THE ROLE OF CERTAIN NON-LEGUMINOUS WOODY SPECIES IN THE NITROGEN NUTRITION OF SOME CONIFER SEEDLINGS

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THE ROLE OF CERTAIN NON-LEGUMINOUS WOODY SPECIES IN THE NITROGEN NUTRITION OF SOME CONIFER SEEDLINGS

INTRODUCTION

The role of leguminous plants acting through a bacterial symbiont in the fixation of nitrogen has been recognized for many years. Using legumes in crop rotations for soil improvement is a well known practice. On the other hand foresters have ignored a similar manipulation of potentially beneficial species that often occur in the forest stand.

On western forest lands, the two most important and widely distributed, nodulated, woody, non-leguminous genera capable of nitrogen fixation are *Alnus* and *Ceanothus*. Although these genera are not utilized directly, except for certain species of *Alnus*, it is thought that they could be managed for soil improvement, because of their nitrogen fixing capacities. However not much is known about the influence these two genera have on conifers with which they are associated. Because of this lack of information and the potential importance of the relationship the following study was proposed with three main objectives. The first was to determine whether symbiotic nitrogen fixation by species of *Alnus* and *Ceanothus* had any appreciable effects on the growth and nitrogen nutrition of certain conifers. The second was to determine the approximate amounts of nitrogen that could be fixed by either *Alnus* or *Ceanothus*, and the third was to evaluate the competitive influence of either *Alnus* or *Ceanothus* when grown in association with certain conifers. With a better understanding of these facts more suitable recommendations regarding the management of associated species in the forest stand could be made.

LITERATURE

For a long period of time forest land use in the United States consisted of exploitation, often leaving in its wake devastated and generally unproductive land. Some of this land was farmed, but eventually much of it was abandoned and natural forest regeneration was slow or non-existent. The situation began changing in the early 1900's. As a result of the crusading spirit of some of the national leaders of that time, a more constructive approach to forest management was taken. Noting the successful use of legumes in agriculture, foresters began to consider the advisability of using certain woody legumes to increase soil nitrogen and growth of forests.

One of the first reports in this country on the effect of a woody legume on the growth of some associated species came from Pennsylvania State College (15, p. 318-319). Ferguson reported that catalpa (*Catalpa speciosa* Ward.), growing near black locust (*Robinia pseudoacacia* L.), a woody legume, made better height and diameter growth than one growing some distance away. He also noted that soil nitrogen under pure locust was higher than under a pure stand of catalpa. Chapman (9, p. 37-60) reported similar findings. As the distance from black locust increased, growth of the associated forest tree decreased. A similar relationship held for total nitrogen in the soil. In still another study (10, p . 1-15), survival of planted seedlings under a black locust plantation was significantly higher than in an old field plantation. Comparisons have also been made of foliar nitrogen and growth in mixed and pure forest plantings (16, p. 31-33). Trees grown in association with black locust were superior to those not grown with black locust, with respect to foliar nitrogen, height, and diameter growth.

More recently data have been presented on the amounts of soil nitrogen accumulating under black locust (22, p. 346-349). In one study, Ike and Stone concluded that the increase of nitrogen in the 0-20" layer during a 16-20 year period was 600 pounds per acre greater in a black locust stand than in a non-locust site. Other benefits including the increase of certain mineral nutrients, such as calcium, magnesium and potassium (18, p. 123), and the improvement of soil structure (4, p. 446) have been observed.

Certain legumes have been suggested as cover crops for forest nurseries (32, p. 170-173). Due to soil deterioration in many areas of the Central Hardwood Region, recommendations have been made for using a preliminary crop of black locust to recondition the site for future hardwoods (4, p. 441).

The fact that certain nodulated, non-leguminous plants also fix nitrogen is not nearly so well known, but may be of much greater significance in forest management. A 1959 report indicated that 8 non-leguminous genera with approximately 190 species (6, p. 59) are active in nitrogen fixation. These genera included Elaeagnus, Hippophäe, Shepherdia, Alnus, Ceanothus, Casurina, Myrica, and Coriaria. During the past year it has been suggested that the genera Discaria (29, p. 945) and Purshia (34, p. 745-752) are also involved in this relationship. Although nitrogen fixation was mentioned as occurring in some of these genera as early as 1910 (26,

p. 213-218), all of the ten genera have not been critically evaluated with present day techniques to ascertain the actual existence of a nitrogen fixing capability.

The early research with some of these genera consisted of attempts to isolate and characterize the symbiont (2, 7, 8). Although these attempts failed, morphological studies of the internal and external features of the root nodules continued (2, 3, 17).

On western forest lands, the most important and widely distributed nodulated, non-leguminous woody plants are members of two genera, Alnus and Ceanothus. There is, however, little information with regard to the influence of these genera on the growth of associated conifers.

While working in the Northern Rocky Mountains, Wahlenberg (35, p. 601-612) noted a more favorable soil moisture regime beneath snowbrush (Ceanothus velutinus Dougl.), than in the open. The soil in the open reached the permenent wilting point 2-3 weeks prior to that beneath ceanothus. He also found that soil temperatures in the open exceeded those under snowbrush by as much as 30°F. Youngberg, (36 p. 1-3) working on cut over lands in Central Oregon has reported similar relationships regarding soil moisture and soil temperatures in the open and under snowbrush ceanothus. Likewise Dyrness, (13, p. 156) noted the same relationships for soil moisture in virgin ponderosa pine (Pinus ponderosa Laws.) stands in another portion of Central Oregon. He also presented data showing higher nitrogen contents in the litter and in the A horizon under snowbrush than under other species.

In 1917 Johnson recognized the potential importance of species of Alnus (25, p. 981-985). From several empirical observations he concluded that better soil physical conditions and more favorable nutrient relations existed under the alder. Recently these early observations have been substantiated by Tarrant (33, p. 238-246) in a more detailed investigation of the soil fertility within a Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco)-alder (Alnus rubra Bong.) stand. He noted greater growth of fir in mixed stands with alder. This improvement in growth was reflected in terms of increased height and diameter growth and form class. Total nitrogen contents of soil and of Douglas-fir foliage were also significantly higher in the plantation containing some alder in the mixture.

Greenhouse trials involving preplanting of Ceanothus spp. to increase soil nitrogen have been successful in two instances. One investigator (31, p. 830-831) used Sierra gooseberry (Ribes roezli Regel.) as a test species and reported twice as much growth for the gooseberry when it was grown in pots that contained the roots of previously active ceanothus, as compared to gooseberry grown in pots without ceanothus roots. Hellmer and Kelleher (21, p. 276) found that tomato plants grown in pots that had supported ceanothus contained nearly twice as much nitrogen as plants grown in fresh soil.

Mikola (27, p. 1-10) found that the addition of alder (Alnus glutinosa Gaertn.) litter increased growth and dry weight production of Scots pine (Pinus sylvestris L.) grown in the greenhouse.

The importance of alder in primary plant succession in the Glacier Bay area of Alaska has been emphasized by Crocker and Major

(11, p. 427-448). Of particular significance is the increase of soil nitrogen that results from nitrogen fixation. Soils associated with alder also had more favorable bulk densities.

Not only are these non-legumes important for improving the growth of forest trees, but it has also been reported that they improve the productivity of lakes (19, p. 287). A recent study indicates that an alder (Alnus tenuifolia Nutt.) plantation increased the nitrogen contents of deep seepage and spring water feeding a nearby lake.

PROCEDURES

During the spring of 1960, subsoil samples were obtained from two different locations in Western Oregon. One sample, a soil developed from granitic residuum was taken from Douglas County along U.S. Highway 99, about 10 miles south of Canyonville. The other, a waterlain pumice, was obtained in Jackson County on the Union Creek Ranger District of the Rogue River National Forest. Additional soil collections were made as necessary.

Well nodulated seedlings of snowbrush (Ceanothus velutinus Dougl.) were collected in the Union Creek area, while the nodulated alder (Alnus rubra Bong.) seedlings came from several locations in Benton County.

Ponderosa pine and snowbrush litters were collected in Central Oregon and Douglas-fir and alder litters were obtained in the Upper Alsea Basin.

Shortly after the soils had been collected, samples were taken from each type, for moisture percentage determinations and for chemical analyses. Approximately 2,500 grams of air dried granitic soil and 1,500 grams of pumice soils were placed in 7-inch diameter polyethylene pots. The same weights were used for subsequent experiments.

Two nodulated alder seedlings were transplanted into the granitic soil and two snowbrush seedlings into the pumice soil. The soil surface was covered with 250 grams of a washed, 30 mesh quartz sand and the pots thoroughly watered. Later, conifer seeds were sown into each pot. One week after the initial sowing, 100 ppm. of phosphorus were applied to each pot. In pots where germination was poor, additional seed was sown, so there would be a minimum of 8-12 pine seedlings per pot. After two months had elapsed, the various nutrient treatments were applied. Each treatment was replicated four times. About one month later the seedlings were thinned to predetermined levels.

Seedlings were grown under greenhouse conditions unless otherwise specified. Daytime temperatures ranged from 70-85° F. and nighttime temperatures from 55-65° F. Throughout each experiment day-length was maintained at 16 hours and when necessary supplementary light from overhead warm and daylight fluorescent bulbs was used. All seedlings were irrigated at irregular intervals in an effort to maintain soil moisture near field capacity. Except for experiment #1 the seedlings remained under these conditions until they were harvested.

