizal.

The specific questions to be addressed in this study were: (1) How do annual and perennial congeneric, sympatric species from these families respond to inoculation with common VAM fungi? (2) Are responses dependent on plant life span or P availability? (3) Do the differences between annuals and perennials vary between a pair in which the perennial does persist into mid-succession and a pair in which the perennial is restricted to recent disturbances?

Materials and methods

Three perennial species typical of forest edges, large forest openings, and waste areas were selected for study: *Silene nivea* (Nutt.) Otth (snowy campion; Caryophyllaceae), *Solanum carolinense* L (Ball-nettle; Solanaceae), and *Solanum dulcamara* L (bittersweet nightshade; Solanaceae). The first two are native to North America, whereas the third was introduced from Europe. In addition, two annual species typical of abandoned agricultural fields were chosen: *Silene noctiflora* (nightflowering catchfly; Caryophyllaceae) and *Solanum nigrum* (black nightshade; Solanaceae); both species were introduced from Europe. The *Silene* seed stocks came from central Michigan (Kellogg Biological Station) and the *Solanum* seed stocks from northern Illinois populations (F and J Seed Service, Woodstock, Ill.).

Glomus intraradices Schenck and Smith is among the most common *Glomus* species in the southeastern United States and will form VAM with a wide range of hosts (Schenck and Smith 1982). The culture was obtained from J. H. Gerdemann at the University of Illinois and was isolated from agricultural field crops. The *G. intraradices* culture had originally been identified as *G. fasciculatum*, but was subsequently re-identified (S. B. Rabatin, personal communication).

Seeds were planted in flats of acid-washed sand and transplanted to 10-cm-diameter pots of 4:1 sand:perlite (v:v) when the first true leaves appeared. Each plant to be VAM-inoculated was given 30 ml of an inoculum slurry which contained at least 300 *G. intraradices* spores. Plants which were to remain free of VAM infection were given an equal amount of a slurry which had been passed through a 15-μm filter to remove VAM spores (Jensen 1982). The *G. intraradices* isolate used was the one which produced strong responses in perennial grasses (Boerner 1992).

The plants were grown for 8 weeks during summer in a glasshouse at ambient temperature and light intensity. They were fed weekly with a nutrient solution (Ruakura Solution) designed for sand culture (Smith et al. 1983) modified so as to supply P at rates similar to that found in relatively fertile and infertile forest sites in Ohio (Boerner 1990); high P: 5.0 mg $P \cdot l^{-1}$ in solution and 11.0 mg P total over the course of the experiment versus low P: 2.0 $mg \cdot l^{-1}$ in solution and 4.4 mg P total addition. Each species/VAM inoculum/P level combination was replicated 20 (*Silene*) or 16 (*Solanum*) times in a completely randomized design.

The plants were harvested 8 weeks after inoculation. At that time, most of the *S. carolinense* and *S. nigrum* plants had flowered and set fruit. The number of open flowers plus set fruit was determined for each plant at harvest. The shoots and a subsample of roots were then weighed fresh, dried at 70° C for 72 h, and weighed again. The remainder of the roots were weighed fresh then preserved in formalin:acetic acid:ethyl alcohol (FAA). The preserved root material was later stained with trypan blue in lactoglycerin and examined microscopically to verify the presence or absence of VAM structures (Giovanetti and Mosse 1970). All VAM-inoculated plants had at least 10% of root length infected; plants from non-VAM treatment combinations which had any detectable infection were discarded. The dried root and shoot material was digested in 30% $H_2O_2 + H_2SO_4$, and phosphate concentrations in digests determined by the stannous chloride method (American Public Health Association 1976).

All response variables were tested for normality, then analyzed by analysis of variance and the Ryan-Einot-Gabriel-Welsch Modified F test (Statistical Analysis System 1985). All significant differences noted are at $P < 0.05$, except where otherwise noted.

