PHOSPHORUS NUTRITION OF MYCORRHIZAL AND NON-MYCORRHIZAL RED OAK SEEDLINGS

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ABSTRACT

Red oak (Quercus rubra L.) seedlings were grown for six months in a potting medium of fumigated sand and soil, the same medium inoculated with mycorrhizal roots, and the medium inoculated with forest soil. Phosphorus was added as either iron, calcium, or organic phosphate. Phosphorus uptake of non-mycorrhizal roots was greater than that of mycorrhizal roots as was the P content of roots fertilized with phosphorus; no difference existed in the P content of seedling tops in either treatment. Differences in the amounts of the three phosphatic fertilizers taken up were not significant. Growth of mycorrhizal seedlings was superior to non-mycorrhizal seedlings, thus emphasizing the importance of mycorrhizae to overall tree development.

In general, the availability of phosphorus in most forest soils in the Ozark Plateau is relatively low. Several investigators have suggested that ectomycorrhizae increase the solubility of poorly soluble phosphates in the soil (Rosendahl 1943, Routien and Dawson 1943, Voigt 1971). The solubilizing effect is thought to be brought about by fungus released organic acids or the higher metabolic activity of mycorrhizal roots in comparison with non-mycorrhizal roots. Increased uptake of phosphorus by mycorrhizal plants has been reported among others by Bowen and Theodorou (1967), Henderson and Stone (1970) and McComb and Griffith (1946); however, conflicting or erratic responses have been reported (Doak 1955; Stone 1950). The role of mycorrhizae in the growth of oaks in the Ozark Region is a problem to be solved before the mineral nutrition, in particular that of phosphorus, of these species can be satisfactorily related to the chemical properties of soils.

The present investigation examined the influence of ectomycorrhizae on the uptake of slightly soluble forms of phosphorus and the growth of red oak, Quercus rubra L.

METHODS

The effects of three soil treatments and three fertilizer treatments upon the growth of red oak were studied in a pot culture experiment in the greenhouse. The soil treatments consisted of (1) fumigated sand plus fumigated soil, (2) treatment 1 inoculated with mycorrhizal roots and (3) fumigated sand inoculated with unfumigated forest soil. No attempt was made to keep the pots sterile. The fertilizer treatments included a control and three forms of slightly soluble phosphate -- iron phosphate ($FePO_4$), calcium phosphate ($CaHPO_4$ dibasic), and an organic phosphate, phytic acid calcium salt ($C_6H_5\ (OPO_3Ca)_6$).

The forest soil used in the study, Weller silt loam, was collected beneath a medium-aged oak-hickory stand. The soil was placed in a $0.21m^3$ metal drum and fumigated with methyl bromide (MC-2). After one week the soil was removed and allowed to air for three days. Sand was fumigated in the same manner. The growth medium consisting of 8000g of sand and 1000g of soil was placed in plastic pots, whose volume was $6380cm^3$. The phosphate fertilizers were mixed with the soil before it was added to the pots. Each pot, other than the controls, received 300 ppm of phosphorus; either 1.316g $CaHPO_4$, 1.463g $FePO_4$, or 1.435g $C_6H_5\ (OPO_3Ca)_6$ were added.

The 12 soil and fertilizer treatments were as follows: uninoculated (U), uninoculated + iron phosphate (U+FeP), uninoculated + calcium phosphate (U+CaP), uninoculated + organic phosphate (U+OrgP), inoculated with roots (IR), inoculated with roots + iron phosphate (IR+FeP), inoculated with roots + calcium phosphate (IR+CaP), inoculated with roots + organic phosphate (IR+OrgP), inoculated with soil (IS), inoculated with soil + iron phosphate (IS+FeP), inoculated with soil + calcium phosphate (IS+CaP), inoculated with soil + organic phosphate (IS+OrgP).

Six acorns, which had been surface sterilized with sodium hypochlorite, were planted in each pot. After germination the trees were thinned to three per pot. The trees were watered regularly with demineralized water. Since the potting mixture was predominantly sand, once a week the trees were supplied with a nutrient solution which contained no phosphorus.

