EFFECT OF NITROGEN AND PHOSPHORUS FERTILIZERS ON ROOT GROWTH AND NODULATION IN BLACK LOCUST (*ROBINIA PSEUDOACACIA* L.) SEEDLINGS

SHAUKAT ALI, DEPUTY CONSERVATOR OF FORESTS, NWFP, FOREST DEPARTMENT

ABSTRACT

Root weight and number of nodules/ seedling of Black locust (*Robinia pseudoacacia* L.) were differently affected by Nitrogen fertilization. Root weight was minimum in N stressed medium while number of nodules were a little less than maximum. Root weight increased significantly with 100 ppm of nitrogen while there was slight increase in nodulation. Frequent application of N resulted in increase in root weight while a reduction in the number of nodules was recorded. Phosphorus fertilization increased root weight but there was no effect on the number of nodules with phosphorus alone.

INTRODUCTION

General

Black locust (*Robinia pseudoacacia* L.) is a leguminous medium sized tree extensively planted for stabilization of soils, erosion control, fuelwood and fodder production. Black locust grown in many parts of the world, rivals poplar

as the second most extensively planted genus after eucalyptus. It has a wide range of economic, aesthetic and ecological uses that make it well suited for agroforestry. In Pakistan black locust is well adapted to the sub-tropical climate of the Northern mountainaous region ranging in elevation from 3000 to 5000 feet. It is widely planted in northern part of Pakistan on private grazing lands for erosion control and along banks of streams and fields for soil stabilization. It is becoming one of the most sought after agroforestry tree species because of its cash value as fuelwood and fodder.

Problem statement

As the single most costly industrial input into agricultural productivity, chemical fixed nitrogen accounts for up to 35% of the total productive capacity of all crops (Gordon et al, 1979). Each pound of industrially fixed nitrogen requires 30 cu.ft. of natural gas. The mass application of fertilizer in agriculture may become more and more expensive in the future; hence intensive research was carried out on inter-cropping systems to maximize crop yield per unit land area with a minimum input of nitrogen fertilizer (Ofori and Stern, 1987).

A growing body of evidence points to the physical benefits of incorporating trees into farming system. The most significant contribution of trees may be their favorable influence on soil fertility (Auten, 1945). Under closed stand conditions one ton of black locust litter liberates to the soil the equivalent of 56 pounds of N per acre annually which is in the form of soluble nitrates and is equivalent to an annual application of over 264 pounds of pure dry ammonium sulphate per acre.

If the effectiveness and efficiency of N_2 fixation by black locust can be increased, it may be possible to reduce the need for nitrogenous fertilizers (Reinsvold and Pople, 1987). One of the primary factors affecting the quality of seedlings is soil fertility maintained through fertilizer and organic amendments. One way to address the problem is to produce seedlings with optimum biomass with application of suitable doses of fertilizer. This study was, therefore, conducted with the objective to evaluate the effect of different levels of nitrogen fertilizer, alone and in combination with phosphorus, and application interval of nitrogen fertilizer on the production of the number of nodules in black locust seedlings that enable the plants to fix atmospheric nitrogen.

LITERATURE REVIEW

In forestry nitrogen fertilization is becoming increasingly important, however, in the competition for chemically fixed nitrogen, forestry will be a lower priority than agriculture. The potential value of black locust for nitrogen fixation is recognized in most parts of the world. With rising oil prices, the importance of black locust as a N fixing species is increasing (Keresztesi, 1980). Nitrogen and phosphorus are often deficient in nursery soils and limiting for growth of trees. Symbiotic nitrogen fixation in black locust seedlings can be affected by the availability of various nutrients (Reinsvold and Pople, 1987).

NITROGEN

All the essential elements are important in the nutrition of plants from a physiological point of view, but N is the most important single element. It forms 2-4% the dry weight of an average pot plant and occurs in all plant proteins and chlorophyll. Deficiency of N causes a marked reduction in plant growth rate. Marshall (1981) reported that root elongation of

young black locust seedlings was inhibited by 10 gm. of a fertilizer/liter concentrate applied shortly after germination. He attributed reduction in nodulation to decrease in root growth. He showed that shoot and root dry biomass also did,nt increase with high fertilization.

