Nutrient Concentration Effects on *Pisolithus tinctorius* Development on Containerized Loblolly Pine (*Pinus taeda* L.) Seedlings

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A trade-off between seedling size and degree of mycorrhizal infection needs to be considered when determining the rate at which to fertilize containerized seedlings infected with ectomycorrhizae. Tree Planters' Notes 37(3):17-22; 1986.

Ectomycorrhizae can increase forest tree survival and growth under many different site conditions (11). Mycorrhizae increase tree root surface area, increase the absorption of water and nutrients, and can exploit forms of phosphorus (P) unavailable to nonmycorrhizal plants (1). *Pisolithus tinctorius* (Pers.) Coker & Couch is a fungus capable of forming ectomycorrhizae on trees growing on acidic, droughty, and infertile soils. It has been used successfully for improving the performance of pines on reclaimed surface mines (6) and on clay soils in North Carolina and Georgia (4).

Much work has been done on methods for inoculating pines with *P. tinctorius* basidiospores (5, 8, 9). Using spores is more convenient and less costly than inoculating with vegetative fungal mycelia. Marx and co-workers (7) found that ectomycorrhizal development was related to soil fertility, with nitrogen (N) and P being the most critical nutrient elements. At high N and P concentrations, *P. tinctorius* infection of loblolly pine appears to be reduced. Lower *P. tinctorius* infections coincide with lower levels of sucrose in the roots. On the other hand, Lamb and Richards (3) found that greater mycorrhizal infection of Monterey pine (*Pinus radiata* D. Don) and slash pine *Pinus elliottii* Engelm. on Australian soils occurred when P was applied. The objective of this study was to examine the relationship between nutrient levels and *P. tinctorius* ectomycorrhizal development on container-grown loblolly pine seedlings.

Materials and Methods

P. tinctorius fruiting bodies were collected in the fall from a 20-year-old loblolly pine (*Pinus taeda* L.) stand in Buchanan County, VA. Basidiospores were harvested, suspended in water, and added to vermiculite and peat (1 :1, v/v) that was being mixed in a cement mixer. Spores were applied at a rate equivalent to 250 grams per hectare. Spencer-Lemaire Rootrainers (Hillson model, 150 cubic centimeter per cavity) were filled with this mixture and two loblolly pine seeds of Virginia Piedmont origin were placed in each container. After germination, seedlings were thinned to one per container.

Four replications (eight seedlings per replication) of five nutrient solution levels (treatments) were used in this study (table 1). The five treatments consisted of a serial dilution of reagent grade chemicals. Molar ratios of 1 :1.2:1.1 :0.3 for $KN0_3$, NH_4S0_4, H_3PO_4, and Fe (as Greenol), respectively, were used to provide the various amounts of nutrients. A serial dilution was used to establish treatment levels so that the most concentrated treatment was 16 times as concentrated as the lowest fertilization rate (table 1). Nutrients were added in 15 milliliters of water every 2 weeks. Additional watering occurred on a regular basis.

Table 1—*Amount of nitrogen, phosphorus, potassium, and iron added every 2 weeks during the 20-week study period*

	Amount of nutrient (mg)			
Solution level	N	P	K	Fe
1×	0.25	0.16	0.21	0.09
2×	0.50	0.32	0.42	0.18
4×	1.00	0.64	0.84	0.36
8×	2.00	1.28	1.68	0.72
16×	4.00	2.56	3.36	1.48

After 20 weeks, seedlings were harvested. The degree of mycorrhizal infection was calculated as the number of individual short roots colonized, divided by the total, and expressed as a percentage. Roots and shoots were separated at the root collar, dried to a constant weight at 65 °C, and weighed. After weighing, needles were stripped from the stems and composited by replicate for foliar N and P analyses. Foliar N was determined by the Kjeldahl technique (2). Phosphorus was extracted with 6 N HCl from 0.5 gram of tissue, following dry-ashing at 450 °C. Phosphorus was determined by the Murphy-Riley ascorbic acid technique (13). Regression techniques were used to analyze the data (12).