<u>Experiment #1.</u>

After the transplanted alder and snowbrush seedlings had been growing for one month, four pots of the granitic soil and eight pots of the pumice soil were selected and one nodulated seedling from each pot was cut off at groundline. Ponderosa pine was sown into these pots and 20 additional pots of each soil and the treatment schedule previously outlined was followed. Table 1 presents the levels and sources of the nutrients applied in this experiment. The pine seedlings were thinned to six per pot in the presence of alder or snowbrush and eight per pot in the absence of these nodulated

Table 1. Treatment levels and nutrient sources for Experiment #1.

Treatment No.	Levels of Nutrients Added (ppm)				Number of Nodulated Seedlings
Granitic	N	P	Mo	B	Alder
1	0	100	2	1	0
2	25	100	2	1	0
3	50	100	2	1	0
4	75	100	2	1	0
5	100	100	2	1	0
6	0	100	2	1	1
Pumice	N	P	Mo	B	Snowbrush
1	0	100	2	1	0
2	25	100	2	1	0
3	50	100	2	1	0
4	75	100	2	1	0
5	100	100	2	1	0
6	0	100	2	1	1
7	0	100	0	0	1

Nutrient Sources:

N – NH_4NO_3

P – H_3PO_4

Mo – Na_2MoO_4

B – $Na_2B_4O_7 \cdot 10H_2O$

seedlings.

Nine months later, the pine seedlings had set buds. Since a chilling requirement is usually necessary to break dormancy, the seedlings were set outdoors in a protected area during mid-December. In March the seedlings were returned to the greenhouse where they subsequently resumed growth and after reaching an age of 15 months they were harvested.

Experiment #2:

Pots with the transplanted alder and snowbrush seedlings not used in experiment #1 were allowed to grow under greenhouse conditions for the next nine months. Four pots of each type were then selected on the basis of the most vigorous and healthy looking alder or snowbrush seedlings. After the seedlings were clipped at groundline these pots, along with 20 additional ones of each soil, were sown with Monterey pine (*Pinus radiata* D. Don) seed. Nutrient treatments and cultural procedures were carried out as previously outlined. Treatment levels and nutrient sources are presented in Table 2. After thinning the pine seedlings to 12 per pot, the seedlings remained under greenhouse conditions until the late summer of 1961, when they were harvested. At this time the seedlings were about 12 months old.

Experiment #3:

Two hundred grams of Douglas-fir litter were added and thoroughly mixed with the granitic soil in four pots. Two hundred grams of alder litter were added and mixed into the same type of soil in four

Table 2. Treatment levels and nutrient sources for Experiment #2.

Treatment No.	Levels of Nutrients Added (ppm)				Number of Root Systems of Nodulated Seedlings
Granitic	N	P	Mo	B	Alder
1	0	100	2	1	0
2	25	100	2	1	0
3	50	100	2	1	0
4	75	100	2	1	0
5	100	100	2	1	0
6	0	100	2	1	2
Pumice	N	P	Mo	B	Snowbrush
1	0	100	2	1	0
2	25	100	2	1	0
3	50	100	2	1	0
4	75	100	2	1	0
5	100	100	2	1	0
6	0	100	2	1	2

Nutrient Sources:

N - NH_4NO_3

P - H_3PO_4

Mo - Na_2MoO_4

B - $Na_2B_4O_7 \cdot 10H_2O$

other pots. Similarly ponderosa pine and snowbrush litters were added to pots of pumice soil at a rate of 120 grams per pot. Monterey pine was sown into these pots and into eight additional pots of each soil. The nutrient treatments and sources for the third experiment are presented in Table 3. Each pot was thinned to 12 seedlings and after ten months in the greenhouse the seedlings were harvested.

Field Investigations:

The influence of snowbrush on root morphology and foliar nitrogen of ponderosa pine seedlings was investigated on the Pringle Falls Experimental Forest in Central Oregon.

Seedlings grown under or in association with snowbrush and in the open were excavated. After the soil was washed off, comparisons were made of the root systems.

Needle samples from terminal leaders were collected from pine growing in the open and in association with snowbrush.

Laboratory:

After harvesting the seedlings were separated into their component parts of needles, stems, and roots. Following drying in a forced air oven at 70° C. for approximately 48 hours, the parts were weighed. Next each part was ground in a small osterizer and stored for future determinations.

Plant tissue and litter samples were analyzed for total nitrogen by a modified micro-Kjeldahl technique using salicylic acid and concentrated H_2SO_4 as the primary digestion reagent. Other

Table 3. Treatment levels and nutrient sources for Experiment #3.

Treatment No.	Levels of Nutrients Added (ppm)				Kind and Amount of Litter Added
Granitic	N	P	Mo	B	
1	0	100	2	1	0
2	25	100	2	1	0
3	0	100	2	1	Douglas-fir - 200 g.
4	0	100	2	1	Alder - 200 g.
Pumice	N	P	Mo	B	
1	0	100	2	1	0
2	25	100	2	1	0
3	0	100	2	1	Ponderosa pine - 120 g.
4	0	100	2	1	Snowbrush - 120 g.

Nutrient Sources:

N - NH_4NO_3

P - H_3PO_4

Mo- Na_2MoO_4

B - $Na_2B_4O_7 \cdot 10H_2O$

determinations made on the litter samples included calcium, magnesium, potassium, and phosphorus. Procedures outlined in the California Agricultural Experiment Station Bulletin 766 were followed (24, p. 24-78).

Soil samples were analyzed for the same nutrients according to the methods in the Oregon State College Agricultural Experiment Station Miscellaneous Paper 65 (1, p. 1-8).

When applicable the data were statistically analyzed by the analysis of variance test and the new multiple range test (23, pp. 205-208, 238-241).

RESULTS AND DISCUSSION

Experiment #1:

It is apparent from Figures 1 and 2 that alder and snowbrush seedlings grown in association with ponderosa pine seedlings did not have a beneficial influence on the pine. Figures 3 and 4 indicate that they had a depressing effect upon growth and nitrogen content in the pine seedling tops. It is probable that these effects are due to competition in the relatively small pots. Beneficial effects have been noted in the field with similar associations where competition is not as severe. A general response to added increments of nitrogen can be noted.

Considering first, the granitic soil (Figure 3 A, B, and C), maximum yield in terms of dry matter occurred at 75 ppm. of added nitrogen with a slight decrease at the highest level. Mitchell working with Pinus strobus L. obtained maximum yields with about 300 ppm. of added nitrogen (28, p. 41). It would also be expected that the ratio of top yield to total yield would increase sharply at the higher nitrogen levels, however this is not the case (Figure 3 C). The sharp increase of yield at the lowest levels of added nitrogen suggests that nitrogen is limiting, however at the higher levels of nitrogen some other factor appears to be limiting. The soil test value for potassium on this soil is unusually low (Table 4) and since phosphorus was added, it appears that potassium has become limiting. If potassium had been added it is probable that the seedlings would have responded to the higher levels of

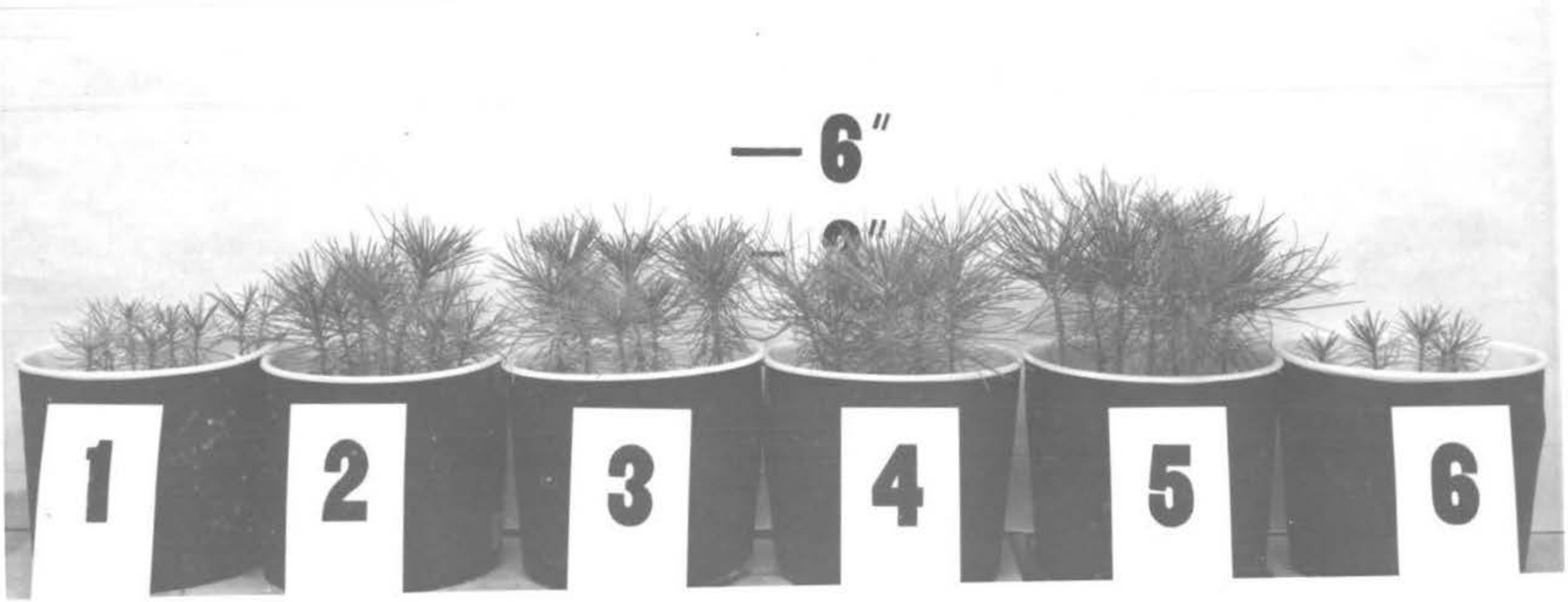


Figure 1. Ponderosa pine seedlings grown on a granitic soil, with varying levels of nitrogen in experiment #1. Treatment levels are (1) 0 - N, (2) 25 ppm N, (3) 50 ppm N, (4) 75 ppm N, (5) 100 ppm N, and (6) 1 alder seedling (removed to show pine seedlings).