Pots in which mycorrhizae were to be grown were inoculated with a slurry of shredded mycorrhizal red oak roots and water.

Trees were harvested after six months and the foliage removed. The root systems were rinsed with deionized water. Root and stem length were determined, and the root systems were examined under a dissecting microscope for visible signs of mycorrhizal infection, i.e., monopodial swellings. Foliage, stems, and roots were dried at 105°C, weighed, ground and analyzed for phosphorus by the molybdovanado-phosphoric acid method.

RESULTS

The detailed examination of the root systems of the seedlings showed that those grown in the uninoculated fumigated potting medium (U) exhibited no signs of mycorrhizal infection, while the root systems in the pots inoculated with mycorrhizal roots (IR) and those inoculated with forest soil (IS) did develop the association. Slightly more than 50% of the roots in the IS treatment became mycorrhizal, while slightly less than 50% of the IR roots became infected. Apparently fumigation effectively eliminated mycorrhizal fungi, while the root and soil inocula successfully reintroduced the fungi. However, roots were considered uninfected if monopodial swellings were not present. As Bowen and Theodorou (1967) have pointed out, such external evidence is not an infallible guide to non-infection.

Growth of Seedlings

Mean seedling stem length, stem weight, foliage weight, and above ground biomass were significantly greater for the forest soil inoculation treatment than for the uninoculated treatment; only stem weight differed significantly from the inoculated with roots treatment (Fig. 1). Though not statistically different, other seedling growth measurements, with the exception of root length and weight, were greater in each case for the IS treatment. Root development was greatest in those seedlings which were not inoculated. As a result, root/shoot ratios were highest, 1.27, for the U seedlings, intermediate, 1.01, for the IR seedlings, and lowest, 0.95, for the IS trees.

The phosphorus fertilizer treatments had little influence on growth with the exception of the calcium phosphate treatment which decreased the length of the seedling root systems (Fig. 2). Although not statistically significant, overall development of the seedlings receiving $FePO_4$ was slightly superior (mean weight 6.11g) to the other treatments, the poorest developed of which were the unfertilized seedlings (5.46g).

The interaction between the mycorrhizal and the phosphorus fertilizer treatments had a significant influence on only one growth attribute -- stem length (Fig. 2). Seedlings in the soil inoculated treatment were the tallest (mean height 19.51 cm), while those which were uninoculated were the shortest (15.48 cm). There were no significant differences between the mean heights of the seedlings in the four phosphorus treatments. While mean heights of the phosphorus treatment seedlings within the uninoculated treatment differed by less than 1 cm, the seedlings in the root inoculated treatment which were fertilized with $FePO_4$ were significantly taller ($P<0.05$) than the seedlings in the other three treatments within that group. They were taller than the $FePO_4$ trees in the uninoculated ($P<0.01$) and soil inoculated groups ($P<0.05$) as well. Within the inoculated with soil treatment, the organic phosphate and control seedlings were significantly taller than those seedlings receiving $FePO_4$ and $CaHPO_4$ ($P<0.05$). In addition, they were significantly larger than the corresponding uninoculated and inoculated with roots treatment seedlings.