Results and Discussion

Fertilizer concentration had a significant effect on degree of infection, shoot weight, root weight (figure 1) and on foliar N and P concentrations (figure 2). The highest rates of fertilizer resulted in larger seedlings, but mycorrhizal colonization was greatest at the lower rates. Maximum root system colonization was about 30 percent, which decreased to about 10 percent at the highest fertilizer level.

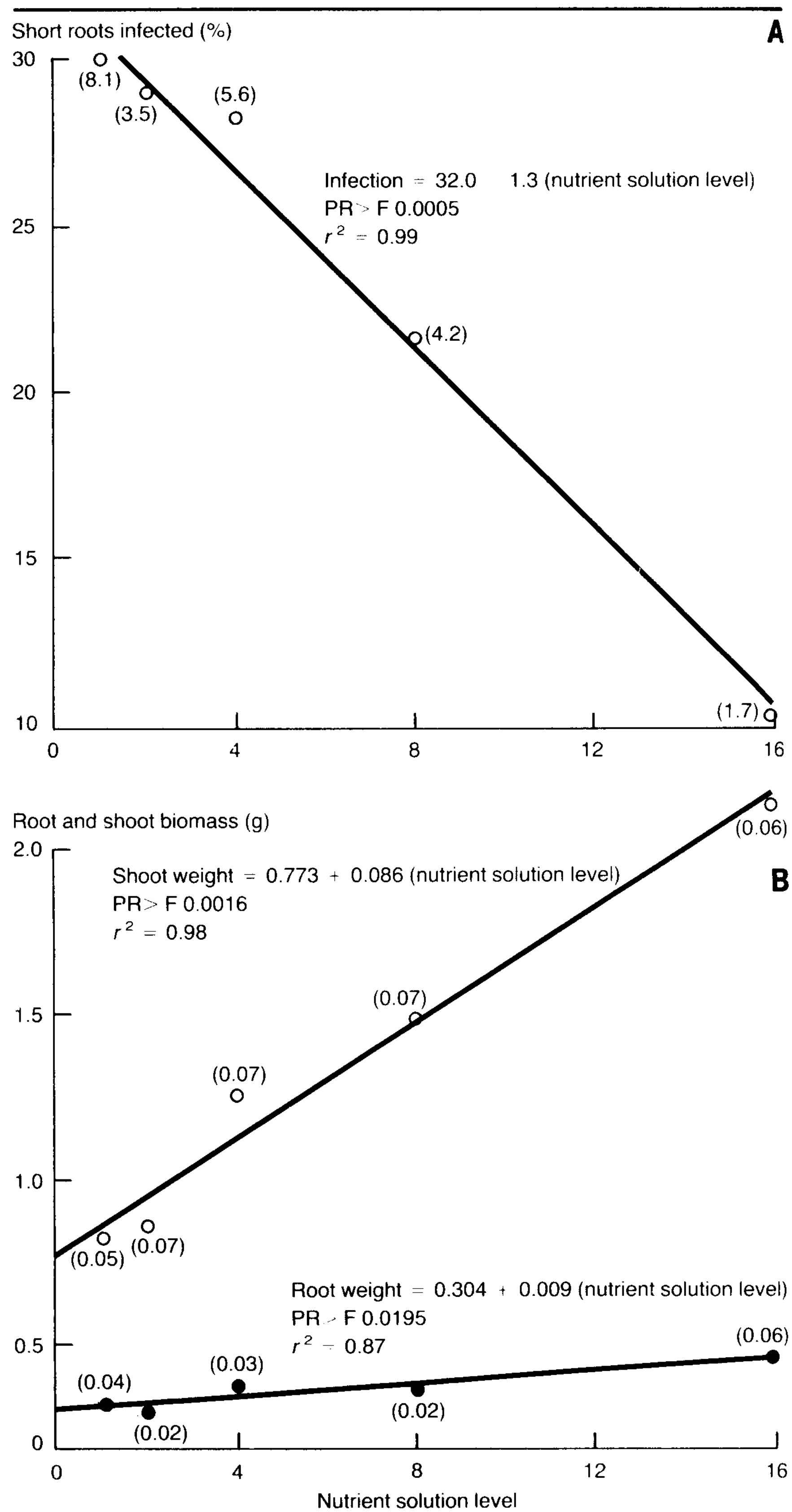

__Figure 1__-Relationship between mycorrhizal infection (__A__), seedling biomass (__B__), and nutrient solution level. Numbers in parentheses are standard deviations.

Foliar nutrient levels increased with fertilizer rate (figure 2). In the most concentrated treatment, foliar N and P levels were 1.02 and 0.14 percent, respectively. Although the N/P ratio (1.56 milligrams per milligram) in the nutrient solution was greater than the N/P ratio (1.21) used by other researchers (9, 10), there seemed to be insufficient N even at the highest treatment level. Nitrogen is generally considered deficient when foliar levels are below 1.2 percent, whereas 0.1 percent is a commonly accepted critical level for P (14).

There were also significant relationships between foliar nutrients and seedling biomass and infection (figures 3 and 4). Shoot weight increased but root infection decreased with increasing foliar N and P levels. Seedling size continued to increase as foliar P levels rose above 0.1 percent. This can be explained by the fact that N was limiting but P was available in sufficient quantities. Additional increments of N and P then resulted in luxury consumption of P, while seedling growth was still responding to added N.

Although root weight was used as a measure of root size, an estimate of total root length or surface area would have been more meaningful. There were distinct morphological differences observed

