

1 Title: Effects of soil moisture on *Lupinus spp.* growth and root nodulation

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11 **ABSTRACT**

12 Soil moisture is highly important in the growth of Lupine (*Lupinus spp.*). *Lupinus*
13 *spp.* in wet plots produces root nodules in response to being nitrogen limited, unlike dry
14 plots that are water limited. As a result of a stated optimal temperature, lupine could
15 produce root nodules around Gothic, CO in the Central Rocky Mountains since the
16 temperature in the summer falls within the range at which root nodules can still exist
17 (Evers 2001). This research involved collecting vegetation data to monitor growth rate,
18 collecting soil cores for gravimetric analysis of soil moisture, digging up the plants after
19 five weeks for root nodules, and collecting wet and dry biomass of the roots, shoots, and
20 nodules if available. It was found that there was a temperature difference of 3⁰C between
21 the higher and lower elevations. The higher elevation recorded very high soil moisture
22 values and 94% root nodule production, only to be succeeded by the mid elevation with
23 22% root nodule production in its wet plots; therefore, suggesting that at least soil
24 moisture dictates nodulation.

25 **INTRODUCTION**

26 Nitrogen is required for plant growth, and in many cases it is a limiting resource.
27 Air is about 79% nitrogen, and some plant species are able to use atmospheric nitrogen
28 through bacteria known as *rhizobia*, in a process called biological nitrogen fixation
29 (BNF). The mediation of bacteria is necessary because plants are unable to use
30 atmospheric nitrogen directly; thus, the need for rhizobia to convert atmospheric nitrogen
31 into a useable nitrogenous compound, which is ammonia, NH₃. Ammonia is the main
32 reactant for *all* nitrogenous compounds found in plants, such as amino acids and vitamins

(Dalton 1999). Therefore, if nitrogen fixation can have an effect on the synthesis of amino acids and ultimately DNA and RNA, it should dictate the growth of Lupine.

The *Lupinus* genus near Gothic, CO is comprised of *L. argenteus* and *L. caespitosus* and it is an example of a legume that utilizes BNF by means of producing root nodules. Each nodule is an individual colony of rhizobia under a mutualistic relationship with Lupine. BNF becomes a necessity when nitrogen is a limiting nutrient for plant growth, and that reduces the need for fertilizer as a source of nitrogen. However, this process is energy demanding, since the lupine has to provide carbohydrates (sugar) for the rhizobia before the rhizobia are able to fix nitrogen for the lupine (Evers 2001).

The energy for BNF comes from the plants' photosynthetic activity (Redmon and Smith 2001). In a global survey of nitrogen fixation energetics, Gutschick found that wild plants devote 2.5% of their photosynthetic energy to fixing nitrogen, and another 5% to oxidizing nitrates that are recycled from the decay of previously fixed nitrogen. Therefore, plants should obtain nutrients through the most energetically efficient means available (1978).

Dependency of legumes on BNF increases as soil nitrogen decreases. Yet, the possibility of a legume to invest its energetic resources in nodulation also depends on temperature. Research conducted on white clover shows an optimum temperature range for BNF between 55⁰F-80⁰F (~12.8⁰C-26.7⁰C), with a sharp decline above and below this range and no occurrence of BNF below 48⁰F (8.9⁰C) (Evers 2001). Root nodulation is dependent upon external and internal causes, and these causes are evident in the growth rate of Lupine. In general, lupine's growth rate relies on the percent soil moisture, temperature, nutrients (nitrate level), and leaf area. Leaf area is taken into consideration

56 as related to the leaf's ability to intercept sunlight for the energy needed to split the N₂
57 gas in the root nodules (Evers 2001).

58 My research will focus on whether lupine's growth rate is limited by soil nitrogen
59 or soil moisture along an elevational gradient near Gothic, CO in the Central Rocky
60 Mountains. I expect that plants in a moist environment will be nutrient limited, rather
61 than water limited, and should therefore produce more root nodules than plants in dry
62 environments as a consequence of using up most of the available soil nitrogen. However,
63 I expect plants in dry sites to be water limited rather than nutrient limited, and should not
64 invest in root nodules. Temperature decreases with altitude; as such, I expect a sharp
65 decline in root nodules if the temperature of my sites lies outside of the optimum
66 temperature. Hence, root nodules at higher elevation should develop more slowly and due
67 to nutrient limitation, plants' growth in general is expected to grow slower.

