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Physiological and Biochemical Studies of Nitrogen Fixation in the Nodules of Sagebrush (Artemisia Ludoviciana) and Other Desert Nitrogen-Fixing Systems

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PHYSIOLOGICAL AND BIOCHEMICAL STUDIES OF NITROGEN FIXATION IN THE NODULES OF SAGEBRUSH (*ARTEMISIA LUDOVICIANA*) AND OTHER DESERT NITROGEN-FIXING SYSTEMS

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ABSTRACT

Ecosystem analysis and subsequent modeling of desert ecosystems require quantitative data on the amount of fixed nitrogen being added to the nitrogen pool by biological nitrogen fixation. Nodulation of *Artemisia ludoviciana* and reduction of acetylene by these nodules indicate this system to be a source of fixed nitrogen in many areas of the desert ecosystem. Field studies were undertaken to determine the distribution in this area of *A. ludoviciana*, and to determine the presence of nodules on this species and other desert plants. Sites investigated were the Pine Valley and Curlew validation sites, Morgan, Utah, the B.Y.U. Farm near Spanish Fork, Utah, and the Manti-LaSal National Forest area. Data were collected on soil temperatures and soil moisture content at depths of 7.5 and 15.0 cm. Soil moisture at Pine Valley and at Curlew Valley at these depths in May was near the 15-atmosphere level, and soil temperatures were at 18-20 C. No nodulation was observed on plant species in the area. Major efforts of investigation were concentrated at Morgan where nodulation had been found previously. Studies were made to determine the factors effective in controlling nodulation. Very slight nodulation was found at the B.Y.U. Farm on *A. ludoviciana* and on July 6 at the Manti-LaSal area. However, the nodules found were effective in acetylene reduction.

No nodulation was observed in 1974 at Morgan. A comparative study on climatic data was made for 1974 in relation to 1967 and 1972 when intense nodulation was found. From the investigation made during 1974, the preliminary findings indicate: 1) A fragile balance between soil temperatures, soil moisture and nodulation; 2) Apparently nodulation occurs when soil temperatures are wtihin the range of 5-10 C for a sustained period of time and soil moisture levels hold within a range of 25 to 80% of the available moisture supply concurrent with the temperature range; 3) The data show that 1974 had a cold, wet spring, with precipitation considerably above normal and temperatures considerably below normal, after which it turned hot and dry; 4) As nodules become old they do not reduce acetylene.

INTRODUCTION

Ecosystem analysis and subsequent modeling of desert ecosystems require quantitative data on the amount of fixed nitrogen being added to the nitrogen pool by biological nitrogen fixation.

Nodulation of sagebrush, *Artemisia ludoviciana*, was first observed by Farnsworth and Hammond (1968). Other desert and forest plants have been found to show nodulation and are probably associated with nitrogen fixation in the desert system (Clawson 1973, Clawson et al. 1972, Farnsworth and Clawson 1972, Holiday 1971, Wallace and Romney 1972, Youngberg and Wollum 1970).

Nonleguminous plant symbiosis has long been recognized (Allen and Allen 1965) but this source of nitrogen fixation has taken on an entirely new perspective since 1951 (Allen and Allen 1965, Farnsworth and Hammond 1968).

In 1973 it was decided that the role of symbiotic nitrogen fixation by nonleguminous plants should be considered as a part of the Desert Biome program. The project was funded for 1974.

OBJECTIVES

The project is designed to include both field and laboratory studies in order to determine the contribution of sagebrush and other similar desert plants to the nitrogen cycle in the desert biome and to provide preliminary data on the nature and properties of the nitrogen-fixing system in sagebrush. The specific objectives follow.

Field Studies

1. Study the extent and distribution of *Artemisia ludoviciana* and the extent of nodulation at two Desert Biome validation sites (Pine Valley and Curlew Valley) and in areas adjacent to Provo, Utah, and to provide data for estimating the magnitude of nitrogen fixed by sagebrush at these sites.
2. Study field measurements of soil and air temperature, soil moisture and available combined soil nitrogen content at the time of nodulation and monitor changes in these conditions and N_2-fixing ability during the "life" of the nodules.
3. Determine the longevity and magnitude of nitrogen-fixing activity at the different geographical locations. Also look for factors which may affect nodulation and nitrogen fixation at these sites.
4. Determine distribution of nodulated plants and calculate amounts of nitrogen fixed by these systems.
5. Examine other desert plants for root and aerial nodules which may contribute to nitrogen fixation.
6. Investigate possible relationships between the root system of non-nodulated desert plants and free-living nitrogen-fixing microorganisms.