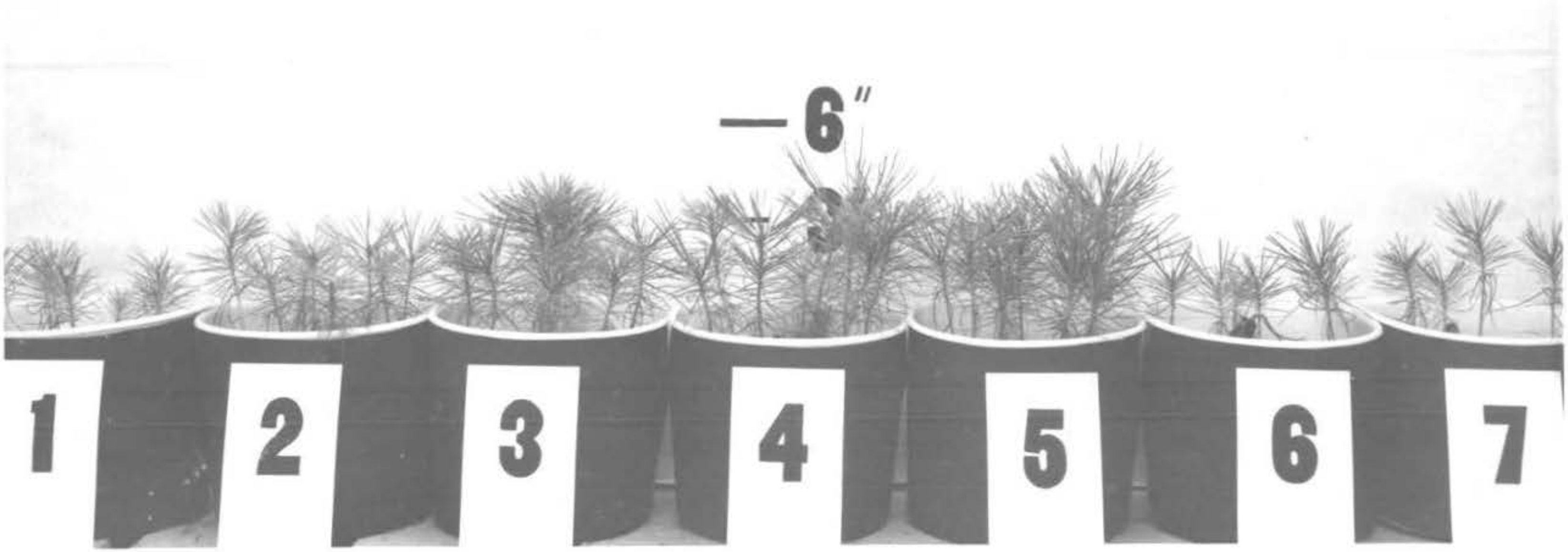


Figure 2. Ponderosa pine seedlings grown on a pumice soil, with varying levels of nitrogen in experiment #1. Treatment levels are similar to those in Figure 1, except snowbrush seedlings were grown in (6) and (7). Treatment (7) contained no molybdenum or boron. Snowbrush seedlings were removed to show ponderosa pine seedlings.

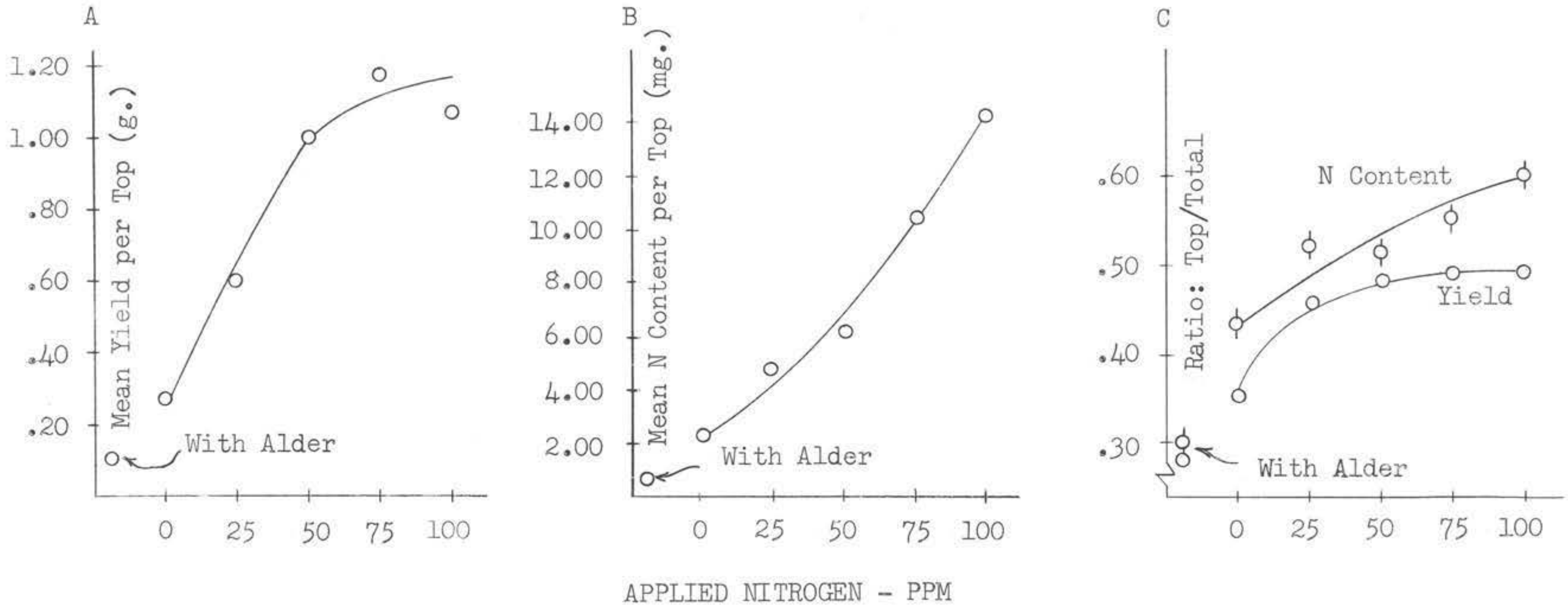


Figure 3. Yield and nitrogen content data for seedlings grown in experiment #1 on a granitic soil. (A) Mean yield per top (grams), (B) Mean nitrogen content per top (milligrams), and (C) Ratios of top yield and top nitrogen content to total yield and total nitrogen content.

Table 4. Chemical analysis of soils in greenhouse experiments.

Soil	pH	P ppm	K	Ca	Mg	TN %
				m.e./100gm.		
Granitic	6.2	3.0	0.09	4.6	1.3	0.008
Pumice	6.3	5.5	1.24	4.0	0.65	0.009

nitrogen. In spite of the optimum conditions of light, temperature, and moisture that prevailed under the greenhouse conditions, alder had a depressing effect on the growth of ponderosa pine. During harvesting, observations made on the alder roots showed they had practically permeated the entire soil mass. It is possible if a larger pot had been used, alder would not have had this adverse influence on the growth of the associated pine.

Nitrogen contents of the tops of the ponderosa pine appear to be related to supply (Figure 3 B). As the external supply of nitrogen was increased, the content found in the top increased in nearly a linear relationship; therefore in the range of applied nitrogen being studied, it is probable that the nitrogen content of the seedling tops is not related directly to growth, but rather is indicative of the external supply. Mitchell (28, p. 77) also reached a similar conclusion with white pine in a comparable range of added nitrogen.

Statistical evaluation of the yield and nitrogen content data by the new multiple range test indicates that the values obtained for the tops of seedlings growing in the presence of alder were not significantly different at the 5% significance level, from those values obtained on the zero nitrogen seedlings. However the yield and nitrogen content of the tops of the seedlings grown on the zero nitrogen treatment were significantly less than the seedlings grown on the other nitrogen treatments (Appendix 1).

The response of ponderosa pine grown on the pumice soil differed as no maximum top yield was reached (Figure 4 A). Also the shape of the top yield to total yield curve was quite different (Figure 4 C).

The soil test values already presented (Table 4) show that the pumice soil has a higher content of exchangeable potassium than the granitic soil. It is likely that this difference has caused the dissimilar response of ponderosa pine on these two soils. Molybdenum and boron or the absence thereof has not affected the yield or nitrogen content of pine seedlings in the presence of snowbrush; consequently the separate values have been combined for plotting (Appendix 2). As previously demonstrated, the nitrogen content of the tops followed the amount of nitrogen supplied (Figure 4 B).

Analysis of the data by the new multiple range test reveals that the top yield and top nitrogen content of the seedlings grown with snowbrush were significantly lower, at the 5% significance level, than the seedlings grown with no nitrogen applied. Similar values obtained for the seedlings grown at the higher nitrogen treatments were significantly greater than the top yield and top nitrogen content of the seedlings grown on the zero nitrogen treatment (Appendix 2).

