ROOT NODULE STUDIES OF A DESERT BROWSE LEGUME GUAJILLA

(Calliandra eriophylla Benth.)

by

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ABSTRACT

Investigations were made into the nature of the nodule production and nitrogen fixation by Calliandra eriophylla Benth. Field, greenhouse and laboratory observations were made.

These investigations confirmed the presence of nodules and suggested that the Rhizobium responsible was nitrogen active. It was usggested that only the mesquite-grassland and oak woodland communities of the Southern Desert Shrub region have sufficient numbers of the species to warrant consideration as a nitrogen supply in range and watershed management proposals.

INTRODUCTION

Range and watershed management plans are often made on the basis of the relative values of the plant species involved. Frequently, however, the values of many of these species are not known. This is particularly true of the native, shrubby legumes of arid and semi-arid grasslands. Nevertheless, ranchers and technicians repeatedly recommend removal of these shrubby species, at great expense, in an attempt to establish relatively pure stands of grass, even though we do not yet know the magnitude of the benefits of these shrubs in terms of forage value, soil conservation, and soil improvement through their suspected nitrogen-fixing abilities. Consequently, the benefits of these shrubby legumes need to be investigated so that adequate management plans can be made.

Since little is known of the ability of desert legumes to fix nitrogen, this study was aimed primarily at determining the nature of nodule production and indirectly of nitrogen fixation on one of these plants, guajilla (*Calliandra eriophylla* Benth.),[1] and its possible role in the nitrogen balance of some semi-desert vegetation types. *C. eriophylla* was chosen from the numerous leguminous desert species because it is generally known as good or excellent forage by ranchers and technicians, and because it is a common and widely distributed species.

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1. Scientific nomenclature after Kearney and Peebles, 1960.

This study was conducted in the field, greenhouse, and laboratory. In the field, a vegetation survey was made of four plant communities to determine the frequency and abundance of guajilla and to determine what species grow in association with guajilla. Measurements were made on the north and south aspects to determine if this legume occurs more on the cooler north slopes or on the warmer, dryer, south slopes. Then, the root systems of several plants on north and sourth slopes were examined for nitrogen fixing nodules. A greenhouse experiment was made to determine the effect of temperature and moisture on the abundance of nodules on plants of different ages. Laboratory studies were then made to determine the genera of bacteria that produce the nodules, and to investigate the histology of the root hairs and nodules.

REVIEW OF LITERATURE

Calliandra eriophylla, a member of the Mimosoideae sub-family, is a non-prickly perennial undershrub, having many long, stout stems to about 1.2 meters in length. The flowers occur in heads and are generally pink or white. Seed pods are thick and have rib-like margins and valves that recurve after dehiscense. Flowering occurs from March to May, depending on the location and yearly variation in the climate. The fruit matures from June to August.

Guajilla is common on slopes and mesas at elevations of 2,000 to 5,000 feet in the Mohave and Chihuahuan deserts (Benson and Darrow, 1954), in the Sonoran Desert (Humphrey, 1960); and in the desert grassland and foothill areas of other grassland types. In these areas the species occur in association with several species of grasses as well as many half shrubs and shrubs such as palo verde (Cercidium microphyllum (Torr.) Rose & Johnson), bur sage (Franserja deltoidea Torr.), mesquite, (Prosopis juilifora (Swartz) D.C.), creosote bush (Larrea tridentata (D.C. Coville), and at higher elevations, live oak. On the more favorable sites, guajilla often make up as much as 25 percent of the ground cover (Kearney and Peebles, 1960; U. S. Forest Service, 1936).

The species occurs on a variety of soils but is characteristically found on gravelly clay soils and sandy gravel soils of granitic origin (U.S. Forest Service, 1936; Van Dersal, 1938; Dayton, 1931; Young et al., 1931).

Throughout its range, guajilla is a valuable forage plant. It is one of the first plants to initiate growth in the spring and often gives a good supply of green forage throughout the year. Chemical analyses of leaves and young twigs show that the fat, oil, and protein content is approximately equal to that of alfalfa hay (U.S. Forest Service, 1936; Dayton, 1931).

Owing to its ability to sprout, guajilla is inherently more well adapted to withstand fire and grazing. Accordingly, it is often able to persist under a degree of use that would be harmful to most plants (Benson and Darrow, 1954; Humphrey, 1960).

Most species of Leguminosae and nine or ten genera of non-leguminous plants, form nitrogen fixing nodules. Martin (1948) lists 26 native desert legumes, including guajilla, on which nodules can be found under natural conditions.

Although nodules on herbaceous legumes have received the major consideration, a few studies have been made on browse and tree species. Allen and Allen (1936) found considerable variation in the size, number, and distribution of nodules on a number of leguminous trees from 1 to 20 years old and from 5 to 40 feet high. As a rule, young nodules were round and had a white, smooth surface. Older nodules assumed variously branched shapes, were commonly black or dark brown, had a rough surface, tending to be more or less of a hard "corky" or "woody" texture. Spratt (1919) observed that nodules persist for long periods of time on woody, perennial, leguminous plants. Allen et al. (1955) reported that elongated nodules on certain woody species of Acacia, Robinia and Saphora, showed indentations indicative of apical seasonal growth periods alternating with winter dormancy.

Nodules which are characteristic of the Leguminosae are produced by bacterial infection of the root hairs and cortex by the genus Rhizobium. The type of nodule formed depends not only upon the anatomical peculiarities of the host plant, but also upon the nature of the environment. Joffee, et al. (1961) established that symbiotic nitrogen fixation is a thermo-sensitive process confined to a relatively narrow range of temperature. Deviations on either side of this range appeared to reduce the ability of Rhizobia to fix adequate amounts of nitrogen. The actual process of nodulation, however, seemed to take place under a wider range of conditions.

Temperatures of the roots also affected the number, size, distribution, longevity, and pigmentation of the nodules. Moore (1905) found the optimum temperature range for the growth of nodule forming organisms to be 23^{o} to 25^{o} C. No appreciable growth occurred above 40^{o} C or below 10^{o} C.

Other factors which influence nodulation are light, alkalinity, aeration, moisture supply, and soil chemistry. According to Moore (1905), both strong sunlight and the lack of air has deleterious affects on bacterial cultures. He also noted that alkaline nitrates having a strength of 1 part in 10,000 could prevent nodule formation. Both Moore (1905) and Selman (1952) suggest that drought is not fatal to the bacteria. However, drought may limit nodulation. Moore also observed that the presence of 0.33 to 1.0 percent of potassium and sodium salts was often sufficient to inhibit the formation of nodules whereas the same amount of calcium and magnesium salts favored nodule production.

Nodulation may also be affected by the physiology of the host plant (Fred and Wilson, 1934). For instance, nitrogen fixation is often limited by carbohydrate synthesis in the plant. This synthesis, a function of the percentage of CO_2 in the atmosphere, is rarely sufficient to allow the maximum development of nodules with respect to both size and number. Any limitation in size and number is usually accompanied by a limitation in the quantity of nitrogen fixed. If carbohydrate synthesis in increased, the number of nodules likewise increases. However, the size of the nodules increases only if the amount of oxygen and nitrogen present are not restricting nitrogen fixation.

It appears also that the normal distribution of nodules, on or near the upper main roots, may be a manifestation of the carbohydrate level in the plant (Fred and Wilson, 1934). This is because the carbohydrates available to the bacteria infecting the lateral roots may be insufficient to allow development of nodules at all the points of invasion. Any factor that tends to raise the carbohydrate level such as a high percentage of CO_2 or a low percentage of oxygen or nitrogen may cause increased nodule formation at invaded points.

Although little is know of the mechanism of infection, the process has often been studied, particularly in herbaceous species. Nutman (1956) reported that before the bacteria starts the infection, the host plant influences the process taking place outside the root by the secretion of material essential for root invasion by bacteria.

Thorton (1929), cited by Nutman (1956), deduced that an effective accumulation of the exudate in alfalfa occurred by the time the seedling had unfolded its first leaf. Nutman added that the bacteria enters the root by way of the root hair. Usually the root hairs are infected, but not invariably, as a consequence of the secretion trypotphane which is transformed to I.A.A. (indole acetic acid) by cytase from Rhizobium (Allen and Allen, 1954). Apparently this reaction is restricted to legumes.

In some legumes, infected root hairs appear to be curled. Nutman (1956) concluded that ... "bacteria at the growing tip of the root hairs induces reorientated growth in the primary cell wall to give rise to an infection thread by invagination." During early stages of infection, the growing thread tips and the numerous blister like swellings along its side consist of a slime-like matrix filled with rod-shaped rhizobia. As rhizobia migrate through the host cytoplasm, the strands become enclosed in a cellulosic sheath. This sheath is absent in the middle lamella at the time strands cross between adjacent cell walls (Allen and Allen, 1954). Nodulation generally starts as an infection of root hairs by the thread tip. The bacteria enter the root hairs by a mechanism that is not well known. Some investigators have observed that bacteria secrete I.A.A. as they enter the root hairs. Inside the hair, bacteria attack rootlet cells. Here, they spread and multiply very rapidly causing the cells to be converted to tetraploids. In this way, cells are altered and multiply to form nodules if the environment is appropriate (McIntosh, 1964).