68 I anticipate that plants in a wet plot will grow up to their water limit only after
69 producing root nodules at their nitrogen limit. Contrary to the wet site, I expect plants in a
70 dry site to go up to their water limit without the need to produce root nodules due to the
71 excess amount of nitrogen present in the soil (Figure 1). I hypothesize that if an increase
72 in the number of root nodules is observed in the wet, and not in the dry environment, then
73 soil moisture is a possible regulator of BNF. However, when an increase in root nodules
74 is observed in both dry and wet environments, then nitrogen is a limiting resource in both
75 sites, and therefore, even the so-called dry sites are not drought limited. Alternatively,
76 were root nodules to decrease in both environments, then nitrogen is not a limiting
77 resource, but water or some other essential resource is the limiting factor.

METHODS

Research Sites

I worked at sites of three different elevations: high elevation—Schofield Pass and Emerald Lake (~3173-3239 m); medium elevation—Gothic, RMBL town site, and Maxfield Meadow (~2859-2878 m); and low elevation—Kettle Ponds (~2837-2850 m). At every site, I had six to eight plots of twelve plants each. I designed a plot system in order to group plants at proximities to one another by their similarity in soil moisture values and soil type (be it rocky, loose, and/or woody) based on my observations with a few exceptions that will be highlighted.

At Emerald Lake, I sampled three plots of *Lupinus spp.* in a meadow, followed by two other plots on either sides of a running stream, and two more plots along a roadside slope. At Schofield Pass, I sampled two plots wherein the west plot receives more sun than the east plot which has a layer of conifer duff up to 10 cm in depth. At the RMBL town site I sampled a slope leading to a running river; another plot with a growth media of piles of conifer duff and a wetter area; the third next to a pond; the fourth on the Gothic roadside, while the rest were at Maxfield Meadow. Kettle Ponds site comprised of six plots. One encircling the pond, another on a gentle slope, the rest were all meadows (Figure 2).

None of the three sites comprised of plots that if all samples were to be uprooted, it would have caused a significant change in the population of the *Lupinus spp.* of that plot. The highest percentage of plants uprooted came from the roadside leading to Schofield Pass, and that is about 25% of the total population in a very localized habitat

(the rest range from about 5-10%). Even so, only about 58% of the focal individuals across the three sites were uprooted for harvest.

Due to the chances that *L. caespitosus* might be included in my data when actually I aimed at the more abundant species, *Lupinus argenteus*; I have generalized by referring to all my samples as *Lupinus spp.* or commonly as Lupine. However, I have observed and/or recorded about 4-5% of relatively outlying heights of plants, difference in leaf color, time of flowering relative to plant height, and hairiness that could be associated with difference in species characteristics.

Data Collection

Field Sampling

I collected three sets of growth data within 5 weeks for about 240 individual plants of Lupine across my three sites. For the first data set of soil moisture, I used a “rapitest Moisture Meter” to measure soil moisture at four sides of the plant while in the second and third set, I used the gravimetric analysis method. The gravimetric analysis involved collecting a 10 cm x 1 cm soil core from 30 cm away from the plant, and then transferring the sample into a Ziploc bag for analysis in the lab later the same day. For accuracy purpose, I scraped off the top 2 mm to remove any litter that otherwise would make its way into the sample.

Plant vegetation data consisted of counts of flowers, stems, and leaves, and representative leaf area, average plant height, and flowering dates. In recording soil temperature, I stationed one temperature recorder, containing two 10 cm-deep soil thermometers and two 10 cm air thermometers, at each of the three sites to determine the soil and air temperature of that site once every 15 minutes for 4 weeks. This gave me an

123 idea of the differences/similarities in soil and air temperatures across the altitudinal
124 gradient.