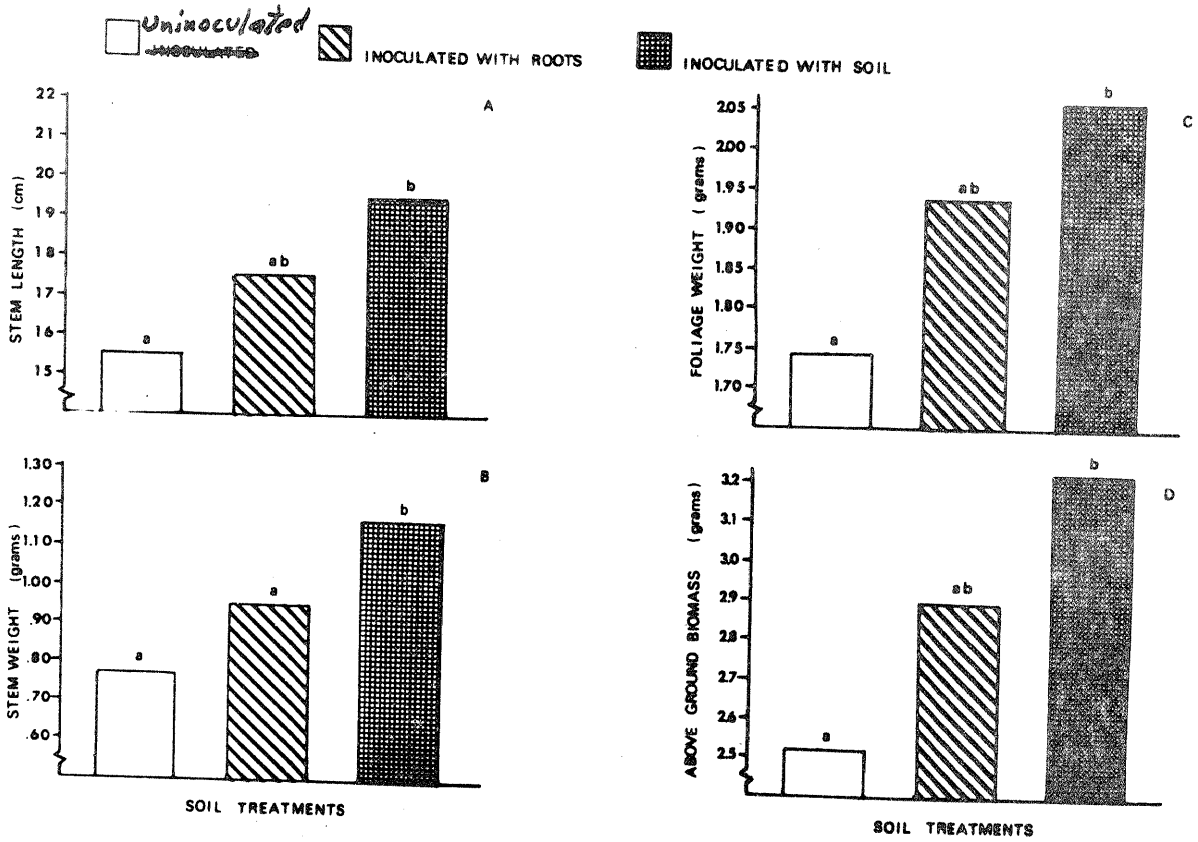

Fig. 1 - Effects of inoculation with mycorrhizal roots, and inoculation with forest soil on (A) stem length, (B) stem weight, (C) foliage weight, and (D) above-ground biomass of red oak seedlings. Comparable bars with same letter are not significantly different (5%) by Duncan's multiple range test. (Means of 27 plants.)