METHODS

Most of the field work was carried out on the Santa Rita Experimental Range in southern Arizona, although one plant community thirty miles north of Sonoita, Arizona was also sampled. Vegetation in this study area is very rich in terms of the numbers of species present (Appendix A) and very diversified in terms of species associations.

In general, the climate is arid to semi-arid. Yearly rainfall averages 10 to 14 inches, most of which comes in the late summer and winter months. Temperatures vary from a low of around 20° F. to a high of about 110° F.

Vegetation Survey

A line-step transect method was used for the vegetative analysis. Eight transects were used to sample the north and south aspects of four different vegetation types. Each line was 400 steps long and a plot was taken every four steps. Plots consisted of a square meter frame placed on the ground at the end of every four steps. In this frame, all the guajilla plants were recorded as to their relative age (young, medium, or old). In addition, all other species growing in the frame were recorded.

The vegetative communities sampled were: 1) palo verde-bursage (Figure 1), 2) creosote bush (Figure 2), 3) desert grassland (Figure 3), and 4) oak woodland (Figure 4).

Figure 1. Example of a typical palo verde-bur sage plant community.

Figure 2. Example of a typical creosote bush plant community.


1


2

Figure 3. Example of a typical mesquite-grassland plant community.

Figure 4. Example of a typical oak woodland plant community.


3


4

Field Root Study

Eight plants of guajilla 4 to 8 years old were removed from the soil on north and south facing slopes of a mesquite grassland vegetation type using the "block-washing" method as described by Weaver (1926). A "direct method", also described by Weaver (1926), was used in an investigation of the rooting habits of guajilla. Figure 5 shows how the plants were removed using the block-washing method. The ball of soil, 1 foot square and 2 feet deep, was burlapped so that the roots would not be broken during transport (Figure 5a). Later, the ball of soil was placed for 16 hours in a barrel containing water (Figure 5b). This allowed the soil to wash from the roots with a minimum of disturbance. The roots, rootlets, and nodules were then carefully cleaned and washed. The nodules were examined, counted, and recorded as to their position on the roots, form, and color. Two plants were taken from a north slope and two from a south slope of two areas having similar vegetation.

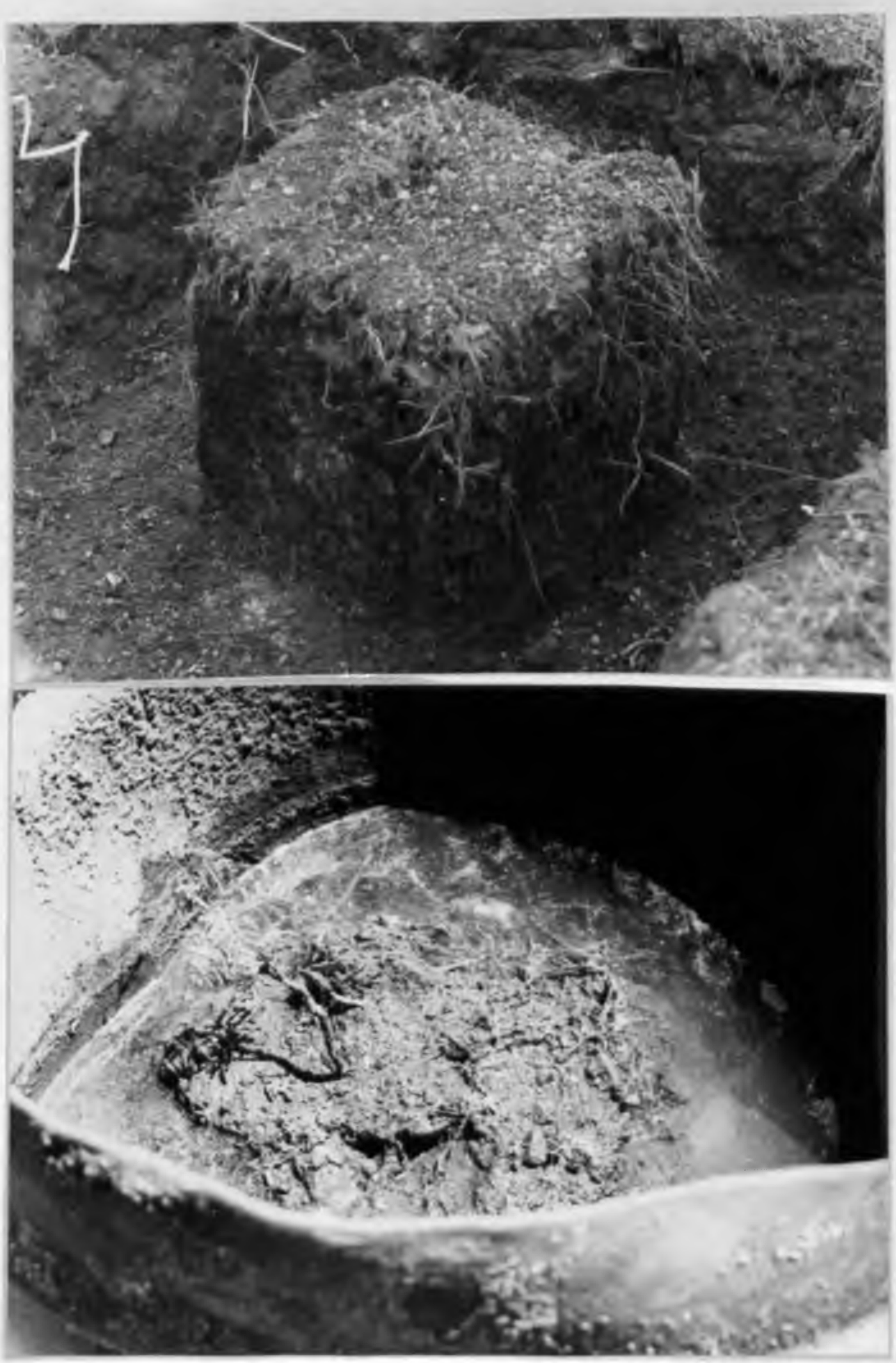

Figure 5. The block washing method used in the root observation study; a) the soil block in place, b) the removal of the soil from the root.

Green House Study

Seeds used in the greenhouse study were collected in Lower Sabino Canyon near Tucson. Most of the seeds were small or medium in size. These were inspected and any hollow or damaged seeds removed. The seeds were scarified with concentrated sulphuric acid for five minutes (Collins, 1963).

A mixture of six parts riverbed sand to one part peat moss was used as soil material in the greenhouse experiments. The sand was sterilized in an autoclave at 280° F. and 15 pounds pressure for about five hours.

Inoculation of the seeds was accomplished by mixing them with soil material known to have contained guajilla roots. They were left in this soil until they assumed a dark brown color at which time they were removed and planted in a germination box. The box was placed in the greenhouse at a temperature that varied between 65° and 80° F. Ninety-seven percent germination was obtained. Most of the seeds germinated the second day, and the first leaves appeared on the fifth day.

When the plants were six days old, they were transplanted to pots which were four, six, and eight inches in diameter. The pots were filled with the same soil mixture described above. A single plant having roots three to six inches long was planted in each pot. Ortho-grow, a commercial fertilizer pellet, was added to the pots as a source of plant food. Six pellets were dissolved in 4.5 liters of water and added, in the amounts specified below, eight days after transplanting and repeated once every two weeks until the end of the experiment.

Two different temperatures (76° F. and 85° F. average) and three different moisture levels (10, 20, and 30 cubic centimeters) were used. Differences in temperature were obtained by locating plants in different areas of the greenhouse. The plants were watered every other day with tap water except on the days listed above when the fertilizer solution was used. These treatments were replicated four times for a total of 218 plants.

One plant under each moisture level and each temperature was removed every ten days, and the nodules counted. The roots and branches were saved and their dry weights determined so that comparisons between treatments could be made.

Histological Observations

Nodules and rootlets from several plants five weeks old were removed and histological examinations made. The techniques used for killing and fixing the material follow those given by Johansen (1940).

The plant material was immersed in a formalin-aceto-alcohol (F.A.A.) preservative for seven days and then dehydrated with tertiary butyl alcohol. The dehydrated material was then embedded in paraffin. Several weeks later, the paraffin was cut into slices of 3, 6, and 8 micron thickness. These were stained with Heidenhin's hematoxylen and safranin-fast green. After staining, cross-sections were mounted and microscopic observations made.