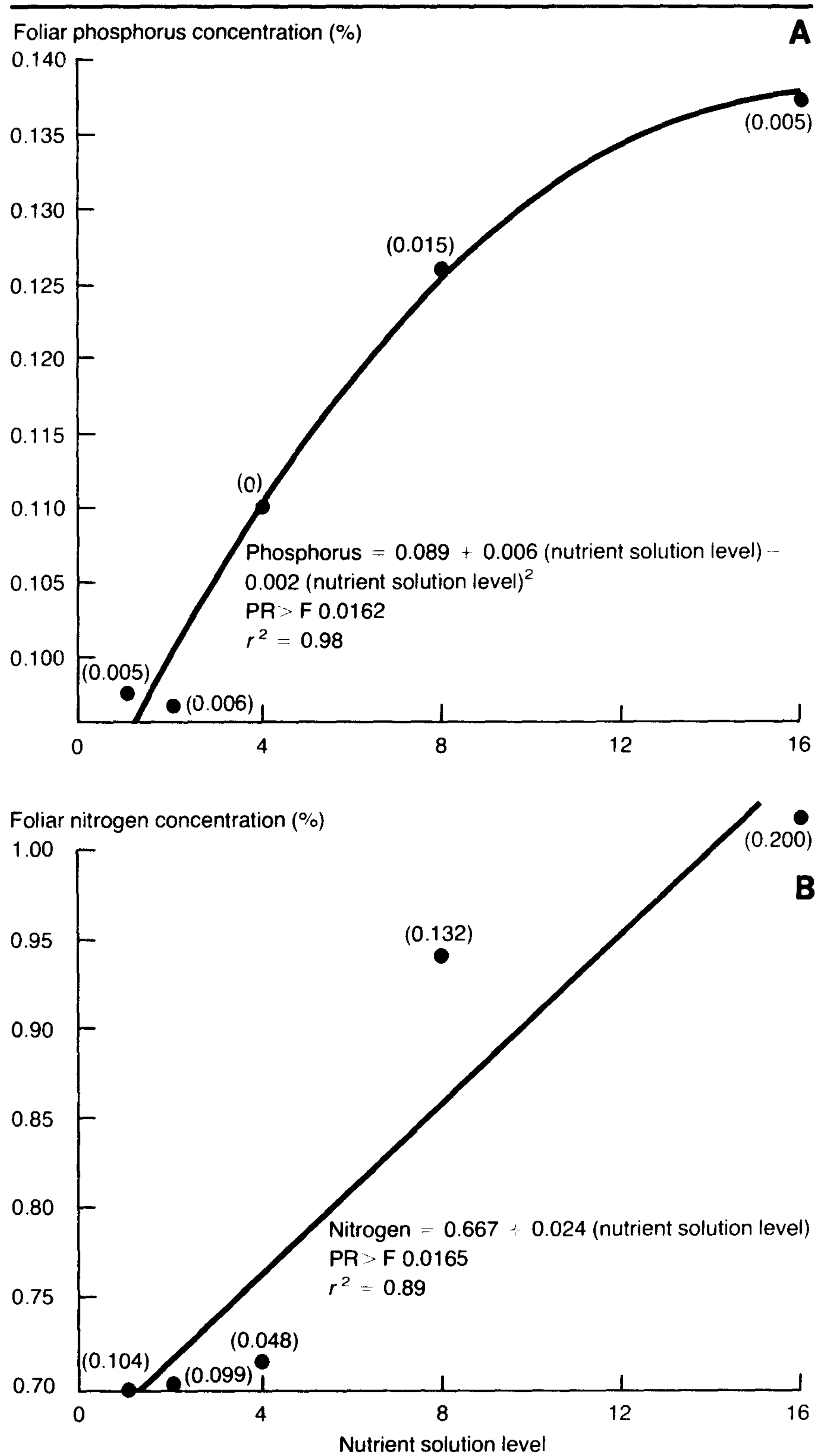

Figure 2—*Relationship between foliar phosphorus (**A**) and nitrogen (**B**) and nutrient solution level. Numbers in parentheses are standard deviations.*

between root systems at different nutrient levels. Root systems of seedlings receiving the highest treatment were short and stocky. Although they weighed less, the root systems subjected to lower nutrient levels were longer, more fibrous, and had a greater degree of branching.

In order for seedlings to survive in harsh environments, they need an adequate root system to absorb moisture and nutrients. Seedlings receiving the greatest amount of nutrients in this study, although larger, could be at a disadvantage when outplanted. They would require greater amounts of moisture to meet the demands of their larger foliar biomass, but their root systems had a smaller surface area and less absorptive capacity. The likelihood that the smaller trees receiving the lowest levels of nutrients would be more successfully established is increased by the greater degree of mycorrhizal infection.

Conclusion

Although nutrients are necessary to develop healthy containerized seedlings, it appears that there is an optimal level above which additional nutrient supplies produce seedlings that may be more sensitive to drought and less able to