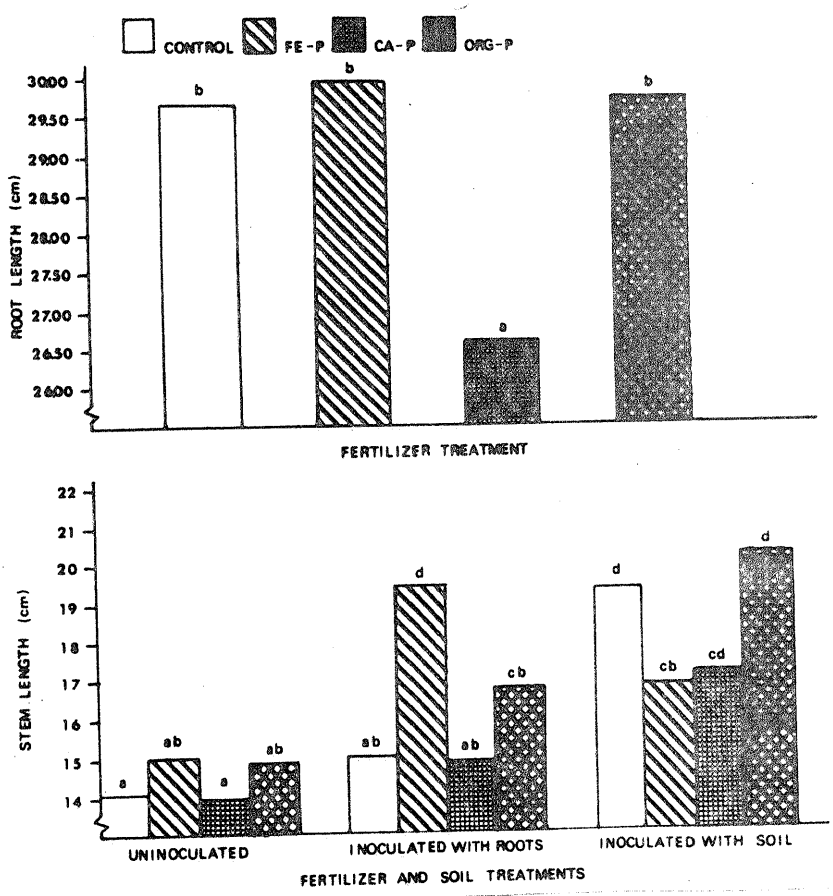

Fig. 2 - Effects of phosphorus fertilization on root length, and of phosphorus fertilization and inoculation with roots and soil on stem length of red oak seedlings. Letters as in Fig. 1. (Means of 27 plants.)

Uptake of Phosphorus

Phosphorus content of the entire seedling and the above-ground portions did not differ significantly between the uninoculated, inoculated with roots, and inoculated with soil treatments; however, the phosphorus content of the root systems of seedlings in these treatments did differ (Fig. 3). Root systems of seedlings in the uninoculated treatment contained a significantly greater amount of phosphorus than those of the seedlings in the soil inoculated groups. Within each of the soil treatments, roots in the three phosphate treatments absorbed significantly more phosphorus ($P<0.05$) than the root systems of seedlings which had received no supplementary phosphorus.

The three types of phosphorus fertilizers significantly increased the phosphorus content of the seedling roots in comparison with the root systems of the control plants, but differences in amounts of phosphorus taken up between the three treatments were not pronounced (Fig. 3).

DISCUSSION

Mycorrhizae had no apparent influence on phosphorus uptake by the entire plant. The uninoculated seedlings had a phosphorus content of 1036 ppm/g as compared to 1026 ppm/g for the inoculated with roots and 933 ppm/g for the inoculated with soil treatments. The same general pattern held for the phosphorus content of the above ground portion of the seedlings. In the case of the P content of the roots, uptake by the uninoculated seedlings was significantly greater than that of the IS seedlings and exceeded that of the IR seedlings. On a weight per seedling basis, the mean phosphorus values were 0.577 mg, 0.579 mg, and 0.570 mg per seedling for the U, IR, and IS treatments, respectively. These results are contrary to a number of reports in the literature (Hatch 1937, McComb and Griffith 1946, Henderson and Stone 1970) which show an increased phosphorus content in mycorrhizal seedlings. However, other investigators (Stone 1950, Mejstrik 1975) have reported erratic responses in which phosphorus uptake by mycorrhizal seedlings was no greater than that of non-mycorrhizal plants.