125 During and/after the third dataset for vegetation and soil moisture, I dug up six
126 representative plots at the high elevation, leaving behind two of three plots at the meadow
127 in Emerald Lake with the assumption, backed up by the vegetation data, that all three
128 plots around the lake are similar. At the middle elevation, I uprooted all surviving plants.
129 I recorded only 9 samples dug at Kettle ponds—7 at one extreme and 2 at the other
130 extreme. In all cases, I was unable to dig any plant in its entirety except that I was closest
131 to that for the two plots on the road side to Schofield Pass, the reason being that the soil
132 there was the loosest of all 21 plots.

133 *Laboratory Sampling*

134 At the laboratory, soil samples were transferred to aluminum tins to get their wet
135 weight on the day of collection, and then were weighed again to determine their dry
136 weight after being placed in an oven of 95-125⁰C. If there was not enough space in the
137 drying oven, the soil samples were not weighed on the day of collection. Instead, they
138 were stored in the refrigerator for up to six days to preserve the amount of moisture
139 present in them until space became available. Dry samples were later sieved after
140 weighing them dry, in a mesh size of 0.85 mm to isolate rock and organic matter.

141 As for the biomass, I either took the wet biomass of each harvested plant on the
142 same day or stored it in the freezer until the drying oven space became available. Of all
143 the harvested parts of the plants, I only took wet biomass data for root nodules. This is
144 because the wet biomass of the plain roots and shoots may not reflect the amount of water
145 taken up by them since the first dataset due to the frequency of rain for two weeks before

harvesting. I peeled off and counted all nodules irrespective of size and weighed them immediately. Then, I divided the rest of the plant into roots and shoots and placed those two parts into different paper bags for drying. I weighed the root nodules and dried them with the rest of the roots in an oven of 95-125⁰C while the shoots were dried at 85⁰C. After 48-72 hours, I weighed the three separate entities.

Data Processing

I used JMP IN 5.1 to process and analyze my data. Just as flowers, stems and leaves can suffer herbivory, the total number of leaves can change when leaves and/or stems that are not living and/or healthy were included in the leaf count. Also, number of stems may increase or decrease as time goes by. The number of stems may increase if one plant was thought of as two in a previous data, but later appeared to be a single individual. Apart from predation or drying, number of stems can also decrease if one stem was counted as two stems in a previous dataset as a result of that stem branching into two secondary stems at the above ground-below ground boundary. Therefore, in the case of an increase in the number of stem, I adjusted the data by having what I call “steam load” which is the ratio of leaves to stem(s), while in the case of a decrease caused by counting error, I adjusted for it by making the last set of data’s number of stems as the ultimate number of stem, and used it in all three sets.

Data Analyses

I determined growth rate by subtracting a later census from an earlier one. This was necessary for correlating my vegetation data, soil moisture, and temperature with growth rate and nodulation. I took into account the difference of the wet biomass from the dry biomass of root nodules to estimate the amount of water contained in the nodules.

When it was possible to uproot the entire root systems, it was found that nodules are evenly distributed along roots. Therefore, even though I was unable to uproot most of the roots of each plant, having a ratio of nodule weight to the weight of the rest of the root that was dug gave a relative value of nodulation. Just to create a clear cut between many small and few big nodules, I have created an index of mass of nodules divided by number of nodules.

Formulas

- $\% \text{ moisture} = \frac{\text{wet weight} - \text{dry weight} - \text{rock weight}}{\text{wet weight} - \text{rock weight}} * 100$
- Representative leaf area = leaf length * leaf width
- Stem load = #leaves / #stems
- Nodule distribution = nodule dry biomass / root dry biomass
- $\text{Growth rate} = \frac{\text{plant height 2} - \text{plant height 1}}{\text{day 2} - \text{day 1}} = \frac{\text{growth (cm)}}{\text{time (days)}}$

where 1 and 2 represent the first and second set of data respectively

RESULTS

There was a decrease in temperature of about 3⁰C as we moved from the low elevation to the high elevation sites (Figure 3). In general, soil moisture varied across sites; with a significant difference between the high elevation site from the other two; the high site was wetter (Figure 4). Nodule production varied dramatically across sites. 94% of plants at the high elevation sites had nodules, about 20% in the mid elevation had nodules, and no nodules were observed at the low elevation (Figure 5). However, when it came to the highest nodule producing site (high elevation), I saw an inconsistent variation in the

ability of a plot to produce nodules when soil moisture was taken into consideration. Plots E, A, and slightly H had an increase in root nodule production with increase in soil moisture, while plots D, F, and slightly G, had a decrease in the nodule production with an increase in percent soil moisture (Figure 6). Leaf area decreased with an increase in altitude (Figure 7). On the other hand, growth rate increased with an increase in altitude (Figure 8).