Laboratory Studies

1. Produce nodules in the laboratory by growing plants in soil from the field, varying temperature, moisture conditions and combined nitrogen content to match conditions observed in the field studies.
2. Examine the sagebrush nodules by various microscopic techniques, looking for infection thread and development of bacteroids, etc. Examine the factors responsible for senescence of sagebrush nodules and the infective

process for involvement of indole acetic acid and other plant hormones.

3. Reisolate and characterize endophyte from sagebrush nodules from field and plants grown under laboratory conditions. Characterize and establish the identity of the endophyte and examine it for nitrogen-fixing ability in a nonsymbiotic environment.
4. Investigate possible cross-inoculation of legumes with the isolated endophyte.
5. Examine the nitrogen-fixing properties of intact nodules.
6. Prepare cell-free extract and examine nitrogenase activity (acetylene and nitrogen reduction) and the gross composition of the enzyme system (number of protein components and metal content).
7. Look for a "leghemoglobin-like" protein in the nodules.

METHODS

The study of nitrogen fixation by nonleguminous plants is a new Desert Biome investigation which began in 1974. The procedures for the study were formulated and set forth prior to the spring growing season of 1974 and are as follows.

Field Studies

Field studies at the Pine Valley and Curlew Valley validation sites will be carried out to determine the distribution of *Artemisia ludoviciana* and the extent of nodulation on these plants. Preliminary visits to the Rock Valley site will likely be made to initiate a collaborative investigation with Evan Romney and Arthur Wallace.

Nitrogen-fixing activity of the sagebrush nodules will be determined at weekly intervals to provide a profile of this activity for the life of the nodules. These data will provide the basis for an estimation of the contribution of biological nitrogen fixation to the nitrogen pool of the desert ecosystems.

Other desert plants will be examined for root and aerial nodulation which may also fix nitrogen. Quantification of the contribution of such systems will be made.

Conditions of soil moisture, temperature and nitrogen transformation will be monitored during the late winter and spring to determine factors influencing, and thereby controlling, nodulation of sagebrush.

A. ludoviciana will be grown under cultivated conditions at the Brigham Young University farms to provide an immediately accessible supply of nodules for both field and laboratory studies.

Laboratory Studies

Reisolation of sagebrush endophyte and complete characterization by standard procedures of identification and nomenclature will be carried out. The endophyte will also be cultured in a nitrogen-free medium to test nitrogen-fixing capacity in a nonsymbiotic environment.

In order to duplicate environmental conditions monitored in the field, a growth chamber will be used to produce nodules under laboratory conditions. After successful production of nodules in a controlled environment, a program to study reinfectivity will be carried on following the procedures of Koch's postulates.

Sagebrush roots will be examined and analyzed during the infective period of nodulation to determine if indole acetic acid or other growth regulators are involved in the infective process in nonlegumes.

Various microscopic techniques will be used in the examination of nodules for infection thread, development of bacteroids, etc. Nodular cross-sections will be prepared for examination by regular light microscopy. Electron microscopy will be employed for more detailed study of nodular material. The general anatomy of the nodules will be carefully studied to determine, in particular, whether or not the infected cells are in medullary position, as in the legume nodules, or in the cortical region, as in all previously described nonlegume nodules.

Common legumes (alfalfa, red clover, sweet clover, ladino clover and soybeans) will be grown in sterile soil and inoculated with the sagebrush endophyte to determine if cross-inoculation is possible. Plants will be inoculated and grown at different temperature levels.

The key to obtaining active nitrogen-fixing subcellular extracts from free-living organisms has been the use of anaerobic conditions during the cell-rupturing process and following manipulations. In the case of legume root nodules, anaerobic conditions plus the use of polyvinylpolypyrrolidone for removal of inhibiting phenolic compounds from the macerated plant tissue during the preparation of bacteroids, have greatly enhanced the nitrogenase activity of the resulting extracts. The root nodules of sagebrush are similar in appearance to the nodules of soybeans; thus, successful techniques utilized with those systems will be employed in the preparation of cell-free extracts from sagebrush nodules. Nitrogenase activity will be determined by supplying the extract with reducing power (sodium dithionite) and a constant low level of ATP (via a creatine phosphate-creatine kinase ATP generating system) and appropriate substrate. With the use of adequate control reactions (minus ATP or reductant) ammonia production measures nitrogen reduction and ethylene production measures acetylene reduction.