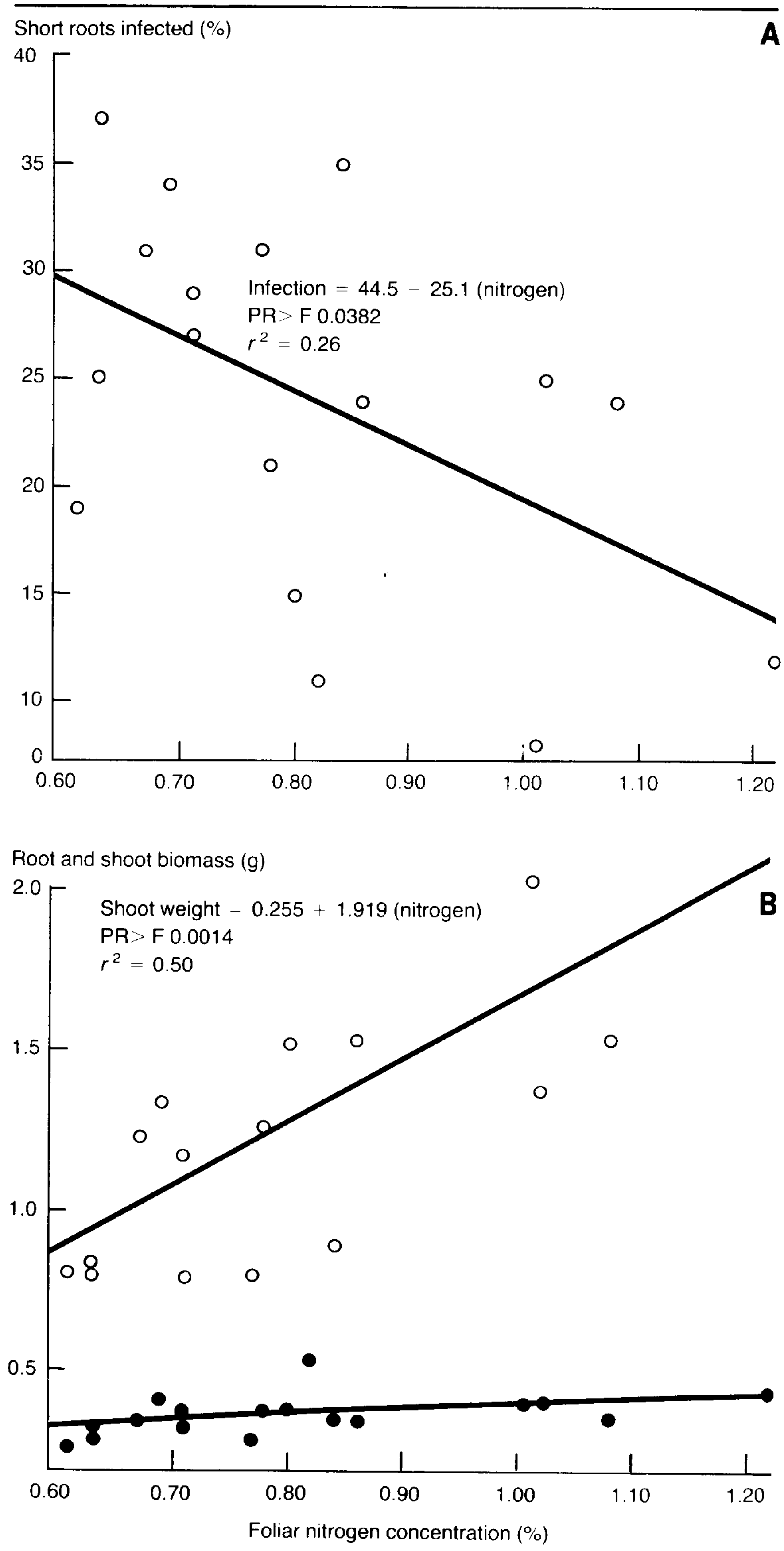

Figure 3—*Relationship between mycorrhizal infection* (**A**)*, seedling biomass* (**B**)*, and foliar nitrogen concentration.*

adapt to stress. Seedling size, root system morphology, and mycorrhizal colonization need to be optimized. An ideal fertilization program might be one that allows the seedlings to attain just enough N and P to reach foliar levels considered adequate for good growth. At higher levels, mycorrhizal infection is reduced and root systems undergo morphological changes resulting in smaller surface areas. In this study, level 4 x (1 .0 milligram N, 0.6 milligram P, and 0.8 milligram K) may represent the best level of nutrient supplementation because infection is still high and seedlings are significantly larger than those receiving lower levels of nutrients. Ultimately, seedlings should be outplanted to determine how these morphological and physiological properties affect establishment and early growth.

