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C. T. Youngberg

Previous observations have shown that snowbrush (Ceanothus velutinus Dougl.) does not nodulate readily on all sites in the Western Cascades. Nodulation appears to increase concurrently with increases in snowbrush biomass, litter accumulation, and N accretion on these sites. The primary objective of this study was to identify some of the soil factors which may be responsible for poor nodulation.

Ten sites in the Western Cascades and Central Oregon were selected to determine some of the soil factors which affect snowbrush nodulation. The changes in soil chemical properties resulting from snowbrush occupancy for periods up to 9 years were determined on three of the sites. It was found that snowbrush significantly increases the base level of the soil on sites slow to nodulate. On the study area, the Ca level in the surface 20 cm of soil was increased by 5 meq over a period of 7 years. Magnesium and K were also increased but not to the same degree.

The nodule potential of three soils obtained in the Western Cascades was determined in a greenhouse study. The three soils were obtained on or near clear cut units designated as Spring Creek 1, Foley Ridge 2, and Toad Creek forest site. The base saturation of the soil was 50.4, 16.3, and 8.3% respectively. The three soils were inoculated with two inoculum sources, Fort Benham soil and Toad Creek soil both obtained directly from under nodulated snowbrush plants. Fort Benham soil had a high inoculum density while the Toad Creek soil was relatively low in inoculum. The nodule potential of the soil with the highest base saturation, namely Spring Creek, was highest. Snowbrush seedlings grown in Spring Creek soil produced the largest number of nodules per plant, Foley Ridge soil was intermediate, and Toad Creek forest soil was lowest. The same number of nodules per plant was produced in Spring Creek soil when inoculated with both the high inoculum density source and the low inoculum density source. Toad Creek forest soil, however, produced significantly more nodules when inoculated with Fort Benham soil than with Toad Creek soil. The difference in nodulation suggests that the endophyte was able to grow in the Spring Creek soil but was unable to grow, or grow in an infective form, in the Toad Creek forest soil.

The frequency of nodulation on ten sites throughout Central Oregon and the Central Western Cascades was correlated with soil base saturation. An equation was calculated ($N = 8.42 + 1.47(B)$ where

N = percent snowbrush nodulation and B = percent soil base saturation) relating percent nodulation to soil base saturation. Seventy-eight percent of the variation in percent nodulation is accounted for by soil base saturation.

Increasing the base status of Toad Creek forest soil, which has a low base saturation (8. 3%) and low nodule potential, with $CaCO_3$ and $CaSO_4$ significantly increased both nodule numbers and nodule mass produced per plant after 40 weeks. Nodule numbers and mass per plant increased approximately two times with $CaCO_3$ and three times with $CaSO_4$. The dry weight of tops and total N content of tops increased with nodulation. In Toad Creek forest soil the addition of Ca as $CaSO_4$ consistently produced larger and better nodulated snowbrush plants than did a similar amount of Ca added as $CaCO_3$.

The addition of up to 220 ppm S as $CaSO_4$ or up to 80 ppm S as H_2SO_4 to Toad Creek forest soil did not improve nodulation. The addition of high levels of elemental S (500, 1000, and 2000 ppm) produced a nodulation response similar to that obtained when 3, 6, and 12 meq of Ca as $CaSO_4$ was added to this soil. (The addition of $CaSO_4$ supplies 480, 960, and 1920 ppm S respectively.) The nodule response which is observed with the high rates of S application is probably due to a pH induced change in nutrient availability. The nutrient or group of nutrients so affected has not yet been determined.

The addition of up to 10 ppm N as NH_4NO_3 stimulated snowbrush nodulation in Toad Creek forest soil. Experiments indicated that a significant increase in nodulation occurs when N and Ca as $CaSO_4$ or $CaCO_3$ are added to Toad Creek forest soil. The addition of 20 ppm N as NH_4NO_3 nearly completely inhibited nodulation in this soil. The addition of $CaSO_4$ at all levels applied appeared to overcome the inhibition produced by the N.

Investigation of inoculum source indicated that Fort Benham soil was a better source of inoculum in Toad Creek forest soil than nodules obtained from plants on Toad Creek 1, an area immediately adjacent to the forest site. The nodules produced by Fort Benham soil inoculum appeared to be more effective in terms of N fixation. Up to four times more N was found in plants inoculated with Fort Benham soil inoculum as compared to plants inoculated with crushed nodules from the Toad Creek site.

The growth and/or infectivity of the endophyte responsible for root nodules on snowbrush appears to be restricted in Toad Creek forest soil. For periods up to 14 weeks, the endophyte did not invade or was non-infective in Toad Creek forest soil placed in contact with either high nodule potential Fort Benham soil or Toad Creek forest soil inoculated with 2% Fort Benham soil. The addition of Ca or Mg with and without N did not facilitate the occupancy of non-inoculated Toad Creek forest soil by the endophyte.

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Professor of Soil Science
in charge of major

Redacted for privacy

Head of Department of Soil Science

Redacted for privacy

Dean of Graduate School

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SOME SOIL FACTORS AFFECTING SNOWBRUSH NODULATION

I. INTRODUCTION

Snowbrush (Ceanothus velutinus Dougl.) has been shown to be of significant benefit in the establishment and early growth of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) in the Western Cascade Region of Oregon (Scott, 1970). The beneficial effects of snowbrush are twofold. First, it creates a more favorable moisture and temperature regime for the establishment of Douglas-fir seedlings. Secondly, snowbrush improves the nutrient status of the site through symbiotic N fixation and the concentration of Ca in the surface soil.

In the Western Cascades, snowbrush, which is shade intolerant, occurs as a pioneer species following logging and slash burning or wildfires. It reaches its maturity in 10 to 15 years after which it becomes decadent (Zavitkovski and Newton, 1968a). Douglas-fir seedlings growing under its canopy overtop the snowbrush in 8 to 10 years and as the coniferous canopy closes it is shaded out. Snowbrush is thus an ideal nurse crop for Douglas-fir seedlings. The brush provides newly established seedlings the necessary shade they require during their establishment and early growth, adds needed N through its nodulation and N fixation capabilities, and is easily removed from the site by competing coniferous species.

It has been observed that snowbrush does not nodulate readily on all sites in the Western Cascades. On some sites nodulation occurs within 1 year after the brush is established, whereas, on other sites under similar previous vegetation and logging practice, nodulation may be delayed for 5 to 8 years. Since snowbrush becomes decadent after approximately 15 years, a significant proportion of its time is spent without the capability of adding needed N to the site. This failure to nodulate could, therefore, be a significant factor influencing soil fertility of the area.

The objective of this investigation was to identify some of the soil factors which may be responsible for poor snowbrush nodulation on some sites in the Oregon Cascades. To meet the overall general objective the following more specific objectives were undertaken:

(1) To determine what changes in the soil nutrient status result from snowbrush occupancy.

(2) To study the influence of soil nutrient changes, caused by snowbrush, on nodulation.

II. LITERATURE REVIEW

The failure of snowbrush to nodulate soon after occupying a logged over area in the Western Cascades has been observed and reported by Youngberg and Wollum (1970). Wollum, Youngberg and Chichester (1968) related the age of previous timber stands to snowbrush nodulation and found that the older the previous stand the poorer the subsequent nodulation. They concluded the endophyte population was probably very low because of the absence of the host plant for long periods of time. With prolonged absence of the host plant, there appeared to be a natural biological decay of the symbiont; thus on these sites snowbrush nodulation was infrequent until the endophyte population increased.

Youngberg and Wollum (1970) found that on sites which were slow to nodulate no plants nodulated in the first 2 years, less than 10% in the 3rd year, approximately 25% in the 4th year, and 40% in the 5th year. The increase in nodulation was correlated with increase in percent snowbrush cover, snowbrush biomass, litter production, and N accretion. Direct cause and effect was not established, thus the question remains as to whether the plants became nodulated first resulting in the increase in biomass or whether the increase in snowbrush biomass with time ameliorates soil conditions thus increasing nodulation and greater snowbrush biomass production.

Legumes

Legumes introduced to virgin lands in Australia have failed to nodulate for a number of reasons. The most common cause of nodulation failure, as reported by a number of workers, may be summarized as follows: low phosphates, high nitrates, water logging, low pH, high pH, low nutrient levels, fertilizer toxicity, root rot, competition from ineffective Rhizobium, excessive drying of agar inoculum, high soil temperatures, insect attacks, root nematodes, and microbial antagonisms (Cartwright, 1967; Damirgi and Johnson, 1966; Gibson, 1963; Holland and Parker, 1966; Hattingh and Louw, 1966; Lie, 1969; Molina and Alexander, 1967; Munn, 1968 a, b, c, d; Philpotts, 1967).

The effectiveness of $CaCO_3$ in improving nodulation on acid soils has been well established in the literature. The influence of $CaCO_3$ on nodulation in acid soils has been shown to be largely due to the effect of $CaCO_3$ on soil properties, such as reducing heavy metal toxicity, improved soil structure, and increased availability of some nutrients, most notably Mo, rather than a Ca effect _per se_. Calcium itself has, however, been implicated in the nodulation of legumes. Calcium is not only required by the host plant but also by Rhizobium for their normal growth and development. The role of Ca in higher plants has been well established, thus a discussion in detail at this time would serve little purpose. The role of Ca and Mg in the nutrition of Rhizobium has been discussed by a number of workers (Norris, 1958, 1959; Vincent, 1962; Vincent and Colburn, 1961).

Of more significance to this study is the role of Ca in nodulation. Banath, Greenwood and Loneragan (1966) found that mild Ca deficiencies caused the N concentration of all plant parts to decrease. The effect of the Ca deficiency was shown to be on nodule efficiency. Lowther and Loneragan (1968) studied the effect of Ca ions on the nodulation of subterranean clover in culture solutions. They found that in the presence of N nodule initiation has a higher Ca requirement than either nodule development or host plant growth. Munns (1970) found that Ca and H interacted on nodulation. Increasing acidity from pH 5.6 to pH 4.8 increased the Ca concentration needed for nodulation. At Ca levels below 0.2 mM or when acidity dropped below pH 4.8 nodulation was completely inhibited. He noted also that either $CaCl_2$ or $CaSO_4$ were equally effective but that Mg could not substitute for Ca in the initiation of nodules. Loneragan and Dowling (1958) found that Ca and H had a compound effect on nodulation of subterranean clover. Nodulation was zero below pH 4.0 regardless of Ca concentration. Similarly, below 0.01 mM Ca nodulation was also zero regardless of acidity. Above these critical values of Ca and acidity a wide range of Ca and H ion would produce maximum nodulation. Because of the effects of Ca and H on both the host and the bacterium they concluded the Ca requirement for nodulated plants was higher than for host or Rhizobium alone.

The effect of Ca on nodulation also has been observed on plants growing in soil. Albrecht and Davis (1929) found that the addition of Ca as $CaCO_3$ or $CaCl_2$ improved nodulation on acid soils already well inoculated with Bacillus radicicola (Rhizobium). They found that 10-day-old plants liberally supplied with Ca nodulated better when transplanted to acid soils than similar plants grown in a medium low in Ca. Studies using clays saturated with Ca or K indicated that nodulation was greater in Ca saturated clay than in K saturated clay. Holding and King (1963) found that the effectiveness of Rhizobium on 48 sites was correlated to the base saturation of the soil in the highland pasture soils in Scotland. Helyer and Anderson (1970) reported that lucerne growing in soils whose pH ranged from 4.9 to 5.4 and with 25 to 50% of the C.E.C. occupied by Al required high levels of Ca for nodulation, but that the need for Ca could be largely removed by the addition of N. They concluded that the main function of Ca in this instance was to improve conditions for nodulation. Robson and Loneragan (1970), while working with Medicago trunculata, observed that under acid conditions $CaCO_3$ improved nodulation and that the effect of $CaCO_3$ could be replaced by increasing the level of inoculum. The results suggest that the endophyte could not survive and/or reproduce under the acid conditions present. Clearly then, the solution culture work and the results of $CaCO_3$ additions to soil indicate that Ca plays an important role in nodulation. The role of Ca appears to be twofold; a direct effect involving Ca in one of the phases of nodule initiation and indirectly through the effect of Ca on soil properties both chemical and physical (Munns, 1970).

The role of nodulated plant species in increasing soil N has been well documented in the past and needs little comment at this time. However, in addition to their ability to increase N levels in the soil, many nodulated species also increase the level of other nutrients, in particular, the basic cation levels in the soil. Garman and Merkle (1938) reported that locust not only increased the N content of the soil but also increased the level of Ca, Mg, and K. In addition to the increase in nutrient status, the soil structure was also improved.

Nonlegumes

A great many of the factors which affect nodulation of legumes also influence nodulation of nonleguminous plants but because the economic importance of the latter group has only recently been realized, there is much less known about the nodulation of this group. Despite this fact much insight may be gained by comparisons made with the legume system. Since little has been reported on the influence of Ca on nodulation of nonlegumes, some of the literature available on the influence of soil acidity may be helpful and pertinent.

Bond (1951), working with *Myrica gale* L., concluded that the endophyte from root nodules on this species must be more acid tolerant than legumes because they occurred naturally in more acid habitats than did legumes. Nodulated plants grew well at pH 5.4 with some nodulation at a pH as low as 3.3. Non nodulated plants supplied with NO_3 - N grew best at pH 3.3 with the growth rate decreasing with decreasing acidity to pH 6.3. Casuarina nodulated best near neutral pH with the nodulation decreasing rapidly with increased acidity. Infection failed to occur at pH 4.0 and was very poor at pH 5.0. The acid tolerance of Casuarina was much lower than Myrica or Alnus (Bond, 1957). Ferguson and Bond (1953) observed that nodule formation on Alnus glutinosa occurred most freely over the range pH 5.4 to 7.0 but that best plant growth occurred from pH 5.2 to 5.4. Thus, the host obviously is more tolerant of acid conditions than is the nodule organism. The same plants grew well in the range pH 3.3 to 4.2 when supplied with NO_3 - N but would not nodulate under these strong acid conditions.

The effect of combined N on nodulation has been reported by many workers. Stewart and Bond (1961) showed that the nodules on Alnus and Myrica grown in water culture tended to be fewer but larger than on plants in solutions free of NH_4 - N. Stewart (1963) reported the same effect of combined N on nodulation of Myrica gale and Casuarina cunninghamiana. He concluded that in these two species nodulation would not be affected by combined N normally present in the soil. Rodriguez-Barrueco, MacKintosh and Bond (1970) reported that NH_4 - N at low levels stimulated nodulation of Ceanothus but at high levels of N nodulation was retarded. Zavitkovski and Newton (1968b) found that nodulation and growth of red alder was favorably influenced by increase in total soil N (TSN). When TSN was low, nodules were few but large and when TSN was high, nodules were numerous but small. Red alder nodulation was adversely affected by urea as a N source but NO_3 - N in the range 15 to 30 ppm stimulated nodulation. Higher levels of NO_3 - N depressed nodulation in red alder.

III. DESCRIPTION OF SAMPLING AREAS

Location

Soils and field data used in this study came from three areas. Two of the sample locations were in the Western Cascade Range of Oregon, the third was located in Central Oregon a few miles south of Bend. The first area was on Foley Ridge which is located approximately 40 miles east of Eugene in the McKenzie River drainage system. The Foley Ridge sampling sites were all located in T16S, R6E and R7E, W.M., Lane County, Oregon. The second sampling area, also located in the Western Cascades, was located in the vicinity of Toad Creek. The map locations may be given as T13S, R6E, Sec. 26 and Sec. 12, W.M., Linn County, Oregon. The third sampling area was located in Central Oregon in T19S, R11E, Sec. 8, W.M., Deschutes County.

Vegetation

The Western Cascade sampling sites are in the "Tsuga Heterophylla" Zone (Franklin and Dyrness, 1968). This region is dominated by a sub-climax _Pseudotsuga menziesii_ association. The climax vegetation would be dominated by a _Tsuga heterophylla_ - _Thuja plicata_ association. The sampling sites in Central Oregon are in a "Pinus Ponderosa" Zone. The ponderosa pine communities are maintained in this region by periodic fires (Franklin and Dyrness, 1968).

The stand on the Toad Creek area was old growth Douglas-fir (300 to 500 years old) with an understory of western hemlock (Tsuga heterophylla (Raf.) Sarg.). The western hemlock is basically in two age classes; one, 250 years old and older and the second, between 100 and 200 years old. The old growth stand is characterized by a closed canopy with very little shrub growth in the understory. In small openings of the canopy vine maple (Acer circinatum Pursh.) may often be found. In depressional areas and next to Toad Creek itself western red cedar (Thuja plicata Don), grand fir (Abies grandis (Dougl.) Lindl.), and western white pine (Pinus monticola Dougl.) may also be found.