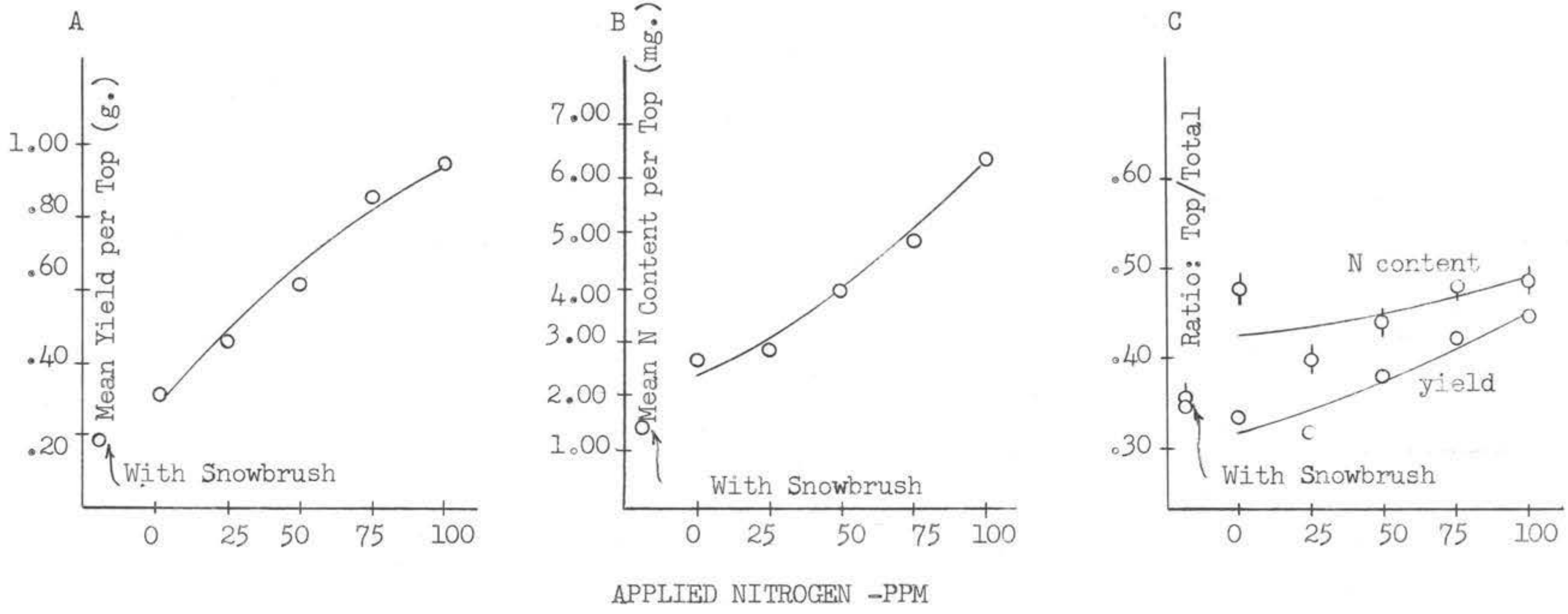


Figure 4: Yield and nitrogen content data for seedlings grown in experiment #1 on a pumice soil. (A) Mean yield per top (grams), (B) Mean nitrogen content per top (milligrams), and (C) Ratios of top yield and total nitrogen content to total yield and total nitrogen content.

A comparison of the initial soil nitrogen content and the final soil nitrogen content after supporting alder or snowbrush reveals several important and interesting facts (Table 5).

Table 5: Effect of snowbrush and alder on soil nitrogen under greenhouse conditions.

Soil and Plant	Increase in Soil Nitrogen per Pot - ppm N. no Mo or B	Mo and B added
Granitic - alder	-	4.8
Pumice - snowbrush	10.0	20.0

With pumice the addition of molybdenum and boron resulted in a greater accumulation of soil nitrogen over the non-molybdenum and non-boron treatment. This might be expected as molybdenum has been shown to be an important factor influencing nitrogen fixation (14,30). It appears that under the conditions of this experiment snowbrush added a greater amount of nitrogen to the soil than alder. Whether this was a result of a greater fixation capacity is not known.

Experiment # 2:

The response of Monterey pine to added nitrogen was similar to that measured for ponderosa pine in the previous experiment (Figures 5 and 6). A notable exception occurred in treatment 6 for both the granitic and pumice soils, where the response of Monterey pine grown in the presence of alder or snowbrush roots instead of in association with actively growing plants, was similar to that of approximately 25 ppm of added nitrogen.

Figure 5: Monterey pine seedlings grown on a granitic soil, with varying levels of nitrogen in experiment #2. Treatment levels are (1) 0 - N, (2) 25 ppm N, (3) 50 ppm N, (4) 75 ppm N, (5) 100 ppm N, and (6) roots of 2 alder seedlings.

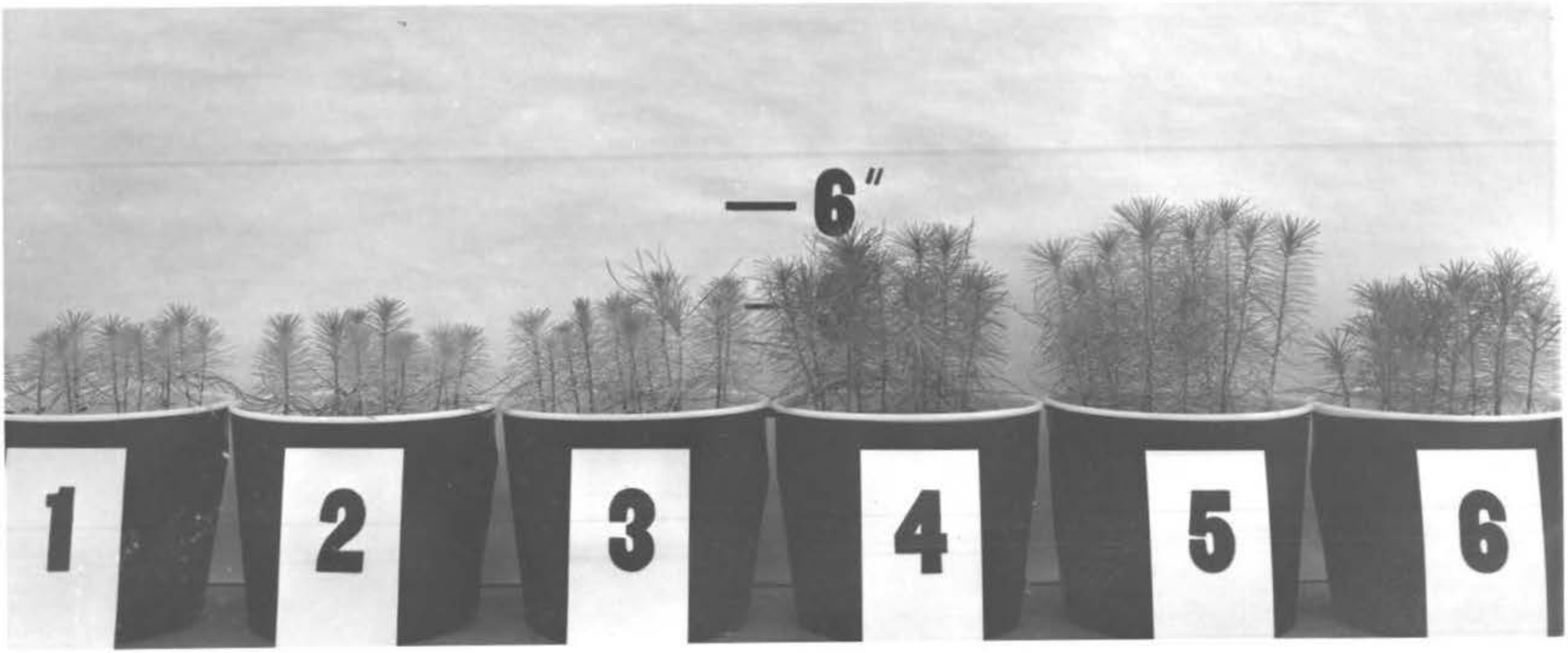


Figure 6: Monterey pine seedlings grown on a pumice soil, with varying levels of nitrogen in experiment #2. Treatment levels are similar to those in Figure 5, except (6) contained the roots of 2 snowbrush seedlings.

The yield data for granitic soil given in Figure 7A are similar to that in Figure 3A. The maximum yield was again reached in the vicinity of 50-100 ppm of added nitrogen, however the top yield and nitrogen content for Monterey pine in the presence of alder roots has shifted upwards. Comparing the yield and nitrogen content data for these seedlings with those having received added nitrogen shows that approximately 15 ppm of the nitrogen fixed by alder, became available for the growth of the pine seedlings (Figure 7A and B).

At the 5% significance level, the zero nitrogen seedlings were significantly smaller, and contained less top nitrogen that the seedlings grown with 25 ppm of nitrogen, which in turn were smaller and contained less nitrogen than the seedlings grown with the higher nitrogen additions. The statistical tests indicated intermediate values for the top yield and top nitrogen content of the seedlings grown with alder roots. Generally these data were significantly greater than comparable data for the zero nitrogen seedlings, but significantly smaller than the top yield and top nitrogen obtained from the seedlings grown with a 25 ppm nitrogen addition (Appendix 3).

The relationships of top yield to total yield and nitrogen content of the tops to total nitrogen (Figure 7C) are similar to the data for the seedlings grown on the granitic soil in the first experiment. Had nitrogen still been limiting at 50-100 ppm of applied nitrogen, there should not be a linear increase in foliar nitrogen at the higher rates of applied nitrogen.