The limited information available on the nitrogen-fixation activity on nonlegume nodules is similar to that obtained with legume nodules. This suggests that the nitrogenase enzyme system of nonlegumes could be similar to the nitrogenase systems previously isolated from legume bacteroids and nonsymbiotic nitrogen-fixing organisms, that is, consisting of two metallo-protein components (an iron-molybdenum component and an iron component). It is therefore anticipated that adaptation of the protamine sulfate fractionation, gel filtration, and DEAE cellulose techniques for purification of those systems could, if successfully used, provide the nitrogenase proteins from sagebrush nodule extracts also. The homogeneity of these proteins can be established by disc gel electrophoresis and analytical ultracentrifugation (which will also provide a preliminary estimation of the molecular weights).

A preliminary indication of the iron content of the protein will be determined by atomic absorption and/or by a modified, unpublished micro-bathophenothroline method used by Bulen and LeComte. Molybdenum present in the protein will be measured with the dithiol reagent of Clark and Axley as described by Bulen and LeComte (1966). Acid-labile sulfur will be determined by the method of Fogo and Popowsky (1949).

Leghemoglobin, the pigment responsible for the pink-reddish color of legume nodules, is easily extracted with water from crushed nodules. It is then readily precipitated from this extract in a 50 to 75% saturated ammonium sulfate fraction. The use of DEAE cellulose provides resolution into the component proteins. The pink to purple color of active nitrogen-fixing sagebrush nodules is suggestive of the presence of a hemoglobin-like chemoprotein in this system. Spectral examination of a water extract and ammonium sulfate fractionated material from crushed nodules should confirm or reject this possibility.

The climatic pattern along the Wasatch Front in the spring of 1974, particularly in April, was one of high precipitation and cold temperatures, followed by a marked increase in temperature and little precipitaion. This pattern complicated the study because it was difficult to find nodulation and much of the investigation was contingent upon location of an ample supply of nodules. This situation made it necessary to deviate from the planned procedures and intensify efforts to locate nodules.

An extensive search for nodules at the Curlew Valley site proved fruitless. A request had been made that digging for nodules should not be carried on within the fenced area of the site. All digging, therefore, was made in the area outside of the north fence. A telephone call was made to Romney and Wallace. They reported not being able to find nodulation at the Nevada Test Site so no trips were made to that area.

Soil moisture contents at Curlew Valley were found to be low and soil temperatures high. This led to a concentration of effort at Morgan and at the Manti-LaSal Forest area where nodules had previously been found in abundance; it was assumed they could again be found there.

Considerable time was spent in developing and constructing cold-root zone growth chambers. It was planned to develop greenhouse conditions to duplicate whatever soil-moisture conditions were found where nodulation occurred in the field.

A study was made to compare climatic data and climatic patterns of 1967 and 1972 (when profuse nodulation had been found) with 1974 data (see Appendix A).

RESULTS

The studies of 1974 proved to be mostly negative so far as finding nodules was concerned. It was a year which, at least, showed climatic and soil patterns of temperature and moisture content under which nodulation did not occur.

Soil moisture and temperature studies were begun at Morgan, Utah, and continued through July 10. These data are shown in Table 1.

Nodules were not found on the roots of either *Artemisia ludoviciana* or *Artemisia tridentata* at this site. This was particularly unexpected because nodulation of *A. ludoviciana* had been observed at Morgan every year since 1967.

Investigation was begun on the plants at the B.Y.U. Farm near Spanish Fork, Utah, on April 17 but no nodulation was observed at that time. On May 2, nodules were beginning to appear on the roots of *A. ludoviciana*. These were near the surface of the soil, 2-4 cm in depth. Acetylene reduction was very slight. On May 8 the surface soil had dried. The nodules were shriveled and brown and did not reduce acetylene at all.

At Curlew Valley on May 14 there was no indication of nodulation on *A. tridentata* . The soils were dry and the soil temperatures were high. The moisture content of the soil from 0- to 7.5-cm depth was below the 15-atmosphere (wilting point) level, and only slightly above this level in the 7.5- to 15.0-cm depth. Soil temperatures were 20.5 and 19 C, respectively, at those depths. These data are shown in Table 2.