Bacteriological Examination

The bacteria were isolated from nodules taken from six-month old guajilla plants. Roots of these plants were washed with fog spray and six healthy nodules having a light brown color were cut from the roots. The method used for the isolation of the bacteria was adapted from Allen (1959).

Each nodule was washed under sterile conditions, first with distilled water, then with a 1:1000 $HgCl_2$ solution and next with a 75% alcohol solution. After a final washing in distilled water, each nodule was crushed in a petri plate containing 1 ml. of sterile water.

Ten petri plates were then prepared as follows: In each petri plate, 1 ml. of sterile water was added and inoculated with one loop of the nodule exudate. Warm liquid mannitol agar was then added and agitated slowly so that the inoculate would be distributed throughout the medium. These plates were then incubated at 28° C for twenty days. After incubation, the five petri plates containing the most uniform colonies were selected for examination. From these colonies, smear slides stained with acid fast and gram stain were made according to the method of Bryan et al. (1962).

RESULTS

Data from the above investigations show that nodule forming bacteria do infect roots of C. eriophylla. Furthermore, bacteriological and histological investigations have, to some extent, shown the nature of this infection. These results are presented in detail below.

Vegetational Survey

Palo verde-bur sage

Two transects, one on a north-facing slope and one on a south-facing slope were taken in a palo verde-bur sage community. The site had a 35 to 45 percent slope and was located on a gravelly sandy loam at 2600 feet elevation.

Palo verde-bur sage was by far the most frequent species, occurring in 82 percent of the plots on the south-facing slope and 76 percent of the plots on the north-facing slope. Guajilla occurred in only 5 and 6 percent of the plots on the north and south slopes respectively. (Table 1). Ten guajilla plants were counted on the south transect and 5 on the north transect (Table 2).

TABLE 1. --Percentage frequency of the important species of a palo verde-bur sage community

Species	South Aspect	North Aspect
Franseria deltoidea	82	76
Janusia gracilis	18	1
Simmonsia chinensis	8	7
Krameria parvifolia	7	18
Cassia bauhinoides	--	15
Carlowrightia arizonica	--	9
Calliandra eriophylla	6	5
Larrea tridentata	--	4
Opuntia spp. (cholla)	3	4
Mammillaria spp.	--	4
Other	6	6

TABLE 2. --Numbers of young, medium and old C. eriophylla plants found on north and south aspects of four plant communities.

	Community							
	Palo verde - bur sage		Creosote bush		Oak woodland		Mesquite grassland	
Age	South	North	South	North	South	North	South	North
Young	3	1	---	---	7	0	25	25
Medium	7	2	---	---	131	2	00	72
old	0	2	---	---	120	0	47	44
TOTAL	10	5	---	---	258	2	152	141

Creosote bush

Because creosote bush typically inhabits the more or less level "bajadas", the data are not presented according to aspect. The site sampled had a sandy clay soil and a deep subsoil. The elevation was approximately 2,750 feet.

In this community, creosote bush was the most frequently occurring plant. Guajilla did not occur in any of the plots on the transect, although this species was present in the area (Table 3).

TABLE 3. --Percentage frequency of the important species of a creosote bush community.

Species	level "bajada"
Larrea tridentata	26
Zinnia pumila	8
Cassia spp.	7
Tridens pulchellas	2
Malvastrum bicuspidatum	1
Calliandra eriophylla	--

Oak Woodland

The oak woodland community was sampled approximately thirty miles north of Sonita, Arizona. The soil in the area was a sandy loam, and the hill sides sampled varied from 30 to 35 percent slopes. Transects were made on both the north and south aspects.

Grass species were the most frequently occurring species on these transects, particularly on the north aspect. A high frequency of guajilla was observed on the south aspect and an extremely low frequency appeared on the north slope. Another species of Calliandra, C. humilis was noted on the north-facing slope (Table 4).

Mesquite Grassland

The Mesquite-Grassland transects were taken at approximately 4,350 feet elevation on a sandy loam soil having a rocky subsoil and a 35 precent slope.

TABLE 4. Percentage frequency of the important species of an oak woodland community.

Species	North Aspect	South Aspect
Bouteloua curtipendula	75	84
Eragrostis intermedia	58	--
Andropogon spp.	35	--
Andropogon babinodes	21	45
Aristida spp.	12	45
Bouteloua hirsuta	16	--
Bouteloua radicosa	16	--
Artemisia spp.	11	--
Calliandra humilis	10	--
Eriogonium wrightii	9	--
Muhlenbergia spp.	6	--
Hilaria belangeri	--	33
Sida spp.	--	12
Other	9	4
Calliandra eriophylla	1	80

As in the oak woodland community, grass species were again the most abundant. However, on these transects gujilla appeared frequently. The numbers of guajilla occurring on the north and south aspects were comparable both in terms of total numbers and percent frequency. The relative ages of these plants were also rather uniform (Table 5).

TABLE 5. --Percentage frequency of the important species on a mesquite grassland community.

Species	North Aspect	South Aspect
Calliandra eriophylla	52	66
Bouteloua filiformis	66	50
Bouteloua chondrosoides	31	62
Bouteloua curtipendula	58	19
Hilaria belangeri	63	4
Mimosa dysocarpa	18	--
Trichachne californica	8	8
Andropogon barbinodis	7	--
Heteropogon contortus	6	7
Bouteloua eriopoda	--	5
Fouquieria splendens	4	3
Eriogonium wrightii	6	--
Artemisia spp.	4	--
Aristida spp.	10	2
Other	13	17

Field Root Study

Observations using the direct method of root study on guajilla plants, which were approximately five to eight years old, indicated an extensive root system (Figure 6). A vertical tap root six or seven feet deep with secondary roots and rootlets within the top one-third of the main root were frequently observed.

A number of different ages and forms of nodules were found on these rootlets. The younger nodules were a brown-red color while the older nodules were black and filled with a black powder. Nodule form varied from spherical to elongated and cylindrical or elongated and branched (Figure 7). However, because of the extensive disturbance to the rootlets, root hairs, and nodules when using the direct method, adequate data as to numbers and locations of nodules were not obtained.

On the older plants, sprouts from secondary roots growing parallel to and just below the soil surface were often noted.

In general, data from observations on the seedlings using the "washed-block" method were more accurate and detailed due to the lack of disturbance to the nodules (Table 6).

Figure 6. Root system of a 5 to 8 year old _C. eriophylla_ plant.