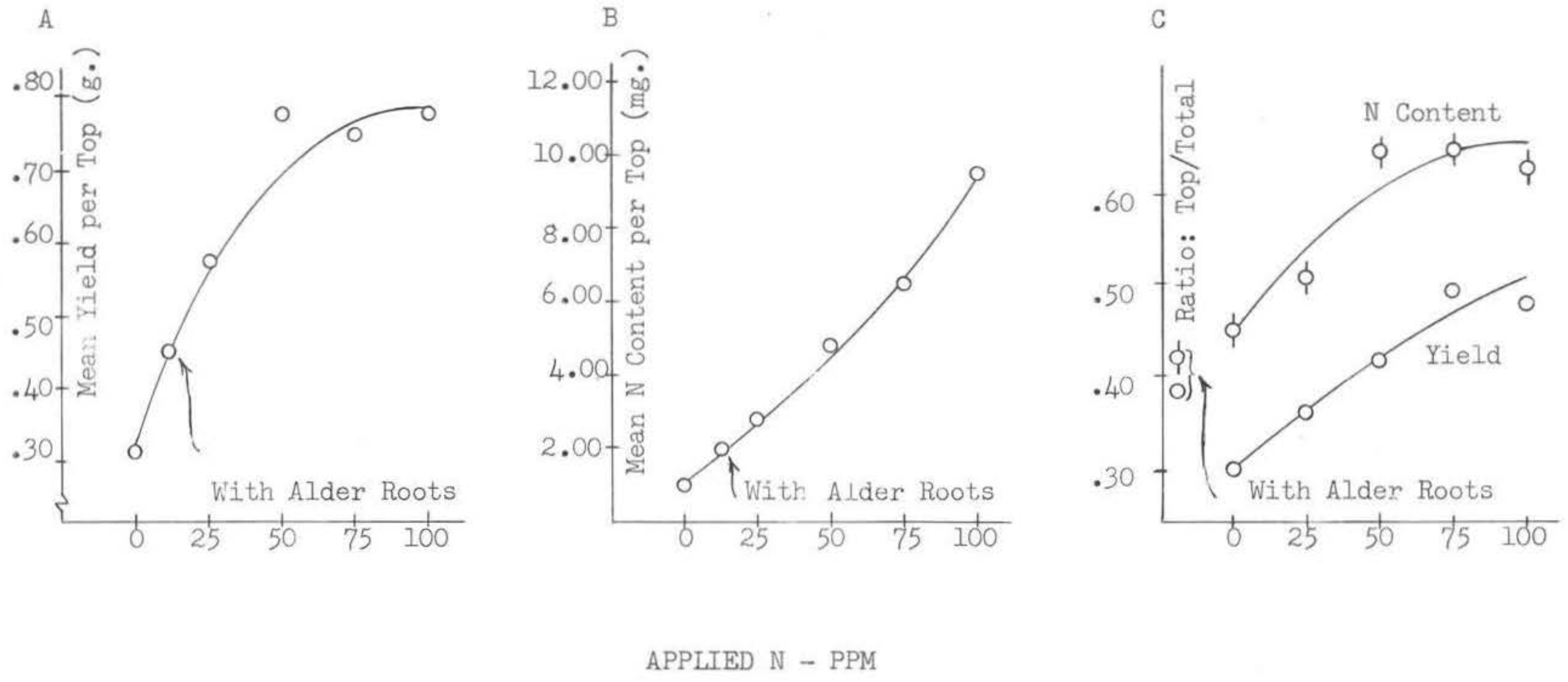


Figure 7: Yield and nitrogen content data for seedlings grown in experiment #2 on a granitic soil. (A) Mean yield per top (grams), (B) Mean nitrogen content per top (milligrams), and (C) Ratios of top yield and top nitrogen content to total yield and total nitrogen content.

Patterns already established for ponderosa pine on the pumice soil in relation to added increments of nitrogen remain essentially unchanged for Monterey pine (Figures 8A, B, and C). The significant difference resulted from the presence of snowbrush roots, that were once a part of an actively growing shrub. It is possible to estimate the amount of nitrogen that has been made available through nitrogen fixation by the snowbrush, for the subsequent utilization of the pine seedlings. According to both yield and nitrogen content data it amounts to 35 ppm of nitrogen (Figure 8A and B). This increase is significant at the 5% level. The top yield and top nitrogen content for seedlings grown with snowbrush roots are significantly greater than the yield and nitrogen content for seedlings grown with 25 ppm of nitrogen added, but are not as great as similar data for those seedlings grown with 50 ppm of nitrogen added to the soil (Appendix 4).

Experiment #3:

The influence of additions of different kinds of litter on the growth of Monterey pine in granitic and pumice soils is illustrated in Figures 9 and 10.

Alder litter which has a high nitrogen content compared to Douglas-fir litter (Table 6) caused a larger increase in top yield for Monterey pine than Douglas-fir litter or an inorganic addition of 25 ppm of nitrogen (Figure 11). Undoubtedly most surprising is the depressing influence of Douglas-fir litter on the growth of

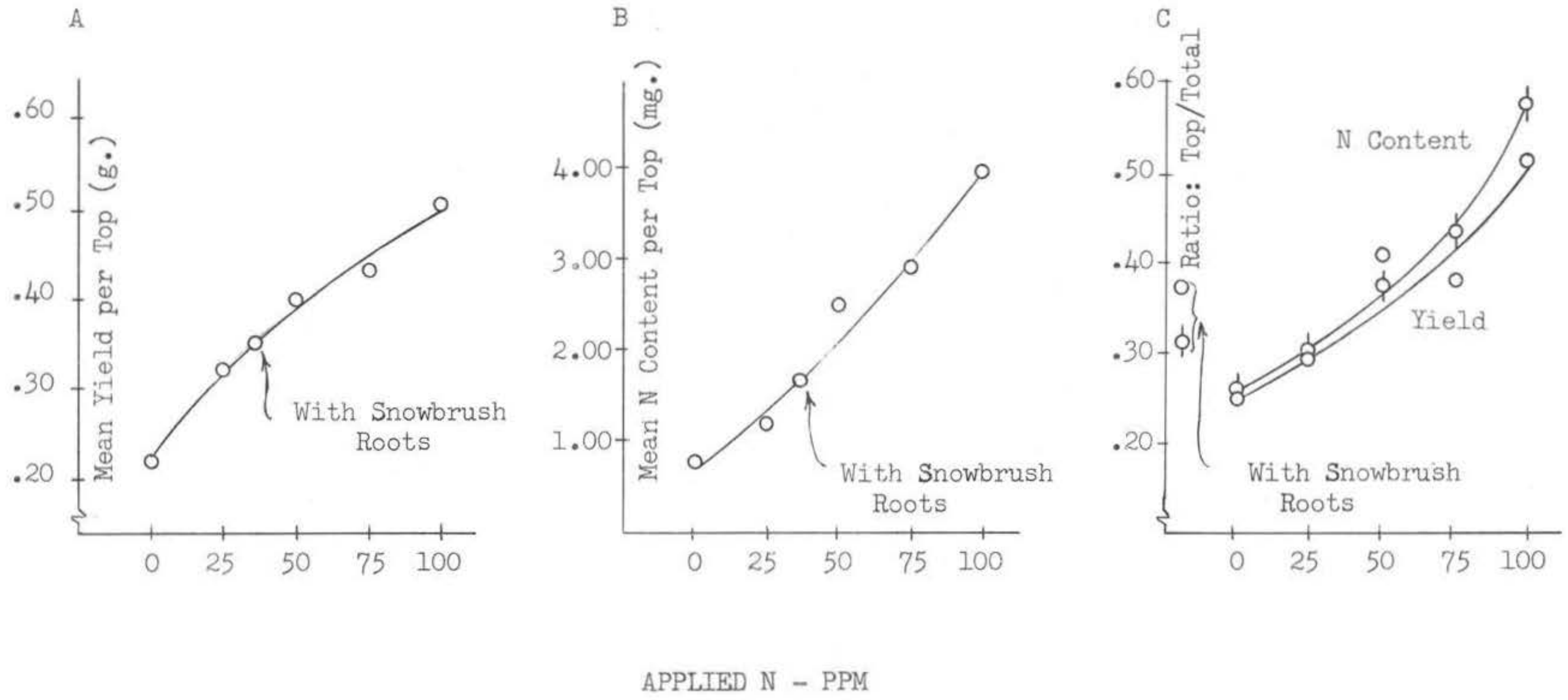


Figure 8: Yield and nitrogen content data for seedlings grown in experiment #2 on a pumice soil. (A) Mean yield per top (grams), (B) Mean nitrogen content per top (milligrams), and (C) Ratios of top yield and top nitrogen content to total yield and total nitrogen content.