DISCUSSION

The middle elevation recorded a large variance when compared to the other two elevations. This was a reflection of sampling from many different plots, ranging from the sides of a pond, the sides of a stream, under a tree with media composed of dead cones, and by the dry roadside.

Root nodulation supported my assumption that wet areas are nitrogen limited and should produce root nodules in response to that (Figure 1). Also, similarity between Gothic and Kettle Ponds in most of my data analyses suggested that there were no true “high,” “mid” and “low” elevations; rather, there was a higher elevation that comprised of Emerald Lake and Schofield Pass and a lower elevation which included Gothic and Kettle Ponds sites. This inference became more vivid when there were no root nodule production on the roadside in Gothic, Maxfield Meadow (all leading to Kettle Ponds), and Kettle Ponds; thus, the reason for digging up only 9 out of 72 possible plants from Kettle Ponds.

It was quite unpredictable to me that there would have been an increase in growth rate with altitude since the higher elevation was colder, and had shorter plants, fewer

stems, and smaller leaf areas. However, the fact that the higher elevation recorded a significantly higher growth rate, could have been a reflection that the tallness of plants, bushiness of stems, and wideness of leaves in the lower elevation were a sign of those samples approaching their growth limit by the time the research was conducted. In essence, the lower elevation might have started growing earlier than the higher elevation as a result of earlier snow melt at the lower elevation. Thus, I may have had the wrong timing.

The soil moisture of my sites increased with altitude. The higher elevation site recorded *only* about 94% of root nodules because 2 plants of the non nodule producing ones had their roots embedded in rocks, thus, not so reliable to convince us of the possibility of having lupine with no nodules at the higher elevation. As for the mid elevation that recorded 22% root nodulation, 8 nodule producing plants came from a plot next to a stream, and 5 plants in a plot next to a pond; thus, supporting the hypothesis that only wet plots can produce nodules.

Plants that did not produce root nodules at the lower elevation could not have been as a result of a higher temperature. This is because the temperature of all three sites fell within range of sharp decline of BNF ($12.8^{\circ}\text{C} > \theta > 8.9^{\circ}\text{C}$) (Evers 2001).

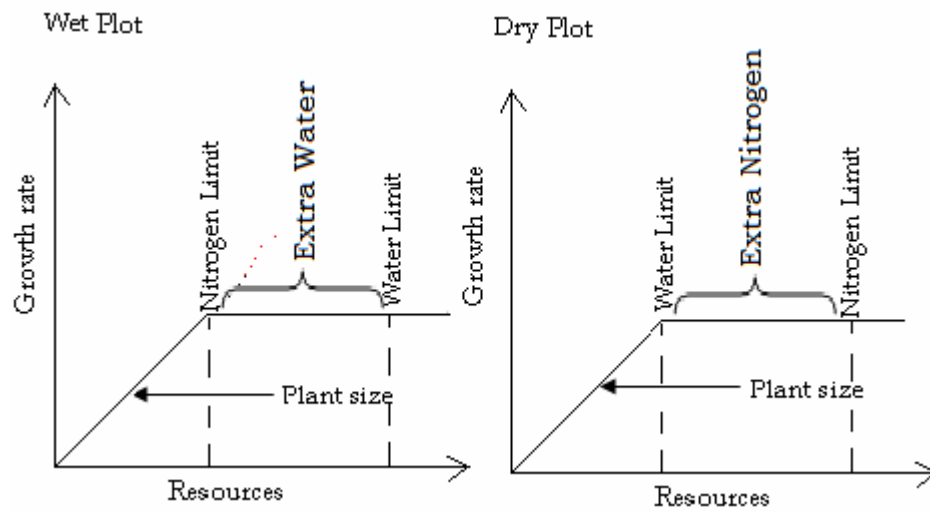
Differences in the trend of root nodule production in response to soil moisture from the high elevation suggested that soil type might have a role to play in root nodulation. Therefore, I recommend further research to understand the effect of soil nitrogen on root nodulation by testing for soil nitrogen directly. Also, to understand how sunlight and soil type (rocky, woody, loose, and muddy) affect nodulation.