The Foley Ridge sample sites, which are in the same zone as the Toad Creek sites, may best be described by dividing the ridge into two parts based on elevational differences. Below about 3000 feet the dominant species is Douglas-fir with minor amounts of western hemlock as an understory. Varying amounts of grand fir and western red cedar are found on the deeper soils and on the more moist sites. Incense cedar (Libocedrus decurrens Torr.) and golden chinquapin (Castanopsis chrysophylla (Dougl.) A. D. C.) are found on the drier sites. The lesser vegetation is comprised of vine maple, Pacific dogwood (Cornus nuttallii Aud.) and some hazel (Corylus cornuta

Marsh. var. californica (A. D. C.) Sharp.). The productivity in terms of Douglas-fir is generally good. The site class is II and the site index ranges from 160 to 170 feet at 100 years. The vegetation above 3000 feet is still dominated by Douglas-fir but contains much more western hemlock. The shrub layer consists of vine maple, salal (Gaultheria shallon Pursh.), rhododendron (Rhododendron macrophyllum D. Don.), Vaccinium spp., and ocean spray (Holodiscus discolor (Pursh.) Maxim). Site quality in terms of Douglas-fir is usually III and IV with site index ranging from 120 to 150 feet at 100 years.

The Fort Benham site, in Central Oregon, is in the "Ponderosa Pine" Zone. The vegetation consists of mixed stands of ponderosa pine (Pinus ponderosa Laws.) and white fir (Abies concolor Lindl.) with smaller amounts of lodgepole pine (Pinus contorta Dougl.). The shrub vegetation consists of snowbrush, green manzanita (Arctostaphylos patula Greene) and bitterbrush (Purshia tridentata (Pursh) DC.) in varying amounts of each species. The amounts of each of the brush species reflect not only the stand density but also the moisture available in the soil.

Soils

The soils of all three of the sample areas have been influenced by ash and pumice to some extent. The percentage of the clay fraction of all three soils is small and is dominated by amorphous constituents.

The soils on Foley Ridge range from shallow stoney soils developed mainly on the steeper terrain with poorly developed, often truncated profiles, to deep, well developed profiles occurring on benches and on the lower parts of gentle slopes. Textures range from coarse gravelly sandy loam to clay loam. Under the existing coniferous forest canopy 2.5 to 5 cm of litter accumulates on the surface. About one-half of the surface organic matter is undecomposed or only slightly decomposed. The remaining one-half is largely well decomposed forming the H horizon. The soils immediately to the west of Foley Ridge have been classified as Reddish Brown Lateritic, Yellowish Brown Lateritic, and Regosols (Stephens, 1964). The soils on Foley Ridge closely resemble the Carpenter series described by Stephens (1964).

The Toad Creek soils are similar to those found on Foley Ridge. Both are developed from similar parent materials, and under similar climatic and vegetative influence. The soil textures are a little coarser being mainly sandy loams. The litter accumulation on the surface is also slightly thicker (2.5 to 7.5 cm). The structure is, however, different. Toad Creek site soils have a weak structural development particularly in the surface horizon. The structure may best be described as fine or very fine granular to single grained. The soil structure of the Foley Ridge soils is generally coarse granular or blocky. The blocky nature is particularly apparent on the deeper soils with the higher productivity.

The soils in the Fort Benham area are much different from the two already described. They are derived almost entirely from ash and pumice. They have developed under a much drier climate with quite different vegetation than that found in the Western Cascade region. Soil profile development is limited to an A and an AC horizon, unlike the soils on the previous sites which have a distinct B horizon. The A horizon is delineated by a slight increase in organic matter. The structure throughout the profile is single grained reflecting the coarse nature of the geologic deposit and the lack of soil development. The texture is generally very coarse loamy sand. Because of the hot, dry climate, organic matter does not accumulate, thus there is an absence of a thick undecomposed litter horizon. The organic horizon is thin and discontinuous. Open areas are devoid of any accumulation of organic matter, whereas under trees it may be 0.5 to 1 cm in thickness.

The chemical differences which exist between these soils and their implications will be discussed in a later section.

Description and Recent History of Sampling Units

The Fort Benham unit (F. B.) was the only unit included in the study which was not logged. The unit was occupied by an open stand of ponderosa pine with some white fir and lodgepole pine. Understory vegetation consisted mainly of snowbrush, manzanita, and bitterbrush.

In the fall of 1962 a wildfire killed all the existing vegetation, thereby preparing the unit for snowbrush germination in the spring of 1963. At present the unit is covered with a dense stand of snowbrush with some manzanita, gooseberry (Ribes velutinum Greene), and some grass species, particularly under the snowbrush crowns.

In the Toad Creek area two clear cuts were selected. The clear cut units are designated Toad Creek No. 1 (T. C. 1) and Lava Lake No. 7 (L. L. 7). T. C. 1 was logged in 1962 and the slash burned that fall. Snowbrush thus became established in 1963. L. L. 7 was logged and burned in 1962 with snowbrush establishing the following spring. Prior to logging, both units were occupied by a 300- to 500-year-old Douglas-fir - western hemlock stand typical of this region. Snowbrush would have been absent from both units for at least 300 years prior to its establishment following the slash fire. Presently T. C. 1 has approximately a 75% cover of snowbrush, whereas L. L. 7 has only about a 30% cover.

The third area used for this study was Foley Ridge. Seven clear cut units were selected. Four of the units were below 3000 feet elevation and three above. The four lower units have the map designation Northwest Foley No. 2 (N. W. F. 2), Southwest Foley No. 2 (S. W. F. 2), Spring Creek No. 1 (S. C. 1), and Spring Creek No. 2 (S. C. 2). N. W. F. 2 and S. W. F. 2 were both logged and the slash burned in 1959. S. C. 1 and S. C. 2 were logged and burned in 1969.

Snowbrush germinated and became established the spring following the fall slash burn on all four units. The forest vegetation prior to logging was dominated by a 300- to 400-year-old Douglas-fir stand with an understory of western red cedar and western hemlock; snowbrush was undoubtedly absent for a prolonged period prior to logging. These four units are the most productive in terms of Douglas-fir site index. N. W. F. 2 and S. W. F. 2 are at present supporting a thick stand of snowbrush, about 90% cover, with a well stocked stand of Douglas-fir emerging through the brush canopy. S. C. 1 and 2 are nearly devoid of cover but a large number of snowbrush germinants are in evidence.

The three remaining units, Foley Trail Cabin No. 1 and No. 2 (F. T. C. 1 and F. T. C. 2) and Foley Ridge No. 2 (F. R. 2) were logged and burned in 1960, 1961, and 1962 respectively. The units are less productive than the four previous units. As on the previous units, snowbrush became established following slash burning operations. The number of snowbrush germinants or their survival was low. Snowbrush cover on these units ranges from approximately 25 to 50%. Survival rate for planted Douglas-fir seedlings was low, thus the units are poorly stocked.

Changes in vegetation on F. B., T. C. 1, and F. R. 2 have been followed by Youngberg and Wollum (1970) since logging or fire removed the previous timber stand and snowbrush became established.

Information on the number of snowbrush seedlings, snowbrush biomass, percent cover, litter production, percent plants nodulated, and N accretion with time has been determined on all three units.

IV. METHODS AND MATERIALS

Effect of Snowbrush on Soil Nutrient Levels

Changes occurring in soil nutrient levels as a result of snowbrush occupancy following logging and/or fire were determined on three units (F. B., F. R. 2, and T. C. 1). These units were selected because the nutrient levels of the soil immediately after logging and slash burning or wildfire were determined by Wollum (1965). Permanent sample plots were also established on these three units by Youngberg and Wollum (1970). The purpose of the permanent plots was to follow changes in vegetation and biomass which occurred on these sites after snowbrush became established. There is, therefore, a substantial amount of information available regarding the vegetation, the changes in vegetation, and the nutrient levels of the soil at the time of snow-brush establishment on these three sites.

To determine the changes which occurred in the soil nutrient status at least nine samples were collected on or immediately adjacent to the permanent plots. The soil was sampled to a depth of approximately 20 cm. This depth was used because most of the nodules occurred within this range. The soil samples on both T. C. 1 and F. B. were obtained under dense stands of snowbrush and only under nodulated plants on T. C. 1. The soil samples on F. R. 2 were likewise obtained from under snowbrush plants but since the unit has less than a

25% cover of snowbrush and the percentage of nodulated plants is also low, it was impossible to limit soil sampling to just those plants which were nodulated.

Soil samples were also obtained from under timber stands adjacent to T. C. 1 and F. R. 2. Nine soil samples were obtained from the timbered area adjacent to F. R. 2 and 11 next to T. C. 1. As in the clear cuts, only the surface 20 cm of mineral soil was sampled. The sample sites under the forest canopy were randomly selected.

The soil samples were returned to the laboratory, air dried, sieved to pass through a 2 mm screen, and the C. E. C., meq of Ca, Mg, and K, pH, percent organic matter, and total soil N were determined. Comparisons were then made between the levels of nutrients found currently under snowbrush stands and the levels of nutrients as reported by Wollum (1965) 1 year after logging and/or fire and the levels of nutrients which exist in the soil under adjacent timber stands.

Soil Base Status and Nodulation

Since it is impossible to follow a problem area from a fresh clear cut to a well nodulated snowbrush stand because of the long time period involved, a number of clear cut areas of various ages were selected and the percent base saturation and frequency of snowbrush nodulation were determined. Ten units, with varying degrees of snowbrush cover and different ages of snowbrush, were selected throughout Oregon. A brief description of the 10 units is given in the preceding section. Of the 10 units, one was in Central Oregon and nine in the Western Cascade region. Of the nine Western Cascade units, three units (T. C. 1, N. W. F. 2, and S. W. F. 2) have a dense cover of showbrush; four units (F. R. 2, F. T. C. 1, F. T. C. 2, and L. L. 7) have a very light cover of snowbrush, and two units (S. C. 1 and S. C. 2) are recent clear cuts with no snowbrush cover as yet. Three of the 10 units were the units on which the permanent sample plots were established. The soil on these units, T. C. 1, F. R. 2, and F. B., was previously sampled as described earlier and, therefore, these units were not sampled again at this time.

At least six soil samples were obtained from each of the seven units. The sampling was confined to the surface 20 cm of mineral soil as in the previous sampling. The samples were obtained randomly throughout each unit. The soil samples were returned to the laboratory, air dried, sieved to 2 mm, and the C. E. C. and percent base saturation determined.

The percentage of snowbrush plants which were nodulated was determined by digging up a minimum of 10 plants on each unit and ascertaining if they had nodules or not. No attempt was made to determine the number or mass of nodules on each plant.

Concurrently with the determination of the percent nodulation, the current year's growth was sampled from each of the plants which were dug up. The samples were returned to the laboratory, dried in a forced air oven at 70°C, and analyzed for major nutrients. An attempt was made to relate nutrient levels in the plant to soil nutrient levels and nodulation.

Nodulation Potential of Three Soils

The rate at which snowbrush plants nodulate following the establishment of the brush varies markedly from site to site in the Cascade region. The base saturation of the soil may be one of the factors which plays a vital role in determining the rate at which snowbrush nodulation may proceed. To further determine if soil base saturation may be implicated in snowbrush nodulation, the nodule potential of three soils, each with a different base level, was compared. The three soils were obtained from the following units or locations: from the timber stand adjacent to T. C. 1 (Toad Creek forest soil), F. R. 2, and S. C. 1. The base saturation of the soils was 8.3, 16.3, and 63.6% on Toad Creek forest soil, F. R. 2, and S. C. 1 respectively. Sufficient soil was obtained from each site to conduct a number of experiments in the greenhouse and growth chamber. The surface 20 cm of mineral soil was screened through a 0.5-cm mesh screen, then stored in plastic bags at field moisture content until it was needed for the various experiments.

The three soils (Toad Creek forest, F. R. 2, and S. C. 1) were inoculated using F. B. and T. C. 1 soil as the inoculum source. The inoculating soils were obtained in both cases from among the roots of nodulated snowbrush plants. The soils were screened using a 2 mm sieve and stored in plastic bags to retain their field moisture content.

Each of the three soils (Toad Creek forest, F. R. 2, and S. C. 1) was inoculated with four levels each of the two inoculum sources. Each of the inoculating soils was added at the rates of 0, 2, 10, 25, and 50% on a dry weight basis. The total weight of the soil in each pot was 2000 g. Each treatment was replicated three times, thus for one soil with both sources of inoculum 27 pots were used.

The soils were brought to field capacity with distilled water and eight snowbrush seeds were sown in each pot. The moisture content was maintained near field capacity by weighing 10 to 12 pots at each watering; the remaining pots were then watered on the basis of an approximate volume of water needed by the pots which were weighed. The plants were grown for 40 weeks in the greenhouse under daylight supplemented by fluorescent lighting to maintain a 16-hour day length.

At the end of 40 weeks the plants were harvested. The tops were oven dried at 70°C and the dry weight determined. The roots were washed free of soil, nodules were counted, removed from the roots, and a fresh nodule weight determined. The results were recorded on a number and weight per pot and per plant basis. The tops were kept for further chemical analysis.

Effect of Calcium on Nodulation

Snowbrush has been found to significantly increase the base level of the surface soil with time. Subsequent experiments and field data indicate that soil base level may be an important factor in snowbrush nodulation in the Cascades. To further determine the effect of base levels on nodulation, the percent base saturation on Toad Creek forest soil, which is slow to nodulate and low in percent base saturation, was increased by the addition of Ca. Two sources of Ca were used, namely $CaCO_3$ and $CaSO_4$. The base level of Toad Creek forest soil was increased by 25, 50, and 75% of the soil C. E. C.; that is, 6, 12, and 18 meq of Ca were added to this soil. The addition of 6 meq of Ca was intended to reflect the base levels found on T. C. 1 after 7 years under a dense snowbrush stand. The addition of both 12 and 18 meq of Ca, that is, an increase of 50 and 75% in the percent base saturation respectively, was intended to bring the base levels up to those found on areas where nodulation did not appear to be a problem.

The experiment was conducted both in the greenhouse and in the growth chamber. The greenhouse experiments were conducted in pots which were 17.8 cm in diameter by 19.1 cm deep, containing 2000 g of soil each.

Appropriate amounts of $CaCO_3$ and $CaSO_4$ were added to each pot and thoroughly mixed with the soil. Each treatment was replicated three times, thus a total of 21 pots was used. Each pot was sown with eight seeds and watered with distilled water as already indicated.

The entire experiment, consisting of 21 pots, was repeated in the growth chamber. A second experiment was also conducted in the growth chamber. The same levels of $CaCO_3$ and $CaSO_4$ were added to Toad Creek forest soil but in addition burnt conifer litter was added to the soil surface in each pot. The coniferous litter was collected under an old growth Douglas-fir - western hemlock stand adjacent to T. C. 1. The litter was air dried then burned using a propane torch. Twenty-five g of the burnt litter was then added to the soil surface of each pot. The pot size used in the growth chamber study was 10. 2 cm in diameter by 11. 5 cm deep. The pots were uniformly filled with 500 g of soil for all trials. Four snowbrush seeds were sown into each pot. Watering was conducted as before. A summary of all the Ca treatments which were applied is given in Table 1.

The seeds were allowed to germinate and grow for about 2 weeks then the soil in each pot was inoculated with a crushed nodule suspension. The crushed nodule suspension was made from nodules which were collected fresh from the field as needed for each use. Nodules were collected from T. C. 1 site, packed in soil, returned to the laboratory, then washed free of soil using tap water. Once the nodules were free of soil they were rinsed in distilled water, dried, and weighed. A crushed nodule suspension was made by grinding 10 g of

Table 1. Summary of experiments used to evaluate the effect of Ca, N, and S on the nodulation of snowbrush in Toad Creek forest soil.

Experiment no.	Source of Ca	Levels of Ca added (meq/100 g)	Source of N	Levels of N added (ppm)	Source of S	Levels of S added (ppm)	Burnt litter added	Growth time (weeks)
1	$CaCO_3$	0, 6, 12, 18	none	0	none	0	0	40
2	$CaCO_3$	0, 6, 12, 18	none	0	none	0	+	40
3	$CaSO_4$	0, 6, 12, 18	none	0	$CaSO_4$	960, 1920, 2880	0	40
4[a]	$CaSO_4$	0, 6, 12, 18	none	0	$CaSO_4$	960, 1920, 2880	0	65
5	$CaSO_4$	0, 6, 12, 18	none	0	$CaSO_4$	960, 1920, 2880	+	40
6	none	0	NH_4NO_3	0, 5, 10	none	0	0	10
7[b]	$CaSO_4$	0, 3, 6, 12, 18	NH_4NO_3	0, 5, 10, 20	$CaSO_4$	0, 480, 960, 1920, 2880	0	20
8[b]	$CaCO_3$	0, 3, 6, 12, 18	NH_4NO_3	0, 5, 10, 20	none	0	0	20
9	none	0	none	0	H_2SO_4	0, 5, 10, 20, 40, 80	0	20
10[b]	$CaSO_4$	0, -, 1.5, 3	NH_4NO_3	0, 5, 10	$CaSO_4$	0, 50, 220, 480	0	20
11[b]	none	0	NH_4NO_3	0, 5	S	500, 1000, 2000	0	20

All treatments were replicated three times on all experiments.

[a]Experiment 4 was conducted in the greenhouse, all others were in the growth chamber.