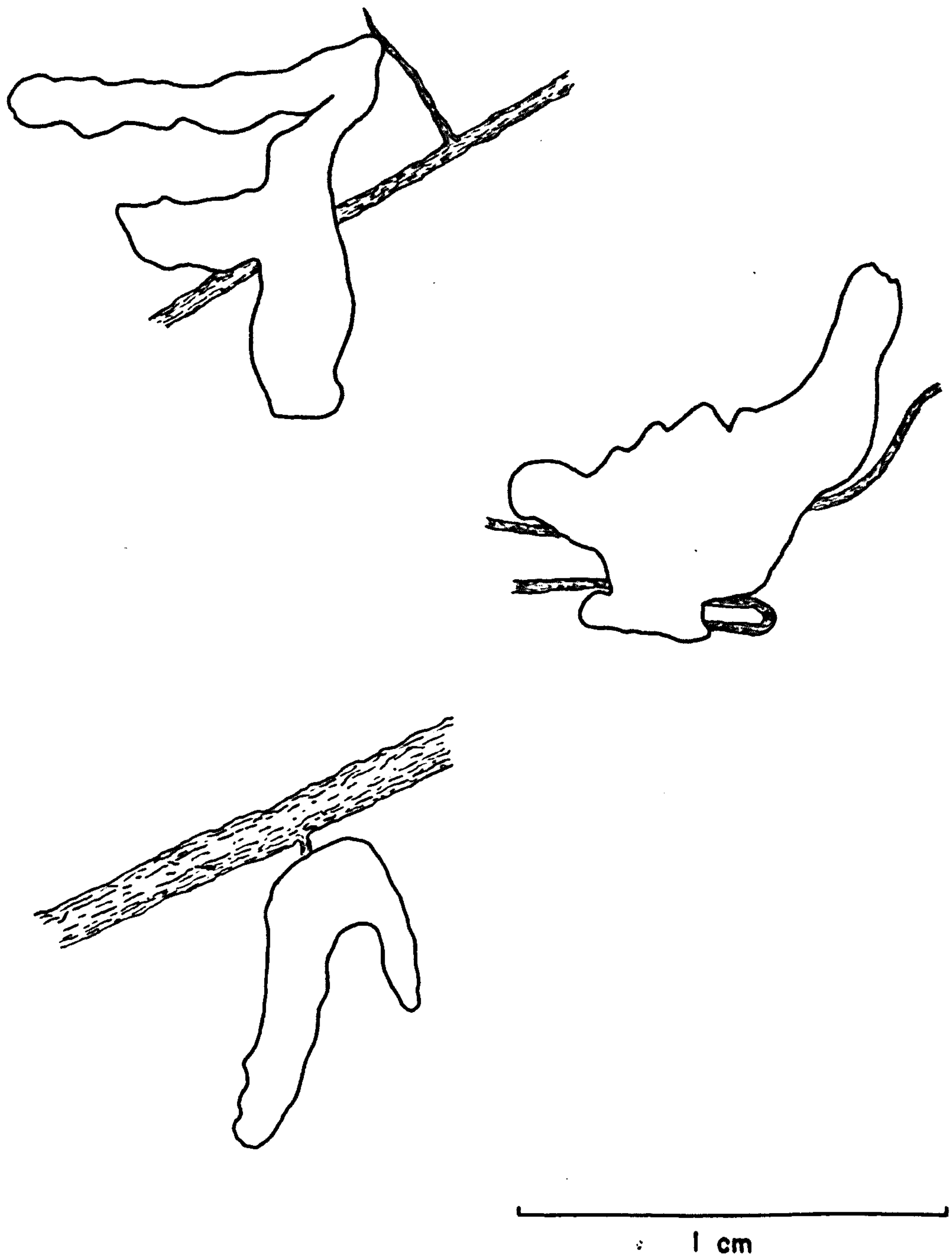

Figure 7. Nodule shapes found on roots of the field plants.

TABLE 6.--Number of nodules observed on C. *eriophylla* seedling roots taken from a desert grassland community.

Replication	Number of nodules per plant North Aspect	South Aspect
1	6	8
2	5	12
3	0	21
4	2	0
Total	13	41
Mean	3.25	12.25

The analysis of variance for these data indicated no significant difference between the number of nodules on plants occurring on different slope aspects, even though there were more nodules per plant on plants from south slopes. (Table 7). Additional sampling would be necessary to make definite statements.

TABLE 7. --Analysis of variances for the field root nodule data.

Source	df	SS	MS	F
Total	7	349.5		
Treatment	1	98.0	98.0	2.34*
Error	6	251.5	41.91	

* not significant at the 5 percent level

Greenhouse Study

The minimum and maximum temperatures occurring on the north and south sides of the greenhouse are given in Figure 8. The analysis of variance of the root nodule data obtained in the greenhouse is presented in Table 8.

As may be seen from the analysis of variance (Table 8), only the age and temperature-age interaction had a significant influence on the number of nodules. Table 9 gives the average number of nodules produced according to age and temperature. Age means were separated by Duncan's Multiple Range test at the 5 per cent level (Table 10).

TABLE 8. --Analysis of variance for the greenhouse root nodule data.

Source	df	SS	MS	F
Block	3	18.39	6.13	
Temperature	1	9.38	9.38	
Moisture	2	1.23	.62	
Age	8	251.51	31.44	4.82*
Temp x Moisture	2	20.58	10.29	
Temp x Age	8	160.58	20.07	3.08*
Moisture x Age	16	80.36	5.02	
Temp x Moisture x Age	16	61.83	3.86	
Error	159	1036.36	6.52	
Total	215	1640.22		

*significant at 5% level

Figure 8. Minimum and maximum temperatures occurring on the north and south sides of the greenhouse.

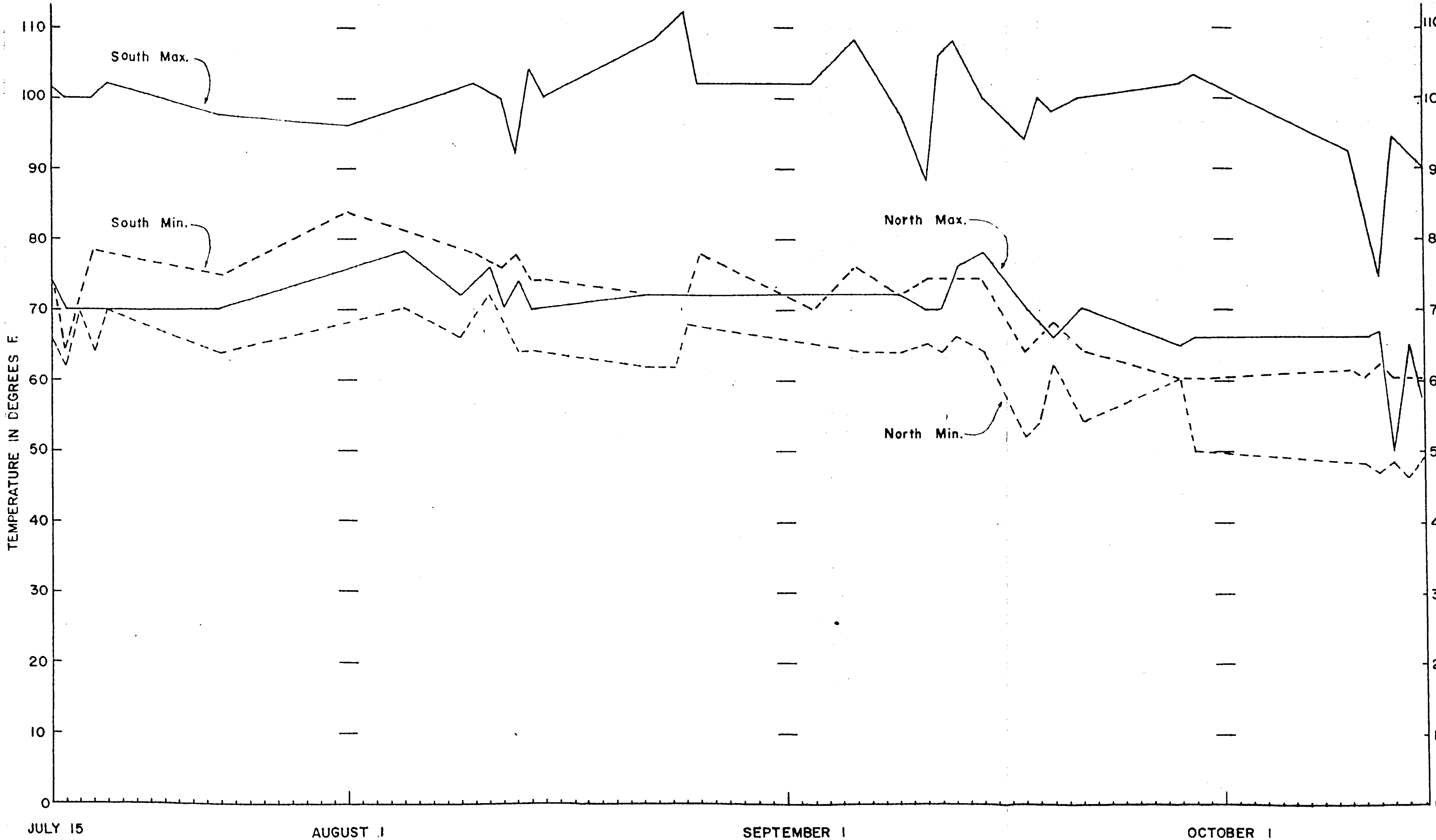
South Max.
South Min.
North Max.
North Min.
TEMPERATURE IN DEGREES F.
110
100
90
80
70
60
50
40
30
20
10
0
JULY 15
AUGUST 1
SEPTEMBER 1
OCTOBER 1

TABLE 9. --Average number of nodules per plant at various days from planting as affected by low, medium, and high moisture levels and by high and low temperature levels in the greenhouse.[1]

Days from planting and temperature levels		Moisture levels		
		Low	Medium	High
10	High	.50	.75	.00
	Low	.75	.50	.00
20	High	2.00	.50	1.50
	Low	.75	.00	.50
30	High	.00	.75	.50
	Low	.75	2.00	.25
40	High	1.25	1.75	1.25
	Low	3.75	1.25	1.00
50	High	3.50	2.50	3.75
	Low	2.50	1.25	3.00
60	High	1.00	1.00	1.25
	Low	.25	.75	3.00
70	High	6.25	8.50	3.75
	Low	2.50	1.50	1.25
80	High	1.75	5.50	1.75
	Low	1.50	1.00	3.25
90	High	1.25	1.00	1.00
	Low	3.75	2.50	3.75

1. These means are the average of four plants.

Root nodules found on the plants in the greenhouse test were located close to the soil surface and had a number of different forms. These nodules occurred primarily on the upper quarter of the roots, indicating that the bacteria forming these nodules are aerobic (McKee, 1962).

Age and temperature-age interaction were the only factors which were significant.

TABLE 10. --Duncan's Multiple Range Test at the 5 percent level for average number of nodules from various age and temperature levels in the greenhouse study.

6.17	3.33	3.25	3.00	2.25	2.00	1.92	1.75	1.42	1.33	1.08	1.00	.42

(Any two means underlined by the same line are not significantly different).

