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EFFECTS OF HIGH AND LOW IRRIGATION ON SYMBIOTIC NITROGEN FIXATION ON COWPEA (VIGNA UNGUICULATA (L.) WALP.)

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EFFECTS OF HIGH AND LOW IRRIGATION ON SYMBIOTIC NITROGEN FIXATION ON COWPEA (*VIGNA UNGUICULATA* (L.) WALP.)

by

Ibrahim Elbashir Mohamed

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STATEMENT BY AUTHOR

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Adjunct Assistant Professor

July 21, 1982
Date

This piece of work is dedicated in
memory of my late sister Fatima and
my late brother Elsiddeig

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ABSTRACT

The response of two cowpea cultivars inoculated with granular inoculum under optimum irrigation and water stress (drought) was evaluated. "California #5" and "Speckled Purple Hull" cowpeas were examined under field conditions for nitrogenase activity, nodule mass and number, and percent nodulation over the growing season. Data were also obtained for stem, leaf, root, pod drys, yield and protein content.

Significant differences among inoculated and non-inoculated plots were found for both cultivars with respect to nodule number, nodule mass and nitrogenase activity. High nitrogenase activity in the irrigated plots was not manifested by increased yields. However, high protein content was recorded with respect to inoculated plots than to uninoculated plots for "California #5" and "Speckled Purple Hull" cowpeas.

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Introduction

Grain legumes are unique because they can meet their nitrogen requirement by fixing atmospheric nitrogen through symbiosis. Therefore, productivity may depend on their nitrogen fixation potential in unfavorable environments. Cowpeas are important sources of protein for peoples of Africa, South America and Asia (Ahmad et al., 1981).

In the northern Sudanian zone of Africa cowpeas are grown as a rainfed crop and are well adapted to drought (Hall and Dancette, 1978). In contrast, cowpeas are grown under irrigation in central California during summer and dry seed yields as large as 6328 kg/ha have been obtained (Turk, Hall and Asbell, 1980).

Nitrogen fixation by this legume requires rhizobia and suitable environmental conditions for nodule formation and activity. The amount of nitrogen fixed is determined by the compatability between the plant and bacteria and by the total environment of which climate and host nutrition are probably the most important (Bell and Nutman, 1971). Nitrogen fixing activity, in many instances, has been considered to vary directly with nodule weight and large nodules were found to be effective. This may not necessarily be true since nodules vary greatly in size and number depending on the plant and the rhizobia (Azu, 1975).

Many observations have been made on the effects of moisture stress on root nodules of various legume species (Sprent, Bradford and Gallacher, 1978; Pate, Gunning and Briarty, 1969; Sprent, 1971). These studies have used the number, size and weight of nodules to evaluate the effect of stress on the amount of nodule tissue that is formed (Wilson, 1931; Gallacher and Sprent, 1978). Water stress (drought) has been shown to affect the occurrence, growth, and survival of nodule bacteria, nodule formation and the nodule function (Doku, 1970; Masefield, 1961; Shimishi et al., 1967; Vincent, 1965). However, Shimishi et al. (1967) did not find any effect of frequency of irrigation on nodulation of peanuts, and suggested that multiplication of and infection by bacteria might take place during the short periods of high soil moisture immediately after irrigation.

The objective of this study therefore was to determine the extent to which continuous exposure to favorable and unfavorable moisture conditions affected nitrogen fixation of two cowpea cultivars grown under (Campbell Ave. Farm) field conditions. This information may provide a basis for the evaluation of the extent by which symbiosis may be improved through irrigation during periods of stress and the contribution of the symbiotic system under stress to the nitrogen nutrition of the plant.

LITERATURE REVIEW

Cowpea plants can be erect, prostrate or trailing. Leaves are pinnately trifoliate while flowers are yellow or purple in color. Cowpeas seed vary in shape, color and size. The common name (black-eyed pea) was given to cultivars which have a white hilum with a dark frame. Some other cultivars have colored seeds which are spotted, marbeled or mottled (Hartwig, H. B., 1953).

Cowpeas, as with other legumes have a reputation for enriching the soil, requiring little fertilizer nitrogen (N) and yielding grain or forage high in protein which enhances the diet (Meisener, Craig and Douglass, 1980). Cowpeas are desirable since they have little dependence upon nitrogenous fertilizer for optimum yields if successfully nodulated with the appropriate Rhizobium spp. (Zablotowicz, Focht and Cannell, 1981).