[b]Experiments 7, 8, 10, and 11 were factorial with nutrients applied in all possible combinations.

nodules in 100 ml of distilled water in a mortar. The greenhouse pots received 20 ml each and the pots in the growth chamber 5 ml each of this nodule slurry.

The plants were grown for 40 weeks. Temperatures in the growth chamber were kept at 26 $\pm$ 2°C daytime and 15 $\pm$ 2°C night temperatures. Greenhouse temperatures were subject to a greater degree of fluctuation and in the late fall never exceeded 24°C and were usually 21°C during the day. After 40 weeks, the plants were harvested and nodulation was determined as in the previous experiment.

Effect of Nitrogen on Nodulation

The effect of N on nodulation in Toad Creek forest soil was determined as below by the addition of NH_4NO_3 at the rates of 0, 5, and 10 ppm N. The NH_4NO_3 was added to 500 g of soil as a solution, the soil was then well mixed and returned to the pot. Each treatment was replicated three times. Three 3-week-old snowbrush seedlings were then transplanted into each pot. Within 72 hours, each pots was inoculated with 5 ml of crushed nodule suspension.

To obtain more uniformity in plants, snowbrush was first grown in a tray of perlite supplied with a full strength Hoagland's solution (Hewitt, 1966). Snowbrush seeds were sown onto a tray of perlite and then covered with a thin layer of perlite and sand. Nutrient solution was added as the need for watering occurred. Seedlings were grown for 3 weeks in the growth chamber. Seedlings which were uniform in size and had a well developed root system were selected and transplanted into pots containing soil.

The seedlings were grown under growth chamber conditions already described for 10 weeks. After 10 weeks the number and weight of nodules on each plant were determined.

The effect of increasing both the N and the base level on snowbrush nodulation in Toad Creek forest soil was determined. The base level of the soil was increased by adding Ca as $CaCO_3$ and $CaSO_4$. The N level was increased by the addition of NH_4NO_3. Nitrogen was added at the rates of 5, 10, and 20 ppm and Ca was added at the rates of 3, 6, 12, and 18 meq/100 g of soil. A summary of all the N and N plus Ca treatments is presented in Table 1.

Three snowbrush seedlings were transplanted from perlite into each pot. Each pot was then inoculated with 5 ml of crushed nodule suspension. The plants were grown in the growth chamber under the conditions previously described. After 20 weeks the plants were harvested and the nodule and plant growth response determined.

Effect of Sulfur on Nodulation

To determine if the increased nodulation observed with the addition of Ca as $CaSO_4$ was a response to S as well as Ca, H_2SO_4 at the rates of 5, 10, 20, 40, and 80 ppm S was applied to Toad Creek forest soil. The experiment was conducted in the growth chamber using 10.2-cm diameter plastic pots each containing 500 g of soil. Appropriate amounts of H_2SO_4 were applied to the soil in each pot, the soil was well mixed, and allowed to stand for 72 hours before three snowbrush seedlings were transplanted into each pot. The soil in each pot was then inoculated with 5 ml of a crushed nodule suspension.

The plants were allowed to grow 20 weeks and then harvested. Nodulation was determined on the basis of number and mass of nodules on each plant.

Sulfur was also added as $CaSO_4$ at the rates of 50, 220, and 480 ppm along with 5 and 10 ppm N as NH_4NO_3 to Toad Creek forest soil. The experiment was carried out in the growth chamber using a total of 24 10.2-cm diameter plastic pots. Appropriate amounts of $CaSO_4$ and NH_4NO_3 were added to the soil in each pot, the soil was thoroughly mixed, three snowbrush seedlings per pot were transplanted into the soil, inoculated as before, and allowed to grow for 20 weeks. After 20 weeks the plants were harvested and nodulation evaluated.

High levels of S were added as elemental S to Toad Creek forest soil. The S was added at the rates of 500, 1000, and 2000 ppm. Appropriate amounts of powdered S were added to 500 g of soil, the soil thoroughly mixed, brought to field capacity with distilled water, and allowed to incubate for 72 hours before plants were introduced into the system. Five ppm N as NH_4NO_3 were added to one block of the S replications. The procedures as outlined in previous experiments were followed in transplanting, inoculating, and harvesting. The plants in this experiment were allowed to grow 20 weeks in the growth chamber prior to harvest. A summary of the S experiments is given in Table 1.

Comparison of Inoculum Sources

The effectiveness of two inoculum sources in effecting snowbrush nodulation in Toad Creek forest soil was determined. The inoculum sources were fresh nodules collected from plants on T. C. 1 and Fort Benham soil. The nodules for crushed nodule inoculum were obtained fresh from the field, returned to the laboratory packed in soil, and prepared in the manner previously described. Fort Benham soil was collected from under nodulated snowbrush plants, sieved to 2 mm, and stored in plastic bags to maintain field moisture content until ready for use.

Both inoculum sources were introduced into Toad Creek forest soil and Toad Creek forest soil supplemented with 10 ppm N as KNO_3, 18 meq of Ca as $CaCO_3$, and 10 ppm N plus 18 meq $CaCO_3$ in the following manner. The Fort Benham soil inoculum was introduced by adding and thoroughly mixing 2% of the inoculating soil with Toad Creek soil. The crushed nodule inoculum was introduced into the soil adjacent to the roots of a newly transplanted snowbrush seedling. Ten ml of crushed nodule suspension were used to inoculate the soil in each pot. The inoculum was evenly distributed between the plants in each pot.

The experiment was carried out in the growth chamber using 14-cm square by 15.3-cm deep plastic pots with 1000 g of soil in each. The plants were allowed to grow 30 weeks before harvesting. Nodulation was evaluated as before.

The entire experiment, using the two inoculum sources and the addition of N and Ca, was repeated with F. R. 2 soil.

Growth of the Endophyte in Toad Creek Forest Soil

To determine if the endophyte would invade and grow in Toad Creek forest soil, a split pot experiment was used. Each pot was divided vertically in half with a piece of cardboard. One half was filled with Toad Creek forest soil inoculated with 2% Fort Benham soil, the other half was filled with Toad Creek forest soil and Toad Creek forest soil plus 6 meq of Ca:Mg in the ratio of 5:1. The Ca and Mg were added as $CaCO_3$ and $MgCO_3$ respectively. Each treatment was replicated three times. The cardboard divider was withdrawn thus allowing the soil in each half to be in contact.

Two 3-week-old snowbrush seedlings were transplanted to each half of the pot and were grown in the growth chamber for 10 weeks. The ease with which the endophyte would invade the Toad Creek forest soil without the 2% Fort Benham was evaluated on the basis of number and size of nodules produced in the non-inoculated half relative to the inoculated half.

The movement of the endophyte out of Fort Benham soil into Toad Creek forest soil was also investigated. A band of Fort Benham soil 1 cm thick was placed within the Toad Creek forest soil so that the top of this band was 2 cm below the soil surface. The Fort Benham soil was, therefore, in contact with Toad Creek soil underneath it and was covered by 2 cm of Toad Creek soil. Three small snowbrush seedlings were transplanted into each pot. Care was taken to select young seedlings with roots no longer than 2 or 3 cm, thereby insuring that the soil layers were not disturbed.

The plants were allowed to grow in the growth chamber for 5 weeks. At the end of this period the soil was carefully removed from around the roots and the distribution of nodules relative to the two soils was observed and recorded.

Laboratory Methods

Soil Analysis

Chemical determinations were made for some of the major nutrients required by plants, namely N, P, K, Ca, and Mg. In addition, soil acidity, percent organic matter, C. E. C., and percent base saturation were also determined. The procedures followed for each analysis are outlined by Roberts et al. (1971) and is the procedure normally used by Oregon State University Soil Testing Laboratory.

Foliar Analysis

Vegetative samples of snowbrush obtained either from field or from greenhouse and growth chamber studies were oven dried at 70°C for at least 48 hours. The samples were ground in a Wiley Mill to pass through a 20 mesh screen. The samples were stored in manila coin envelopes until needed for analysis.

Prior to analysis, samples were again placed in a 70°C oven for 24 hours. Appropriate amounts of material were then weighed out for the various analyses.

Nitrogen. Total N content of the samples was determined by micro-Kjeldahl procedure as outlined by Jackson (1958). One-tenth to 0.5 g samples were digested using H_2SO_4 and a $CuSO_4$ - Se catalyst. After digestion, NH_3 was distilled into a H_3Bo_3 solution and titrated with 0.1 or 0.05 N HCl.

Determinations of Ca, Mg, K, P, Mn, Fe, Zn, and Co. One-half or 1 g samples of dry plant material were weighed out and placed in a 125 ml Erlenmeyer flask. Ten ml of HNO_3 were added and the solution allowed to stand 12 hours. The solution was then heated until dense brown NO_2 fumes ceased. The samples were allowed to cool and 6 ml of 70% $HClO_4$ were added. The samples were returned to the hot plate and digested for 10 minutes after dense white fumes were coming off. The flasks were cooled and 10 to 20 ml of water were added. The solution was filtered through a No. 50 Whatman filter paper into a 50 or 100 ml volumetric flask depending on initial sample size. Aliquots of this solution were then taken for analysis of specific elements.

All the elements, except P, were determined on a Perkin-Elmer Atomic Absorption Spectrophotometer, Model 303 or 306. Standard conditions were used for each element determined as suggested by Perkin-Elmer Corporation (1971). Calcium and Mg were determined in the presence of 5 ml per 50 ml of a 15,000 ppm $SrCl_2$ to prevent interference from Al and P. Potassium was determined in the presence of 2000 ppm NaCl to minimize K ionization.

Phosphorus content was determined using the vanadomolybdo-phosphoric method. Ten ml of the standard digest were diluted to 50 ml (1/500). The determination was done on a Bausch and Lomb Spectronic 20 at a wavelength of 420 nm. The readings were compared to a standard curve formed over the range from 0 to 7 ppm P (Jackson, 1958).

<u>Sulfur</u>. The total S content of the snowbrush samples was

determined by a turbidimetric method. Two-tenths of a g of sample was weighed out in a 50-ml beaker. Four ml of 15 N nitric acid were added and evaporated to dryness. Then 2 ml of a 50% (w/v) $Mg(NO_3)_2 \cdot 6H_2O$ were added and evaporated to dryness. The sample was placed in a muffle furnace for at least 3 hours at 450°C. The ash was dissolved in 4 to 5 ml of 2 N HCl then quantitatively transferred to a 50-ml volumetric flask and brought to volume. A 20-ml aliquot was drawn and placed in a 50-ml volumetric flask and 5 ml of 50% acetic acid, 5 ml of 25% nitric acid, and 1 ml of orthophosphoric acid were added. The flask was brought to approximately 45 ml and well shaken. One g of $BaCl_2$ crystals was added without disturbing the contents of the flask. The flask was set aside 10 minutes, then inverted slowly twice. The inversion was repeated after another 5 minutes, and 5 minutes later each flask was inverted 10 times slowly. One ml of gum acacia (5 g/l of hot water) was added, the flask was brought to volume, inverted twice, and allowed to stand 1-1/2 hours. After 1-1/2 hours, each flask was inverted 10 times and the optical density was measured immediately on a Bausch and Lomb Spectrophotometer 20 at 490 nm. Standards were prepared from 0 to 12 ppm S.

V. RESULTS AND DISCUSSION

Past studies have shown that snowbrush nodulation is often delayed for several years following its establishment in the Western Cascades. The delay in nodulation is not, however, observed in Central Oregon in the ponderosa pine communities. Table 2 gives a summary of snowbrush nodulation in these two areas with time following a burn or clear cut and burn (Youngberg and Wollum, 1970). It is apparent, in some instances, that significant nodulation is delayed for at least 5 years in the Western Cascades. Several reasons have been advanced for the slow nodulation rate which is observed. Wollum _et al._ (1968) postulated that the lack of endophyte is caused by prolonged absence of the host plant from the area. Doubtless a prolonged absence of snowbrush from these sites probably causes a decrease in the endophyte population, but it is also possible that the endophyte loses its infectivity during this period. The question still remains as to why it takes so long for the population to build up again on some sites and not on others and which soil factors influence the growth and infectivity of the endophyte on these sites.

Wollum and Youngberg (1970) have shown that there is a marked increase in snowbrush biomass, dry matter production, percent snowbrush cover, litter production, and N accretion on sites slow to nodulate and this increase occurs at a point in time when percent nodulation increases. The question still remains, however, what is the cause of the sudden increase in nodulation?

Table 2. Percent snowbrush nodulation with time after its establishment on sites in the Western Cascades and Central Oregon.

Years after logging and / or fire	Percent nodulation		
	Ponderosa pine burn	Douglas-fir clear cut and slashburn	
	Fort Benham	Toad Creek	Foley Ridge
1	77.3	0	0
2	-	-	0
3	83.3	6.6	0
4	84.5	25.0	4.2
5	92.5	41.8	-
6	-	-	16.6
7	100.0	70.8	-

Influence of Snowbrush on Soil Nutrient Status

Some of the changes which occur in nutrient status of soils as a result of logging and snowbrush occupancy have been determined on three units (T. C. 1, F. R. 2, and F. B.). The changes in some of the major nutrients have been determined by comparing the soil chemical analysis made by Wollum (1965), who established permanent sample plots on these units immediately after logging and slash burning or wildfire, with a current soil analysis. A summary of these analyses for each unit is presented in Table 3.

Table 3. Summary of chemical analysis of soils from three units under various conditions.

Unit and site history	No. samples	Base status (meq/100 g) CEC	Ca	Mg	K	Percent base saturation	pH	Percent organic matter	Percent total N
Toad Creek 1									
350-yr-old DF-H	11	24.2	1.56	0.29	0.16	8.3	5.8	3.49	0.084
1 yr after logging	6	19.3	1.22	0.27	0.15	8.5	6.0	4.40	0.096
7 yrs after logging	9	24.7	6.47	0.38	0.21	28.6	6.1	4.76	0.116
		(13.1)[a]				(54.0)[a]			
Foley Ridge 2									
300-yr-old DF-H	8	23.0	1.09	0.18	0.13	6.1	6.0	4.27	0.093
1 yr after logging	7	25.0	0.76	0.31	0.21	5.1	6.1	5.64	0.098
6 yrs after logging	10	22.7	2.69	0.54	0.47	16.3	5.8	6.01	0.090
		(9.6)[a]				(38.5)[a]			
Fort Benham									
1 yr after fire	10	14.5	3.72	1.25	1.19	42.5	6.7	2.44	0.041
9 yrs after fire	13	10.5	3.35	0.87	1.12	50.8	6.8	1.21	0.036
		(7.5)[a]				(71.0)[a]			

[a]C. E. C. and percent base saturation determined at pH 6.0.

As was discussed previously, the F. B. unit is much different in terms of climate, vegetation, and physical soil characteristics than either T. C. 1 or F. R. 2. It is also apparent from Table 3 that the F. B. soil properties also differ from those of the other two units. F. B. is lower in organic matter and total soil N but has a higher pH and a higher base saturation. The differences in base levels are most apparent when comparing the sites immediately after logging or fire. The difference observed is not surprising when considering the differences in the climatic regime between F. B. and the other areas.

Perhaps of more significance is the change in the soil chemical characteristics which occurs after logging and burning and the occupancy of the area by snowbrush for a number of years. On both T. C. 1 and F. R. 2 it is apparent that there is an increase in base level of the soil under snowbrush stands. The largest single increase is in Ca but there are also increases in K and Mg. A similar increase in base level is not observed on the F. B. unit, despite the presence of snowbrush. Since this soil has a much higher initial level of Ca, Mg, and K, it is possible that the equilibrium in the cycling of Ca and other bases is less disturbed by removal of cover in the drier Fort Benham area than in the higher rainfall areas of the Cascade region. The increase in the base level of the soil on T. C. 1 is approximately 5.5 meq during the 7-year period after logging and burning. Similarly there is an increase in the base level of about 2 meq on F. R. 2. The difference in the amount of the increase between T. C. 1 and F. R. 2 is undoubtedly due to the difference in percent snowbrush cover on these two units. T. C. 1 has about a 75% cover of snowbrush, whereas the cover on F. R. 2 is less than 25%. The difference in snowbrush cover probably explains the difference in the amount of bases in the surface of the soil of these two units. An increase in the soil base level under snowbrush compared to areas devoid of snowbrush on the same clear cuts was shown by Scott (1970). Snowbrush is an important factor in the cycling of bases in this area.

Despite the shift in base level which occurs on the Western Cascade sites, there is very little change in the measured acidity. The reason for this is the nature of the clay fraction. The clay fractions of soils in the Western Cascades are high in amorphous constituents. Between 60 and 90% of the clay fraction is amorphous (based on personal unpublished data). The high amorphous clay and organic matter content results in a highly pH dependent C. E. C. The soil C. E. C. was determined at pH 7 which is higher than the pH of the soil in the field; therefore, the C. E. C. obtained is higher than it would be under field conditions. The base saturation thus appears too low for the measured pH. This does not, however, negate the observed increase in cations. This method of determining soil C. E. C. was used in this study to maintain uniformity with the previous work done by Wollum (1965) on these same sites.