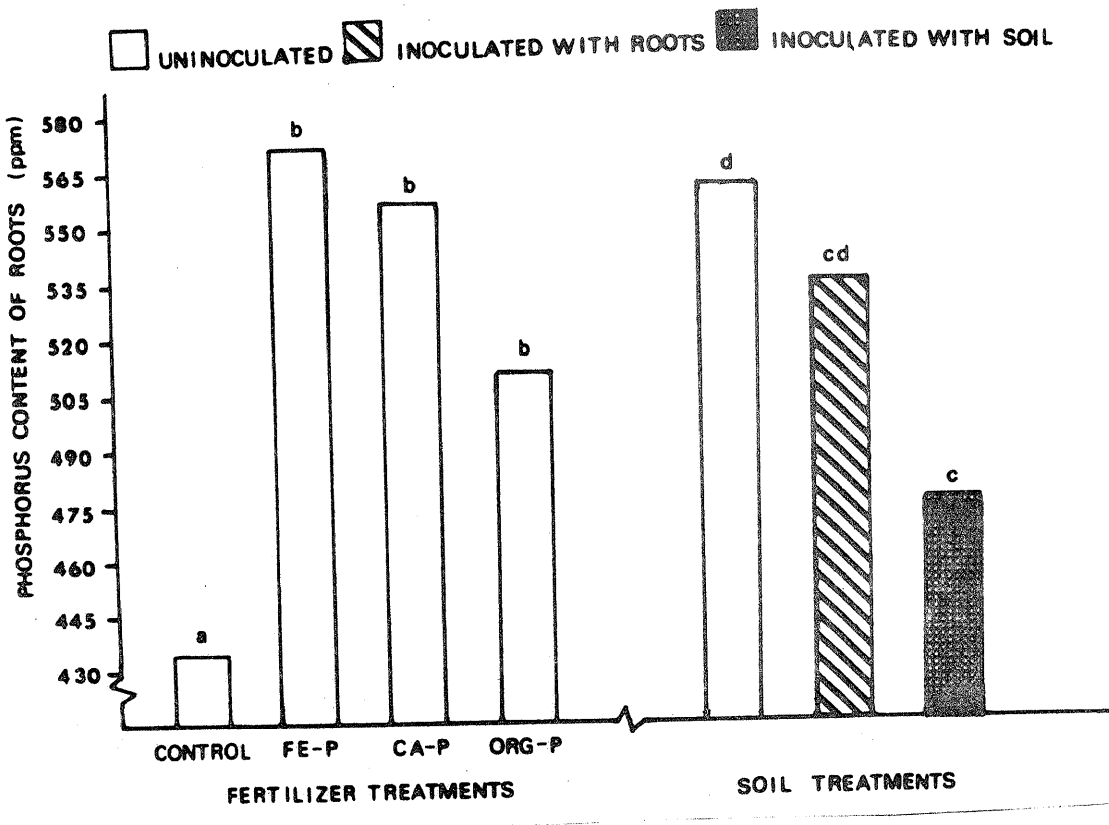

Fig. 3 - Effect of phosphorus fertilization, inoculation with roots, and inoculation with soil upon the phosphorus content of red oak seedlings. Letters as in Fig. 1. (Means of 27 plants.)

The reason for the reduced uptake of P by the mycorrhizal seedlings in comparison with the non-mycorrhizal seedlings is not apparent. The observations on P content of the seedlings are based on the assumption that the mycorrhizae observed had been present long enough to affect phosphorus uptake. This may not have been the case since development of mycorrhizae throughout the course of the study was not followed. However, above-ground growth of the mycorrhizal seedlings was significantly greater than that of the non-mycorrhizal seedlings (Fig. 1); consequently, the mycorrhizae do seem to have been present long enough to have had an effect.

Bowen and Theodorou (1967) report that some mycorrhizae may be no more efficient in phosphate uptake than uninfected short lateral roots. Mean root length was 29.56 cm, 28.78 cm, and 28.69 cm for the U, IR, and IS treatments, respectively. Since the organisms present in both inocula were not identified, quite possibly the fungal components of the mycorrhizae were species which were relatively inefficient in phosphorus uptake.

A further possibility is that seedlings in the uninoculated treatment became contaminated even though examination of the root systems under a dissecting microscope did not reveal any monopodial swellings or the presence of fungal sheaths (Bowen and Theodorou 1967, Slankis 1973). Bowen (1973) has pointed out that microorganisms of the rhizosphere may play a role in phosphorus solubilization and the uptake of P by higher plants.

The media, sand plus soil, in which seedlings were grown may have had an influence. Stone (1950) found that in pot culture studies Monterey pine grown in a synthetic soil containing ground phosphatic minerals failed to demonstrate any effect of mycorrhizae on phosphorus uptake by the seedlings.