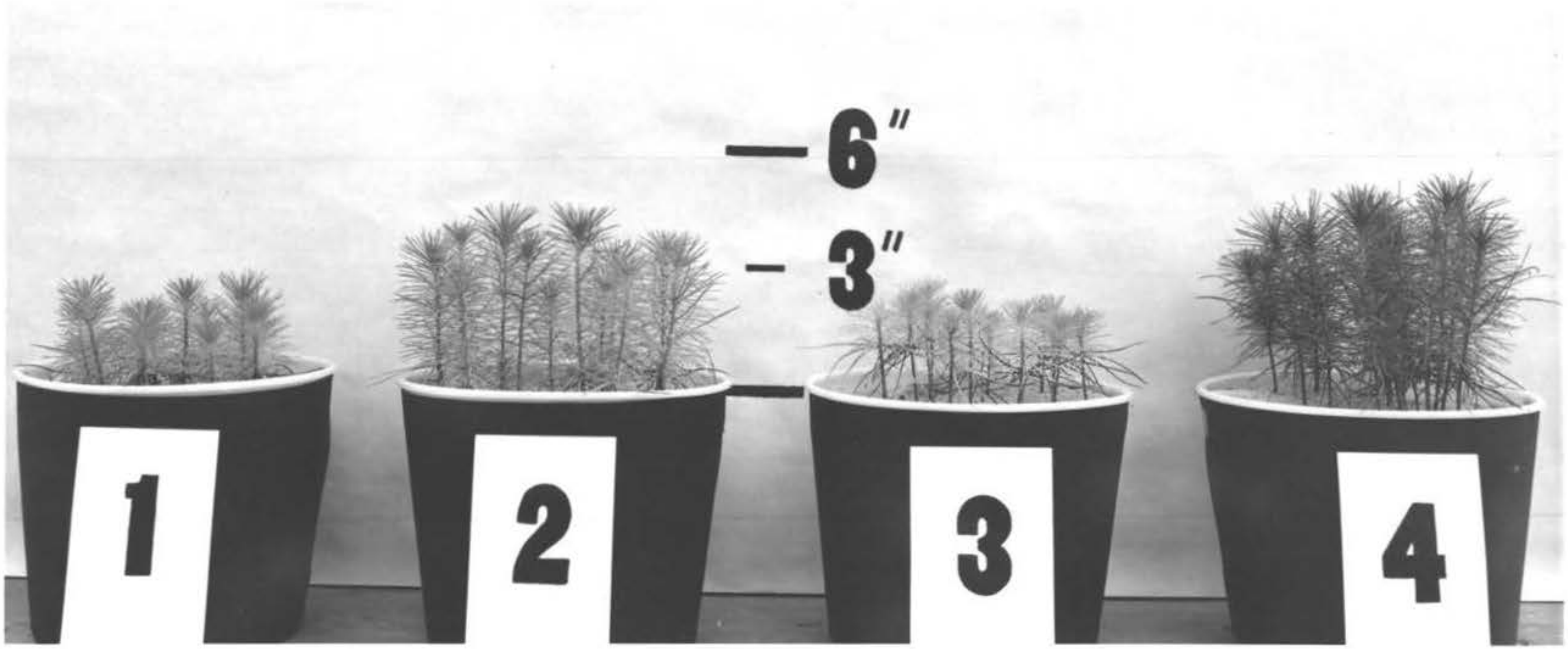


Figure 9: Monterey pine seedlings grown on a granitic soil, with different forms of nitrogen, in experiment #3. The different forms are (1) 0 - N, (2) 25 ppm N from an inorganic source, (3) 200 g. of Douglas-fir litter, and (4) 200 g. of alder litter.

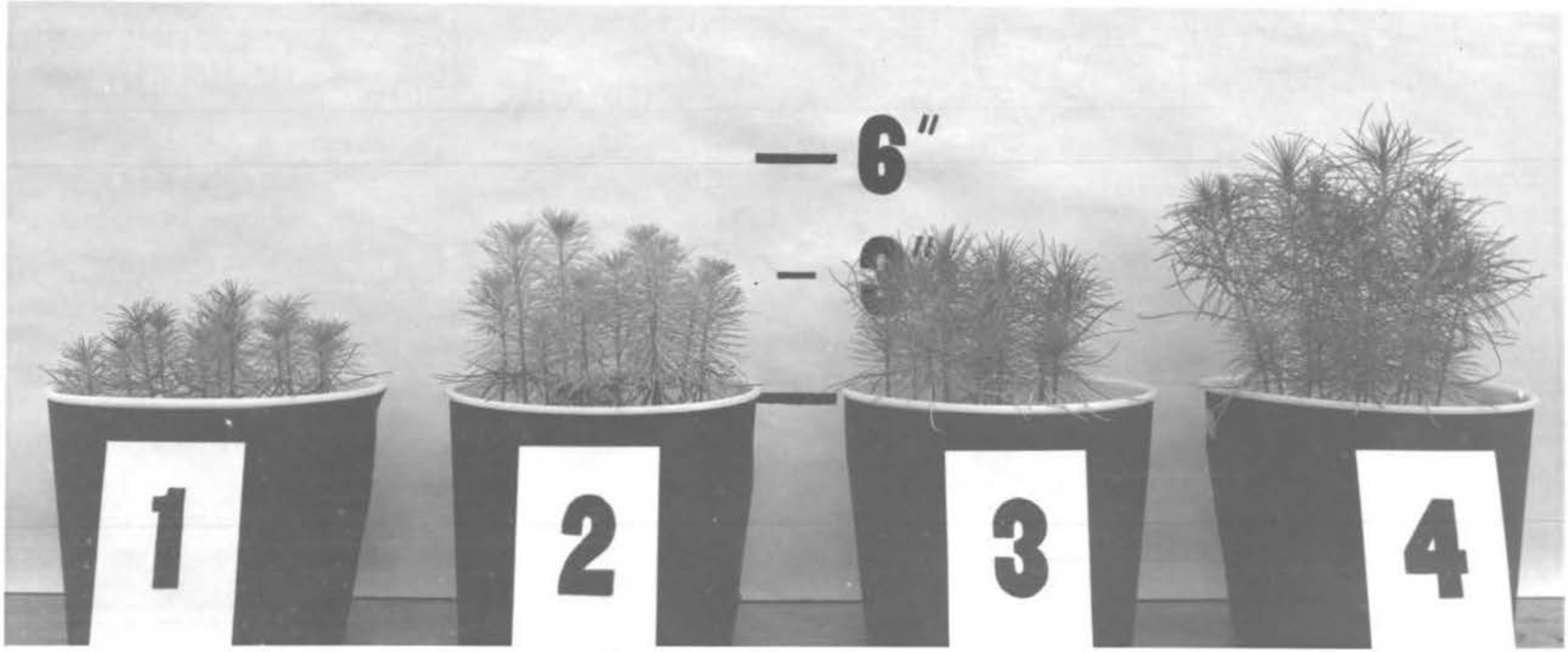


Figure 10: Monterey pine seedlings grown on a pumice soil, with different forms of nitrogen in experiment #3. The different forms are (1) 0 - N, (2) 25 ppm N from an inorganic source, (3) 120 g. of ponderosa pine litter, and (4) 120 g. of snowbrush litter.

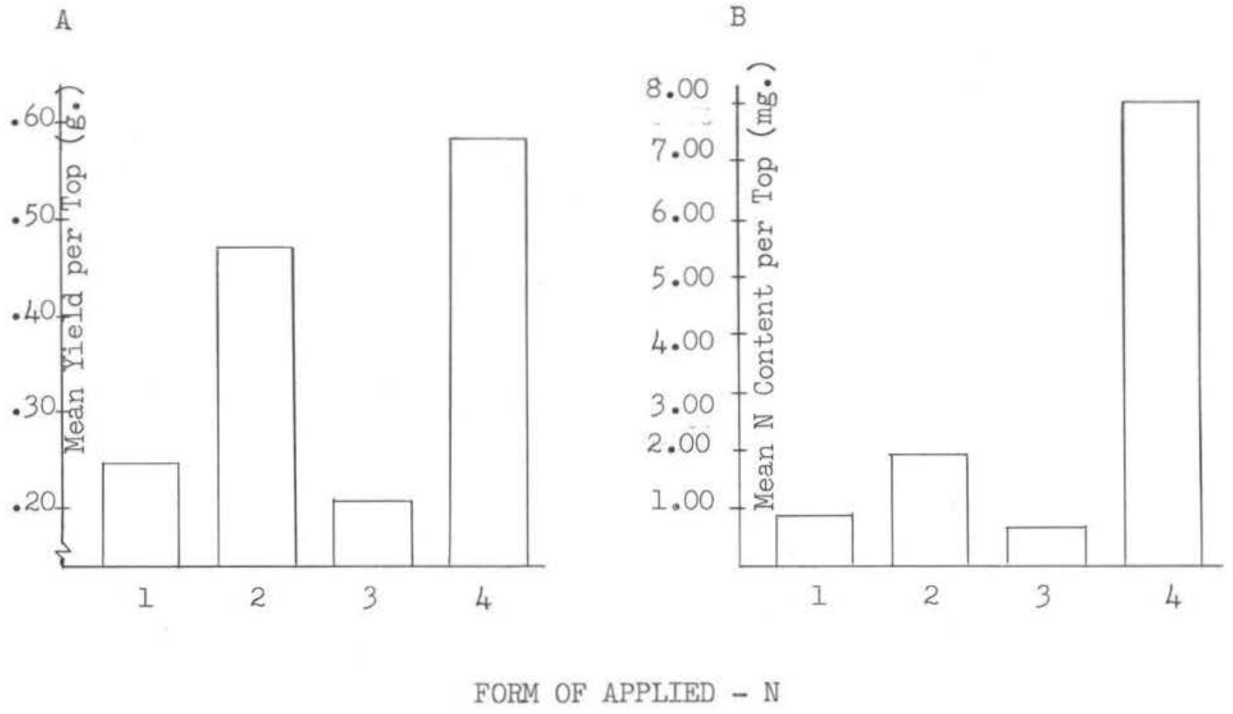


Figure 11: Yield and nitrogen content data for seedlings grown in experiment #3 on a granitic soil. (A) Mean yield per top (grams) and (B) Mean nitrogen content per top (milligrams). The forms of applied nitrogen are (1) 0 - N, (2) 25 ppm N from an inorganic source, (3) 200 g. of Douglas-fir litter, and (4) 200 g. of alder litter.