On May 16 in the Manti-LaSal Forest area above Ephraim, no nodulation was observed at the Snowberry exclosure at an elevation of 2560 m. The road on up to Bluebell (elevation 2720 m) had not yet been opened through the snow. On June 11 the soils at the Snowberry exclosure had dried so much that they could not be dug with a shovel. At Bluebell the roots of *Artemisia michauxiana* were heavily nodulated. Plants and nodules were collected for greenhouse study and laboratory isolation of the microorganism. Soil temperatures were 10 C. At Philadelphia Flat (elevation 2940 m) the shoots of *A. michauxiana* were just beginning to appear above the soil surface. Indications were that both Bluebell and Philadelphia Flat might be productive areas in 1974. Visits to these sites should have been made regularly at this time because the weather turned hot and dry.

On June 24 when the next trip was made to this area, the soil temperatures at Bluebell had risen to 28 C. The nodules were shriveled and brown. The surface soils at Philadelphia Flat were dry within 25 to 50 cm of the melting banks of

Table 1. Soil moisture content and soil temperature at Morgan, Utah, February 28 to July 10, 1974, on dates as shown

Date	Soil Moisture Content (Percent-Wt.) 0-7.5 cm	7.5-15.0 cm Depth	Soil Temperature (° Centigrade) 7.5 cm	15.0 cm Depth	Air Temp.
February 28, 1974	22.8	19.3	1.0	1.5	10.0
March 15, 1974	24.4	23.1	11.5	9.0	11.0
March 22, 1974	23.6	21.6	2.0	1.0	3.5
March 29, 1974	24.8	21.4	4.5	4.0	10.5
April 4, 1974	23.4	21.0	9.0	5.0	14.5
April 11, 1974	HEAVY SNOWSTORM				
April 18, 1974	23.6	20.5	9.0	7.0	20.0
April 30, 1974	21.9	20.6	13.0	9.0	19.5
May 7, 1974	14.6	17.7	16.0	11.0	21.5
May 22, 1974	7.2	10.5	15.0	11.0	18.0
May 30, 1974	1.8	1.5	19.5	16.0	18.5
June 15, 1974	4.7	3.6	24.0	19.5	33.0
July 10, 1974	1.6	1.6	31.0	25.5	30.5
Moisture Content Determined at 1/3 atmosphere*(1)	23.2	21.7			
Moisture Content Determined at 15 atmosphere*(1)	8.7	8.0			

*Average of 5 samples

(1) Considered upper limit of available moisture (approx. field capacity).
(2) Considered lower limit of available moisture (approx. wilting point).

Table 2. Soil moisture content and soil temperature at Curlew Valley site, May 14, 1974

Date	0-7.5 cm	7.5-15.0 cm Depth	7.5 cm	15.0 cm Depth	Air Temp.
May 14	13.2	17.8	20.5	19.0	22.5
Moisture Content Determined at 1/3 atmosphere(1)*	30.9	34.4			
Moisture Content Determined at 15 atmosphere(2)*	14.5	16.2			

(1) Considered upper limit of available moisture.
* Average of 10 determinations.

(2) Considered lower limit of available moisture.
* Average of 5 determinations.

snow. The soil temperature at this distance from the snow was also 28 C. The plants in the area were now 10 to 15 cm in height. There were no nodules on the roots.

In the greenhouse and laboratory studies difficulties were encountered in the temperature control of the cold-root zone growth chambers. Temperatures could not be maintained below the desired 10 C level. No nodulation was obtained from the roots of greenhouse plants which had been moistened with cultures of organisms isolated from the nodules obtained at Bluebell on June 11.

DISCUSSION

From the studies made thus far it appears that nodulation of sagebrush in the desert, range and forest areas is a fragile system which is very sensitive to narrow limits and very slight changes in the microclimate of soil moisture and temperature. The data obtained and observations made indicate that nodulation is most likely to occur when soil temperatures are within the range of 5 to 10 C, concurrent with a soil moisture content only slightly below field capacity and about 25% of the available soil moisture level above wilting point.

The data from Morgan, Utah, when plotted within these ranges (Fig. 1), show that after April 30, at which time the plants were only 5.0 to 7.5 cm in height, the temperature of the soil at the 7.5-cm depth was above 10 C. Soil moisture content was decreasing rapidly. Plant growth and photosynthetic activity at this date might also be at a restricted level of carbohydrate production.

It appears that there must be sufficient plant growth, with corresponding photosynthetic activity, for a period of time within the range of suitable temperature and soil moisture if conditions are to be suitable for nodulation. The length of the period of nodulation would then be determined or correspond to the time that these interrelationships existed.

In the study of comparative climatic data of 1967 and 1972 at Morgan (when extensive nodulation was known to have occured) with those of 1974, it appears that 1974 was considerably different from the other two years (Table 3). These differences are more evident when shown as "deviations from normal" (Table 4). Both February and March 1967 were warm, followed by a cool April and May with a high level of precipitation. Nodulation was observed on May 28.