The percent organic matter present in the soils shows very little change except on the F. B. site (Table 3), where an apparent decrease of 50% was observed. The high organic matter content on the F. B. site shortly after the wildfire may have resulted from the rapid decomposition of unburned litter from the fire killed vegetation. The organic matter content of the soil after 9 years probably more truly reflects the levels in equilibrium with the climate in this region. The percent soil organic matter on both T. C. 1 and F. R. 2 does not appear to have changed significantly with snowbrush occupancy. Despite the lack of change in the amount of organic matter present in the soil it is probable that the nature of the organic components has changed. Any coniferous litter left after slash burning has decomposed and is not evident under the snowbrush canopy. Snowbrush litter, unlike the coniferous litter, does not accumulate under dense stands of snowbrush. It decomposes more rapidly than coniferous litter and is incorporated into the soil quickly. The high N content (Youngberg, 1965) coupled with a more favorable microclimate created by the snowbrush canopy (Scott, 1970) contribute to the rapid decomposition of the litter. Snowbrush has been found to contain high levels of Ca; the levels are about 50% greater than those in other species commonly found in this area. The rapid decomposition of the snowbrush litter would then quickly alter the nutrient status of the soil in the area.

Snowbrush in the Western Cascades, therefore, appears to be instrumental in altering the base status, N level, and the nature of the organic matter of these soils. These changes may then be associated, at least in part, with the observed increase in nodulation.

Soil Base Status and Nodulation

The increase in base saturation which occurs with time under snowbrush cover may influence nodulation. Holding and King (1963) found a positive correlation between the percent base saturation and effectiveness of Rhizobium in white clover pastures in Central Scotland. The possibility that a similar relationship may exist with snowbrush in the Western Cascades has been considered.

Nine clear cut units in the Western Cascades and one in Central Oregon were selected and the percent base saturation and percentage of snowbrush plants nodulated were determined. A summary of the nodulation and base saturation on the various units is given in Table 4. Figure 1 shows the relationship between percent soil base saturation and percent snowbrush nodulation. A positive correlation appears to exist between the base saturation and percent nodulation in the field. A simple correlation analysis indicates that 78% of the variation in the data may be accounted for by the relationship of nodulation to base saturation. On the bases of the equation calculated $N = 8.42 + 1.47(B)$

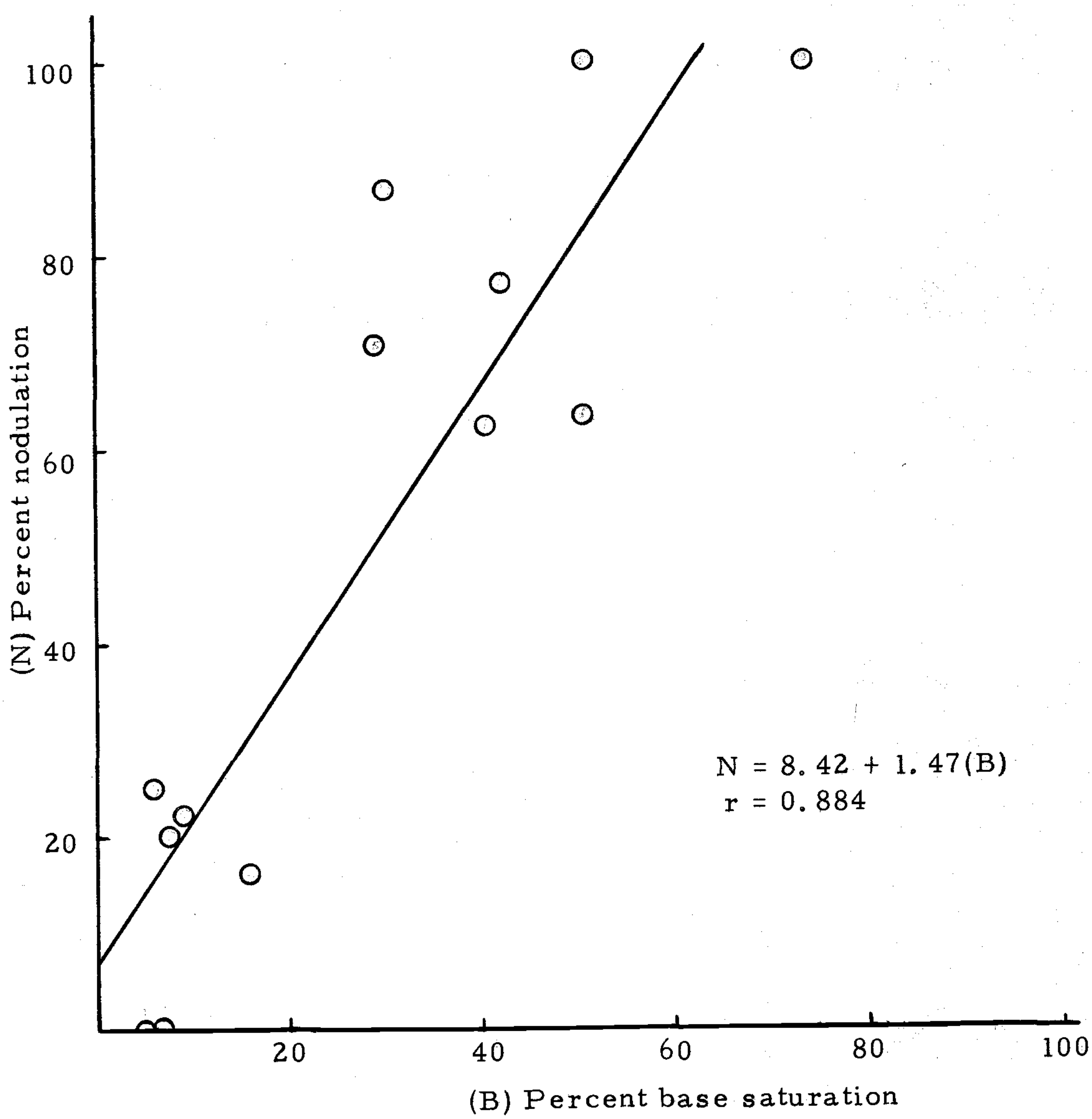

Figure 1. Relationship between percent base saturation of the soil on various units and percentage of nodulated snowbrush plants.

Table 4. Relationship between the percent base saturation of the soil and percent nodulation of snowbrush on various sites throughout Oregon.

Area	Logging unit	No. yrs. after snowbrush established	% Base saturation of soil	% Plants nodulated
Central Oregon	F. B.	1	42.4	77.3
	F. B.	5	50.8	100.0
Toad Creek	T. C. 1	1	8.5	0
	T. C. 1	5	28.6	70.8
	L. L. 7	8	8.8	23.1
Foley Ridge	F. R. 2	1	5.1	0
	F. R. 2	6	16.3	16.6
	F. T. C. 1	9	6.2	25.0
	F. T. C. 2	7	7.5	20.0
	N. W. F. 2	10	73.9	100.0
	S. W. F. 2	10	29.5	87.5
	S. C. 1	1	50.4	63.6
	S. C. 2	1	40.3	62.5

(where N = percentage of snowbrush plants nodulated, and B = percent base saturation of the soil), the nodulation of snowbrush may be predicted by determining the base saturation of the soil.

The units which were selected ranged from old clear cuts, such as N. W. F. 2 and S. W. F. 2 cut 10 years ago, to recent clear cuts, such as S. C. 1 and S. C. 2. The ages of the previously logged timber ranged from 300 to 500 years on all units.

A high percentage of snowbrush plants were nodulated on units with a high base saturation regardless of time elapsed since the unit was cut. Nodulation rate on S. C. 1 and S. C. 2 was high. One year after slash burning nodulation was 64 and 62% respectively, whereas on

T. C. 1 and F. R. 2 nodulation was zero at the same point in time. The base saturation on these units 1 year after logging was 50. 4, 40. 3, 8. 5, and 5. 1 on S. C. 1, S. C. 2, T. C. 1, and F. R. 2 respectively. The base saturation 5 years after logging on T. C. 1 was 28. 6% and after 6 years on F. R. 2 was 16. 3%, while the percent nodulation was 70. 8 and 16. 3% respectively.

On a unit such as N. W. F. 2, which was 100% nodulated 10 years after logging (Table 4), nodulation probably began 1 year after logging and burning. A comparison of base saturation in timber stands adjacent to this unit reveals the soils to have a base saturation of 20. 1% (Appendix I, Table 2). Thus this unit may not have been as well nodulated as S. C. 1 and 2 but much better nodulated than T. C. 1 or F. R. 2 at the same time following slash burning.

The high base levels found under forest stands adjacent to S. C. 1, S. C. 2, and N. W. F. 2 may be due to the presence of western red cedar in the understory in these stands. Western red cedar has a much higher Ca content in its foliage than either Douglas-fir or the other western conifers found in this area (Gessel, Walker and Haddock, 1950). Since western red cedar may represent up to 50% of the understory species in these stands, it may be responsible for maintaining the high Ca levels found in the surface soil in this area.

That a correlation between soil base saturation and percent nodulation exists should not be surprising. Previous work has indicated that soil pH is strongly related to nodulation in both legumes and nonlegumes (Bond, 1951, 1957; Ferguson and Bond, 1953; Lie, 1969; Munns, 1968 a, d, 1970). Thus since pH and base saturation are closely related, it might be expected that a relationship should also exist between base saturation and nodulation. Because it has been impossible to follow any one site from coniferous cover through to a well nodulated snowbrush situation, a great many variables are included in the correlation which could significantly influence the relationship and which are not properly evaluated. The different units not only vary in their base status, but also in many other factors, including fertility level, soil physical conditions, and elevation, all of which may influence nodulation. Since 78% of the variation in nodulation is apparently explained on the basis of soil base saturation, it is highly probable that base status is significantly influencing nodulation in the Western Cascades.

Nodulation Potential of Three Soils

The nodulation potential of three soils, each with a different base saturation, may provide further evidence that the percent base saturation of the soil is implicated in the nodulation of snowbrush in the Western Cascades. The three soils were from Toad Creek forest site, F. R. 2, and S. C. 1, with a base saturation of 8.3, 16.3, and 63.6% respectively. The three soils were inoculated with five levels, ranging from 0 to 50%, of Fort Benham soil or Toad Creek soil taken directly from under nodulated snowbrush plants.

The number and mass of nodules per plant produced are summarized for all three soils and both inoculum sources in Table 5. Both nodule numbers and mass per plant were greatest in the soil with the highest base saturation, namely S. C. 1, then followed by F. R. 2, and lastly Toad Creek forest soil. On all three soils nodulation increased with increased levels of inoculum soil added. The Fort Benham soil was a better source of inoculum than T. C. 1 soil for all the soils except S. C. 1. S. C. 1 produced essentially the same number of nodules per plant regardless of the source of inoculum (Table 5 and Figure 2). The mass of nodules produced, however, did appear to be influenced by inoculum source. Fort Benham soil as the inoculum generally produced larger nodules in all except S. C. 1 soil where the reverse was true (Table 5).

Spring Creek soil appeared to provide a more favorable medium for snowbrush nodulation than did the other two soils used. Spring Creek soil produced nodulated snowbrush seedlings even when an inoculum was not added. The fact that 1.6 nodules per plant were produced in a soil taken from under a 400-year-old conifer stand would suggest that either the endophyte was present in the soil despite the long absence of the host or that the endophyte was capable of rapid multiplication in this soil once the host was growing in the soil.

Table 5. Number and mass of nodules per plant produced in three Western Cascade soils of different base status using Fort Benham soil and Toad Creek clear cut soil as inoculum sources.

Percent inoculating soil added	T.C. forest soil: Resultant base saturation	T.C. forest soil: No. nodules/ plant	T.C. forest soil: Wt. nodules/ plant (mg)	F.R. 2: Resultant base saturation	F.R. 2: No. nodules/ plant	F.R. 2: Wt. nodules/ plant (mg)	S.C. 1: Resultant base saturation	S.C. 1: No. nodules/ plant	S.C. 1: Wt. nodules/ plant (mg)
				Inoculated with Fort Benham soil					
0	8.3	0	0	16.3	0	0	50.4	1.6	16.4
2	8.7	0.5	1.5	16.7	3.5	26.8	53.0	5.0	22.0
10	10.2	5.0	15.7	18.0	8.9	39.1	55.4	9.4	45.8
25	13.7	8.4	25.5	21.0	12.2	52.4	50.8	15.4	54.6
50	21.4	12.8	28.9	26.8	9.4	44.0	52.5	32.5	53.3
100	50.8	10.3	45.1	50.8	10.3	45.1	50.8	10.3	45.1
				Inoculated with Toad Creek clear cut soil					
0	8.3	0	0	16.3	0	0	50.4	1.6	16.4
2	8.3	1.3	9.1	16.6	0.7	6.2	52.5	4.8	47.5
10	10.4	1.3	6.9	17.6	2.3	16.6	50.6	13.7	80.7
25	13.6	2.1	6.4	19.5	5.0	30.8	45.2	16.1	84.8
50	14.4	2.6	11.1	22.4	5.5	44.2	40.8	-	-
100	28.6	8.9	66.1	28.6	8.9	66.1	28.6	8.9	66.1

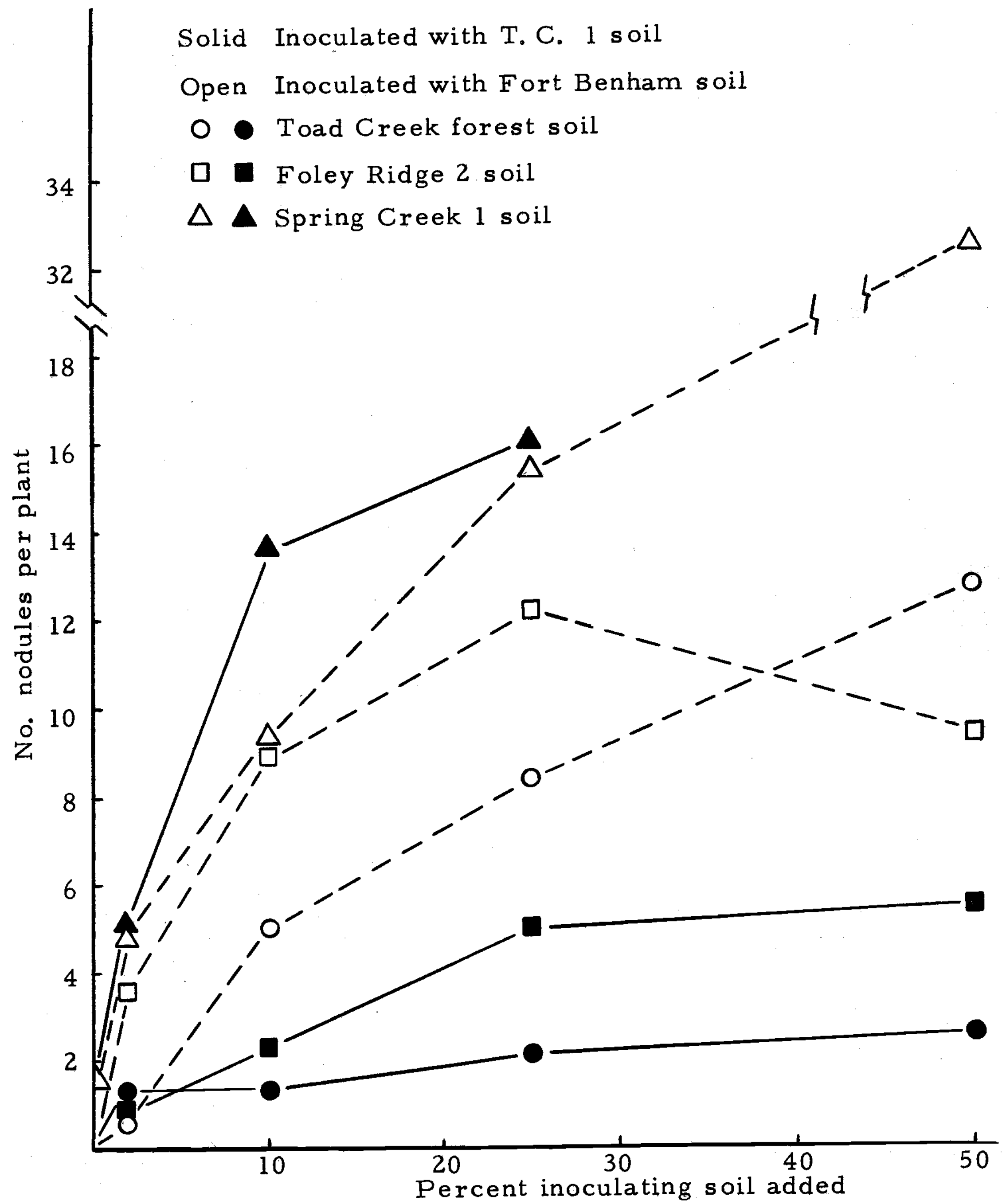

Figure 2. Number of nodules per plant produced in three soils, each with a different base level, by two inoculum sources.