Most inoculants supply far too few viable rhizobia for maximum benefit particularly under stress conditions. Burton (1981) reported that the number of viable effective rhizobia applied per seed is far more important than the actual weight of inoculum applied because inoculants vary widely in rhizobium content.

In a recent conference on irrigated soybeans in arid and semi-arid lands, Hamdi, Alaa Eldin and Abdel Wahalay (1979) noted that with 28×10^6 rhizobia per seeds all the soybean plants were nodulated, but with smaller numbers failures were common.

Bezdicek et al. (1978) evaluated peat and granular inocula for effectiveness in nodulation, nitrogen fixation and yield. They found that higher soybean yields and better nodulation were obtained with granular inoculant. These workers concluded that applying rhizobia in granular carrier may be superior to peat carrier when more rhizobia are needed. Reddy and Tanner's (1980) justifications for using the granular formulation were that: 1) granular inoculant eliminates seed treatment using "sticker" (sugar, milk, water) which may cause damage to seed coat and reduce germination, 2) allows use of standard field equipment and 3) it protects the rhizobia from the heat and desication, providing large numbers of bacteria to rhizosphere.

Cowpeas were reported intolerant of waterlogged conditions and should therefore be grown on free-draining soil (Acland, 1972). Sensitivity to waterlogging stress has been reported for several temperature legumes (Minchin and Summerfield, 1976; Minchin and Pate, 1975) and is ascribed largely to reduce symbiotic nitrogen fixation as a result of impaired oxygen transport to and within the nodules. For soybean, lenticel protuberances on the nodule surface have been implicated as physical agencies in the transport of oxygen to internal bacterial tissues (Dart, Day and Harris, 1972; Pankhurst and Sprent, 1975). However, lenticel type structures are reduced if present at all on cowpea nodules.

Water availability is important for the growth and development of cowpea nodules. Doku (1970) working on cowpeas found that nodules and plant dry weight were increased with increasing water content. However, waterlogging had an adverse effect on nodule development.

He pointed out that nodules did badly in waterlogged conditions because contact with water caused them to decay prematurely as the result of the anaerobic condition in the region.

In addition cool wet conditions delay early nodulation of soybean at lower application of inoculum (Bezdicek et al., 1978). Doku (1970) reported that nodule weight of cowpeas was significantly decreased during long days under the wettest soil conditions.

However, Gallacher and Sprent (1978) working with Vicia faba found that plants in excess water regimes grew best, produced more root nodules and fixed more nitrogen. In the same experiment they showed that average nodule weight and specific activity were unaffected by excess water, while nodules showed progressive increase in size and a more open structure as water supply was increased.

In cowpeas Minchin and Summerfield (1976) found that waterlogging depressed nitrogen fixation and also reduced shoot growth. Similar findings have been reported by Schwinghamer, Evans and Dawson (1970) with pea and Sprent (1969) with soybean. Waterlogging affected the pattern of nodule development. As a result a new nodules were formed in clusters and masses upon young rootlets near the surface of the soil (Edwin, Baldwin and McCoy, 1932).

Cowpeas are usually grown in arid and semi-arid regions and they are found to be drought tolerant. Shackel and Hall (1979) found that vertical leaflet movement affected canopy architecture and significantly reduced percentage cover at midday. Manual movement of the vertical leaflets of droughted plants caused substantial changes in leaflet temperature, indicating that leaflet movement could substantially reduce heat load, water use and water deficits in cowpeas.

Shouse et al. (1981) found that seed yield of cowpeas was particularly sensitive to water deficits and high temperature during the flowering period. They found also that yield was significantly reduced by water deficits at the pod filling stage, while yield was less sensitive to water stress during the vegetative stage.

On the other hand, Sprent, Bradford and Gallacher (1978) reported that water stress (drought) is the environmental factor most frequently associated with lowered nodule activity. According to Sprent (1971) when the fresh weight of detached nodules of soybean was reduced below 80% of its maximum value by dessication, irreversible changes in nodule function occurred. Acetylene reduction and nitrogen fixation activities ceased, respiratory rates became very low and there were structural changes in the nodules.