February and March 1972 were warmer than 1967, followed by a cool April. Precipitation was high in March and April. Nodules were found on April 29 of that year. On May 4 they were highly effective in acetylene reduction but this activity decreased each week until May 30. February 1974 was extremely cold, 11.7 C below normal. March was warm but was followed by a cold, wet April and a hot May. It is unfortunate that soil moisture and soil temperatures were not carefully monitored for these periods in 1967 and 1972.

EXPECTATIONS

Variables of soil moisture, soil temperature, precipitation and air temperature will be more completely monitored in 1975. Work will continue in the development of cold-zone root growth chambers. Conditions at Curlew Valley and Pine Valley will be more thoroughly investigated. An intensive program will be conducted to locate nodules throughout the entire desert area.

Table 3. Comparison of climatic data for Morgan, Utah, for 1967, 1972 and 1974 for the period of February through June

Total Monthly Precipitation (Centimeters)

Month	1967	1972	1974
February	3.8	2.2	4.2
March	3.9	6.2	4.8
April	10.2	12.3	13.8
May	7.3	.4	2.8
June	10.5	1.6	1.1

Mean Monthly Temperature (° Centigrade)

Month	1967	1972	1974
February	-1.1	-.8	-5.3
March	4.7	6.0	4.8
April	5.8	7.0	6.4
May	10.8	13.0	3.1
June	15.0	18.0	20.6

Date When Intensive Nodulation Was Observed

1967	1972	1974
May 28	April 24	None

Table 4. Deviation from normal of mean monthly precipitation and mean monthly temperatures at Morgan, Utah, for months indicated in 1967, 1972 and 1974

Deviation from Normal of Monthly Precipitation (Centimeters)

Month	1967	1972	1974
February	- .02	-1.62	+ .62
March	- .20	+2.07	+ .47
April	+6.77	+8.87 (2)	+9.22
May	+3.85 (1)	-3.02	-1.27
June	+7.97	- .95	-2.72

Deviation from Normal of Monthly Temperature (° Centigrade)

Month	1967	1972	1974
February	+3.60	+6.12	-11.70
March	+7.56	+11.70	+10.98
April	-5.94	-3.06 (2)	- 3.60
May	-4.14 (1)	+2.70	+ 3.42
June	-4.32	+5.32	+ 7.02

(1) Nodulation was first observed May 28, 1967.
(2) Nodulation was observed on April 24, 1972. No nodulation was found in 1974.

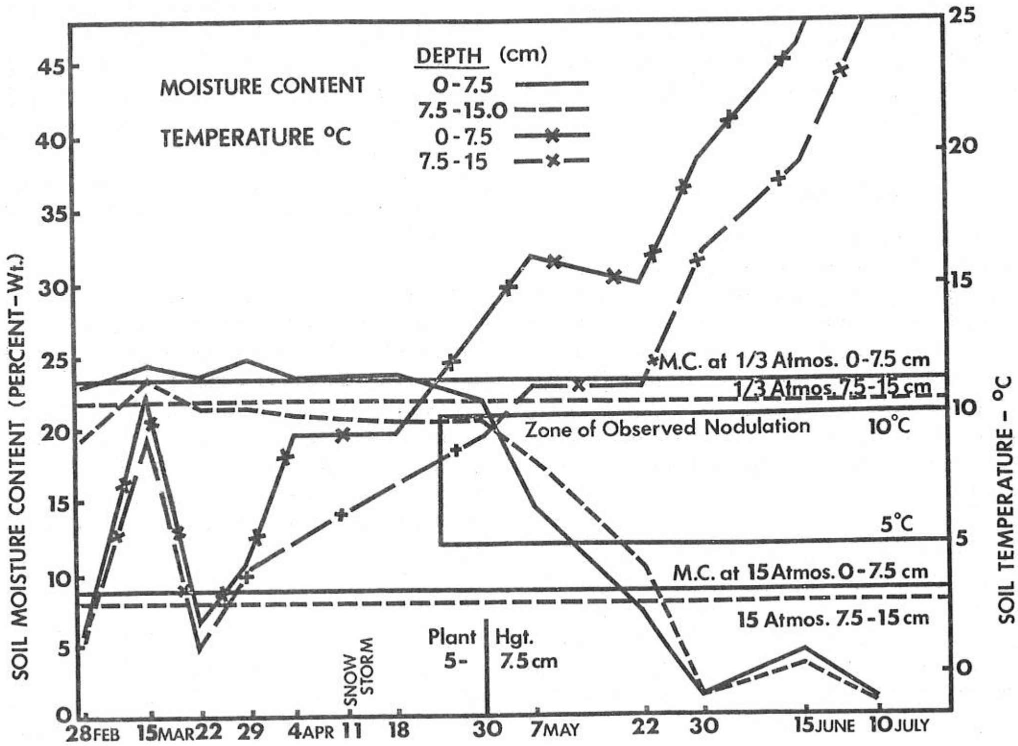

Figure 1. Soil moisture-temperature-nodulation interrelationships, Morgan, Utah, February 28 to July 10, 1974.