Neither Toad Creek forest soil nor F. R. 2 soil produced nodulated plants in the absence of an inoculum source. The lack of nodulation on F. R. 2 soil suggests the inability of the endophyte to grow and multiply in this soil since the unit was logged and burned 6 years previously and supports only a poor stand of snowbrush at present.

The ability of the endophyte population to grow in each of these three soils may be deduced from the results in Figure 2. When Toad Creek forest soil or F. R. 2 soil is inoculated with T. C. 1 soil, both the nodule mass and numbers are less than when Fort Benham soil is the inoculating medium. Thus it may be safe to assume that the level of inoculum as supplied by T. C. 1 soil is lower than that supplied by Fort Benham soil. However, when T. C. 1 soil was added to S. C. 1 soil the same number of nodules per plant was produced as with Fort Benham soil inoculum. Since the level of endophyte in the T. C. 1 soil is lower than in Fort Benham soil, conditions for rapid increase of the endophyte population must exist in the S. C. 1 soil.

The addition of Fort Benham soil to the other soils particularly at the 25 and 50% rates probably significantly changed the chemical characteristics of the system (Table 5). The addition of 2 and 10% Fort Benham soil would not significantly alter the major nutrient status of these soils but it might significantly influence the micronutrient status. The use of T. C. 1 as an inoculum source would, therefore, minimize the chemical changes at the lower levels.

The results of this experiment do not prove that increased base saturation was responsible for the increased nodulation observed. The evidence presented by this experiment, coupled with the field observations, certainly support the hypothesis that base saturation of the soil in this region is associated with snowbrush nodulation.

Effect of Calcium on Nodulation

Evidence obtained from field data suggests that the base status of the soils in the Western Cascades may be a significant factor in snowbrush nodulation. Laboratory analysis of soils from areas where nodulation occurs readily indicates a high base saturation as well as a higher absolute level of bases in the soils (Table 4 and Appendix I, Tables 1 and 2). Similarly, on sites such as T. C. 1 where significant nodulation does not occur for several years following snowbrush establishment, an increase in the soil base status appears to occur concurrently with an increase in frequency of nodulation.

In an attempt to determine the role which soil base status may have on snowbrush nodulation, the base level of soil, collected under an old growth conifer stand adjacent to T. C. 1, was increased by the addition of Ca. Three levels of Ca were applied to the soil in experiments carried out in both the greenhouse and growth chamber. The levels of Ca applied were intended to increase the base saturation of this soil to levels observed on T. C. 1 after snowbrush occupancy for

7 years and to the levels found on sites where nodulation is not a problem (Table 4 and Appendix I, Tables 1 and 2). In these first experiments only the level of Ca was increased since it was shown to be the cation that increased most markedly on the T. C. 1 site (Table 3). Two sources of Ca were used, $CaCO_3$ and $CaSO_4$. Both sources of Ca were used because of their obvious effects on soil acidity.

The effect of the increased levels of Ca was evaluated on the basis of the number and mass of nodules produced. The results are given in Table 6. Both Ca sources significantly increased the nodule mass per plant. Nodule mass per plant was increased by a maximum of 127 mg per plant with the addition of $CaCO_3$ and by 212 mg per plant with $CaSO_4$. The nodule mass was significantly higher under all levels of Ca with $CaSO_4$ having a greater effect than $CaCO_3$. The increase in nodule mass with $CaSO_4$ compared to $CaCO_3$ was as high as 130 mg per plant.

Nodule numbers followed the same trends as did nodule mass. With both $CaCO_3$ and $CaSO_4$ additions, the number of nodules per plant was increased. The maximum increase was about 7 and 11 nodules per plant with $CaCO_3$ and $CaSO_4$ respectively. The difference in nodule numbers was significant at the 5% level with $CaSO_4$ but not with $CaCO_3$ (Appendix IV, Tables 1 and 2). As with nodule mass, there were more nodules produced with $CaSO_4$ as compared to $CaCO_3$. The maximum difference was about 8 nodules per plant but because of the variation within each replication, the difference was not statistically significant.

Table 6. Effect of Ca added as $CaCO_3$ and $CaSO_4$ to Toad Creek forest soil on the number and mass of nodules produced per plant after 40 weeks.

Meq. Ca added	$CaCO_3$		$CaSO_4$	
	No. nodules / plant	Fresh wt. nodules / plant (mg)	No. nodules / plant	Fresh wt. nodules / plant (mg)
0	3.8	102.5	3.8	102.5
6	6.5	176.3	14.5	306.6
12	10.1	192.8	14.0	314.5
18	10.7	229.6	10.2	279.6
LSD 5%	-	61.1	3.7	56.8

The increased nodule mass was the result of increases in nodule number rather than nodule size. The average weight per nodule ranged from 20 mg to 27 mg for both treatments. Thus the effect of increasing the Ca levels appears to be to initiate more infection points rather than just a difference in growth. The fact that more nodules form would indicate that the conditions for endophyte growth and/or infection have been enhanced by the addition of Ca to this soil.

Two other experiments carried out in the greenhouse and growth chamber tend to confirm the observations just reported. The results of a greenhouse experiment confirm the increase in nodule mass and numbers with $CaSO_4$ additions. The results of the greenhouse experiment are given in Table 7.

Table 7. Effect of Ca added as $CaSO_4$ to Toad Creek forest soil on snowbrush nodulation after 18 months.

Meq. Ca added	No. nodules / plant	Fresh wt. nodules / plant (mg)
0	2.7	276.6
6	11.2	406.8
12	14.5	743.1
18	12.6	730.5
LSD 5%	5.9	225.1

As in the growth chamber experiment, the greenhouse experiment, after 18 months, showed the same trend. The largest increase in nodule numbers and mass occurred with the addition of 12 meq of Ca with a slight decrease at 18 meq. The addition of 12 meq of Ca would represent an increase of 50% in the base saturation based on the C. E. C. of the soil as determined at pH 7.

The number of nodules per plant which were observed in both the 40 week and the 18 month experiments are remarkably similar. Both controls produced about three nodules per plant and a maximum of 14 nodules per plant were observed in both cases with the addition of $CaSO_4$. The nodule mass was, however, much greater after 18 months, but the increase compared to the control was quite similar.

The increase in nodule mass is about 2.5 times the control after 18 months and is three times the control after 40 weeks.

A similar comparison with $CaCO_3$ was lost because of disease. Since more than 50% of the seedlings were infected with powdery mildew and had to be removed, the results of this experiment were discarded.

A second experiment conducted in the growth chamber and grown for a period of 40 weeks will perhaps serve to confirm the results previously reported. The same soil as previously used with the same levels of Ca added as both $CaSO_4$ and $CaCO_3$ but in addition 25 g per pot of burnt conifer litter was applied as a mulch to the soil surface of each pot. The addition of the burnt litter was intended to approximate conditions that might be found in the field following logging and a slash burn. The results of this experiment are given in Table 8.

The addition of burnt conifer litter as a mulch to the surface of Toad Creek forest soil caused an increase in nodule numbers and mass about equivalent to the addition of 6 meq of Ca as $CaCO_3$ alone. The addition of Ca as both $CaCO_3$ and $CaSO_4$ in addition to the burnt litter produced a further increase in nodule numbers and mass. A comparison of these experiments is presented in Figures 3 and 4. Both the nodule numbers and mass per plant increased at all levels of added Ca. The increase in nodule mass appeared to be more pronounced with the higher levels of Ca, particularly with the $CaSO_4$

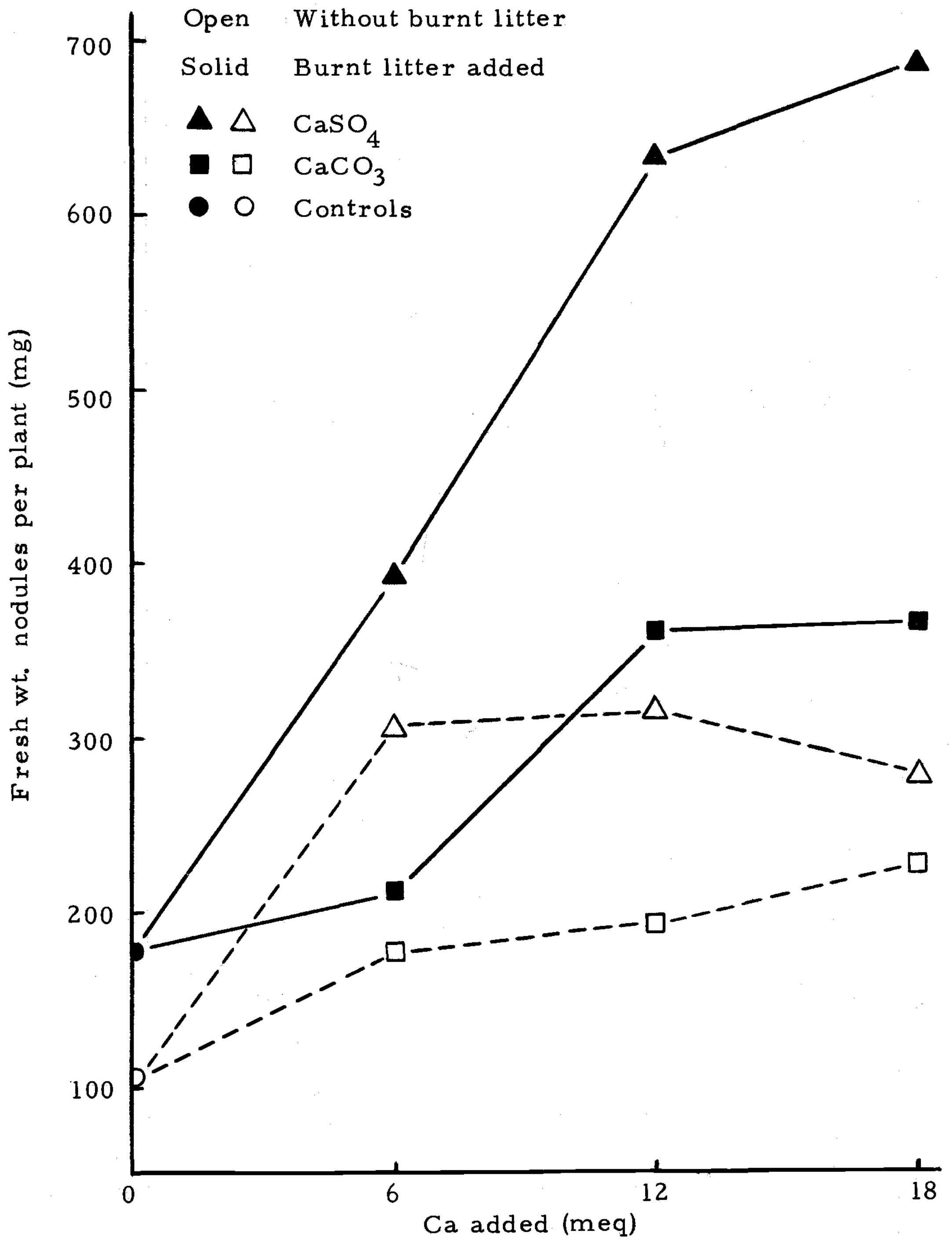

Figure 3. The effect of Ca added as $CaCO_3$ and $CaSO_4$ with and without burnt conifer litter on the weight of nodules per plant produced in Toad Creek forest soil.

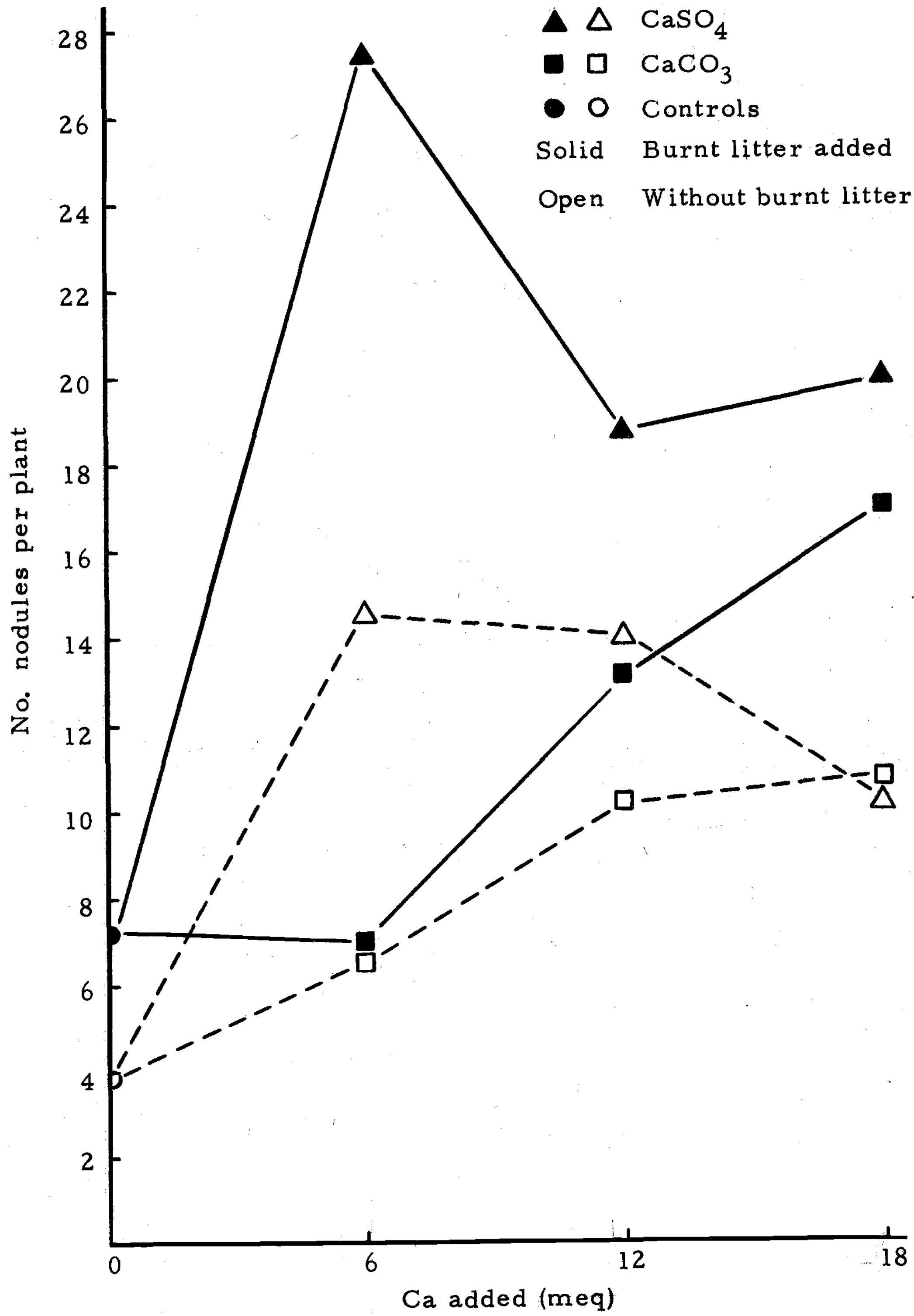

Figure 4. The effect of Ca added as $CaCO_3$ and $CaSO_4$ with and without burnt conifer litter on the number of nodules per plant produced in Toad Creek forest soil.

treatment. Nodule numbers increased but not to the same degree as the nodule mass.

Table 8. Effect of Ca added as $CaCO_3$ and $CaSO_4$ to Toad Creek forest soil with a surface application of burnt conifer litter on snowbrush nodulation after 40 weeks.

Meq. Ca added	$CaCO_3$		$CaSO_4$	
	No. nodules / plant	Fresh wt. nodules / plant (mg)	No. nodules / plant	Fresh wt. nodules / plant (mg)
0[a]	3.8	102.5	3.8	102.5
0	7.2	176.9	7.2	176.9
6	7.0	212.2	27.5	393.4
12	13.1	360.0	18.7	633.1
18	17.0	365.3	20.0	687.3
LSD 5%	-	63.5	4.7	159.0

[a]no burnt litter added

An analysis of the burnt litter indicates that the nutrient content was as follows: Ca, 1.30%; Mg, 0.75%; K, 0.20%; and N, 0.70%. Thus, in terms of increasing the base level approximately 16 meq of both Ca and Mg and 1.3 meq of K were added per pot. Since not all was available an estimate of the exchangeable Ca, Mg, and K reveals that 4.3, 1.0, and 0.75 meq were available via exchange respectively. The addition of burnt litter no doubt increased the level of all the bases not just the level of Ca. Thus the increased nodulation may be because of a better base balance. The possibility that other nutrients such as N, S, P, and some of the micronutrients may also have been added in sufficient quantities to influence nodulation should not, however, be discounted.