According to the Duncan test, only the 70-day samples deviated significantly from the general trend. This was probably due to only one plant that had an "abnormally" high number of nodules (25 as compared to a trend of below 6).

An interesting relationship also occurred in the dry weight data (Figure 9). This was that the plants growing at the cooler temperature had comparatively higher weights.

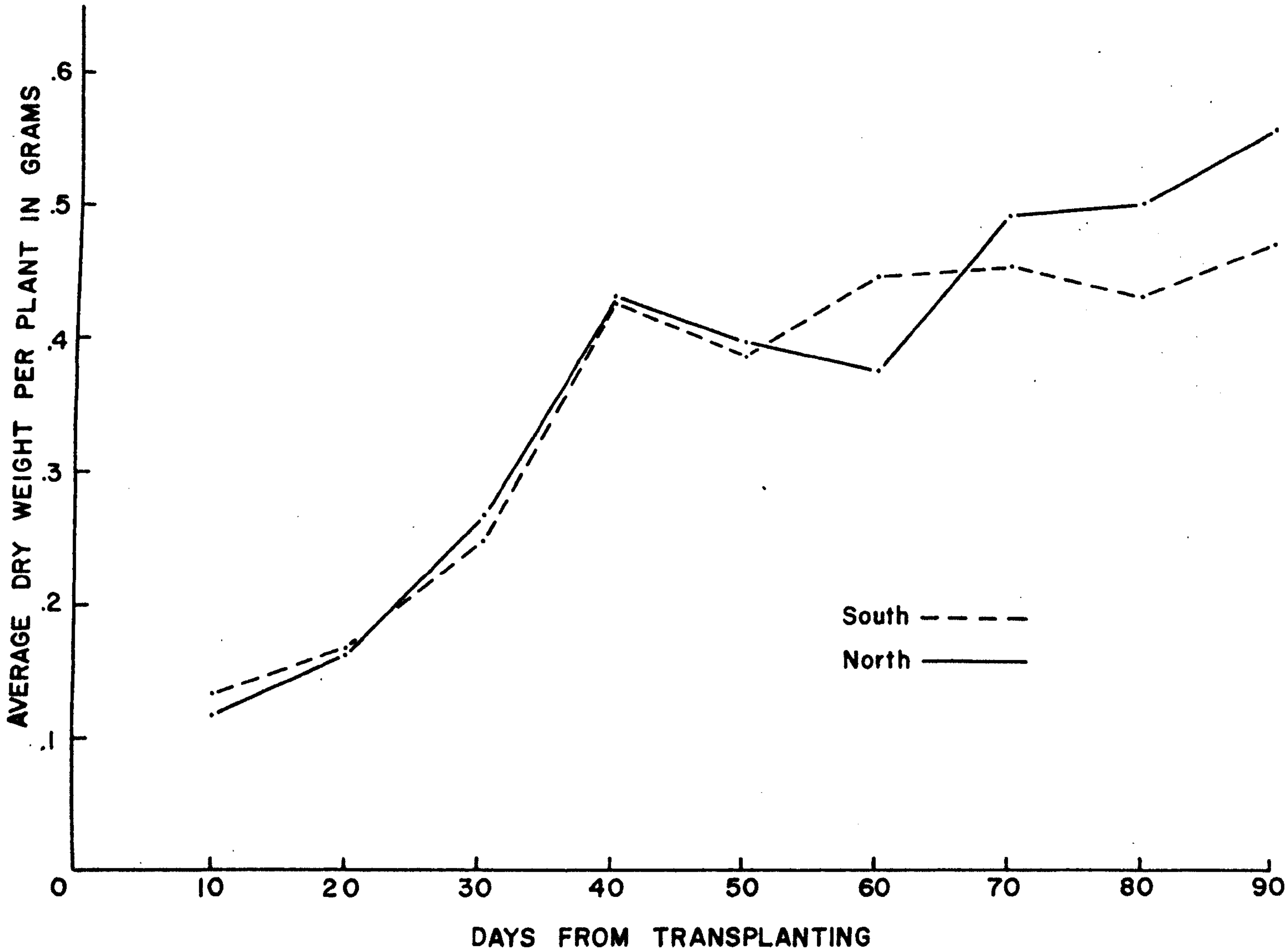

Figure 9. Dry weight of greenhouse plants.

Bacteriological Examination

Bacteria from the nodules of guajilla plants stained with the gram-stain did not retain a blue color; therefore, indicating a gram-negative reaction. Bryan et al. (1962), using Rhizobium, showed that a blue color with acid fast stain indicated a non-acid fast bacteria. The guajilla nodule bacteria staining properties indicated that the bacteria were probably in the genus Rhizobium. The stains also showed several forms of the bacteroids that are also characteristic of Rhizobia. These are cocci, ellipsis, and irregular x-, y-, star-, and club-shaped forms (Figure 10).

Histological Examination

Nodules stained with Safranin fast green showed several distinct areas. Those tissues which could be identified were: 1) cork (dark red stain), 2) cortex (orange red), 3) vascular tissue (green), 4) cell wall (dark green), 5) nucleus (reddish brown), 6) infection thread (green), and 7) the cells infected with bacteria (brown).

The Hematoxilin stain showed only brown and black colors. Corky tissues and the cortex region were black. The cell walls were light brown. Bacteria were stained black.

Cross sections of the nodules showed four major areas: (cork, cortex, vascular system, and bacteriodal region) (Figure 11). The number of vascular bundles found in the cross sections was variable (Figure 10, a and b). Longitudinal sections showed these same four areas in addition to a meristematic area (Figure 12). The vascular region was shown to connect the nodule to the rootlet (Figure 13).

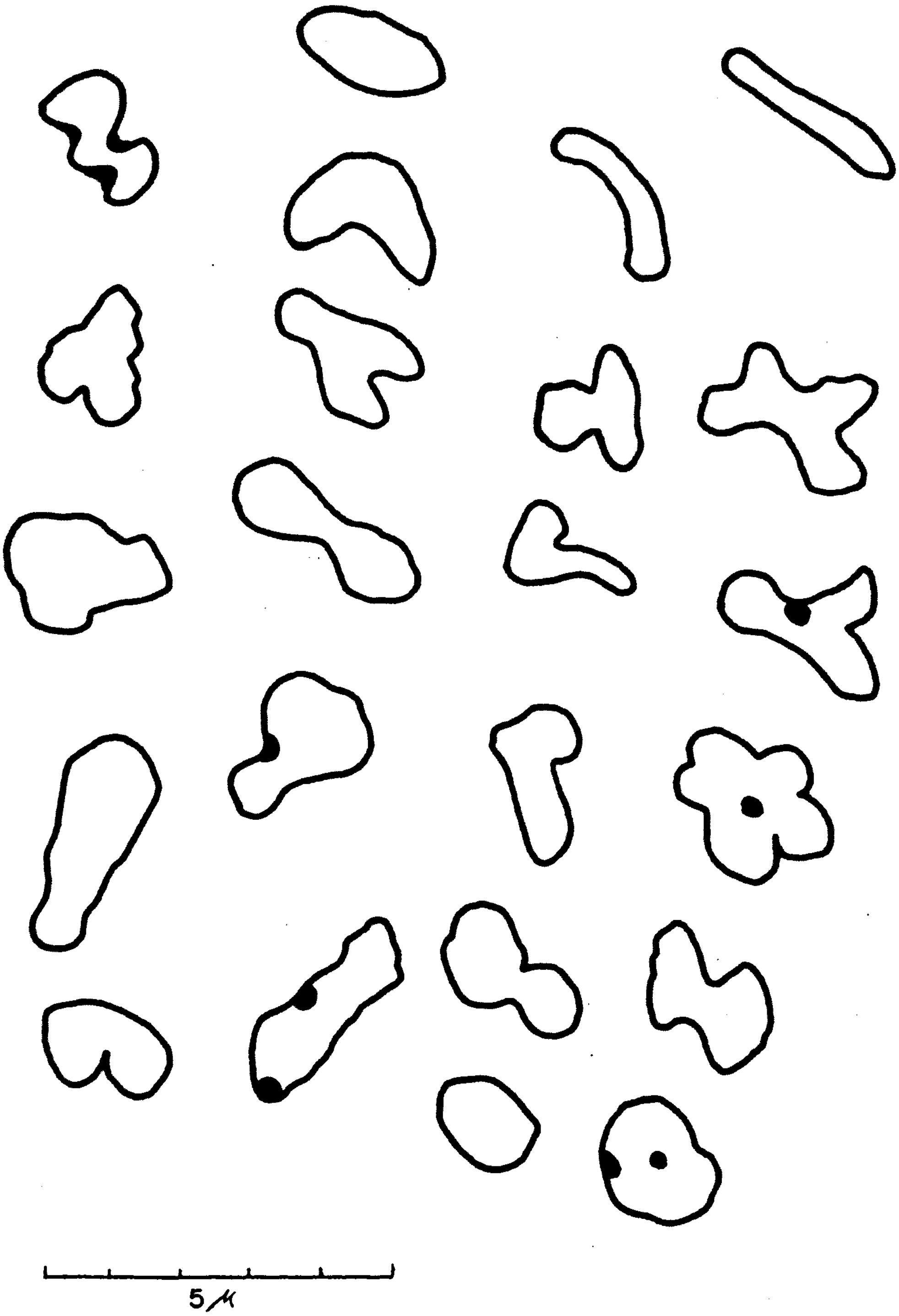

Figure 10. Bacteroid forms of Rhizobium using acid-fast stain.