The low growth reesponse of black locust seedlings to N fertilization on an N deficient field site suggested that only minimal amount of soil N are required in addition to the N fixed to support maximum growth (Plass, 1972). One reason nitrogen fertilization did not promote growth may be its inhibitory effect on nodulation. Nitrogen fixation by black locust-Rhizobium was inhibited when grown in a nutrient solution containing NH_4NO_3 as compared to solution without NH_4NO_3 (Roberts et al, 1984). This reduction in N fixation was due to smaller nodule size and less nodule activity.

Phosphorus

The effect of different nutrients on the number of nodules/plant has been demonstrated by Lynd and Ansman (1989). It was shown that number of nodules/plant was more in plants given 100 mg of phosphorus per kg of soil (100 ppm) than the control in *Arachis hypogaea* (1989 A) and *Canavalia eusiformis* (1989 B). On the other hand Hart (1989) reported extremely varied effect of phosphorus concentration on the number of nodules in white clover plants but showed that the nodule weight/ plant increased significantly with P supply from 1 to 100 mm/$(m)^3$. Caradus and Sanydon (1988) found that root and shoot dry weight and root elongation rate was higher in white clover populations treated with P than untreated.

Combined effect

The combined effect of soil nitrogen and phosphorus was determined on nodulation and and growth of black locust plants by Reinsvold and Pope (1987). It was demonstrated that with increase in level of P there was increase in mean number of nodules per plant and plant dry weight. On the other hand considerably more variation was demonstrated on nodulation and plant dry weight with additon of N alone. Seedlings with medium level of 50 mg/kg (50 ppm) of added N produced more nodules and nodular dry weight when compared with two extreme levels. For number of nodules and nodular dry weight, the interaction between N and P factors and the interaction between seedling age and the level of added P were found to be significant.

MATERIAL AND METHODS

The Experiment

The study was conducted in the shade house of Forest Research Laboratories of Oregon State University, Corvallis under uniform temperature and irrigation conditions. Seed was procured from an unknown source in California. Seeds were soaked in concentrated H_2SO_4 for 40 minutes and then thoroughly washed in running water for 5 minutes just before sowing. Growth medium was lightweight consisting of 10 parts of mineral soil (loam and pumice) and one part each of peat moss and vermiculite. Half pound of commercial Lilly Miller Trace elements was thoroughly mixed with the medium. A total of 1176 white Ray leach super cells (a container system consisting of 98 cells in a tray) each 21 cm.long and 4 cm. top diameter (160 cm^3) were filled with the lightweight medium and one pre-treated seed was sown in each container on June 30, 1990.

There were a total of 14 treatments, with 12 treatments getting 6 levels of fertilizers i.e. 100, 200 and 400 ppm of nitrogen alone and each N level associated with 50 ppm of phosphorus added once with the first fertilizer application. Each N level was applied at intervals of 5 and 10 days making a total of 12 doses. The 13th treatment consisted of adding of 50 ppm of P alone at the time of first fertilization. Ammonium nitrate and phosphoric acid were the sources for N and P respectively. The 14th treatment was an unfertilized control.

The following abbreviations will be encountered: N_o, N_{100}, N_{200}, and N_{400} will refer to the 0, 100, 200, 400 ppm and also called zero low, medium and high levels of nitrogen fertilizer respectively. Similarly P_o and P_{50} will refer to 0 and 50 ppm of phosphorus respectively whereas D_5 and D_{10} will refer to the interval of 5 and 10 days respectively at which N fertilizer was applied.

In first fertilizer application, required quantity of phosphoric acid was added to half of the N treatments before nitrogen application. Liquid N fertilizer (ammonium nitrate) was applied to each container in each treatment, except N_o+P_o and N_o+P_{50}, with a beaker in a way to completely saturate the growing medium and flush out excess fertilizer salts (Landis, 1989). The treatment N_o+P_{50} was given phosphoric acid only. Subsequent fertilization (without P) was continued every 5th and 10th day according to schedule. The plants were watered when needed to avoid any water stress.