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250 **FIGURE LEGENDS:**

251 **Figure 1: a comparison between the growth rates of lupine in a wet plot versus a dry**
252 **plot showing that plants in a wet plot are nitrogen limited, and need to produce**
253 **nodules instead of those in a dry plot with limited water.**



255 **Figure 2: a GIS map of the three sites showing closeness of the Middle and Low**
256 **elevation while the High elevation is farthest away.**

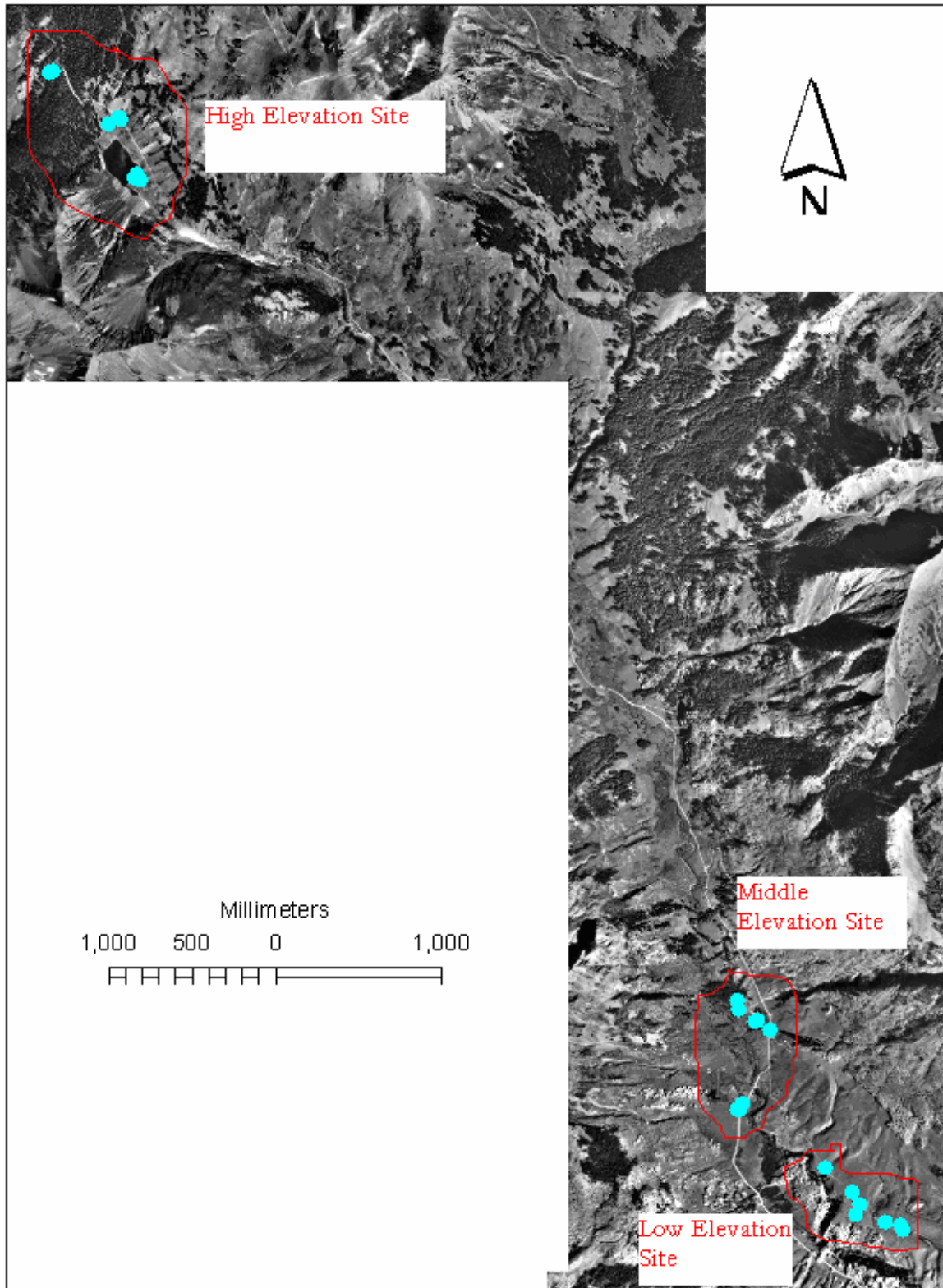


Figure 3: a oneway analysis of soil temperature by site showing a huge variance in the temperature of the low elevation and small variance at the mid and high elevation. the graph also showed that soil temperature at the high elevation is significantly lower than the other two.

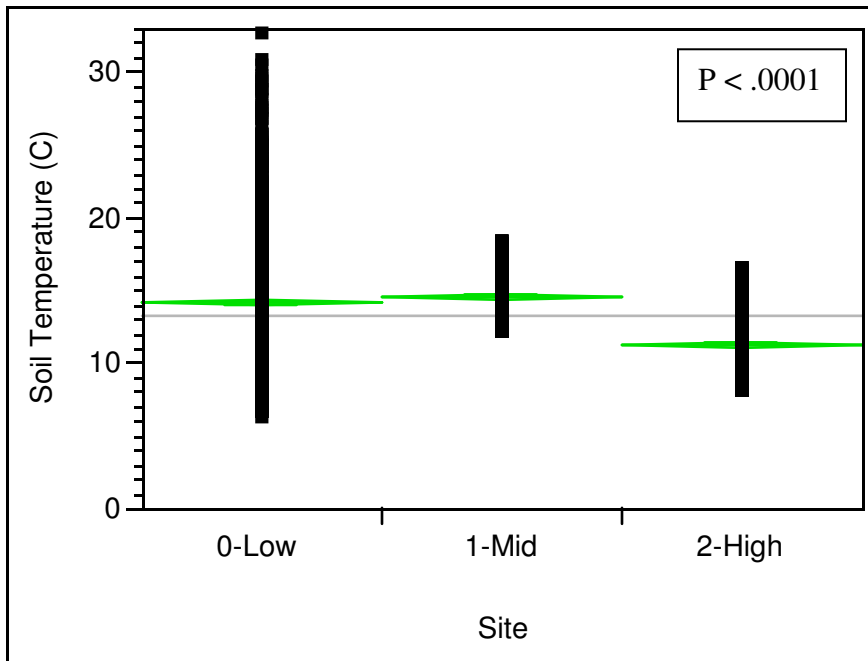


Figure 4: a oneway analysis of soil moisture by site indicating a slight overlap of soil moisture values between the mid and low elevation and a significant difference of mid and low elevation from the high elevation. Soil moisture roughly increases with altitude.