Wilson (1931) working with Phaseolus vulgaris reported that plants shed their nodules under water stress. He noted that shedding of nodules occurred more readily on small and fibrous roots than on the tap roots or from locations near the tap root. In addition reduction in soil moisture from 20 to 12.5% caused bean roots to shed on the average about 36% of their nodules. Fifty-seven percent shedding was recorded for some individual plants.

Likewise, Gallacher and Sprent (1978) reported that established Vicia faba plants were far more likely to be adversely affected by water stress than by excess water. They noted that the weights of stressed plants were lower in comparison with both the excess water plants and control plants, however root dry weight was increased by water stress, whereas the development, size and number of nodules were retarded by water stress. In general older plants were more likely to be adversely affected by water deficits than waterlogging.

Irrigation to supplement soil water content is practiced with many crops. Pronounced increases in yield are obtained with many crops. However, irrigation has been found to increase nodule development. Masefield (1961) showed that irrigation increased nodulation of Vicia faba, Phaseolus vulgaris and Pisum sativum.

Tewari (1966) reported that when the frequency of watering was increased plant size and vigor increased, resulting in increased nodulation. However, Doku (1970) noted thatunder the normal day-length in the tropics cowpeas should nodulate well provided enough water is available to permit a rate of growth that is capable of supporting the degree of rhizobial activity that will produce high nodulation. Sprent, Bradford and Gallacher (1978) found that under dry conditions irrigation can stimulate nodule activity of Vicia faba. Similar findings were shown by Doku (1970) working on cowpeas. Reddy and Tanner (1980) reported that inoculant application increased nodulation and nitrogen fixation. They concluded that irrigation treatment did not affect nodule number or nodule dry weight. Irrigation did however, increase nitrogen fixation where granular inoculant was used.

Zablotowicz et al. (1981) working on cowpea (California #5) observed that maximum nodulation and nitrogenase activity occurred during flowering. Well-irrigated plots have high nodulation and nitrogen fixation as compared with droughted plots. But a significant reduction in total nitrogenase activity did not decrease seed yields of droughted plants below those of well-watered plants. From the above discussion it is clear that inoculation and high water content but not waterlogging are desirable for maximum nodulation and nitrogen assimilation.

Under field conditions factors related to soil moisture stress such as temperature and salt content must be taken into consideration. Balasubramanian and Sinha (1976) felt that salinity (NaCl) retarded growth of leaves, stems, and roots of cowpeas and mungbeans. They noted that the total nodule number, nodule weight and nitrogen content per plant decreased due to salt treatment. High salt concentrations interfered with nodule initiation but not with their development.

Temperature is another factor which has been implicated by many investigators to influence legume symbiosis. Gibson (1961, 1963) working on four varieties of Trifolium subterraneum L. and two strains of Rhizobium trifolii in agar tube culture, observed that root temperature of approximately 22^{o}C was optimum for both nitrogen fixation and ammonium nitrate (NH_4NO_3) uptake. All plant varieties followed a similar pattern of reduction of nitrogen fixation and ammonium nitrate uptake at temperatures above and below optimum. Plants grown in varying temperature regimes showed decreases in growth, nodule weight and nitrogenase activity that were inversely proportional to temperature (Day et al., 1978).

Dart and Mercer (1965) reported that primary and secondary root nodulation of cowpeas (Vigna sinensis Endl. Ex. Hassk.) were significantly affected by temperature, NH_4NO_3 level and light intensity. They noted that fresh weight and size of nodules were adversely affected more by temperature than by the level of NH_4NO_3. On the other hand, Mahdi and Habish (1975) stated that nodulation and growth of cowpea and hyacinth bean were best at moderate temperatures. They concluded that cowpea tolerated warm more than cool temperatures.

MATERIALS AND METHODS

Two cultivars of cowpea, (Vigna unguiculata (L.) Walp.), California No. 5 blackeye (Ca#5) and Speckled Purple Hull (SPH) were grown during the summer, 1981 at the University of Arizona Research Farm (Campbell Ave. Farm), Tucson, Arizona.

The two cultivars were grown on (Gila) soil type, which is a very fine sandy loam and is classified by the Soil Conservation Service as 6J. The field was nearly level, well drained with high moisture capacity and moderate permeability.