Results

Responses of Silene species and G. intraradices

The perennial *S. nivea* exhibited no significant biomass response to VAM inoculation (Fig. 1). Total P uptake and tissue P concentrations were significantly greater in non-VAM *S. nivea* plants than in VAM-inoculated plants at the high P supply rate, whereas there were no differences in P uptake or concentration attributable to VAM inoculation at low P.

S. noctiflora (annual) growth was significantly inhibited by VAM inoculation: VAM-inoculated plants were only 63–73% the size of non-VAM plants (Fig. 1). Although tissue P concentrations did not differ significantly between VAM-inoculated and non-VAM plants, total P uptake by VAM-inoculated plants was significantly lower than by non-VAM plants (Fig. 1).

The mortality rate of *S. nivea* (perennial) was ≤5% over the course of the experiment. In contrast, in *S. noctiflora* (annual), mortality in the VAM-inoculated/low P treatment (30%) was significantly greater than the mortality in the other three treatment combinations (0–6%).

Responses of Solanum species and G. intraradices

VAM inoculation produced no significant biomass effects on the herbaceous perennial *S. carolinense* at high P. In contrast, at low P, VAM-inoculated plants were significantly larger than non-VAM plants (Fig. 2). Total P uptake and tissue P concentrations were both significantly greater in VAM plants than non-VAM plants at both P supply rates.

The woody perennial *S. dulcamara* grew much more slowly than did the other two *Solanum* species in all treatment combinations (Fig. 2). VAM inoculation had no significant effect on any *S. dulcamara* biomass or nutrient parameter.

S. nigrum (annual) plants attained the greatest final biomass among the three *Solanum* species, but both biomass accumulation and P uptake were unaffected by VAM inoculation (Fig. 2). Non-VAM, low P *S. nigrum* plants produced significantly fewer flowers plus fruits, and had significantly lower tissue P concentrations than did both VAM and non-VAM, high P plants.