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APPENDIX A

Utah climatological data, daily precipitation and temperatures for Morgan, Utah (1967, 1972, 1974)

Date	Daily Precip.	Max. Temp.	Min. Temp. (° F)
	FEBRUARY 1967		
1	-	47	26
2	-	41	15
3	-	39	16
4	-	38	17
5	-	40	13
6	.02	39	19
7	-	37	8
8	-	38	5
9	-	48	17
10	.02	42	32
11	-	42	28
12	-	51	16
13	-	44	17
14	.02	47	26
15	.18	36	12
16	.21	34	17
17	.85	41	25
18	-	45	26
19	-	38	8
20	-	31	-1
21	-	43	-3
22	-	45	4
23	-	54	26
24	-	58	18
25	.02	48	31
26	.20	42	26
27	-	41	17
28	-	49	18
TOTAL	1.52	DAILY AVE. 42.7	17.1
		MONTHLY AVE. (29.9)	
DEVIATION FROM NORMAL	-.01	+2.0	

[1]Utah Climatological Data, U.S. Dept. of Commerce, Vol. 69, 74, 76, 1967, 1972, 1974.

Appendix A, continued

Date	MAY 1967 Daily Precip.	Max. Temp.	Min. Temp.	JUNE 1967 Daily Precip.	Max. Temp.	Min. Temp. (° F)
1	.22	42	21	.02	70	44
2	-	48	23	-	72	41
3	-	58	28	-	80	45
4	-	59	27	-	84	44
5	.35	54	36	.02	82	49
6	.05	61	37	-	71	39
7	-	69	38	.16	66	47
8	-	71	35	.32	67	43
9	-	77	40	-	66	38
10	.47	69	38	.11	64	42
11	.40	46	32	.14	67	39
12	.05	45	30	.03	63	47
13	-	50	27	.84	62	42
14	-	55	27	.25	59	46
15	-	66	29	.10	65	43
16	-	72	35	1.05	69	38
17	-	77	34	-	75	40
18	-	78	35	-	81	42
19	-	76	40	.08	76	46
20	-	74	39	.21	73	52
21	-	86	39	-	77	45
22	-	86	41	.43	83	49
23	-	88	43	.41	56	51
24	.02	84	44	-	73	41
25	.34	65	50	-	78	43
26	-	71	45	-	82	49
27	-	74	49	-	84	48
*28	-	73	44	.05	73	49
29	.55	65	47	-	81	44
30	.46	61	43	-	85	50
31	-	65	35	X	X	X
TOTAL	2.91	DAILY AV. 66.6	36.5	4.22	DAILY AV. 73.5	44.5
		MONTHLY AV. (51.6)			MONTHLY AV. (59.0)	
DEVIATION FROM NORMAL	+1.54	-2.3		+3.19	-2.4	

*Date of nodule collection

Date	MARCH 1967 Daily Precip.	Max. Temp.	Min. Temp.	APRIL 1967 Daily Precip.	Max. Temp.	Min. Temp. (° F)
1	-	59	22	.50	45	25
2	-	50	20	.04	50	20
3	-	46	21	-	66	29
4	-	41	22	-	69	38
5	.03	37	18	.30	63	36
6	-	46	14	-	62	22
7	-	45	15	-	62	22
8	-	50	19	.27	57	30
9	-	60	27	-	56	27
10	-	59	42	-	63	26
11	-	56	41	-	60	39
12	-	53	40	.70	50	32
13	.12	46	33	-	54	31
14	.35	40	26	.10	58	32
15	-	50	12	.15	59	31
16	-	69	32	.12	60	30
17	.06	63	32	-	63	24
18	.24	58	33	-	69	37
19	.22	41	32	.14	67	33
20	-	53	27	.70	45	26
21	-	56	26	.16	45	23
22	-	66	29	-	44	25
23	-	67	42	.20	47	25
24	.13	49	33	-	50	23
25	-	51	19	-	52	24
26	-	46	30	.10	59	33
27	-	57	31	-	63	34
28	.13	62	37	-	50	42
29	.02	51	38	.10	45	30
30	.26	38	21	.51	37	26
31	-	46	19	.51	37	26
TOTAL	1.56	DAILY AV. 52.0	27.5	4.09	DAILY AV. 55.7	29.3
		MONTHLY AV. (39.8)			MONTHLY AV. (42.5)	
DEVIATION FROM NORMAL	-.08	+4.2		+2.71	-3.3	