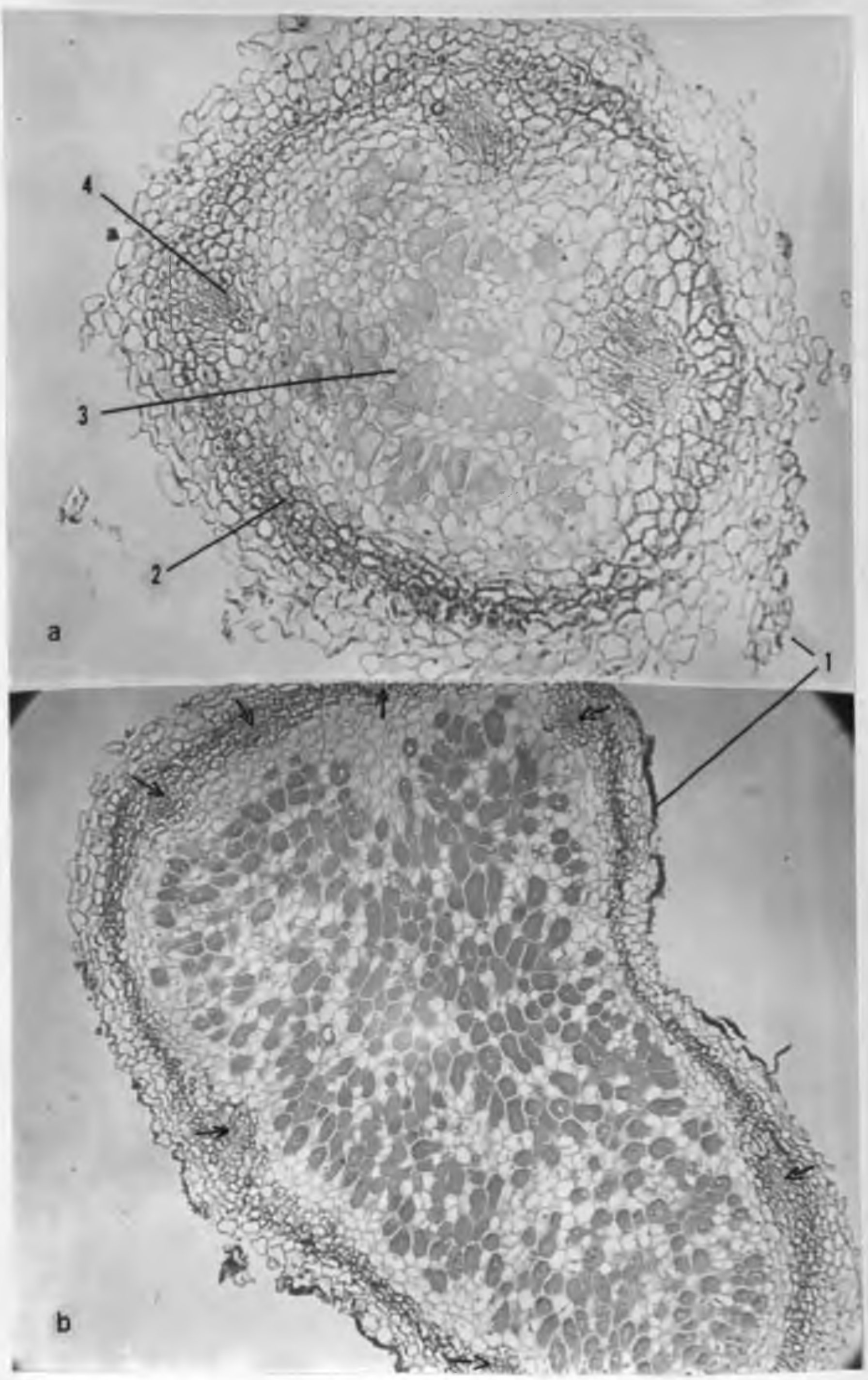

Figure 11. Cross section of nodules showing four major areas: 1) cork, 2) cortex, 3) bacteroidal region and 4) vascular system where a) has three bundles and b) has seven bundles.

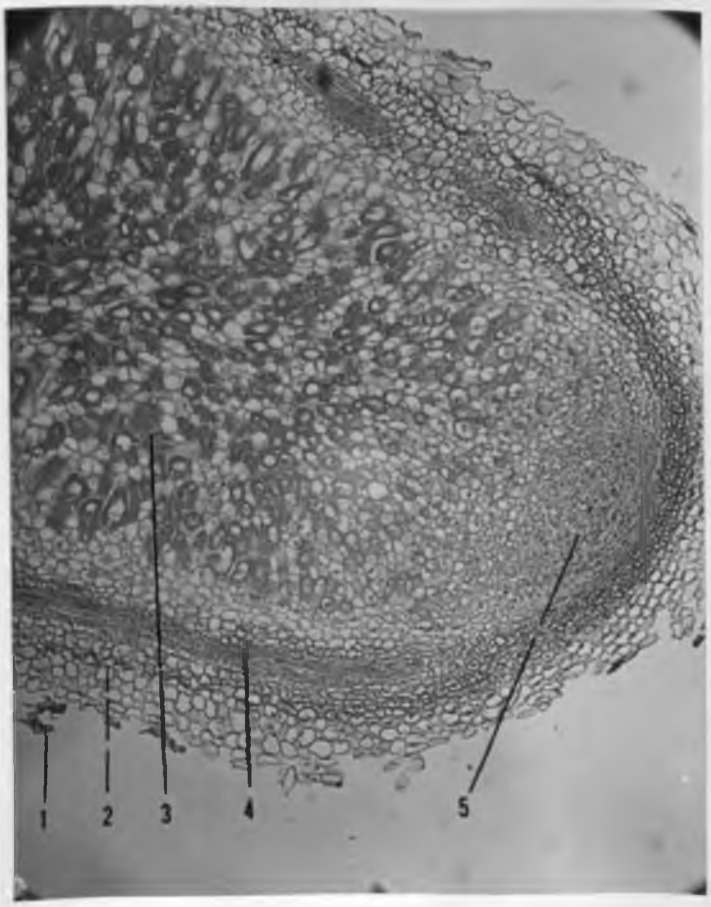

Figure 12. Longitudinal section of a nodule showing five major areas: 1) cork, 2) cortex, 3) bacteroidal region, 4) vascular system, and 5) the meristematic area.

The infection thread could be seen in both the longitudinal and cross sections of the nodule. The thread was noted in the middle lamella and the cytoplasmic area of young cells. Two types of threads were observed; one having the shape of a funnel (Figure 14) and the other resembling hyphae tips (Figure 15). Both of these types stained green or brownish green.

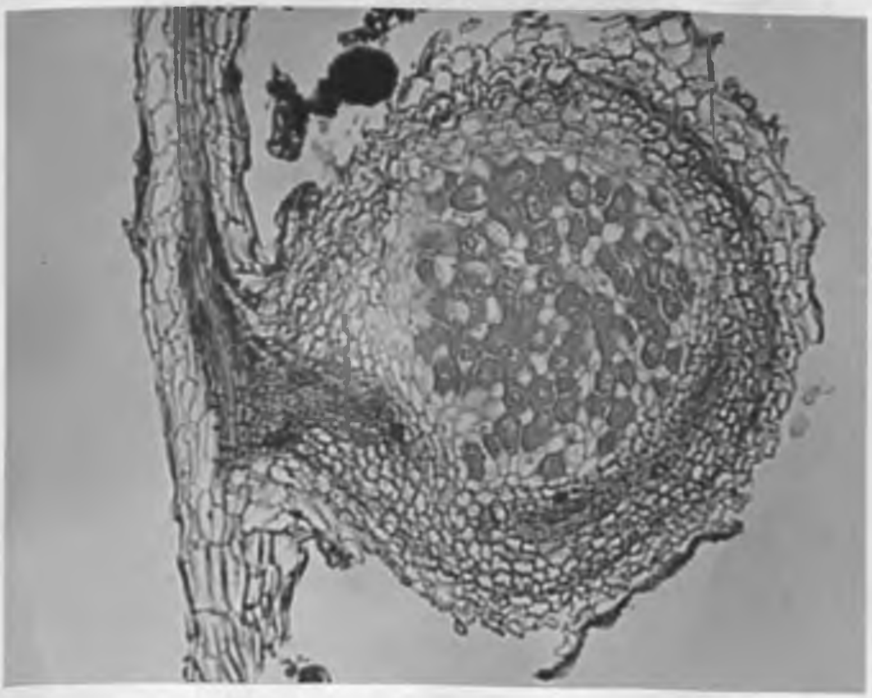

Figure 13. Longitudinal section of nodule showing link between the nodule and the root.