Five seedlings were selected from each treatment for measurement for various growth parameters including counting of nodules. The date was collected after 45 (late summer), 80 (early fall) and 100 days (mid-fall) of first fertilizer application. The seedlings alongwith the cone of medium were carefully washed in a water container. The nodules were severed from the roots and counted regardless of their size.

Data analysis

This was a randomized block design. Levels of N fertilizer were duplicated by applying them with and without phosphorus. Then each fertilizer level was applied after each 5 or 10 days interval except the N_o level with and without P. Since N application interval (days) was not involved in two N_o treatments, therefore, a factorial combination was not possible. Analysis was conducted using the GLM procedures of the SAS personal computers package to find out the significance of treatments.

Contrasts were run to find out the significant effect of main factors i.e. N, P and D and their interaction. For comparison of treatment means of interest, Waller-Dunkan K ratio (100) was used once the contrast showed a factor and interaction of factors to be significant.

RESULTS

Treatments had generally significant effect on root weight as well as nodulation but in reverse order. Results of the last two samples indicated an abrupt increase in root weight in plants that were supplied with low N level. Mixed trend was, howver, found in case of nodulation when the number increased or decreased, though not significantly, with respect to application interval of nitrogen. Additional application of N slightly increased root weight but significantly decreased number of nodules/ seedling. High level of N had almost similar effect on root weight and nodule formation when slight decrease was recorded.

More growth in root weigtht was attained at each level of N in plants that had been supplied with Phosphorus as compared with pants without Phosphorus. The effect of P was not significant at N_{200} and N_{400} levels in case of nodulation.

Application interval of low level of nitrogen affected the number of nodule formation as significant increase was noticed in P_{50} plants getting N less frequently (P_{50} D_5). This trend continued in all the three samples i.e. till the end of the experiment. Root weight, however, increased in plants getting N more frequently at least in the early fall sample. At rest of the N levels there was no significant effect on either of the two variables.

DISCUSSION

To maintain a high photosynthetic capacity, N and P are required in relatively high amounts compared to other nutrients (Landis, 1985). Nitrogen is needed for synthesis of proteins and enzymes and ultimately for growth while P is needed in ATP, an energy source for biochemical reactions.

Seedlings at zero level were nutrient stressed. With the addition of low doze of nitrogen, root mass was strongly stimulated indicating severe deficiency of nitrogen at zero level. When N was increased beyond low level, root mass remained at steady state. Roberts et al, (1983) obtained larger root mass in black locust seedlings grow in an environment where N was available. Since they used only two levels of N, further comparison with our results is not possible.

Root weight had the opposite trend with relation to P fertilization indicating that specific root length (root length/root dry weight) was more in P_o seedlings. Since one of the major nutrients i.e. phosphorus was deficient, therefore, roots had to grow more to explore for the limiting nutrient. Roots in P_{50} seedlings were thicker due to storage of dry matter and therefore heavier per unit root length.