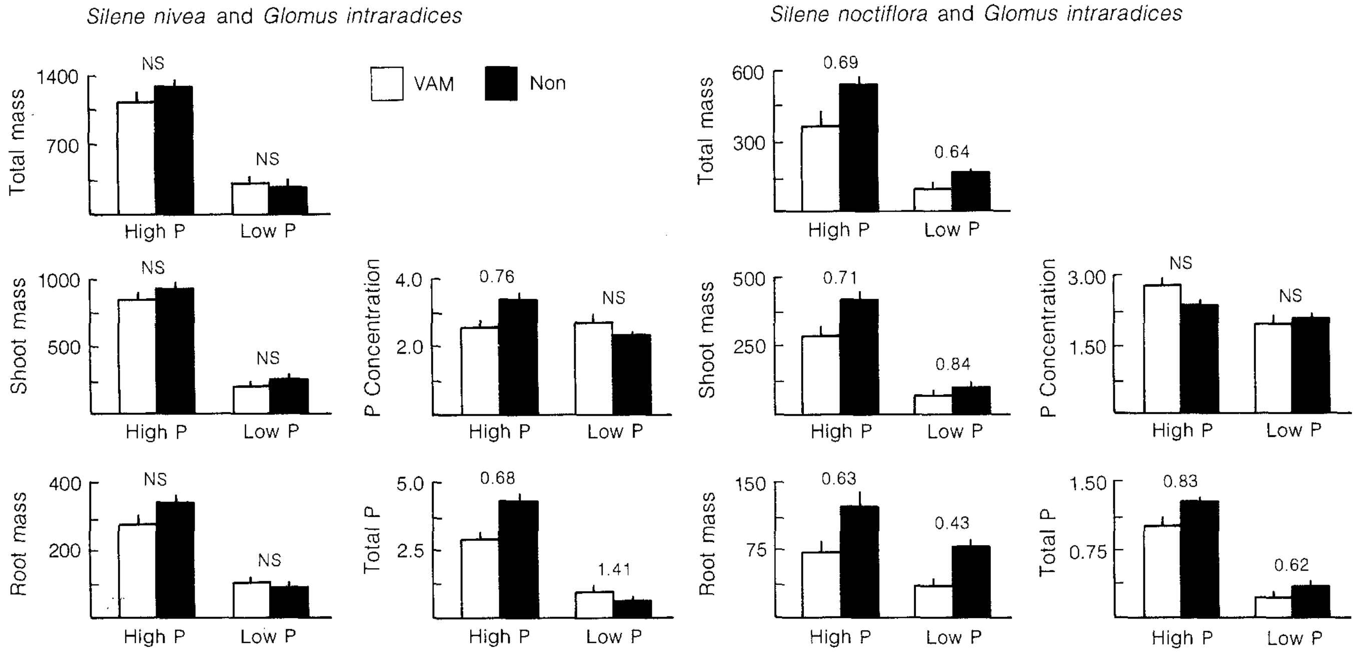

Fig. 1. Responses of *Silene nivea* (perennial) and *S. noctiflora* (annual) to inoculation with *Glomus intraradices* and to P level. *Histogram bars* represent the means of 20 replicates; standard errors are indicated by the *vertical line*. Levels of significant responsiveness are indicated between the appropriate histogram bars. *NS*, No significant responsiveness. Units are mg·plant^{-1} except for tissue P concentration (mg·g dry mass^{-1})

Comparison of responses to VAM inoculation

The three perennial species exhibited a wide range of responses to VAM inoculation, ranging from a strong positive response of *S. carolinense* at low P to a moderate negative response of *S. nivea* at high P (Table 1). Within each genus, the annual plants responded less positively to VAM inoculation than did the herbaceous perennials.

Discussion

Of the five species of putatively weakly mycotrophic plants examined in this experiment, only one (*S. carolinense*) exhibited either dependence on or positive responsiveness to mycorrhizal inoculation. This perennial was positively responsive to inoculation with *G. intraradices* at both P levels tested, though more so at low P than high P. The mean responsiveness of *S. carolinense* over all response parameters tested was well within the range for mycotrophic plants (Table 2 in Boerner 1992); in contrast, the mean responsiveness of the two other perennials, *S. dulcamara* and *S. nivea*, fell below even the mean responsiveness of annuals given by Boerner (1992).

The least responsive species in this group was *S. noctiflora*, an annual. The growth of this species was significantly inhibited by inoculation with *G. intraradices* at both P levels, and mortality was increased at least fivefold after inoculation. Microscopic examination of the roots of dead and dying plants showed circular to ovoid areas of browning around VAM penetration areas, similar to those described following VAM inoculation of non-mycotropic plants (review by Allen 1991). The negative responsiveness of *S. noctiflora* was similar in magnitude to the response of the non-mycotrophic *Salsola kali* to inoculation measured by Allen and Allen (1984). In contrast, the mean responsiveness of *S. nigrum*, the second annual examined here, was well within the range for mycotrophic annuals given by Boerner (1992).

The life history approach of Grime (1979) predicts, in general, that longer-lived, later successional species within a closely related group should show a syndrome of adaptations including lower growth rate, higher root : shoot ratio, increased dependency on and responsiveness to mycorrhizal infection. This syndrome was present among the four species of grasses discussed in the previous paper in this series (Boerner 1992), but is not fully apparent among these five species from weakly mycotrophic families. Though both *Silene* species did have a greater root : shoot ratio under low P conditions than high, the annual *S. noctiflora* had a greater overall root : shoot ratio over all treatments, and demonstrated greater plasticity in root : shoot ratio among treatments than did the perennial *S. nivea*. Under our experimental conditions, *S. nivea* was also able to support greater growth rates under both high and low P than was *S. noctiflora*. Though neither *Silene* species benefited from VAM inoculation, the perennial *S. nivea* was less inhibited by inoculation than was *S. noctiflora*. Neither species in this pair persists past the first decade of old-field succession in our region. Thus, the observations that the perennial had the greater growth rate, the lower root : shoot ratio, and the lower response to infection