Cropping history of the field showed that in 1977 the area was cultivated with sudangrass and ryegrass. In 1978, barn yard manure was added to the area. Sudangrass and ryegrass were planted in 1979 and the area was left fallow in 1980. These cover crops were grown to maturity and harvested to extract excess soil nitrogen. Soil analysis showed that N_2, P_2 and K content in the soil were 183.37 ppm, 63.49 ppm, and 9.00 ppm respectively.

The experimental design consisted of two main complete randomized blocks (wet and dry). Each irrigation level consisted of four treatments two cultivars, Ca#5 and SPH and inoculated and uninoculated plots. The experimental subplots were replicated four times. Cowpeas were planted in 4 row plots on May 15, 1981. Each row was 15 meters long. Spacing between rows was about one meter. The rows were double planted by means of cone planter mounted on a tractor.

A commercial granular inoculant formulated with rhizobia strain 176A50 was provided by Nitragin Company, Milwaukee, Wisconsin. Inoculant was added with the seeds at planting time. Inorganic fertilizers, insecticides or herbicides were not used. Weeds were controlled by cultivation before planting and by hoeing after stand establishment. Irrigation was applied to all treatments to insure a good crop stand. Water stress was imposed on July 10, 1981 for one of the blocks when Ca#5 was in early bud stage. The wet block was irrigated every two weeks by means of furrow irrigation. The dry block received no further irrigation.

Inoculated and non-inoculated plots were sampled on June 19, July 17 and August 20, 1981. Ten plants/plot were harvested from the third row (one of the two in the middle) to study nitrogenase activity by the use of acetylene reduction technique, number and mass of nodules, percent of plant nodulated, shoot and root dry weight, and pod dry weight.

Nitrogenase activity was measured by modification of the acetylene reduction technique (Hardy et al., 1968). Nodulated plants were separated from non-nodulated ones, and the nodulated roots were cut from the rest of the plant by means of clippers. The roots were unwashed, but free of the soil put into jars. The size of the jar for the first, second and third sets of samples was 490 ml, 960 ml, and 960 ml, respectively.

Fifty ml of air was removed out the jars by means of a syringe. Fifty ml of acetylene was added to each jar and thoroughly mixed.

After a 30 minute incubation period, 10 ml of gas from each jar was transferred into 10 ml evacuated vacutainer tubes. These samples were used for gas chromatographic analysis to detect ethylene production. Samples for standard curves were prepared in a similar fashion, however, there were no nodulated roots in the jar. A Varian Aerograph series 1700 gas chromatograph with a flame ionization detector was used for C_2H_2-C_2H_4 assay for nitrogen fixation activity. Ethylene peak heights obtained from gas chromatographic analysis of field sample were compared with the standard curves peak heights versus ethylene content of injected samples. These values were converted into amounts of nitrogen fixed $N_2(C_2H_2)$ using the following equation:

((Sample unit sq.) (Attentuation) - Acetylene std. unit/ml)
(Ethylene std. u mole/unit) (Jar size) = u mole/hr./plant.

The same equation was used to determine the amount of nitrogen fixed during the growing season (Miller, 1980).

At each sampling date, plastic bags containing the nodulated roots wereput on ice. They were brought to the laboratory and placed into the freezer until studied. The nodulated roots were washed, photographed and the nodules were removed, counted, and weighed to determine the fresh weight. Then, they were dried at 100 C degree in an oven for 24 hours and the dry weight was determined.

The tops (stem and leaf) of the nodulated and non-nodulated plants were bagged, labelled and placed in the drier for 72 hours to dry and to achieve constant weight. The total dry weight was determined. Leaves, stems and pods were separated bagged and put in the drier to determine assimilate partitioning. Roots were dried, separated, and counted. Then, they were dried again to constant weight and the dry weight determined. Pods of the two cultivars were separated from the rest of the plant. The percent of mature and immature pods were calculated for each sampling date. Then, pod dry weights were determined.