Effect of Nitrogen on Nodulation

Total soil N has been found to increase after snowbrush occupancy. One year after logging on T. C. 1, total soil N was 0.096% and 7 years later under a dense stand of snowbrush it was 0.116% (Table 3). The increase in total soil N appears to have occurred concurrently with the increase in frequency of snowbrush nodulation on this site. The detrimental effects of high N levels on nodulation has been well documented. Quispel (1958) reported that 3.75 meq of NO_3 per l (52.5 ppm) reduced the number of nodules formed on *Alnus glutinosa*. Zavitkovski and Newton (1968b) reported that with red alder when total soil N was low few but large nodules formed and when total soil N was high many but small nodules formed. They found that 15 to 30 ppm NO_3-N stimulated nodulation in red alder, but higher levels depressed nodulation. Rodriguiz-Barrueco *et al.* (1970) found that NH_4-N at low levels stimulated nodule growth in *Ceanothus*. It is apparent that nodulation is greater at low levels of N. A certain level of N is probably necessary to produce a vigorous host plant which, no doubt, is necessary if nodulation is to occur. Thus the initial level of N in the soil may be one of the determining factors in nodulation in Toad Creek forest soil. The increase in nodulation above that observed with increased Ca levels which was noted on Toad Creek forest soil with the addition of burnt conifer litter, containing 0.70% total N, may have been a response to N. The effect of N after 10 weeks on snowbrush nodulation in Toad Creek forest soil is given in Table 9.

Table 9. Effect of N added as NH_4NO_3 to Toad Creek forest soil on snowbrush nodulation after 10 weeks.

N added (ppm)	No. nodules / plant	Fresh wt. nodules / plant (mg)
0	2.0	7.8
5	6.3	15.8
10	7.8	23.3

The effect of up to 10 ppm N as NH_4NO_3 appeared to stimulate nodulation in the Toad Creek soil. Both nodule numbers and mass were increased by a factor of approximately four with the addition of 10 ppm N. Thus the availability of N may also be an important factor in snowbrush nodulation on this soil.

Since both N and Ca as $CaSO_4$ or $CaCO_3$ appear to stimulate nodulation in this soil, the effect of N and $CaCO_3$ and $CaSO_4$ combined was further investigated. Three levels of N as NH_4NO_3 and four levels of Ca were applied to Toad Creek forest soil. The results are given in Tables 10 and 11.

Both nodule numbers and mass increased with the addition of

Table 10. Fresh weight of nodules in mg/plant produced in response to increasing levels of NH_4NO_3 and $CaSO_4$ in Toad Creek forest soil after 20 weeks.

Ca added (meq)	ppm N 0	5	10	20	LSD 5%
0	5. 1	10. 2	14. 6	0. 1	6. 2
3	9. 1	35. 1	38. 9	21. 4	15. 4
6	21. 0	54. 5	50. 6	38. 6	9. 7
12	29. 3	60. 6	99. 7	59. 7	20. 5
18	39. 5	72. 3	89. 2	65. 2	NS
LSD 5%	15. 4	21. 6	23. 2	12. 7	

Table 11. Number of nodules per plant produced in response to increasing levels of NH_4NO_3 and $CaSO_4$ in Toad Creek forest soil after 20 weeks.

Ca added (meq)	ppm N 0	5	10	20	LSD 5%
0	1. 8	2. 2	3. 2	0. 2	1. 1
3	2. 9	6. 6	8. 8	5. 4	2. 9
6	5. 5	9. 8	9. 4	12. 2	NS
12	3. 8	5. 6	13. 8	12. 2	4. 3
18	2. 8	7. 4	8. 8	9. 2	NS
LSD 5%	NS	1. 7	3. 5	4. 5	

$CaSO_4$ at all levels of applied N. These results confirm the results obtained in previous experiments. Direct comparison is not possible because of the difference in growing times between the two experiments. The trends are, however, very similar. Nodule mass increased with each level of added $CaSO_4$. The increase diminished at the higher levels of $CaSO_4$. Nodule numbers, on the other hand, increased up to the 6 meq rate with both 0 and 5 ppm N, then they decreased at the higher rates of $CaSO_4$ application. With 10 and 20 ppm N applications, nodule numbers appeared to increase to a maximum at 12 meq of Ca as $CaSO_4$ (Table 11).

The addition of up to 10 ppm N as NH_4NO_3 increased nodule numbers and mass as in the previous experiment (Table 9). Nodulation was reduced with the application of 20 ppm N as NH_4NO_3 (Figures 5 and 6). The addition of $CaSO_4$ and N produced the same trend as N alone except that with each successive increase in $CaSO_4$ level nodulation was increased. Maximum production of nodules occurred when 10 ppm N as NH_4NO_3 and 12 meq of Ca as $CaSO_4$ were added to this soil (Figure 5).

An analysis of variance for both nodule mass and nodule numbers indicates that both $CaSO_4$ and NH_4NO_3 produced differences which were significant at the 1% level. The analysis indicated that an interaction between $CaSO_4$ and NH_4NO_3 treatments was also significant at the 1% level of probability (Appendix IV, Table 6). It would appear, therefore, that N up to 10 ppm enhances the effect of $CaSO_4$.

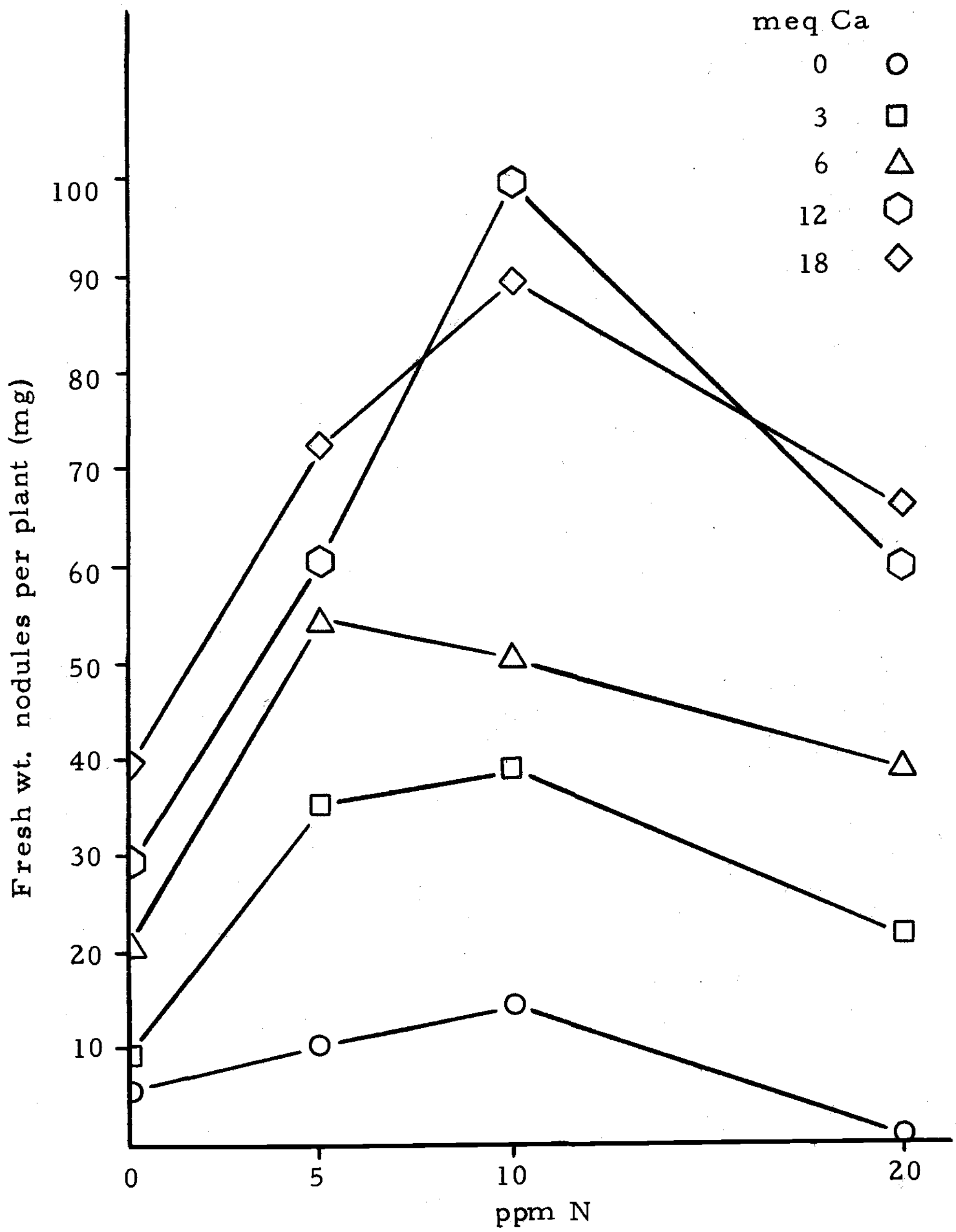

Figure 5. Fresh weight of nodules per plant produced in response to additions of NH_4NO_3 and $CaSO_4$ in Toad Creek forest soil.

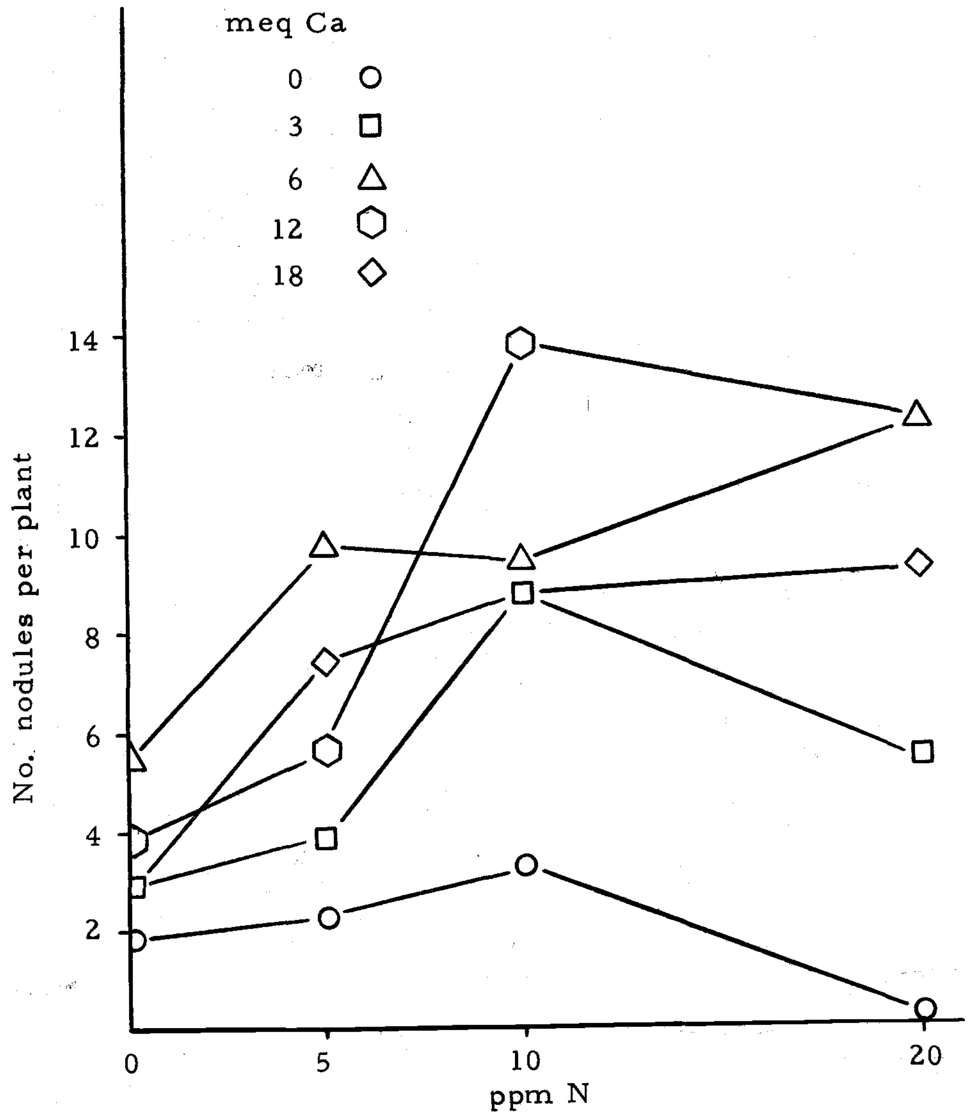

Figure 6. Number of nodules per plant produced in response to additions of NH_4NO_3 and $CaSO_4$ to Toad Creek forest soil.

Nodules failed to form in a similar study with NH_4NO_3 and $CaCO_3$. The complete lack of nodulation suggests that the inoculum source was not effective. This failure, plus several others, as well as certain inconsistencies which have been observed in the experiments, suggests the need for a more detailed investigation of the inoculum sources.

Effect of Sulfur on Nodulation

Snowbrush nodulation has been observed to be consistently better when $CaSO_4$ was used as a Ca source to increase the soil base level than when $CaCO_3$ was used. A definite possibility, therefore, exists that the S added by the addition of $CaSO_4$ may have an effect on the growth and nodulation of snowbrush in this soil.

Responses to S have been observed with many crops on many Oregon soils. Some of the largest increases have occurred with legume crops (Neller, 1925; Reimer and Tartar, 1919). Legumes show larger growth responses to applied S than grasses. The grasses probably do not respond appreciably to applied S because of limiting N supply (Jones and Martin, 1964). It is possible that nodulated non-legumes may also respond to S application in the Toad Creek soil.

The levels of S supplied in previous experiments in which 6, 12, and 18 meq of Ca as $CaSO_4$ were added were 960, 1920, and 2880 ppm S respectively (Table 1). At these high rates, it is certain, sufficient

S was supplied for any plant requirement at even the lowest level of applied $CaSO_4$. To attempt to determine if a S response was obtained, S at the rates of 10, 20, 40, and 80 ppm as H_2SO_4 was added to Toad Creek forest soil. The results are given in Table 12. It did not appear that the application of up to 80 ppm S had a significant effect on nodulation. Nodule numbers varied from 1. 6 to 2. 4 nodules per plant while the weight ranged from 8. 1 to 21. 1 mg/plant. Variations within replications were large. From the results of this experiment it is impossible to state that a response was obtained to the addition of S.

Table 12. Effect of S added as H_2SO_4 to Toad Creek forest soil on snowbrush nodulation after 20 weeks.

S added (ppm)	No. nodules / plant	Fresh wt. nodules / plant (mg)
0	2. 3	12. 9
10	2. 4	21. 1
20	2. 0	14. 4
40	1. 6	11. 8
80	2. 1	8. 1

The effect of S was further investigated using both $CaSO_4$ and elemental S as a source of S. Sulfur as $CaSO_4$ was added to Toad Creek forest soil at the rates of 50, 220, and 480 ppm S, the latter two rates are equivalent to 1. 5 and 3. 0 meq of Ca respectively. Two levels of N were applied to each S treatment, namely 5 and 10 ppm N as NH_4NO_3. This experiment was conducted as a continuation of the N and $CaSO_4$ study reported in Tables 10 and 11. The results are given in Table 13. A significant difference in nodulation did not occur until 480 ppm S was added. This level of S corresponds to 3 meq of Ca. Thus as already discussed, the response could be to increase base status as well as S. Since no significant response appears to occur with the addition of 50 or 220 ppm S, it is probably because there is no response to S and the increase in base level is too small to effect nodulation.

Table 13. Effect of NH_4NO_3 and low levels of S as $CaSO_4$ additions to Toad Creek forest soil on nodulation of snowbrush after 20 weeks.

S added (ppm)	5 ppm N		10 ppm N	
	No. nodules / plant	Fresh wt. nodules / plant (mg)	No. nodules / plant	Fresh wt. nodules / plant (mg)
0	2.2	10.2	3.2	14.6
50	4.1	16.5	3.0	14.7
220[a]	3.9	17.9	3.9	29.8
480[b]	6.6	35.1	8.8	38.9

[a] 1.5 meq Ca
[b] 3.0 meq Ca

The addition of 500, 1000, and 2000 ppm of elemental S to Toad Creek forest soil produced a response quite similar to that observed when 3, 6, and 12 meq of Ca as $CaSO_4$ was added. The $CaSO_4$ added supplied 480, 960, and 1920 ppm S respectively. The effect of the elemental S on nodulation is given in Table 14. A comparison of these results with those given in Tables 10 and 11 reveals that nodule numbers are quite similar but the weights with elemental S are generally higher. The effect of N did not appear to be as marked as with the $CaSO_4$ but a slight increase in nodule numbers, if not nodule weight, is apparent.

Table 14. Effect of high levels of elemental S and NH_4NO_3 additions to Toad Creek forest soil on nodulation of snowbrush after 20 weeks.

S added (ppm)	No N		5 ppm N	
	No. nodules / plant	Fresh wt. nodules / plant (mg)	No. nodules / plant	Fresh wt. nodules / plant (mg)
0	2.3	6.5	2.2	10.2
500	6.1	68.4	7.2	55.8
1000	6.2	51.1	8.3	66.4
2000	4.9	75.8	-	-
LSD 5%	NS	10.8	-	-

From the evidence thus obtained, it would appear that the addition of high levels of S to Toad Creek forest soil improves nodulation. The levels are such that it is most difficult to believe that the response is to satisfy any S deficiency which may exist in this soil. The addition of either elemental S or sulfate has caused an increase in soil acidity. The pH of the soil at harvest, with the addition of 0, 500, 1000, and 2000 ppm S, was recorded as 6.1, 5.9, 5.8, and 5.6 respectively. In previous experiments, $CaSO_4$ caused a similar increase in measured acidity. The increase in acidity was probably the result of a salt effect rather than an actual increase in soil acidity.