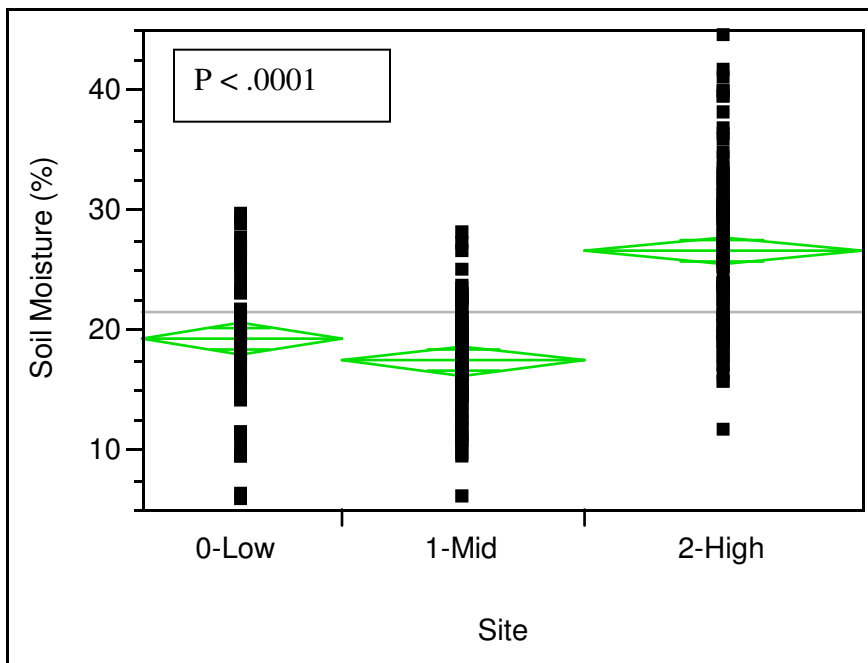
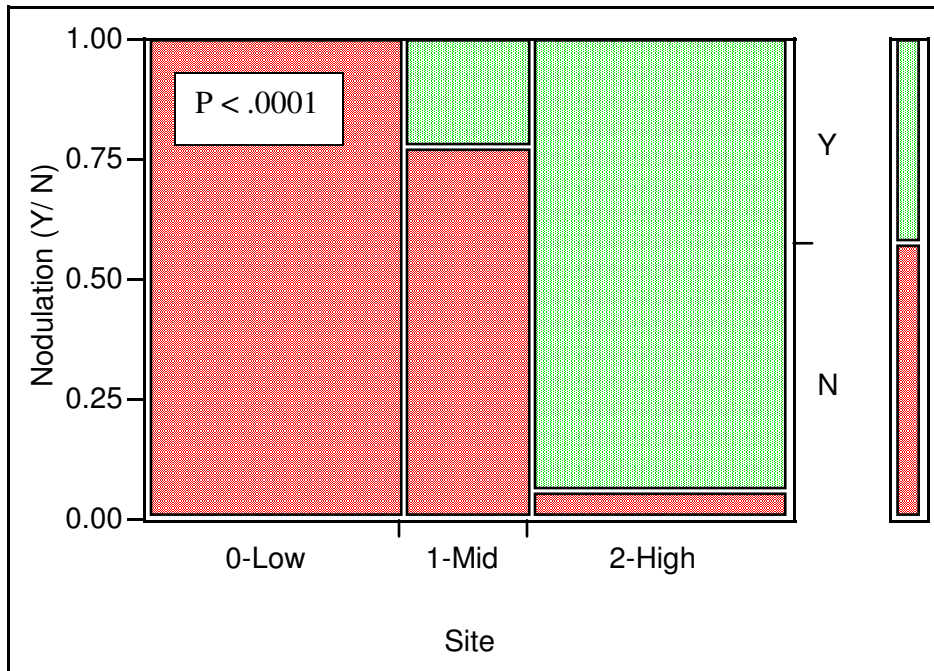