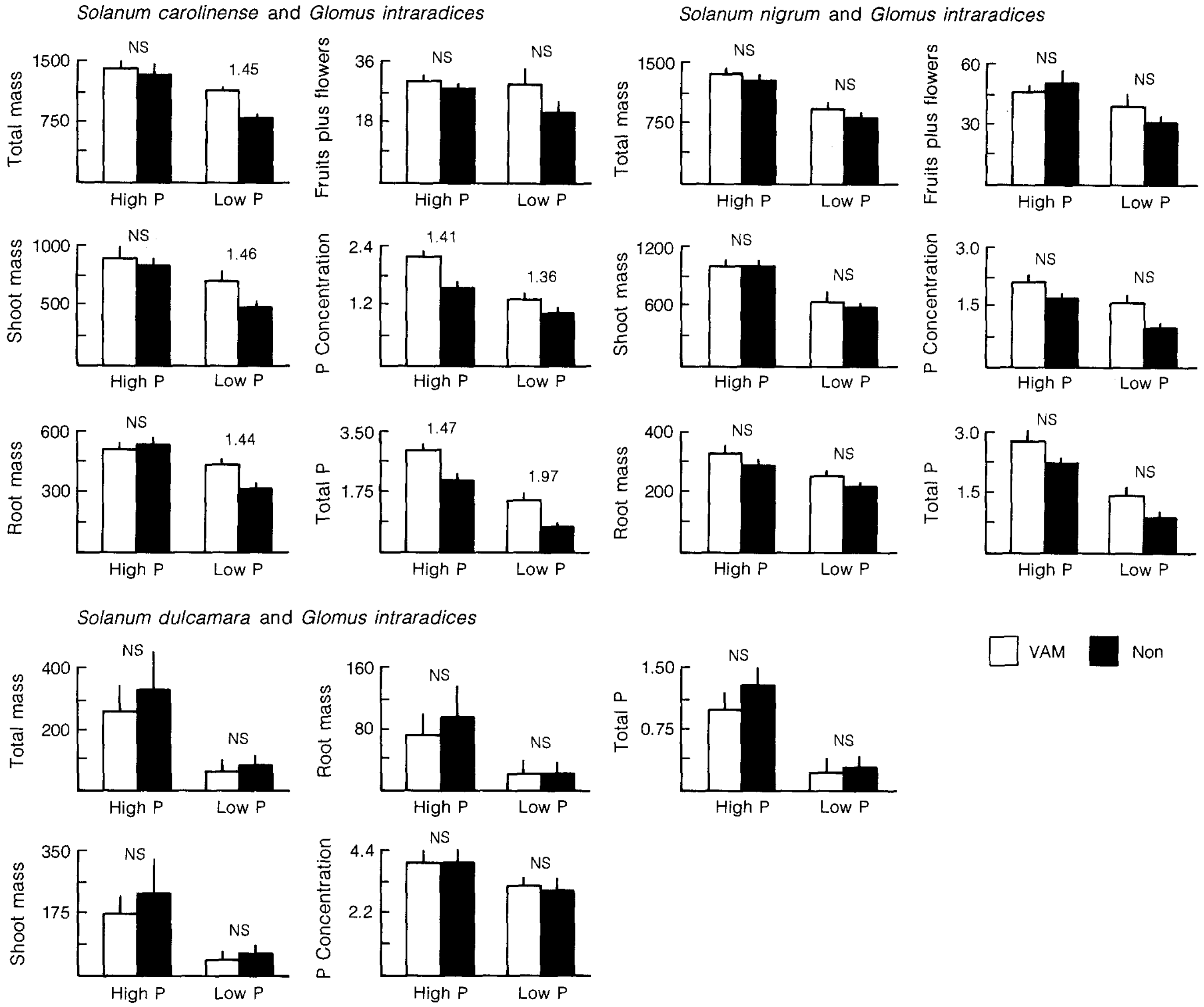

Fig. 2. Responses of *Solanum carolinense* (herbaceous perennial), *S. dulcamara* (woody perennial), and *S. nigrum* (annual) to inoculation with *Glomus intraradices* and to P level. *Histogram bars* represent the means of 16 replicates; standard errors are indicated by the *vertical line*. Levels of significant responsiveness are indicated between the appropriate histogram bars. *NS*, No significant responsiveness. Units are mg·plant^{-1} except for tissue P concentration (mg·g dry mass^{-1}) and fruits plus flowers (*n*)

than the annual, all putative attributes of short-lived species, may have little importance in determining relationships between plant life span, mycorrhizal infection, and succession.