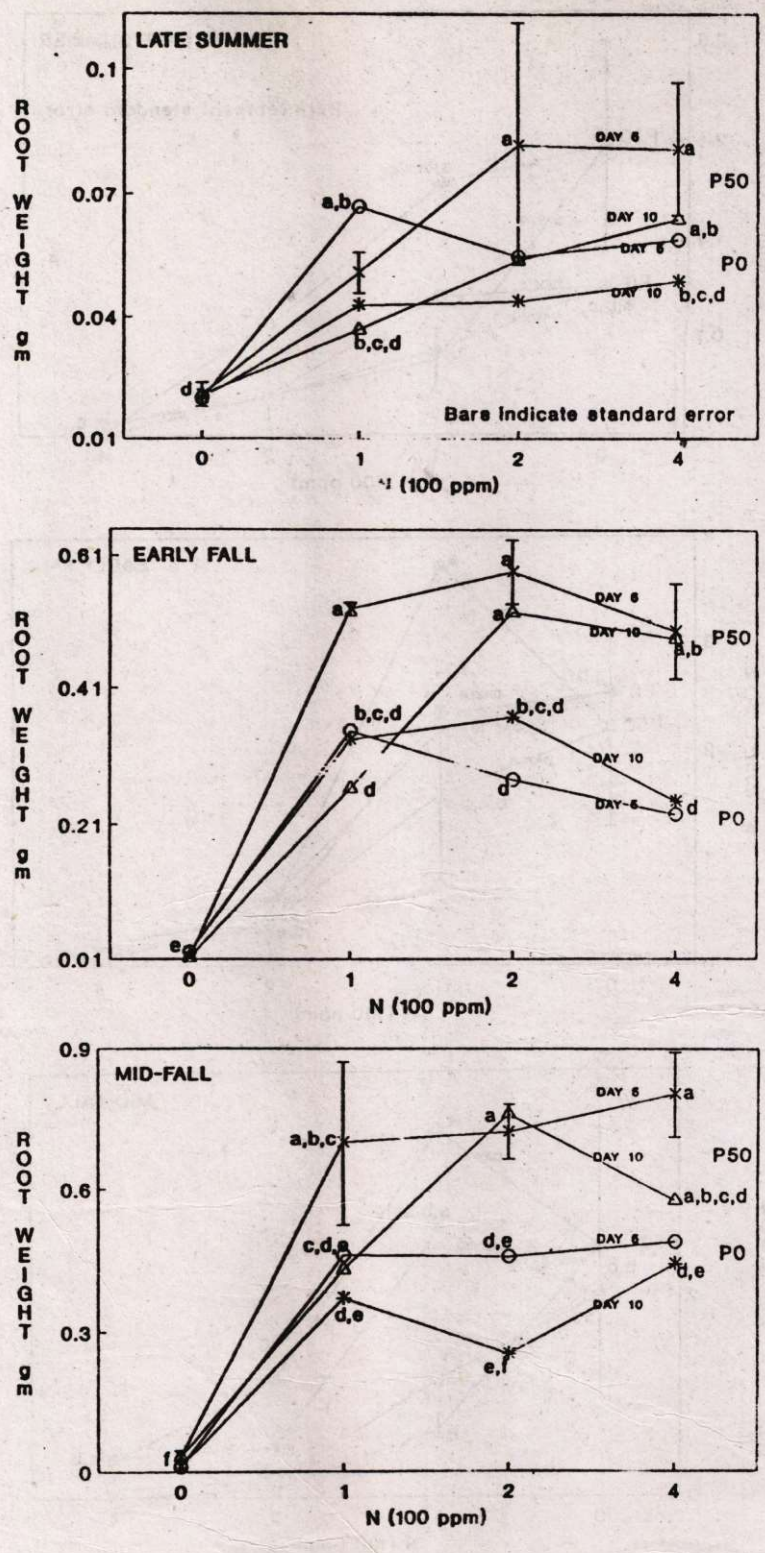
Nodulation was maximum at zero and low levels of N and was inhibited by increasing N level. Earlier findings suggest that nodule activity declines when soil N is readily available. Similar result was obtained by Koo (1989) for nodule dry weight in red alder. Roberts et al, (1983) attributed the negative growth response with the increase in N level to the inhibitory effect on nodulation. This indicated that nodule formation was stimulated more in N deficient plants in order to supplement N availability through symbiosis.

On the other hand, P favors nodule formation (Reinsvold and Pope, 1987; Lynd and Ansman, 1989 A) while negative effects of N fertilization are reduced by P fertilization (Koo, 1989). The results of this study confirmed the previous findings. It was observed that the effect of P on nodulation, as demonstrated in earlier studies, was suppressed by higher N levels. However, low N (D_{10}) level was not strong enough to reduce P effect. Therefore, maximum nodulation was found at N_{100} P_{50} D_{10} level followed by N_o P_{50} level seedlings.