[1]Utah Climatological Data, U.S. Dept. of Commerce, Vol. 69-73

Date	Daily Precip.	Max. Temp.	Min. Temp. (° F)
	FEBRUARY 1972		
1	.04	22	8
2	-	23	-15
3	-	21	-15
4	-	28	8
5	-	37	7
6	T	39	23
7	.03	41	33
8	-	42	27
9	-	38	15
10	-	33	9
11	-	34	4
12	-	47	27
13	-	38	20
14	.19	35	19
15	.09	43	29
16	-	51	33
17	-	52	34
18	-	50	36
19	-	55	24
20	-	58	28
21	-	55	28
22	-	57	34
23	-	49	25
24	-	45	33
25	T	44	32
26	.41	37	23
27	.12	38	12
28	-	59	20
29	-	65	33
TOTAL	.88	DAILY AVE. 42.6	19.9
		MONTHLY AVE. (31.3)	
DEVIATION FROM NORMAL	-.65	+3.4	

Appendix A, continued

Date	MARCH 1972 Daily Precip.	Max. Temp.	Min. Temp.	APRIL 1972 Daily Precip.	Max. Temp.	Min. Temp. (° F)
1	.02	56	22	-	63	27
2	.22	46	33	.53	56	36
3	1.05	50	39	-	59	30
4	-	45	32	-	65	28
5	-	60	33	-	70	35
6	-	64	34	.24	69	38
7	-	54	33	-	66	25
8	-	65	22	-	63	30
9	-	72	25	.25	62	29
10	-	71	28	.45	54	41
11	-	64	29	.87	52	32
12	-	66	26	.70	43	28
13	-	64	34	.36	52	27
14	.30	61	33	.07	54	29
15	-	58	29	-	56	28
16	-	66	26	-	65	30
17	-	70	26	.13	60	33
18	-	50	33	.63	50	27
19	.15	56	31	.21	43	31
20	-	73	25	-	52	26
21	-	70	34	-	60	27
22	-	60	35	-	55	33
23	.26	61	33	*	69	25
24	-	58	26	-	71	35
25	.23	50	26	.26	68	32
26	.24	34	16	.23	54	20
27	-	38	13	-	60	25
28	-	39	14	-	70	27
29	-	40	16	-	53	20
30	-	45	18	-	58	18
31	-	57	20	X	X	X
TOTAL	2.47 DAILY AV.	56.9	27.3	4.93 DAILY AV.	59.1	29.1
	MONTHLY AV.	(42.1)		MONTHLY AV.	(44.1)	
DEVIATION FROM NORMAL	+.83		+6.5	+3.55		-1.7

* Date of first observed nodulation

Date	MAY 1972 Daily Precip.	Max. Temp.	Min. Temp.	JUNE 1972 Daily Precip.	Max. Temp.	Min. Temp. (°F)
1	-	60	24	-	89	47
2	-	65	26	-	82	46
3	-	73	28	-	80	52
4	-	79	38	.55	71	51
5	-	73	40	.06	75	45
6	-	75	45	-	77	53
7	.06	67	41	-	75	54
8	-	66	45	-	72	58
9	-	67	42	-	82	45
10	.10	63	34	-	84	46
11	-	67	28	-	88	49
12	-	65	27	-	82	43
13	-	71	30	-	79	44
14	-	75	33	-	85	45
15	-	82	36	-	86	40
16	-	84	37	-	87	55
17	-	80	45	-	82	58
18	-	74	42	.02	85	45
19	-	75	43	.02	73	39
20	-	69	42	-	80	37
21	-	67	34	-	83	39
22	-	71	31	-	82	50
23	-	72	36	-	81	55
24	-	76	45	-	80	53
25	-	73	44	-	80	48
26	-	68	37	-	80	42
27	-	79	36	-	85	41
28	-	82	39	-	86	43
29	-	84	33	-	87	42
30	-	85	46	-	93	42
31	-	87	42	X	X	X
TOTAL	.16 DAILY AV.	73.4	37.4	.65 DAILY AV.	81.7	46.9
	MONTHLY AV.	(55.4)		MONTHLY AV.	(64.3)	
DEVIATION FROM NORMAL	-1.21		+1.5	-.38		+2.9