Monterey pine seedlings. The exact mechanism of the depression

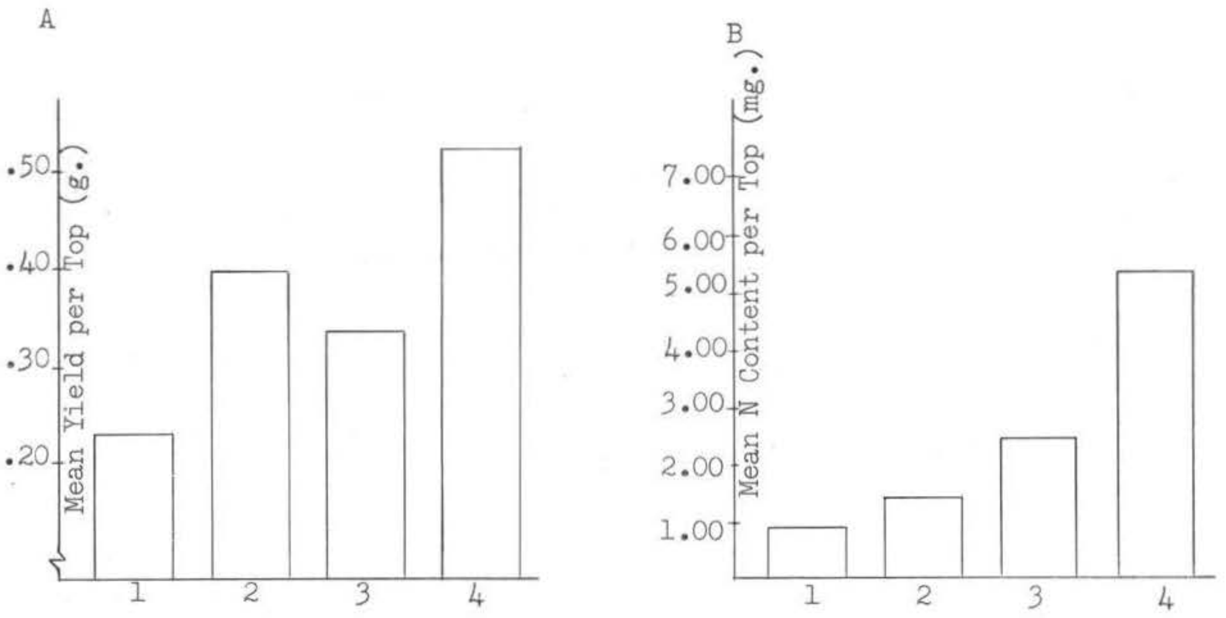
Table 6: Total nitrogen in litters used for third experiment.

Species	TN%
Alder	1.92
Douglas-fir	0.79
Snowbrush	1.12
Ponderosa pine	0.56

is not understood, but it is possible a toxic substance has been produced in the decomposition of the pure fir litter, or it may be a matter of the nutrient balance of the combined soil and litter. Whatever the mechanism, this effect is real and merits further investigation.

Nitrogen contents (Figure 11B) for the tops appear to be consistent with the other work presented in this study. Statistically only the nitrogen content for seedlings grown with alder litter were significantly greater than the nitrogen contents of the seedlings grown with no nitrogen (Appendix 5).

The second part of this experiment on pumice soil differs in several respects from the initial part with the granitic soil. Rather than using alder and Douglas-fir litters, ponderosa pine and snowbrush litters have been used. The responses were also different (Figure 12A and B). Snowbrush litter, like the alder litter produced the largest increase in growth and nitrogen content of the seedling tops. On the other hand ponderosa pine litter,



FORM OF APPLIED - N

Figure 12: Yield and nitrogen content data for seedlings grown in experiment #3 on a pumice soil. (A) Mean yield per top (grams) and (B) Mean nitrogen content per top (milligrams). The forms of applied nitrogen are (1) 0 - N, (2) 25 ppm N from an inorganic source, (3) 120 g. of ponderosa pine litter, and (4) 120 g. of snowbrush litter.

instead of having a depressing effect on growth produced a significant increase in growth over the zero nitrogen level, yet not as large an increase as an addition of 25 ppm of nitrogen. These increases in growth were all significantly different at the 5% significance level (Appendix 6). It should be emphasized that the seedlings grown on a soil supplemented with pine litter, while being somewhat smaller than those seedlings having had an inorganic source of nitrogen, had a much healthier appearance (Figure 10). The nitrogen content of the tops of seedlings grown on soil supplemented with pine litter, was 2.5 milligrams, compared to a nitrogen content of 1.8 milligrams for the tops of seedlings grown on soil with an addition of 25 ppm of inorganic nitrogen, however this difference is not statistically significant (Appendix 6).

Field Observations:

In some areas it is difficult to find ponderosa pine regeneration without the overhead cover of snowbrush. An example of this can be seen in Figure 13. The pine may remain under the snowbrush cover for some time, but eventually it will dominate the site with the snowbrush being confined to small openings in the ponderosa pine canopy. Figure 14 shows an example of some ponderosa pine beginning to emerge from beneath the snowbrush cover.

From outward appearances seedlings of approximately the same size growing in two distinct positions with respect to snowbrush are the same. Examination of the root systems of a pine grown with

Figure 13: Ponderosa pine seedlings growing beneath a dense cover of snowbrush.

snowbrush and a pine grown in the open, reveals that there are a greater number of fine roots in the surface horizons on the pine growing with the snowbrush (Figure 15). It has been established that more favorable moisture conditions exist in the surface horizons under snowbrush and that soil temperatures are also less severe. These conditions could be responsible for the greater amount of fine roots found on ponderosa pine grown under snowbrush.

Analysis of foliage of ponderosa pine growing under snowbrush reveals that the nitrogen content of this foliage is higher than that of open grown pine (Table 7). This difference may have resulted from a greater surface available for nutrient uptake or

Table 7: Nitrogen content of the terminal foliage of ponderosa pine grown in two positions with respect to snowbrush.

Position	TN%
Under snowbrush	1.06
Open	0.94

it may have resulted from an actual difference in the nutrient content of the soil. Dyrness has shown that the nutrient content of the soil and litter under snowbrush is higher than the nutrient content of the soil and litter in the open (13, p. 171). In the final analysis the observed and measured differences between open grown pine and pine grown under snowbrush are probably not attributed to any single factor, but have resulted from a combination

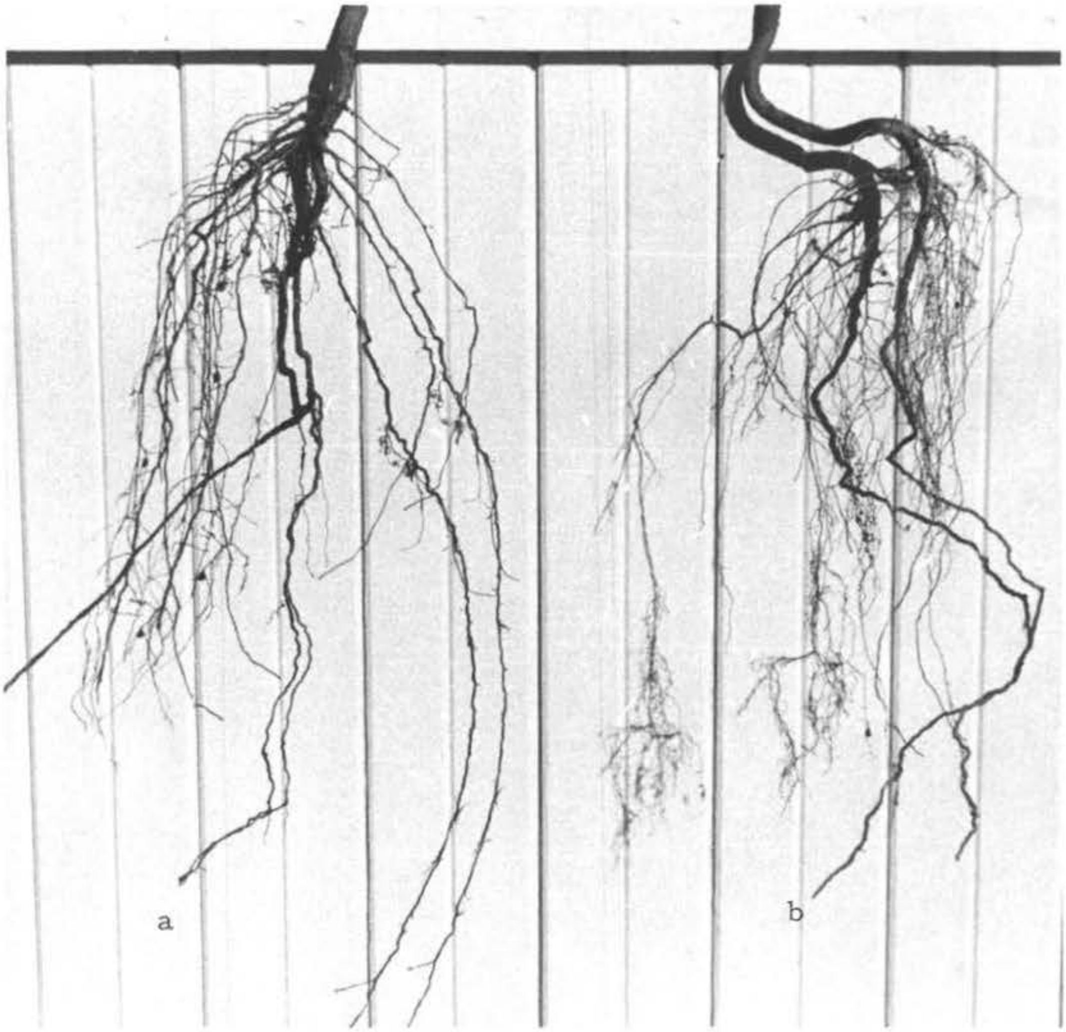


Figure 15: Root systems of two typical ponderosa pine seedlings, (A) grown in the open and (B) grown under snowbrush (The crook in the upper root of (B) was not limited to the under snowbrush position).

of improved environmental conditions that are known to exist under snowbrush.