The three species of *Solanum* examined here do vary in their successional status, from an annual found predominantly in chronically disturbed areas and agricultural fields (*S. nigrum*), to a persistent perennial of old-fields and hedgerows (*S. carolinense*), to a woody perennial found everywhere from waste places to forest canopy gaps (*S. dulcamara*). Consistent with Grime's (1979) predictions, the annual *S. nigrum* had a greater growth rate, lower root:shoot ratio, and less response to mycorrhizal infection than the more persistent perennials. The woody perennial *S. dulcamara* did, as predicted, have the lowest growth rate, but it was also relatively insensitive to mycorrhizal infection, and changed root:shoot ratio little in response to differences in P supply. However, as different VAM-fungus isolates may produce considerable variation in plant responsiveness (Boerner 1990, 1992), it is still possible that *S. dulcamara* might exhibit considerable response to VAM infection if allowed to form mycorrhizae with a more compatible isolate.

Among the four herbaceous species discussed here, the species which persists longest through old-field succession was the most responsive to mycorrhizal infection and had the greatest root:shoot ratio. Both of these traits are indications of greater ability to compete for the below-ground resources which become progressively more limited through succession. This pattern was also consistent with that among four annual and perennial grasses described by Boerner (1992). Such correlational patterns suggest that mycorrhizal and below-ground

Table 1. Comparisons of responses of three *Silene* species and three *Solanum* species to inoculation with *Glomus intraradices.* Positive growth responses are indicated by +, negative responses by −, and the lack of significant response by 0; responses to high and low P treatments are separated by "/". Mean responsiveness ratios are given. ND, No data

Perennials	*Solanum carolinense* and *G. intraradices*	*Solanum dulcamara* and *G. intraradices*
Total plant mass	0/+	0/0
Shoot mass	0/+	0/0
Root mass	0/+	0/0
Fruit flowers	0/0	ND/ND
P uptake	+/+	0/0
Tissue P concentration	+/+	0/0
Totals	2+/5+	0/0
Mean responsiveness	1.18/1.51	0.83/0.86
	Silene nivea and *G. intraradices*	
Total plant mass	0/0	
Shoot mass	0/0	
Root mass	0/0	
P uptake	−/0	
Tissue P concentration	−/0	
Mortality	0/0	
Totals	2−/0	
Mean responsiveness	0.82/1.02	
Annuals	*Solanum nigrum* and *G. intraradices*	*Silene noctiflora* and *G. intraradices*
Total plant mass	0/0	−/−
Shoot mass	0/0	−/−
Root mass	0/0	−/−
Fruit flowers	0/0	ND/ND
P uptake	0/0	−/−
Tissue P concentration	0/0	0/0
Mortality	ND/ND	0/−
Totals	0/0	4−/5−
Mean responsiveness	1.10/1.31	0.81/0.70

competition may be more important in successional replacement in humid ecosystems than has been assumed (see also Janos 1980).

If the patterns described here are widespread among species of humid environments and important in succession, the mechanism by which differential responsiveness (dependency affects successional replacement may fit either the inhibition or tolerance model of Connell and Slatyer (1977). Both of these mechanistic models for succession rely on early colonists being able to alter their immediate surroundings in such a manner as to make it impossible for subsequent colonists to successfully establish. One example of this might be the ability of a more VAM-responsive perennial to grow faster and to reduce the local soil nutrient or water status below the threshold necessary for subsequent, less-responsive colonists. Experimental analysis of the impact of plant and VAM fungal growth on below-ground resources, particularly P and water, and the effect of this on the growth of subsequent colonizers is necessary, however, to establish the existence of such a mechanism.

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