Appendix A, continued

Date	FEBRUARY 1974 Daily Precip.	Max. Temp.	Min. Temp. (° F)
1	.26	39	27
2	-	39	23
3	.34	37	11
4	-	43	3
5	-	37	22
6	-	30	2
7	.07	27	7
8	-	28	-4
9	-	27	-4
10	-	30	-3
11	-	31	-4
12	-	34	-1
13	-	42	19
14	-	37	7
15	-	38	11
16	-	39	8
17	.07	43	18
18	-	35	4
19	.50	37	27
20	.04	33	10
21	-	31	-3
22	.26	30	-4
23	.16	29	-2
24	-	24	-11
25	-	31	-7
26	-	36	3
27	-	48	25
28	-	54	25
TOTAL	1.70 DAILY AVE.	35.3	7.5
	MONTHLY AVE.	(21.4)	
DEVIATION FROM NORMAL	+.25	-6.5	

Date	MARCH 1974 Daily Precip.	Max. Temp.	Min. Temp.	APRIL 1974 Daily Precip.	Max. Temp.	Min. Temp. (° F)
1	-	56	38	.30	45	29
2	.55	54	29	1.15	43	30
3	.24	34	17	.28	41	22
4	-	33	-4	.06	44	22
5	-	49	21	-	57	24
6	.03	50	37	.11	56	37
7	-	54	37	.04	50	29
8	-	52	34	-	67	25
9	-	51	25	-	65	45
10	-	52	25	1.31	50	29
11	-	60	26	.26	50	32
12	-	60	27	.65	50	25
13	-	58	40	.07	45	25
14	.46	56	35	-	52	25
15	-	51	34	-	60	25
16	-	63	33	-	66	29
17	-	62	36	-	65	29
18	.04	57	35	-	70	30
19	-	50	22	T	62	40
20	-	50	17	.99	51	37
21	-	44	13	-	54	30
22	-	45	27	-	70	31
23	-	43	33	-	74	38
24	-	53	17	-	76	36
25	-	64	25	-	73	35
26	-	59	37	-	61	36
27	.10	60	38	.31	51	32
28	.09	54	33	-	48	29
29	.10	54	30	-	52	25
30	.33	59	35	-	59	28
31	T	54	33	X	X	X
TOTAL	1.94 DAILY AVE.	52.9	28.6	5.53 DAILY AVE.	56.9	30.6
	MONTHLY AVE.	(40.8)		MONTHLY AVE.	(43.8)	
DEVIATION FROM NORMAL	+.19		+6.1	+3.69		-2.0

Appendix A, continued

Date	MAY 1974 Daily Precip.	MAY 1974 Max. Temp.	MAY 1974 Min. Temp.	JUNE 1974 Daily Precip.	JUNE 1974 Max. Temp. (°F)	JUNE 1974 Min. Temp. (°F)
1	-	74	30	.-	78	33
2	-	69	51	-	82	36
3	.12	59	39	-	81	39
4	-	72	39	-	84	39
5	-	75	36	.35	68	50
6	-	77	36	T	64	42
7	-	75	39	.04	71	37
8	-	80	40	.07	63	35
9	-	79	44	-	74	32
10	-	74	45	-	81	35
11	-	70	29	-	85	40
12	-	78	30	-	91	43
13	-	72	34	-	92	48
14	-	72	34	-	96	49
15	-	70	35	-	96	49
16	-	63	32	-	96	52
17	-	64	30	-	94	53
18	-	73	52	-	95	49
19	.11	64	38	-	94	50
20	.90	43	32	-	86	57
21	-	59	29	-	85	44
22	-	70	30	-	92	46
23	-	72	37	-	96	48
24	-	79	42	-	95	46
25	-	80	39	-	95	48
26	-	84	43	-	90	50
27	-	88	54	-	86	46
28	-	84	49	-	88	44
29	-	82	43	-	93	43
30	-	73	35	-	94	43
31	-	75	32	X	X	X
TOTAL		DAILY AVE. 72.5	38.0	.46	DAILY AVE. 86.2	44.2
		MONTHLY AVE. (55.3)			MONTHLY AVE. (65.2)	
DEVIATION FROM NORMAL	-.51		+1.9	-1.09		+4.9