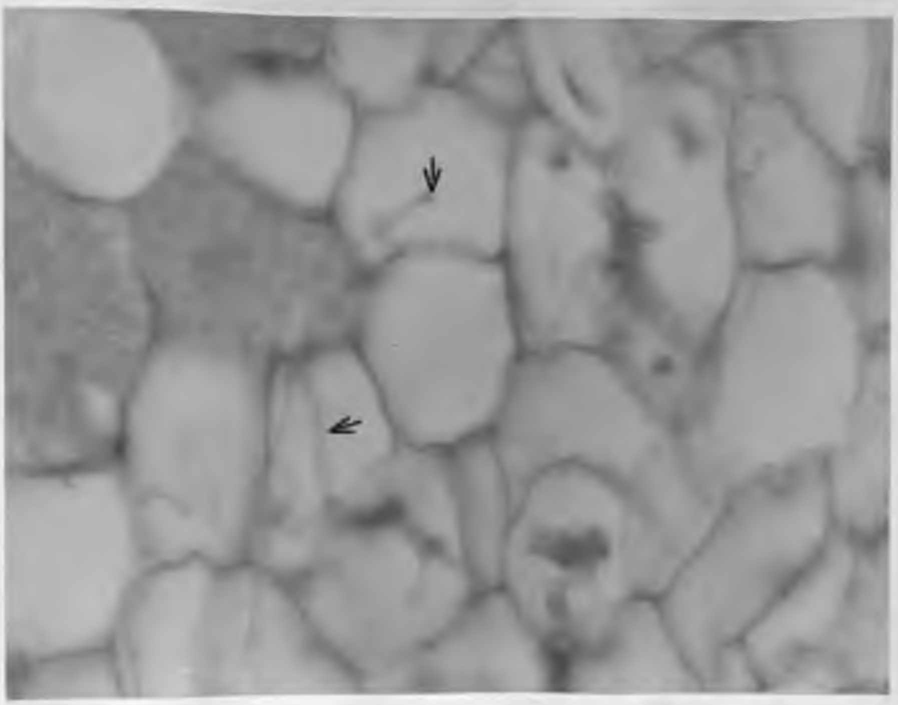

Figure 14. Cross section showing a funnel shaped infection thread.

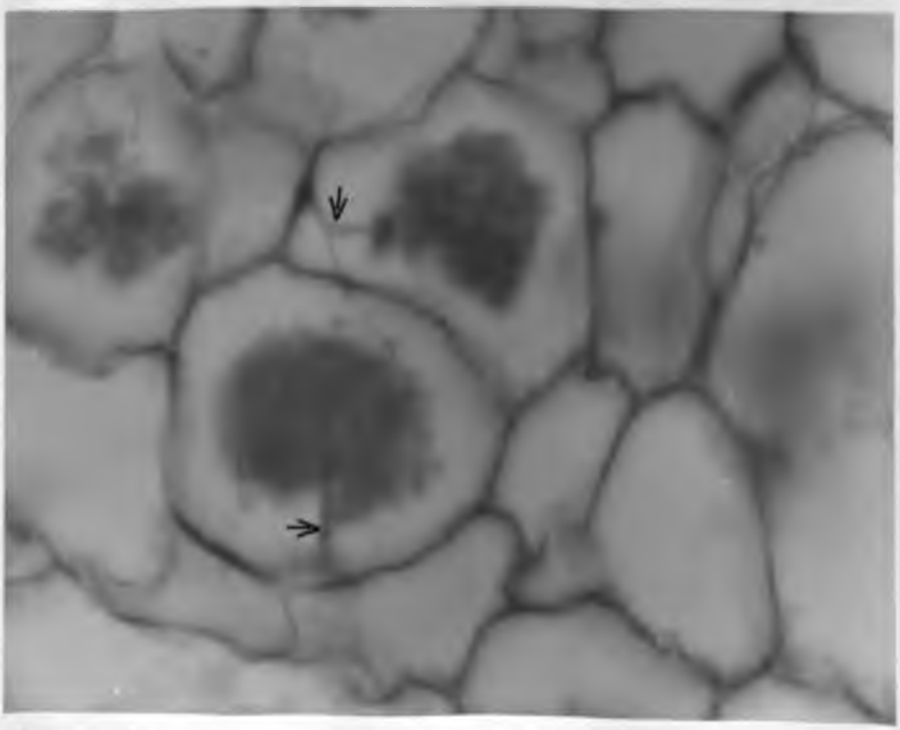

Figure 15. Cross section showing a hyphae tip shaped infection thread.

DISCUSSION

Field and greenhouse observations on *C. eriophylla* roots have confirmed the presence of nodules. Most of these nodules were located on the upper portion of the lateral roots suggesting that aerobic bacteria are responsible for the nodulation. The morphological and staining characteristics of these bacteria were checked with other investigators (Bergerson, 1955; Alexander, 1961; McIntosh, 1964), and, using these comparisons, were identified as a member of the genus *Rhizobium*. Although no direct observation of nitrogen fixation using N^{15} or other such techniques were used, the nature of the infection, that is, the presence of infection thread, bacteroids, etc., and the rose color of many of the nodules suggested active nitrogen fixing bacteria.

The Nature of the Infection

At the point of infection by the nodule forming bacteria, the roots were slightly curved. This seemed to be due to a slight curling of the rootlets at the time of infection. Later, as the rootlet continued growth, a slight curving of the root resulted (Nutman, 1956).

Tissues that can be observed in the cross and longitudinal thin sections of the nodules are generally the same as those occurring in the roots. These are:

1) An outer corky tissue made up of dead cells that were in direct contact with the soil.

2) The cortex which is the area immediately inside the corky layer and which is made up of meristematic tissue and small areas of vascular bundles. The connecting link between the root and the nodules appear to arise from one vascular strand. Also, in the cortex region may be seen non-infected parenchymatous cells of small size.

3) The bacteroid tissue, located in the center of the nodule inside the cortical, vascular, and corky areas. Both infected and non-infected Parenchymatous cells make up the bacteriodal tissues. Mature infected cells are 2 to 4 times the size of the neighboring bacteria-free cells. This is in contrast with Allen and Allen (1954) who found an increase of 4 to 8 times in the size of infected cells. He also noted a lack of continuity of the infection thread and explained this by saying that the threads may become broken during cell enlargement.

The infection thread, as described by Nutman (1956), is a cellulosic sheath around the bacteria, which confines them within a narrow tube. Cross sections of the meristematic area of guajilla root nodules also show the infection thread. However, these threads are located primarily in the middle lamella with a few present in the host cell cytoplasm near the cell nucleus. According to Allen and Allen (1954), the infection thread attacks the cell nucleus first. The bacteria then reproduce within the cell and spread throughout the cytoplasm.

A few cells contain an infection thread but show no sign of an infection (Figures 13 and 14). Other investigators have also noticed this phenomenon and explained it on the basis of certain infection inhibitory factors within the cells. (Allen and Allen, 1954).

Both in the field and in the greenhouse, nodules having many different forms were noted. According to Nutman (1956), this depends primarily upon the size and shape of the meristem and its tendency towards bifurcation. Allen and Allen, (1954) also suggested that nodule shape depends upon the shape of the nodule meristem and that there are essentially three types which influence nodule shape. These are: 1) the hemispherical or bowl-shaped type which produce spherical nodules, 2) the apical type which produces elongated, cylindrical nodules, and 3) the laterally divided type which produces lobate or digitate forms. The flat leaf-shaped nodules are presumably caused by their growing between closely spaced rocks.

Normally, the nodule meristematic cells do not contain either bacteria or the infection thread. They are active and continue to divide; thus, offering a continuous supply of young cells which differentiate into the outer vascular or cortical tissues which may never become infected. Cells which divide to form the inner tissues do become infected and continue the symbiotic functions (Allen and Allen, 1954).

The number of vascular bundles occurring within a nodule has been discussed as being: 1) genetically controlled; that is, different strains of bacteria and host plants develop nodules having different numbers of vascular bundles (Raggio and Raggio, 1962), or 2) they are a function of the nodule growth; the older and larger nodules having more bundles due to division within the vascular cells (Allen and Allen, 1954).