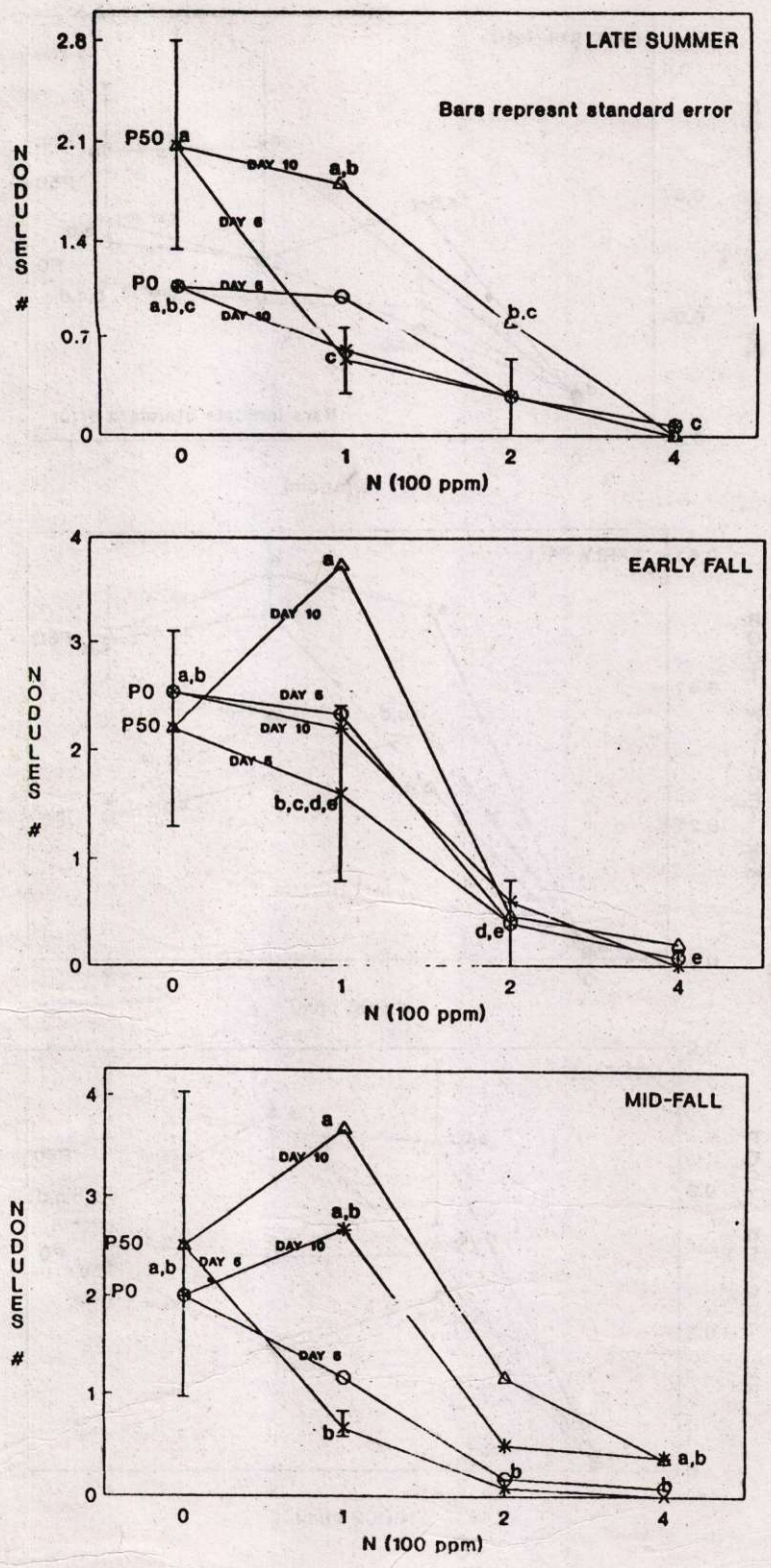
CONCLUSION

The experiment negated the postulation that number of nodules should be more in the seedlings with fibrous roots. Root weight and for that matter root fibrosity was maximum at N_{200} P_{50} level but nodulation on the other hand was touching minimum. Similarly root weight was minimum at zero level of N, whether associated with P or not, but nodulation was