On September 7, 1981, 12 meters of the second row of the two middle rows of each plot was harvested to determine the final seed yield. Each row was 1 meter wide and the row was double planted. The 16 rows of Ca#5 were hand harvested in the field. While, SPH seeds in the dry block were hand harvested, for ease of handling, mature plants in the wet block were cut and left for 5 days to dry under the field conditions. Afterwards, the harvested material from the cultivars was threshed by means of a belt thresher and cleaned. Finally, the total seed yield was calculated. Protein analysis of seed from the wet and the dry blocks was determined by the Kjeldhal Method A.O.A.C. Handbook (1965).

Meterological Conditions

The distribution and amounts of rainfall at Campbell Ave. Research Farm (Tucson, Arizona) is shown in Figure 1. In July, August, and September 2.72, 1.17 and 0.35 inches of rainfall were recorded, respectively. These months represent the major part of the growing season. Thus, in the absence of irrigation there was substantial water deficiency in the field.

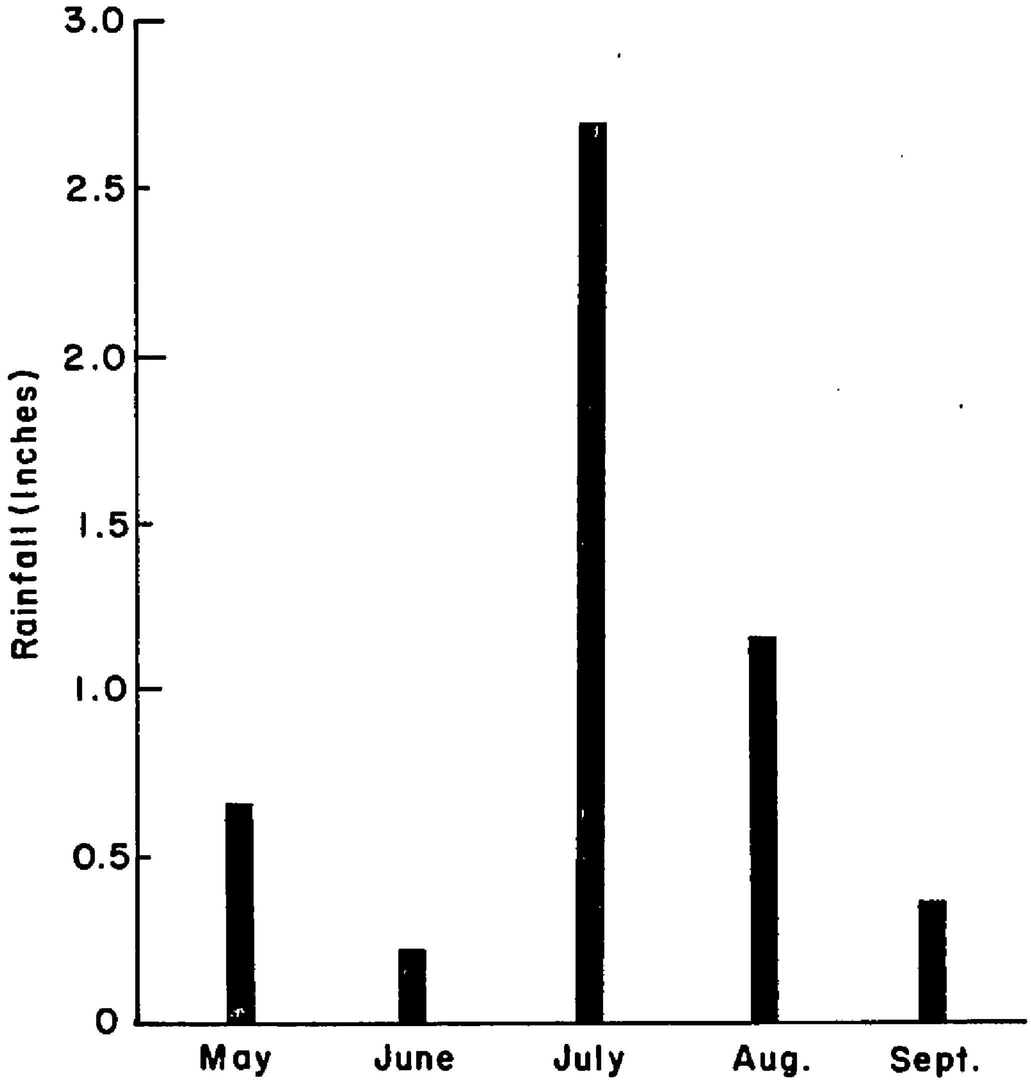


Figure 1. Mean rainfall distribution at Campbell Avenue Farm during cowpeas growing season (May-Sept. 1981).