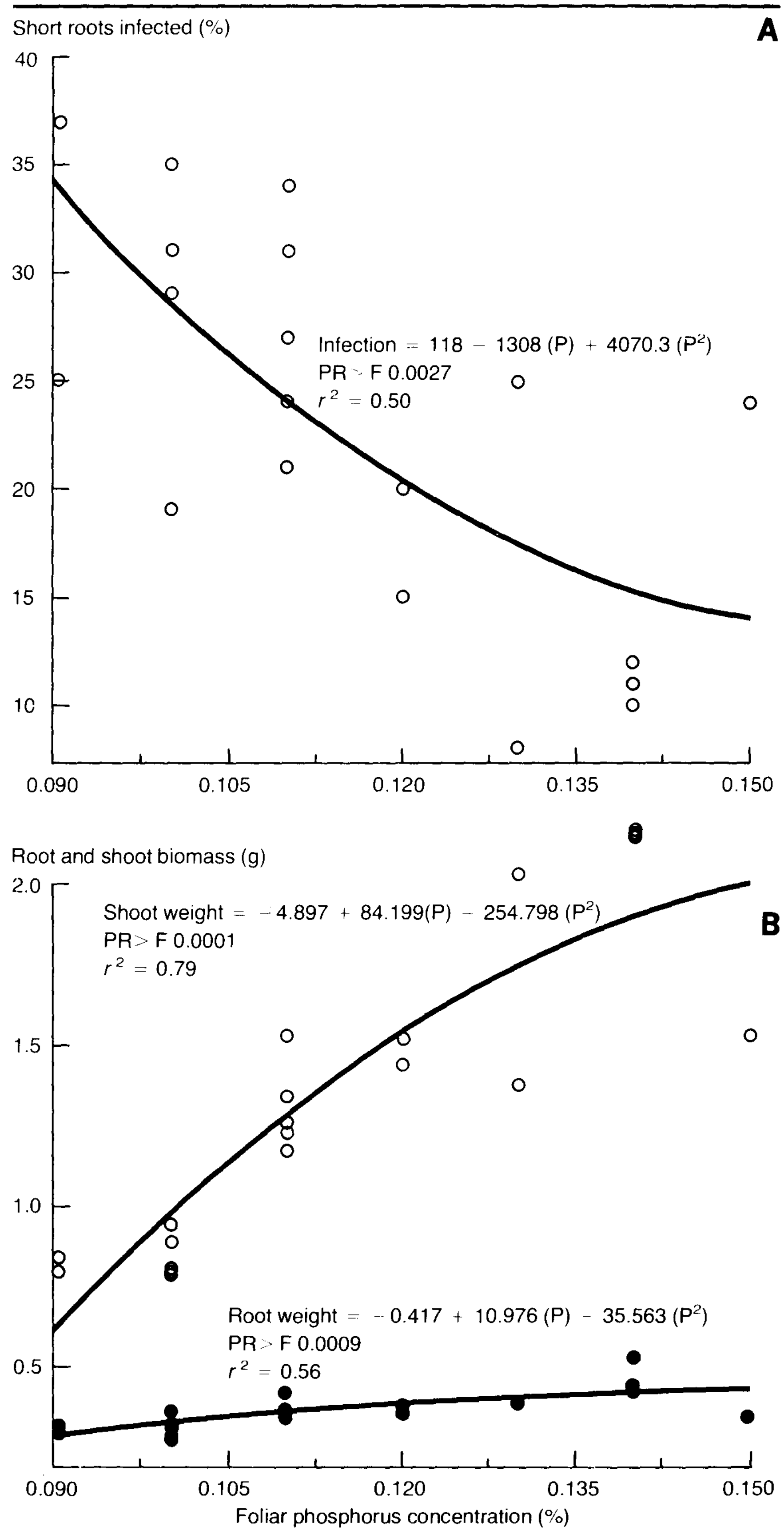

Figure 4—*Relationship between mycorrhizal infection (**A**), seedling biomass (**B**), and foliar phosphorus concentration.*

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