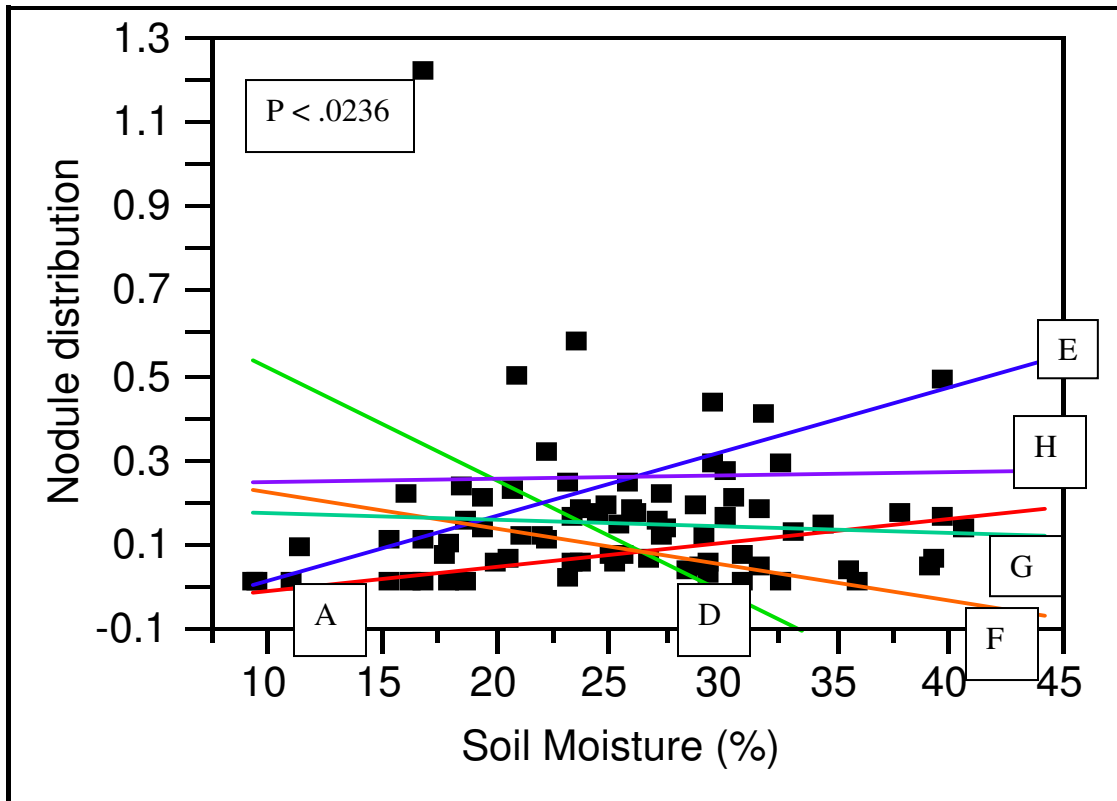