Mean maximum and minimum temperatures is reported in Figure 2. June, July and August were the hottest months during the growing season (May to September 1981).

Analysis of Variance

The data were analyzed as a randomized complete block design. Four factors: Plant cultivar, Rhizobia, irrigation and harvesting dates with all their interactions were considered. Interactions were analyzed at the 5 percent level of significance using Least Significant Difference (LSD).

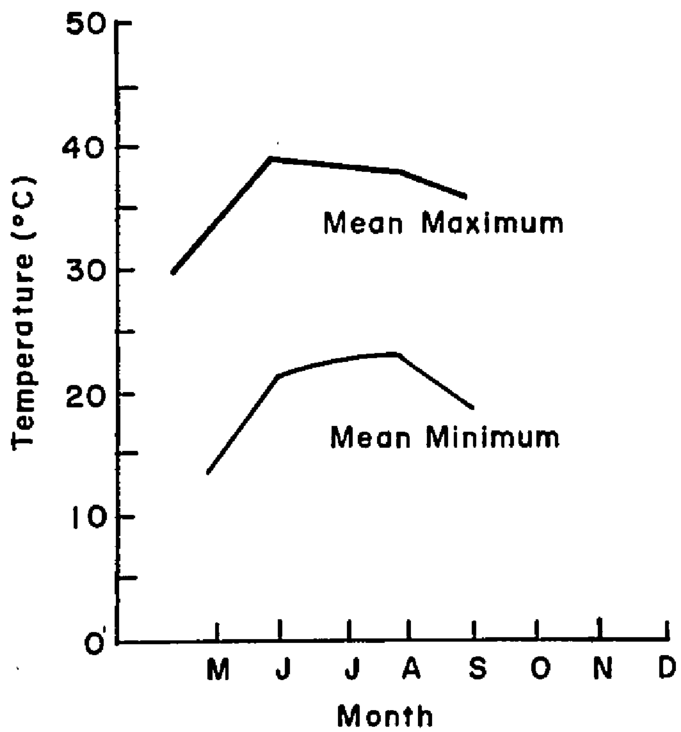


Figure 2. Mean maximum-minimum temperatures of Campbell Avenue Farm during cowpeas growing season (May-Sept. 1981).

RESULTS AND DISCUSSION

The two cowpea cultivars California #5 blackeye (Ca#5) and Speckled Purple Hull (SPH) developed at about the same rate through developmental stage 1. After the vegetative stage, gradual differences in varital development were evident, and cultivar Ca#5 was in full bloom sooner than cultivar SPH (Table 1).