Since the effects of nutrient uptake by mycorrhizae on plant development is usually interpreted to mean that the mycorrhizal response is a nutrient uptake response only, Bowen (1973) has suggested the desirability of comparing the nutrient content of mycorrhizal plants in a nutrient-deficient soil to that of non-mycorrhizal plants of the same size in a fertilized soil. We compared the phosphorus content of seven seedlings from the unfertilized IS treatment, whose total dry weights ranged from 5.01 to 5.69 g (mean 5.28 $\pm$ 0.25 g) to seven seedlings from the uninoculated and fertilized with $FePO_4$ treatment whose weights varied from 5.08 to 5.73 g (mean 5.45 $\pm$ 0.25 g). While the mean weight of phosphorus taken up by the unfertilized mycorrhizal seedlings, 1.201 $\pm$ 138 mg/g, was greater than that of the non-mycorrhizal fertilized seedlings, 0.944 $\pm$ 136 mg/g, the difference was not significant. Thus, under the conditions of this study (a pot culture) no marked superiority of mycorrhizae is shown in the uptake of phosphorus from a phosphorus-deficient medium in comparison with the uptake of phosphorus by non-mycorrhizal oak seedlings from a phosphorus-enriched medium.

While the phosphorus uptake of the roots of seedlings growing in the phosphorus-enriched treatments was significantly greater than that of seedlings which received no additional phosphorus (Fig. 3), the phosphorus content of the seedling tops differed very little. The reason for the difference appeared to be the small amount of P contained in the root systems of forest soil inoculated seedlings which received no additional phosphorus. The mean P content of these roots was 346 ppm/g as compared with the P content of the two other groups of seedlings which received no additional phosphorus--478 ppm/g for the uninoculated and 490 ppm/g for the inoculated with roots treatments. Differences between the P content of the above-ground portion of the same seedlings in these three treatments were not pronounced and, hence, were not significant.

The form of phosphorus, whether $FePO_4$, $CaHPO_4$, or $C_6H_5(OPO_3Ca)_6$, seemingly had no marked influence on the amount of P taken up by the seedlings. Phosphorus content of the tops ranged from 481 ppm/g for the OrgP treatment to 494 ppm/g for the CaP seedlings. The roots showed somewhat more variation--phosphorus content varied from 528 ppm/g for seedlings in the OrgP group to 570 ppm/g for those in the FeP seedlings. Apparently all three forms of phosphorus are equally available to the roots of red oak seedlings. Moreover, since the non-mycorrhizal seedlings had the highest phosphorus levels, apparently the mycorrhizae did not, by themselves, increase the availability or uptake of these slightly soluble forms of phosphates.

Growth response to the phosphorus amendments was erratic. Total dry weight, weight of tops, and root weight of the seedlings were not significantly affected (Table 1). The lengths of the roots in the $CaHPO_4$ treatment were less than those of the seedlings in the other treatments and height growth was generally poorer (Fig. 2). Voigt (1966) has reported that calcium may interfere with the uptake of phosphorus by young pitch pine, but uptake of phosphorus from $CaHPO_4$ by the red oak seedlings was second only to the $FePO_4$ treatment. Consequently, neither an excess nor a lack of P appear to be involved.

Growth of the tops of the mycorrhizal oak seedlings was greater than that of the non-mycorrhizal seedlings, but the reverse was true of the root systems (Table 1, Fig. 1). However, differences in root length and weight between the inoculated and uninoculated plants were not significant. In all cases the seedlings in the forest soil treatment exhibited greater growth than the inoculated with roots treatment. This may indicate that rhizosphere organisms other than mycorrhizae played a beneficial role in seedling development. Such organisms could have been present in the unfumigated forest soil but absent on the mycorrhizal roots which were used for inoculation.

The superior growth of the mycorrhizal seedlings emphasizes the importance of mycorrhizae to overall tree development.

Table 1. -- Statistical significance of mycorrhizal inoculation and phosphorus fertilization on the development of red oak seedlings.

Growth Characteristics	Mycorrhizae	Phosphorus	Mycorrhizae-Phosphorus Interaction
Stem Length	**	NS	*
Root Length	NS	**	NS
Foliage Dry Weight	*	NS	NS
Stem Dry Weight	**	NS	NS
Root Dry Weight	NS	NS	NS
Stem and Foliage Total Dry Weight	**	NS	NS
Seedling Dry Weight	NS	NS	NS
Root-Shoot Ratio	**	NS	NS
Phosphorus Content of Shoot	NS	NS	NS
Phosphorus Content of Root	*	**	NS

* Significant ($P < 0.05$)
** Highly Significant ($P < 0.01$)

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