Field and Greenhouse Data

There was no significant difference in the number of nodules occurring on plants on south-facing slopes and plants on north-facing slopes. However, because of the small sample size, it was believed that these findings may not have indicated the actual field conditions. General observations indicated that there is indeed a difference in the numbers of nodules per plant between slopes; the greater number occurring on the south-facing slopes. This finding seems to be widely held (Moore, 1905; Joffee, et al., 1961; Allen and Allen, 1954); the reason being that higher temperatures and greater light intensity are responsible for higher photosynthetic rate. This in turn increases carbohydrate production, a factor that favorably influences nodule efficiency.

If it is true that plants on more xeric south-facing slopes tend to have more nodules, then this may also indicate that differences in available moisture between north and south slopes is not a limiting factor in nodule production in desert grassland communities. Moore (1905) reported that bacteria infect root hairs to a greater degree at low moisture levels though undoubtedly there would be a limit to this generalization.

Unfortunately, the greenhouse experiments designed to investigate the relationship between temperature, moisture, light and age and nodulation could not be interpreted adequately due to a number of confounding factors. Although temperatures were greater on the south side of the greenhouse, there was more direct sunlight on the north side. Therefore, seedlings on the north side of the greenhouse had cooler temperatures but more light, while those on the south had higher temperatures and less light.

The confounding effects of these different temperatures and light conditions showed up to a degree in the dry weight comparison between plants on the north and plants on the south side of the greenhouse. Plants on the north had higher dry weights, possibly because of the higher light intensity which caused a greater carbohydrate production and because cooler temperatures resulted in less carbohydrate use in respiration. If this is true, the higher carbohydrate levels would favor nodule production for plants on the north side, and this would tend to confound the temperature treatments.

Genetic variation may also have been a contributing factor. Nutman (1956) discussed the genetic structure of the host plant and bacteria strains. He recognized several genetic factors which influence the number and efficiency of the nodules.

In spite of these confounding factors there was a slight, but non-significant, higher nodule production on plants exposed to higher temperatures and a slight trend in increasing nodule production with decreasing moisture level.

In the plant communities sampled, guajilla occurred in measurable amounts in the palo verde-bur sage, mesquite-grassland and oak woodland communities. Even though guajilla occurred in the palo verde - bur sage community, its numbers were small; and it probably does not contribute significantly to the nitrogen balance of these areas. In the mesquite-grassland and oak woodland areas, however, the species is abundant enough so that it could make a contribution to the nutrient status of these communities. And, since these types represent much of the area of the southern desert shrub region, guajilla may well be one of the more important of the native desert legumes not only because of its forage value, but also because of its nitrogen fixing ability. As such, it should be considered in any recommendations made pertaining to shrub control in the mesquite-grassland and oak woodland areas of the southwest.

SUMMARY

Range and Watershed Management plans are often made without adequate knowledge of the plant species involved. A study was designed to investigate the role of *Calliandra eriophylla* Benth, a native desert leguminous shrub, as a nitrogen producer in desert areas. The investigation involved:

1. A field study to determine the relative abundance of the species in four different communities of the Southern Desert Shrub region: (1) palo verde - bur sage, (2) creosote bush, (3) oak woodland and (4) the mesquite-grassland.
2. A field root-study to determine the extent of nodulation under different field conditions.
3. A greenhouse study to determine the relationship between nodulation and temperature, moisture level, light and age.
4. Histological and bacteriological examination to determine the nature of the infection.

These studies confirmed the presence of nodules on *C. eriophylla* and suggested that the bacteria (a member of the genus *Rhizobium*) was active in fixing nitrogen. Of the communities sampled, only the mesquite-grassland and oak woodland were believed to have sufficient numbers of the species of *Calliandra* to warrant consideration as a nitrogen supply in range and watershed management practices. The greenhouse experiments were inconclusive.

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APPENDIX A

Plant Species Found on the Study Area

Scientific Name	Common Name
Acacia constricta Benth.	White-thorn
A. greggii Gray.	Catclaw acacia
A. schottii Engelm.	Amole
A. palmeri Engelm.	Maguey loco
Aloysia wrightii (Gray) Heller	
Amoreuxia palmatifida Moc. & Sesse.	
Andropogon barbinodis Lag.	Cane beardgrass
Andropogon spp.	
Aristida adscensionis L.	Six weeks three-awn
A. orcuttiana Vasey.	Single-awn aristida
A. glabrata (Vasey) Hitchc.	Santa Rita three-awn
Artemisia annua L.	Sweet worm wood
Artemisia spp.	Sage
Arctostaphylos pringlei Parry.	Manzanita
Baccharis brachyphylla Gray.	
Boerhaavia coulteri (Hook.f.) Wats.	Culter spiderling
Bouteloua filiformis (Fourn.) Griffiths.	Slender grama
B. curtipendula (Michx.) Torr.	Sideoats grama
B. trifida Thurb.	Red Grama
B. chondrosiodes (H.B.K.) Benth.	Sprucetop grama

Scientific Name	Common Name
B. radicosa (Fourn.) - Griffiths.	Purple grama
B. hirsuta Lag.	Hairy grama
B. gracilis (H.B.K.) Lag.	Blue grama
B. eriopoda (Torr.)	Black grama
B. aristidoides (H.B.K.) Griseb.	Needle grama
Brickellia coulteri Gray.	Brickell bush
Bromus rubens L.	Red brome
Calliandra eriophylla Benth.	Guajilla
Calliandra humilis Benth.	
Carlowrightia arizonica Gray.	
Carnegiea gigantea (Engelm) Britt & Rose	Sahuaro
Cassia lindheimeriana Scheele.	Senna
C. bauhinoides Gray.	
Ceanothus greggii Gray.	Desert mallow
Ceanothus spp.	
Cercidium microphyllum (Torr.) Rose & Johnson	Palo verde
Encelia farinosa Gray.	Brittle-bush
Eragrostis intermedia Hitchc.	Plains lovegrass
Eriogonum wrightii Torr.	Wild buckwheat
Erythrina flabelliformis Kearney.	Western coral bean
Euphorbia spp.	Milk weed
Ferocactus covelli Britt & Rose.	Barrel cactus
Fouquieria splendens Engelm.	Ocotillo

Scientific Name	Common Name
Franseria ambrosiodes Cav.	
F. deltoidea Torr.	Bur sage
Gossypium thurberi Todaro (Gray).	Wild cotton
Heteropogon contortus (L.) Beauv.	Tangle head
H. melanocarpus (Ell.) Benth.	Sweet tangle head
Hibiscus coulteri Harv (Jones).	
Hilaria belangeri (Steud.) Nash.	Curly mesquite
Janusia gracilis Gray.	Janusia
Jatropha cardiophylla (Torr.) Muell. Arg.	Sangre de drago
Kallstroemia grandiflora Torr.	Arizona poppy
Krameria parvifolia Benth.	Range ratany
Krameria grayi Rose & Painter.	White ratany
Koeberlinia spinosa Zucc.	Crucifixion thorn
Larrea tridentata (DC.) Coville.	Creosote bush
Leptoloma cognatum (Schult.) Chase.	Fall witchgrass
Leptochloa dubia (H.B.K.) Nees.	Green springletop
Lycium Parishii Gray.	Desert thorn
Lycurus phleoides H.B.K.	Wolftail, Texas timothy
Malvastrum bicuspidatum (Wats.) Rose.	
Mammillaria spp.	Bisnaga
Mimosa biuncifera Benth.	Wait-a-minute
Mimosa dysocarpa Benth.	Handsomest mimosa
Muhlenbergia rigens (Benth.) Hitchc.	Deer grass
Muhlenbergia porteri Scribn.	Bush muhly

Scientific Name	Common Name
Muhlenbergia repens (Presl.) Hitchc.	Aparejo grass
Opuntia spp.	Prickly pear
Opuntia spp.	Cholla cactus
Opuntia leptocaulis DC. (Knuth).	Christmas cactus
Panicum hallii Vasey.	Hall's panicum
Parthenium incanum H.B.K.	Mariola
Psilostrophe cooperi (Gray) Greene.	Paper flower
Prosopis juliflora (Swartz) DC.	Mesquite
Quercus oblongifolia Torr.	Mexican blue oak
Setaria macrostachya H.B.K.	Plain bristle grass
Sida spp.	
Simmondsia chinensis (Link.) Schneid.	Jojoba
Solanum elaegnifolium Cav.	White horse-nettle
Sphaeral Cea spp.	Globe-mallow
Talinum angustissimum (Gray) Woot. & Standl.	
Trachypogon secundus (Presl.) Scribn.	Crinkle-awn, trompillo
Tridens pulchellus (H.B.K.) Hitchc.	Fluffgrass
Trichachne californica (Benth.) Chase.	Arizona cottontop
Trixis californica Kellogg.	
Zinnia pumila Gray.	Zinnia