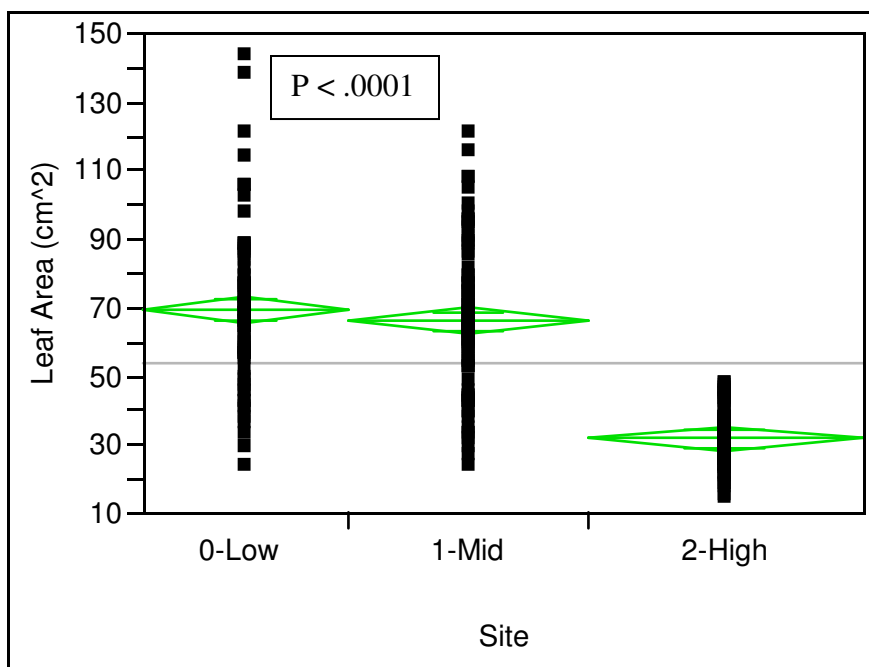
Figure 5: A contingency analysis of nodule production by site representing no nodule production in the low elevation, about 22% production in the mid elevation, and up to 94% root nodulation in the high elevation.



272 **Figure 6: a regression plot of root nodule distribution against soil moisture of plots**
273 **in the high elevation showing the complexity of finding a trend within a site as**
274 **opposed to across sites.**



276 **Figure 7: a oneway analysis of leaf area (cm²) by site suggesting that leaf area**
277 **declines with altitude.**



278

279 **Figure 8: a oneway analysis of the rate of plant height growth by site showing an**
280 **increase in growth rate with altitude; Yet, with no significant difference between the**
281 **low and mid elevation.**