To further emphasize the difference among vegetation sites, the nutrient contents of the litters from stands dominated by 4 different species in Oregon are compared in Table 8. These differences are of such a magnitude that one would expect these species to exert a profound influence on any associated growth of conifers. The differences in nitrogen and calcium contents are

Table 8: Chemical analysis of the litters of several species in Oregon.

Species	TN	Ca	Mg	K
		%		
Alder	1.92	1.29	-	0.1
Douglas-fir	0.79	1.00	-	0.23
Snowbrush	1.12	1.92	0.07	0.07
Ponderosa pine	0.56	0.66	-	0.07

probably the most important. The seedlings grown for experiment #3 certainly reflect these nutrient differences in the litter.

CONCLUSIONS

Under greenhouse conditions an increase in soil nitrogen has been demonstrated in the presence of certain nodulated, woody, non-leguminous species, however due to several factors this nitrogen has been unavailable for simultaneous growth of conifers. First of all the competitive effects of the associated growth of the two different kinds of vegetation have been great. Secondly it is possible the use of a very limited soil mass has intensified this competition. The question naturally arises as to whether or not the effects of competition are as severe under field conditions. where a greater mass of soil is available. Preliminary observations indicate that ponderosa pine is able to successfully compete with snowbrush under certain field conditions. Whether these seedlings are comparable to those grown in the open is not entirely known, but a conifer seedling that has succeeded in overcoming a nodulated, nitrogen fixing shrub, should grow with increased vigor.

In this day however we are not confined to the course of natural development in a plant community. Man possesses within his means various methods of releasing desirable species in the forest stand. It is not the purpose of this study to discuss these methods of release, except to mention that a form of mechanical release was utilized in the second experiment. After competition had been eliminated, the conifers were established and grown at an accelerated rate. An alternative method would be to initially establish the desirable conifer and grow it concurrently with the nitrogen fixing shrub, then after a period of time had elapsed, release the coniferous seedlings by either mechanical or chemical means. Both of these release techniques have been successfully tested under field conditions (12, 20), however widespread practice is not common. The third alternative would be to establish the conifers and without mechanical or chemical aids, allow them to dominate the site by natural stand development. This method usually takes longer than the other two.

Beneficial aspects of mixed stands or utilization of lesser shrubs are not limited to their capability to fix nitrogen, as the rooting environments are continuously being modified through additions of their own litter. This may lead to a number of changes in nutrient status, in microbial activity (33, p. 244), in soil physical conditions (11, p. 427-448), or in microhabitats (13, p. 156,171; 35, p. 601-612; 36, p. 1-3). The importance of the mixed stand has been emphasized where the litter from a pure, unmixed Douglas-fir stand depressed the growth of a selected conifer, when that litter was added to the soil.

Within the scope of this study, it has been possible to estimate the amount of nitrogen that can become available for plant growth, through fixation. This might be classed as a "bioassay" for the effectiveness of certain species to fix nitrogen under greenhouse conditions. Two snowbrush plants in less than a year's time were able to fix a minimum of 35 ppm of nitrogen which became available for plant growth after decomposition of the roots. And this was within a limited soil mass. Alder on the other hand under these same conditions was able to fix a quantity of available nitrogen that was about half the amount similarly fixed by snowbrush. While field fixation has been demonstrated for alder (5, p. 147-153), as yet there is no quantitative measurement of the amount of field fixation of nitrogen by either alder or snowbrush.

The idea of forest stands of mixed composition is not new; therefore in light of this study and other recent developments, it would now seem appropriate to begin, at least, to reevaluate our present forest management policy regarding the associated species in the forest stand complex. It should be recognized that some of these species could play an important role in stimulating forest growth, primarily through improved nitrogen nutritional relationships and secondarily through improved physical conditions and increase of other nutrient levels. In areas of low fertility, these nitrogen fixing species would become especially important.

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APPENDICES

Appendix 1

Yield and nitrogen content data for ponderosa pine seedlings grown on granitic soil in experiment # 1, ranked by the new multiple range test.

Mean weight (g.) per seedling part*:

Treatment

Stem	6	1	2	3	4	5
	.04	.09	.15	.21	.25	.25**
Needles	6	1	2	3	4	5
	.13	.23	.50	.74	.87	.93
Roots	6	1	2	5	3	4
	.47	.59	.77	1.15	1.26	1.28

Mean nitrogen content (mg.) per seedling part:

Stem	6	1	2	3	4	5
	.23	.37	.730	.92	1.48	2.6
Needles	6	1	2	3	4	5
	.98	1.67	3.85	5.15	8.61	11.39
Roots	1	6	2	3	4	5
	2.76	2.79	4.21	5.85	8.17	9.57

* Mean of 4 replications

** Significance level 5%

Appendix 2

Yield and nitrogen content data for ponderosa pine seedlings grown on pumice soil in experiment # 1, ranked by the new multiple range test.

Mean weight (g.) per seedling part*:

Treatment

Stem	6	7	1	2	3	4	5
	.06	.06	.10	.14	.17	.21	.22 **
Needles	6	7	1	2	3	4	5
	.18	.19	.26	.36	.50	.68	.76

Mean nitrogen content (mg.) per seedling part:

Stem	6	7	1	2	3	4	5
	.25	.30	.45	.51	.60	.80	1.12
Needles	6	7	2	1	3	4	5
	1.14	1.19	2.24	2.28	3.25	4.00	5.24
Roots	7	6	1	2	3	4	5
	2.67	2.80	2.97	4.15	5.00	5.23	7.03

*Mean of 4 replications

** Significance level 5%

Appendix 3

Yield and nitrogen content data for Monterey pine seedlings grown on granitic soil in experiment # 2, ranked by the new multiple range test.

Mean weight (g.) per seedling part*:

Treatment

Stem	1	6	2	4	3	5
	.08	.12	.17	.20	.22	.22 **
Needles	1	6	2	4	5	3
	.22	.34	.41	.55	.56	.56
Roots	6	1	4	5	2	3
	.53	.72	.75	.85	1.03	1.07

no significant differences

Mean nitrogen content (mg.) per seedling part:

Stem	1	6	2	3	4	5
	.26	.49	.78	1.42	1.92	4.03
Needles	1	2	6	3	4	5
	.82	1.85	1.92	3.44	4.27	5.33
Roots	1	2	3	6	4	5
	1.33	2.53	2.67	3.25	3.38	5.59

* Mean of 4 replications

** Significance level 5%

Appendix 4

Yield and nitrogen content data for Monterey pine seedlings grown on pumice soil in experiment # 2, ranked by the new multiple range test.

Mean weight (g.) per seedling part*:

Treatment

Stem	1	6	2	3	4	5
	.05	.07	.08	.09	.10	.13**
Needles	1	2	6	3	4	5
	.18	.25	.27	.31	.33	.39
Roots	5	6	3	1	4	2
	.47	.56	.59	.68	.69	.78

no significant differences

Mean nitrogen content (mg.) per seedling part:

Stem	1	6	2	3	4	5
	.13	.26	.30	.73	1.02	1.27
Needles	1	2	6	3	4	5
	.64	.91	1.39	1.75	1.96	2.70
Roots	1	6	5	2	3	4
	2.30	2.65	2.86	3.00	4.30	4.32

* Mean of 4 replications

** Significance level 5%

Appendix 5

Yield and nitrogen content data for Monterey pine seedlings grown on granitic soil in experiment # 3, ranked by the new multiple range test.

Mean weight (g.) per seedling part*:

Treatment

Stem	3	1	4	2
	.05	.06	.10	.12 **
Needles	3	1	2	4
	.15	.18	.35	.47
Roots	3	4	1	2
	.27	.31	.32	.67

Mean nitrogen content (mg.) per seedling part:

Stem	3	1	2	4
	.16	.18	.34	1.62
Needles	3	1	2	4
	.52	.65	1.53	6.46
Roots	1	3	2	4
	1.32	1.35	2.81	4.59

* Mean of 4 replications

** Significance level 5%

Appendix 6

Yield and nitrogen content data for Monterey pine seedlings grown on pumice soil in experiment # 3, ranked by the new multiple range test.

Mean weight (g.) per seedling part*:

Treatment

Stem	1	3	4	2
	.06	.06	.09	.09**
Needles	1	3	2	4
	.17	.25	.29	.42
Roots	3	1	4	2
	.20	.22	.27	.34

Mean nitrogen content (mg.) per seedling part:

Stem	1	2	3	4
	.17	.28	.28	.48
Needles	1	2	3	4
	.69	1.51	2.22	4.84
Roots	1	3	4	2
	1.06	1.17	1.80	1.81

* Mean of 4 replications

** Significance level 5%