The oxidation of inorganic S has been found to reduce soil pH with a subsequent increase in soluble bases and soluble phosphates (McNeur, 1954). Ligon (1935) found that soluble Ca and K had increased as a result of lower pH caused by S oxidation. In addition to the bases and phosphates, the availability of Al, Mg, and Mn are also increased. Ammonification is increased and nitrification is decreased with application of elemental S or some sulfates (Cornfield, 1953; Fife, 1926).

The availability of trace elements is effected by soil acidity (Lucus and Davis, 1961). Liming increases the uptake of Mo but reduces that of Co, Zn, Fe, and Mn. The uptake of Mn begins to fall off above pH 5.5 to a minimum at pH 7.5, above which availability increases to a second maximum at pH 8.5. The behavior of B is similar, except that it begins to fall at pH 6.0. The availability of Cu reaches a maximum between pH 5.0 and 6.0. Zn availability falls off slowly above pH 6.0.

It is apparent from the foregoing discussion that the addition of S or sulfate could significantly effect the availability and uptake of a great many nutrients. Analysis of the snowbrush seedlings has not yielded any information which would suggest any single or group of nutrients which were effected by the addition of high S levels and which would produce the nodule response observed.

Nutrient Levels of Snowbrush

The nutrient levels of snowbrush seedlings grown in Toad Creek soil with various levels of $CaCO_3$ and $CaSO_4$ are given in Tables 15 and 16. The Ca level in all seedlings was increased as a result of $CaCO_3$ and $CaSO_4$ applications to the soil. The levels of Ca were nearly double those found in the controls. The levels of Mg and K were slightly depressed when compared to the levels found in the controls. The reduction in Mg and K levels is probably due to ion competition resulting from the addition of Ca. The addition of burnt conifer litter caused a slight increase in the level of all bases. The levels of Mg and K were not depressed even at the high levels of Ca additions. The Mg and K levels were not depressed when burnt litter was included because both of these ions were added via the litter. The addition of the burnt litter probably established a better base balance in the soil.

The N levels in general increased with the addition of both $CaCO_3$ and $CaSO_4$ (Tables 15 and 16). The concentrations were quite variable and a statistically significant trend could not be established. The N content of the plants did, however, follow the trend in nodulation. The dry weight of tops produced after 40 weeks did increase with

Table 15. Nutrient levels and dry weight of tops of 40-week-old snowbrush seedlings grown in Toad Creek forest soil with three levels of $CaCO_3$ with and without additions of burnt conifer litter.

Ca added (meq)	Dry wt. tops/ plant (g)	Nutrient levels									
		Percent						ppm			
		N	Ca	Mg	K	P	S	Mn	Fe	Zn	Cu
		Without burnt litter									
0	0.29	1.38	0.80	0.22	0.99	0.14	0.14	242	64	42	10
6	0.56	1.54	1.41	0.15	0.79	0.15	0.12	80	50	19	9
12	0.66	1.47	1.21	0.18	0.70	0.20	0.11	90	20	22	7
18	0.75	1.75	1.62	0.22	0.84	0.16	0.12	167	42	20	12
		With burnt litter added									
0	0.58	1.43	0.96	0.14	0.74	0.13	0.15	178	90	24	6
6	0.51	1.80	1.47	0.14	0.94	0.17	0.12	84	55	26	13
12	0.75	1.75	1.77	0.20	0.88	0.21	0.14	90	30	22	12
18	0.96	1.42	1.54	0.16	1.06	0.22	0.13	110	45	23	16

Table 16. Nutrient levels and dry weight of tops of 40-week-old snowbrush seedlings grown in Toad Creek forest soil with three levels of $CaSO_4$ with and without additions of burnt conifer litter.

Ca added (meq)	Dry wt. tops / plant (g)	Nutrient levels									
		Percent						ppm			
		N	Ca	Mg	K	P	S	Mn	Fe	Zn	Cu
		Without burnt litter									
0	0.29	1.38	0.80	0.22	0.99	0.14	0.14	242	64	42	10
6	0.76	1.39	1.34	0.15	0.92	0.12	0.12	237	115	22	6
12	0.82	1.48	1.37	0.18	0.78	0.08	0.16	269	60	25	7
18	0.91	1.50	1.25	0.22	0.73	0.08	0.29	264	85	30	9
		With burnt litter added									
0	0.58	1.43	0.96	0.14	0.74	0.13	0.15	178	90	24	6
6	1.11	1.63	2.02	0.24	0.85	0.12		580	115	45	9
12	1.31	1.57	1.28	0.15	0.69	0.08		440	90	30	5
18	1.96	1.48	1.30	0.20	0.84	0.08		290	75	36	6

the increased levels of both $CaCO_3$ and $CaSO_4$ (Tables 15 and 16). Dry weight was increased to an even greater extent with the burnt litter addition. This increase probably reflects the increased levels of nutrients supplied by the burnt litter. Thus with the slight increase in N concentration and the increase in the dry weight of tops, the total N content increases significantly with the addition of both Ca sources. The increase in total N suggests that N fixation did occur and that more N was probably fixed with the increased levels of $CaCO_3$, $CaSO_4$, and burnt litter additions. The increase in total N does parallel the increase in nodulation.

Phosphorus levels did not appear to be affected by the addition of $CaCO_3$. They did, however, increase slightly when both $CaCO_3$ and burnt litter were applied to this soil (Table 15). With the high levels of $CaCO_3$ and the resultant pH increase, the availability of P could be reduced. The fact that P levels were not reduced even with the high levels of $CaCO_3$ may suggest that P levels in this soil are adequate. Phosphorus levels did show a decrease when $CaSO_4$ was applied (Table 16). The decrease in plant P levels with high rates of $CaSO_4$ may suggest anion competition.

Sulfur levels in the plant tops increased slightly with $CaSO_4$, but no differences in S could be found with $CaCO_3$ additions (Tables 15 and 16). The S levels in the plant tops were not well correlated with the $CaSO_4$ additions. The lack of correlation may be due to the methods of S analysis which are not very satisfactory. The lack of sufficient plant material, particularly with the controls, made it difficult to properly determine what changes might have occurred in the S content of the plants.

The micronutrient levels do not appear to indicate any trends resulting from the Ca additions. The levels of Mn, Fe, and Zn are generally higher in the plants which received $CaSO_4$ additions.

A comparison of nutrient levels found in snowbrush seedlings grown in the growth chamber and those from the field on various sites in Oregon reveals that the concentration of N, P, and Ca is markedly different (Tables 15 and 16, and Appendix II, Table 1). The percent N in field samples ranged from 2.04 to 3.12%, whereas the growth chamber samples ranged from 1.38 to 1.80%. Similarly Ca ranged from 0.43 to 0.63% in field samples and 0.80 to 2.02% in the growth chamber. The level of P ranged from 0.24 to 0.28% in field samples and from 0.08 to 0.22% in the growth chamber. The differences encountered are probably due to differences in plant parts sampled and age of plants. The field samples are from more mature plants and consisted exclusively of leaf material, whereas the plants from the growth chamber are much younger and consist of the entire plant. The higher proportion of woody material in the growth chamber samples would explain the lower N and P concentrations and, in part, the higher Ca levels. The addition of high levels of Ca did, however, contribute to the increase in Ca observed. This is evident by comparing the control samples with those receiving high rates of Ca (Tables 15 and 16).

There did not appear to be any significant differences in the nutrient concentration of snowbrush samples from the various sites throughout Oregon (Appendix II, Table 1). The major nutrient concentrations were very consistent regardless of the site. Manganese levels for all Cascade sites were higher than those in the Fort Benham area, as would be expected.

Effect of Inoculum Source on Nodulation

In several experiments which were conducted in both the greenhouse and the growth chamber, snowbrush seedlings failed to nodulate either completely or nodulation was very sporadic despite inoculation with a crushed nodule suspension. In each case, the crushed nodule inoculum was made with nodules less than 24 hours after collection. Previous work conducted by Wollum (1965) in this laboratory did not indicate any difficulty in obtaining nodulation using crushed nodule inoculum. Bond (1967), on the other hand, reported that crushed nodule inoculum was unreliable in effecting the nodulation of snowbrush. In view of the inconsistent responses obtained from crushed nodules a further investigation was conducted on the effectiveness of crushed nodules as a source of inoculum for Toad Creek soils.

Toad Creek forest soil was inoculated using both a crushed nodule suspension and Fort Benham soil as inoculum. In addition both the N and the base level of the soil were increased. The results are given in Table 17. In all cases the number of nodules per plant was significantly higher when Fort Benham soil was used as the inoculating medium. The nodule mass was also significantly greater with Fort Benham soil as inoculum except when both 10 ppm N and 18 meq Ca as $CaCO_3$ were added to the Toad Creek soil.

The effect of 18 meq of $CaCO_3$ has been to increase both nodule numbers and mass regardless of the inoculum source. The addition of $CaCO_3$ increased nodule numbers by 2.6 and 12.2 nodules per plant and nodule mass by 103.4 and 298.1 mg per plant with crushed nodule and Fort Benham soil as inoculum respectively. The effect of 10 ppm N has been to increase nodule numbers and mass though the increase is not as great as with $CaCO_3$. Nodule numbers increased by 0.8 and 3.6 nodules per plant and nodule mass increased by 20.8 and 91.0 mg per plant with crushed nodule and Fort Benham soil as the inoculum respectively. The addition of $CaCO_3$ and N together produced the largest increase in nodulation. Nodule numbers increased by 4.8 and 15.3 nodules per plant while nodule mass was increased by 294.5 and 322.3 mg per plant with crushed nodules and Fort Benham soil as inoculum respectively (Table 17).

The results of this experiment confirm the conclusions of

Table 17. A comparison of the effectiveness of two inoculum sources in Toad Creek forest soil on snowbrush.

Soil treatment	Not inoculated		Inoculated crushed nodules		Inoculated 2% Fort Benham soil	
	No. nodules / plant	Fresh wt. nodules / plant (mg)	No. nodules / plant	Fresh wt. nodules / plant (mg)	No. nodules / plant	Fresh wt. nodules / plant (mg)
Control	0	0	0.2	3.9	7.8	44.0
18 meq Ca - CO_3	0.5	23.6	2.8	107.3	20.0	342.1
10 ppm NO_3 - N	0	0	1.0	24.7	11.4	135.3
10 ppm N + 18 meq Ca	0	0	5.0	298.4	23.1	366.3

previous experiments that $CaCO_3$ benefits nodulation in this soil. The experiment also shows that there is an increased benefit in terms of nodulation when both N and Ca as $CaCO_3$ are added to Toad Creek soil. This experiment may, in part, replace the $CaCO_3$ - NH_4NO_3 study in which all the snowbrush seedlings failed to nodulate. Thus, although the amount of information is limited, the addition of $CaCO_3$ and N gave a response similar to that obtained by the addition of $CaSO_4$ and NH_4NO_3. This experiment, in addition to the results of previous experiments, further supports the conclusion that Ca with and without added N substantially influences nodulation in Toad Creek soil.

A similar experiment conducted with F. R. 2 soil produced identical results (Appendix III, Table 1). Fort Benham soil was a better inoculum source when compared to crushed nodules. The addition of $CaCO_3$ and $CaCO_3$ plus N significantly increased nodulation regardless of the inoculum source.

Despite the greater number and mass of nodules per plant which was produced with Fort Benham soil as the inoculum source, the size of the individual nodule or nodule clump was smaller than those produced by Toad Creek crushed nodule inoculum. The individual nodules produced by the crushed nodule inoculum ranged in weight from approximately 20 to 60 mg each while the nodules produced by Fort Benham inoculum ranged in weight from approximately 6 to 17 mg each. Nodule size may have significant implications regarding N fixation.

Erdman (1926) reported that large soybean nodules contained about twice as much N as did medium size nodules and 11 times as much as small nodules. Thus in this case the fewer but larger nodules may be just as effective in terms of N fixation as the more numerous smaller nodules.

The effectiveness of the two inoculum sources in producing effective nodules may be shown by the amount of N found in the plants themselves. Table 18 gives a summary of the dry weight of plant tops and their N content. Fort Benham soil supplied a more effective inoculum source than did Toad Creek crushed nodule suspension. Without nutrient additions to Toad Creek forest soil, crushed nodule suspension did not show an increase in the N content or dry weight of plants, whereas Fort Benham soil inoculum increased the N content four times and the dry weight of the plants three times.

The addition of both $CaCO_3$ and NO_3 - N to Toad Creek forest soil improved the nodulation of snowbrush regardless of the inoculum source. Fort Benham soil inoculum continued to be more effective than crushed nodule inoculum. With $CaCO_3$ added, the N content of plants inoculated with Fort Benham soil increased approximately six times, while with crushed nodule inoculum the increase was approximately two times. With both $CaCO_3$ and N added to Toad Creek forest soil, the dry weight of plants increased by approximately 15, 19, and 17 times and the N content increased by approximately 10, 15, and 5

Table 18. Dry weight and total N concentration and content of snowbrush plant tops grown in Toad Creek forest soil inoculated with two sources of inoculum plus additions of $CaCO_3$ and NO_3 - N.

Soil treatment	Average dry wt. of top/plant (g)	Percent total N	Total N content/plant (mg)
Control	0.17	1.34	2.28
Crushed nodules	0.17	1.29	2.20
2% Fort Benham	0.57	1.66	9.47
18 meq Ca-$CaCO_3$	0.74	0.85	6.29
Crushed nodules	0.92	1.50	13.80
2% Fort Benham	2.41	1.47	35.42
10 ppm NO_3-N	0.66	0.86	5.68
Crushed nodules	0.62	1.31	8.14
2% Fort Benham	1.28	1.55	19.84
18 meq Ca + 10 ppm N	2.59	0.90	23.30
Crushed nodules	3.20	1.06	33.92
2% Fort Benham	3.26	1.54	50.20

times when no inoculum, crushed nodule inoculum, and Fort Benham soil inoculum was added respectively. The increase in N content with Fort Benham soil inoculum when both $CaCO_3$ and N were supplied is about two times, whereas the crushed nodule inoculum only increased the N content by a half. Fort Benham soil, without a doubt, supplied the more effective inoculum in all cases.

The effectiveness of Fort Benham soil inoculum is also illustrated by the percent N in the plants. With the large increase in plant growth, particularly when both $CaCO_3$ and N were added to this soil, the N concentration decreased in the controls and those plants inoculated with crushed nodule inoculum, whereas only a very slight decrease was observed with Fort Benham soil as the inoculum. A comparison of Tables 17 and 18 clearly indicates that N fixation was associated with the treatments which produced the greatest number and mass of nodules.

The size and distribution of the nodules produced by the two inoculum sources may be related to the method used to introduce the inoculum into the Toad Creek soil and the growth and/or survival of the endophyte once it is placed in the Toad Creek soil. The crushed nodule inoculum was injected with a syringe into the soil among the roots of a newly transplanted snowbrush seedling. Some of the inoculum was in contact with a root immediately. The distribution and number of nodules per plant suggest that infection must have occurred soon after the injection of the crushed nodule inoculum and that further infection was limited. The implication then is that the endophyte did not reproduce and occupy the remainder of the soil or at least was not in an infective condition. Munns (1968 b, c, d), working with Medicago sativa, and Nutman (1952) reported that the first crop of nodules inhibited formation of subsequent nodules. To what extent this may have occurred in this instance is unknown. It is difficult to accept this explanation in light of the number of nodules formed using Fort Benham soil as the inoculum source.

Two percent by weight of Fort Benham soil was used to inoculate Toad Creek forest soil. The Fort Benham soil was well mixed with the Toad Creek soil; therefore, the endophyte was likewise mixed throughout the entire soil volume. The nodules which formed in this case occurred along the entire root system of each plant. The larger nodules usually occurred on the larger, older roots and the small nodules on the small lateral roots. On a number of occasions the larger particles of Fort Benham soil were found adjacent to a nodule. The question is whether or not the endophyte, which was associated with the Fort Benham soil, left the Fort Benham soil particle and invaded the Toad Creek soil or not. The distribution of nodules seemed to suggest that infection may have occurred as the growing roots made contact with a Fort Benham soil particle.

The difference in the nodule morphology may be the result of a strain difference. In the field, the nodules found in the Toad Creek soil occur most commonly as large clumps. The nodule clumps on the older plants may be 5 to 9 cm in diameter. The nodules found in the Fort Benham area are most frequently small clumps less than 1 cm in diameter or individual bi-lobed structures scattered along much of the root system. Thus the morphology observed in the growth chamber study may be the result of a strain difference which may exist between the two inoculum sources.