Graph


LATE SUMMER
ROOT WEIGHT g m
0.1
0.07
0.04
0.01
DAY 6
DAY 10
DAY 5
P50
P0
a,b
b,c,d
Bars Indicate standard error
0
1
2
4
N (100 ppm)
EARLY FALL
0.61
0.41
0.21
0.01
MID-FALL
0.9
0.6
0.3
0
a,b,c
c,d,e
d,e
e,f
a,b,c,d

Graph


LATE SUMMER
Bars represnt standard error
NODULES #
2.8
2.1
1.4
0.7
0
P50
a
DAY 10
a,b
DAY 5
P0
DAY 5
a,b,c
DAY 10
b,c
c
c
0
1
2
4
N (100 ppm)
EARLY FALL
NODULES #
4
3
2
1
0
a
DAY 10
a,b
P0
DAY 5
P50
DAY 10
DAY 5
b,c,d,e
d,e
e
0
1
2
4
N (100 ppm)
MID-FALL
NODULES #
4
3
2
1
0
a
DAY 10
a,b
P50
DAY 10
a,b
DAY 5
P0
DAY 5
b
a,b
b
b
0
1
2
4
N (100 ppm)

next to maximum. This study suggests that application of some optimum quantity of phosphorus is essential for activation of black locust rhizobium for nodule formation. Likewise some level of N is also required in early stages of seedlings in N deficient medium.

REFERENCES

Auten, J.T. 1945. Relative influence of sassafras, black locust and pines upon old-field sites. Jor. For. 43: 441-446.

Caradus, J.R., and R.W. Sanydon. 1988. Aspects of the phosphorus nutrition of the white clover populations. IV. Root growth and morphology. Jour. Plant Nutr. 11(3): 277-287.

Gordon, J.C., C.T. Wheeler and D.A. Perry. 1979. Symbiotic Nitrogen fixation in the management of temperate forests. Proceedings of the workshop held April 2-5, 1979. For. Res. Lab. O.S.U., Corvallis OR.

Hart, A.L. 1989. Distribution of phosphorus in nodulated white clover plants. Jour. plant Nutr. 12(3): 159-171.

Keresztesi, B. 1980. The black locust. Unasylva. 32:23-33.

Koo, C.D. 1989. Water stress, fertilization and light effects on the growth of nodulated mycorrhizal red alder seedlings. Ph.D thesis. Oregon State University, Corvallis OR.

Landis, T.D. 1985. Mineral nutrition as measure of seedling quality. In Duryea, M.L. (ed). Evaluating Seedling Quality: Principles, procedures and predictiveabilities of major tests. Forest Research Laboratories, O.S.U. Oregon.

Landis, T.D., R.W. Tinus., S.E. McDonald and J.P. Barnett. 1989. Seedling nutrition and irrigation. Vol. 4. The container tree nursery manual. Agric. Handbook. 674. Washington, U.S.D.A. Forest Service 119 P.

Lynd, J.Q., and T.R. Ansman. 1989(a). Effect of P, Ca with four K levels and nodule histology, nitrogenase activity and improved "spanced" plant yield. Jour. Plant Nutr. 12(1):65-84.

Lynd, J.Q., and T.R. Ansman. 1989(b). Soil fertility effects on growth, nodulation, nitrogenase and seed lectin compounds of Jack bean. Jour. Plant Nutr. 12(5):563-579.

Marshall, P.E. 1981. Seedling response to fertilization shortly after germination. Tree Planters Notes. 32:3-8.

Ofori, F., and W.R. Stern. 1987. Cereal-legume intercropping system. Advances in agronomy. 41:41-90.

Plass, W.T. 1972. Fertilization treatments increase black locust growth on extremely acid surface mine spoils. Tree Planters Notes. 23:10-12.

Reinsvold, J. Robert and Phillip E. Pope. 1987. Combined effect of soil nitrogen and phosphorus on nodulation and growth of *Robinia pseudoacacia* Can. J. For. Res. 17:964-969.

Roberts, D.R., R.W. Zimmermann., J.W. Stringer and S.B. Carpenter. 1983. The effects of combined nitrogen on growth, nodulation, and nitrogen fixation of black locust seedlingts. Can. J. For. Res. 13:1251-1254.