Inoculum density may also be a factor in the nodulation differences observed. Quispel (1954) found, with water cultures of *Alnus glutinosa*, that at low levels of inoculum the number of nodules increases with increased inoculum density until a maximum is reached where the number of nodules is independent of the inoculum density. Despite differences in the amount of inoculum which may have initially been introduced by each of the inoculum sources, these differences should have disappeared after 30 weeks if Toad Creek soil is providing a suitable environment for the endophyte.

Another factor which may be of importance in explaining the differences in nodulation which occurred is the obvious difference in the endophyte form which must have existed at the time of inoculation. The form in which the endophyte occurs in the nodule is quite different from its form outside the nodule (Furman, 1959; Uemura, 1964; Wollum, Youngberg and Gilmour, 1966). Thus the Fort Benham soil introduced a form which was probably more adapted to a soil environment than the crushed nodule source. However, since crushed nodules can and do produce a viable endophyte capable of infection, the difference should have been minimal after 30 weeks provided that the soil in which the endophyte was placed or some other external factor did not prevent the growth of the endophyte.

Growth of Endophyte in Toad Creek Forest Soil

The differences in the number of nodules and their distribution along the roots produced by Fort Benham soil inoculum and crushed nodule suspension suggest that untreated Toad Creek forest soil does not provide a suitable habitat for the development of the endophyte responsible for forming root nodules on snowbrush. Two experiments conducted in the growth chamber confirm this observation.

In one experiment, a band of Fort Benham soil 1 cm thick was placed between layers of Toad Creek forest soil. The location of the nodules produced, relative to the Fort Benham soil, is given in Table 19. All the nodules which were formed after 5 weeks were found in the Fort Benham soil. No nodules were found in the Toad Creek forest soil.

The second experiment, in which the soil in each pot was divided vertically in half, with half the pot containing Toad Creek forest soil inoculated with 2% Fort Benham soil and the other half containing either

Table 19. Location of nodules relative to a band of Fort Benham soil placed in Toad Creek forest soil.

Pot no.	Depth to top Fort Benham soil (cm)	Depth to bottom Fort Benham soil (cm)	Plant no.	Depth of nodule from surface (cm)
1	2.0	3.2	1	2.5, 2.2
			2	2.9
			3	-
2	2.5	3.5	1	3.0
			2	2.6
			3	-
3	2.0	3.0	1	2.4
			2	3.0, 2.1
			3	-

Toad Creek forest soil alone; Toad Creek forest soil with 5:1 Ca:$MgCO_3$ or Toad Creek forest soil with 10 ppm N plus 5:1 Ca:$MgCO_3$, showed a limited occupancy of the non-inoculated soil by the endophyte. The results are given in Table 20.

Despite contact for 14 weeks with an inoculated soil, the endophyte did not appear to occupy the adjacent non-inoculated Toad Creek forest soil. From 5 to 8 nodules were found in the soil inoculated with Fort Benham soil but only one nodule in one pot was found in the non-inoculated soil. This one nodule was, however, immediately adjacent to the contact zone between the two soils. The addition of bases with and without N did not appear to improve the conditions for endophyte occupancy and/or growth in an infective form. The addition of bases to the non-inoculated soil in each pot caused a marked increase in

Table 20. Number and mass of nodules produced in inoculated and non-inoculated Toad Creek forest soil.

Pot no.	No. of nodules		Weight of nodules (mg)	
	Left side of pot Inoculated[a]	Right side of pot Not inoculated	Left side of pot Inoculated[a]	Right side of pot Not inoculated
		Untreated		Untreated
1	6	0	11.5	0
2	8	1	17.1	-
3	5	0	10.5	0
		6 meq Ca:$MgCO_3$		6 meq Ca:$MgCO_3$
4	19	1	53.2	9.2
5	24	0	68.0	0
6	14	4	65.5	12.6
		6 meq Ca:$MgCO_3$ 10 ppm N		6 meq Ca:$MgCO_3$ 10 ppm N
7	12	1	48.3	2.5
8	17	2	41.1	6.1
9	15	1	47.4	3.0

[a]Toad Creek forest soil inoculated with 2% Fort Benham soil.

the number and mass of nodules produced in the inoculated half (Table 20). Nodule numbers increased from an average of approximately 7 to 19 and nodule mass from 13 to 62 mg with the addition of 6 meq of base. On the other hand, NH_4NO_3 appeared to inhibit nodulation slightly. The addition of 10 ppm of N with the base produced an average of approximately 15 nodules with a weight of about 45 mg. From 0 to 4 nodules were found in the non-inoculated soil when N and/or bases were added to this soil. In every case, however, the nodules were always adjacent to the zone of contact, thus indicating that any movement of the endophyte into the non-inoculated Toad Creek forest soil was minimal.

The results of these two experiments indicate that the endophyte does not readily invade Toad Creek forest soil, at least not in an infective form. The evidence, however, also suggests that despite the unfavorable conditions for growth and/or infectivity, the endophyte is not completely inhibited in this soil.

VI. SUMMARY AND CONCLUSIONS

Snowbrush plays an important role in nutrient cycling during the post harvest and regeneration periods in forest ecosystems in the Oregon Cascades. Substantial increases in the base levels in the surface 20 cm of soil occur where dense stands of snowbrush develop after logging and burning. In the study areas the levels of Ca in the surface 20 cm of soil were increased by approximately 5 meq over a period of 7 years. Total soil N increased by 0.02% in the same period.

Nodulation on many sites throughout the Western Cascades has been delayed for 5 to 7 years after logging. This appears to be related to prolonged absence of the host plant from the old growth forest. The rate at which nodulation will occur on a given site appears to be related to the base saturation of the soil. Observations indicate that frequency of nodulation is correlated with percent base saturation of the soil. It was found that 78% of the variation in nodulation could be accounted for by percent base saturation of the soil in the Western Cascades.

Soils high in base saturation were shown to have a higher nodule potential than those with a low base saturation. The growth and/or infectivity of the endophyte was much greater in Spring Creek soil than in Toad Creek forest soil. The major difference between the two soils was in the base status.

Increased levels of Ca as $CaCO_3$ or $CaSO_4$ increased nodule numbers and mass in Toad Creek forest soil. The increase in nodulation was consistently greater with $CaSO_4$ than with $CaCO_3$. The increase in nodulation with the addition of Ca is consistent with the hypothesis that base saturation is an important factor in the nodulation of snowbrush in the Western Cascades.

The total soil N content has been observed to increase concurrently with increases in nodulation. The addition of up to 10 ppm of N as NH_4NO_3 enhanced both nodule numbers and mass in Toad Creek forest soil. Additions of NH_4NO_3 above 10 ppm N sharply reduced nodulation. The addition of both $CaSO_4$ and NH_4NO_3 to Toad Creek forest soil substantially increased nodulation above that observed by the addition of $CaSO_4$ or NH_4NO_3 alone. The number and mass of nodules was greatest with the addition of 10 ppm N as NH_4NO_3 and 12 meq of Ca as $CaSO_4$. When no $CaSO_4$ was added, 20 ppm N as NH_4NO_3 almost completely inhibited nodulation in Toad Creek forest soil. At 20 ppm N, the addition of all levels of $CaSO_4$ improved nodulation. Thus, to some degree $CaSO_4$ overcame the inhibition produced by the 20 ppm N.

The addition of up to 220 ppm S as $CaSO_4$ or 80 ppm S as H_2SO_4 to Toad Creek forest soil did not improve nodulation of snowbrush. Additions of 500, 1000, and 2000 ppm elemental S produced a similar response in terms of nodulation as did 3, 6, and 12 meq of Ca as

$CaSO_4$, which added, in addition to Ca, 480, 960, and 1920 ppm S respectively. The levels of S at which a nodule response appears to occur are so high that it is difficult to believe that the response is to satisfy a possible S deficiency. The nodule response which is observed with the high rates of S application is probably due to pH induced changes in nutrient availability. The lowered pH of the soil will increase the availability of the bases, phosphates, and many of the micronutrients. The effect of high levels of elemental S may thus be to make any number of nutrients, which could influence nodulation, more available.

In Toad Creek forest soil, Fort Benham soil was a better source of inoculum than crushed nodules from T. C. 1. An average of 0.2 nodules per plant were produced with crushed nodule inoculum, whereas 7.8 nodules per plant were produced with the Fort Benham soil inoculum. Additions of $CaCO_3$ and 10 ppm N improved nodulation regardless of inoculum source. Fort Benham soil inoculum produced more effective nodules than did crushed nodule inoculum. Both the N content and concentration in snowbrush plants were higher when Fort Benham soil was used to inoculate Toad Creek forest soil than when crushed nodules were used. With addition of $CaCO_3$, total N content in plants produced in soil inoculated with Fort Benham soil inoculum increased six times, while those with crushed nodule suspension increased two times.

The prolonged absence of the host plant from sites in the Western Cascades may cause a decrease in population and/or a decrease in infectivity of the endophyte. The growth and/or infectivity of the endophyte in Toad Creek forest soil appears to be restricted. The endophyte did not invade Toad Creek forest soil placed in contact with either the high nodule potential Fort Benham soil or Toad Creek forest soil inoculated with 2% Fort Benham soil. Increasing the base level and N content of Toad Creek forest soil did not enhance the movement of the endophyte into this soil. Thus despite the presence of an adequate inoculum level in the Fort Benham soil, there did not appear to be any movement of the endophyte after 14 weeks into the adjacent Toad Creek forest soil.

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APPENDICES

APPENDIX I

Table 1. Summary of some soil chemical properties on various units throughout the Central Oregon Cascades under snowbrush cover.

Area	Unit	Meq/100 g				Percent base saturation	pH	Percent organic matter	Percent total N
		Ca	Mg	K	CEC				
Central Oregon	F. B.	3.35	0.87	1.12	10.5	50.7	6.8	1.21	0.036
Toad Creek	T. C. 1	6.47	0.38	0.21	24.7	28.8	6.1	4.76	0.116
	L. L. 7	1.66	0.38	0.15	24.9	8.8			
Foley Ridge	F. R. 2	2.69	0.54	0.47	22.7	16.3	5.8	6.01	0.090
	F. T. C. 1	0.84	0.23	0.22	25.8	6.2	5.9		0.106
	F. T. C. 2	1.18	0.29	0.23	22.8	7.5	5.8		0.113
	N. W. F. 2	17.83	3.31	1.22	30.4	73.9	6.2	7.06	0.115
	S. W. F. 2	5.91	0.54	0.35	23.0	29.5	6.0	5.41	0.111
	S. C. 1[a]	11.50	0.95	0.72	26.1	50.4	6.1	5.80	0.160
	S. C. 2[a]	8.62	0.85	0.66	25.0	40.3	6.0	5.55	0.118

[a] One year after logging

Table 2. Summary of some soil chemical properties under forest cover adjacent to various logging units in the Central Western Cascades of Oregon.

Area	Unit	Meq/100 g				Percent base saturation	pH	Percent organic matter	Percent total N
		Ca	Mg	K	CEC				
Toad Creek	T. C. 1	1.56	0.29	0.16	24.2	8.3	5.8	3.49	0.084
Foley Ridge	F. R. 2	1.09	0.18	0.13	23.0	6.1	6.0	4.27	0.093
	F. T. C. 1	0.65	0.16	0.11	22.2	4.2	5.8	5.30	0.094
	F. T. C. 2	1.01	0.27	0.21	21.0	7.1	5.7	4.16	0.090
	N. W. F. 2	3.43	0.48	0.50	21.6	20.1	6.1	6.65	0.099
	S. W. F. 2	1.39	0.28	0.27	26.1	7.5	6.0	5.75	0.101
	S. C. 1	10.01	0.65	0.68	24.0	47.2	6.0	5.70	0.120

APPENDIX II

Table 1. Foliar nutrient levels in snowbrush collected on various logging units in the Western Cascades and Central Oregon.

Unit	Nutrient levels							
	Percent						ppm	
	N	Ca	Mg	K	P	S	Mn	Fe
Fort Benham	2.57	0.63	0.14	0.85	0.24	0.11	115	109
Toad Creek 1	3.12	0.60	0.14	1.06	0.27	0.16	184	194
Lava Lake 7	2.36	0.53	0.18	1.19	0.28	0.14	224	240
Spring Creek 1	2.68	0.60	0.16	1.26	0.25	0.17	229	468
Foley Ridge 2	2.04	0.63	0.15	1.22	0.25	0.13	175	80
Foley Trail Cabin 1	2.90	0.45	0.15	1.18	0.28	0.13	198	68

APPENDIX III

Table 1. A comparison of the effectiveness of two inoculum sources in Foley Ridge 2 soil on snowbrush nodulation.

Soil treatment	Not inoculated		Inoculated crushed nodules		Inoculated 2% Fort Benham soil	
	No. nodules/ plant	Fresh wt. nodules/ plant (mg)	No. nodules/ plant	Fresh wt. nodules/ plant (mg)	No. nodules/ plant	Fresh wt. nodules/ plant (mg)
Control	0	0	0.5	13.4	16.3	109.7
18 meq Ca - $CaCO_3$	1.0	127.8	6.2	329.5	47.2	549.6
10 ppm NO_3-N	0	0	2.5	152.3	40.0	408.4
10 ppm N + 18 meq Ca	0	0	10.0	426.1	50.6	670.1

APPENDIX IV

Table 1. Analysis of variance for $CaCO_3$ additions to Toad Creek forest soil on mass and number of nodules produced after 40 weeks.

Source of variation	Degrees of freedom	Sum of squares	F value
(a) Mass of nodules produced after 40 weeks			
Total	11	35, 953. 03	
Treatment	3	24, 636. 13	5. 80*
Error	8	11, 316. 90	
LSD 5% = 61. 1			
(b) Number of nodules produced after 40 weeks			
Total	11	189. 20	
Treatment	3	93. 47	2. 60 NS
Error	8	95. 93	

Table 2. Analysis of variance for $CaSO_4$ additions to Toad Creek forest soil on mass and number of nodules produced after 40 weeks.

Source of variation	Degrees of freedom	Sum of squares	F value
(a) Mass of nodules produced after 40 weeks			
Total	11	98, 951. 45	
Treatment	3	89, 190. 36	24. 37**
Error	8	9, 761. 09	
LSD 5% = 56. 8			
(b) Number of nodules produced after 40 weeks			
Total	11	259. 56	
Treatment	3	218. 23	14. 08**
Error	8	41. 33	
LSD 5% = 3. 7			

Table 3. Analysis of variance for $CaSO_4$ additions to Toad Creek forest soil on mass and number of nodules produced after 18 months.

Source of variation	Degrees of freedom	Sum of squares	F value
(a) Mass of nodules produced after 18 months			
Total	10	546, 026. 76	
Treatment	3	418, 702. 74	7. 67*
Error	7	127, 324. 02	
LSD 5% = 225. 1			
(b) Number of nodules produced after 18 months			
Total	10	267. 51	
Treatment	3	180. 70	4. 86*
Error	7	86. 81	
LSD 5% = 5. 9			

Table 4. Analysis of variance for $CaCO_3$ plus burnt conifer litter additions to Toad Creek forest soil on mass and number of nodules produced after 40 weeks.

Source of variation	Degrees of freedom	Sum of squares	F value
(a) Mass of nodules produced after 40 weeks			
Total	14	130, 697. 59	
Treatment	4	110, 460. 46	13. 64**
Error	10	20, 237. 13	
LSD 5% = 63. 5			
(b) Number of nodules produced after 40 weeks			
Total	14	299. 93	
Treatment	4	144. 93	2. 34 NS
Error	10	155. 00	

Table 5. Analysis of variance for $CaSO_4$ plus burnt conifer litter additions to Toad Creek forest soil on mass and number of nodules produced after 40 weeks.

Source of variation	Degrees of freedom	Sum of squares	F value
(a) Mass of nodules produced after 40 weeks			
Total	14	957, 238. 86	
Treatment	4	829, 794. 18	16. 28**
Error	10	127, 444. 69	
LSD 5% = 159. 0			
(b) Number of nodules produced after 40 weeks			
Total	14	1, 251. 43	
Treatment	4	1, 139. 43	25. 43
Error	10	112. 00	
LSD 5% = 4. 7			

Table 6. Analysis of variance for $CaSO_4$ and NH_4NO_3 additions to Toad Creek forest soil on mass and number of nodules produced after 20 weeks.

Source of variation	Degrees of freedom	Sum of squares	F values	LSD 5%
(a) Mass of nodules produced after 20 weeks				
Total	59	51, 568. 06		
Treatment	19	44, 502. 72	13. 55**	8. 40
Calcium	4	9, 801. 68	14. 18**	16. 79
Nitrogen	3	3, 820. 90	7. 37**	18. 76
Ca - N	12	30, 880. 14	14. 89**	10. 85
Block	2	499. 61	1. 45	
Error	38	6, 565. 74		
(b) Number of nodules produced after 20 weeks				
Total	59	1, 062. 23		
Treatment	19	851. 12	8. 07**	1. 50
Calcium	4	140. 69	6. 33**	3. 01
Nitrogen	3	84. 76	5. 09**	3. 36
Ca - N	12	625. 67	9. 39**	1. 94
Block	2	0. 12	0. 01	
Error	38	210. 99		