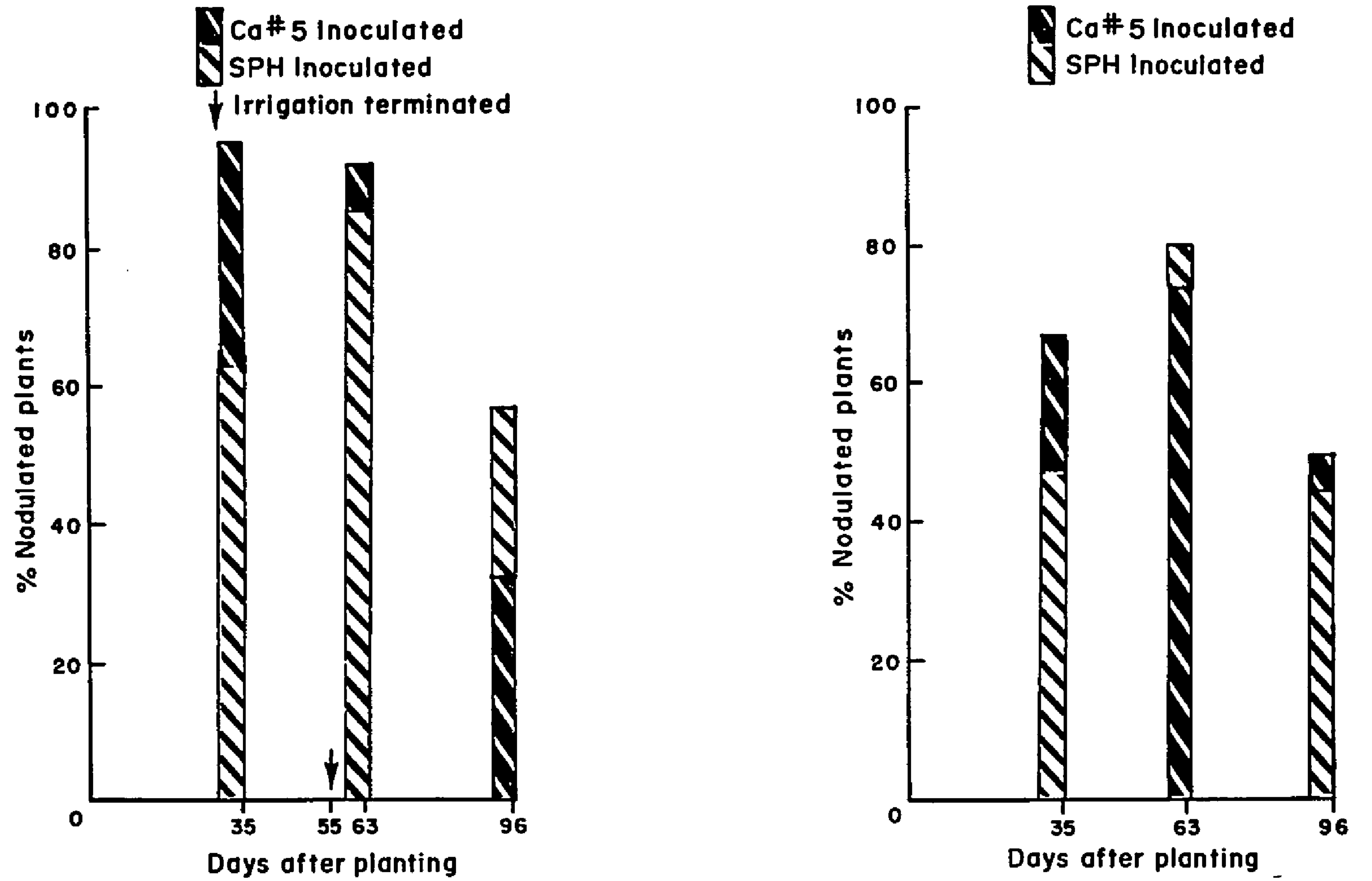
Ten plants/plot were sampled 35, 63 and 96 days after planting and examined for nodulation. Nodules were first observed on inoculated plants twenty two days after planting in both irrigation regimes and the majority of the nodules were on the central tap root. Nodulation was essentially lacking in the uninoculated plots. Percent nodulation of cultivars over the growing season is shown in Figures 3a and 3b. In the non-irrigated regime 97%, 92% and 30% were recorded for Ca#5 in the vegetative, flowering and pod-fill stages respectively. While 60%, 85% and 56% were reported for SPH during the three stages of growth (Fig. 3a), percentage of nodulated plants of irrigated cowpeas (Fig. 3b) shows that Ca#5 again had a higher percentage nodulated plants than SPH.

Nodule number of cowpea cultivars Ca#5 and SPH is shown in Figs. 4a and 4b. Examination of nodule number in the dry and irrigated regimes shows inoculation of cowpeas will insure nodulation and will increase nodule number. The three-way interaction between harvest time, incoulation and cultivar showed no significant differences (Tables 2a and 3a).

Table 1. Growth stages of cowpea cultivars California #5 (Ca#5) and Speckled Purple Hull (SPH) at sampling dates for nitrogenase activity.

Harvest	D.A.P.	Cultivar	Growth Stages	
			Irrigated	Non-Irrigated
1	35		vegetative vegetative	vegetative vegetative
2	63		full flowering early flowering	early bud full flowering
3	96		pod fill full flowering & early pod	pod yellowing pod fill

DAP = days after planting.



a) Non-irrigated treatment

b) Irrigated

Figure 3. Mean percent nodulated plants of inoculated cowpeas over the growing season.

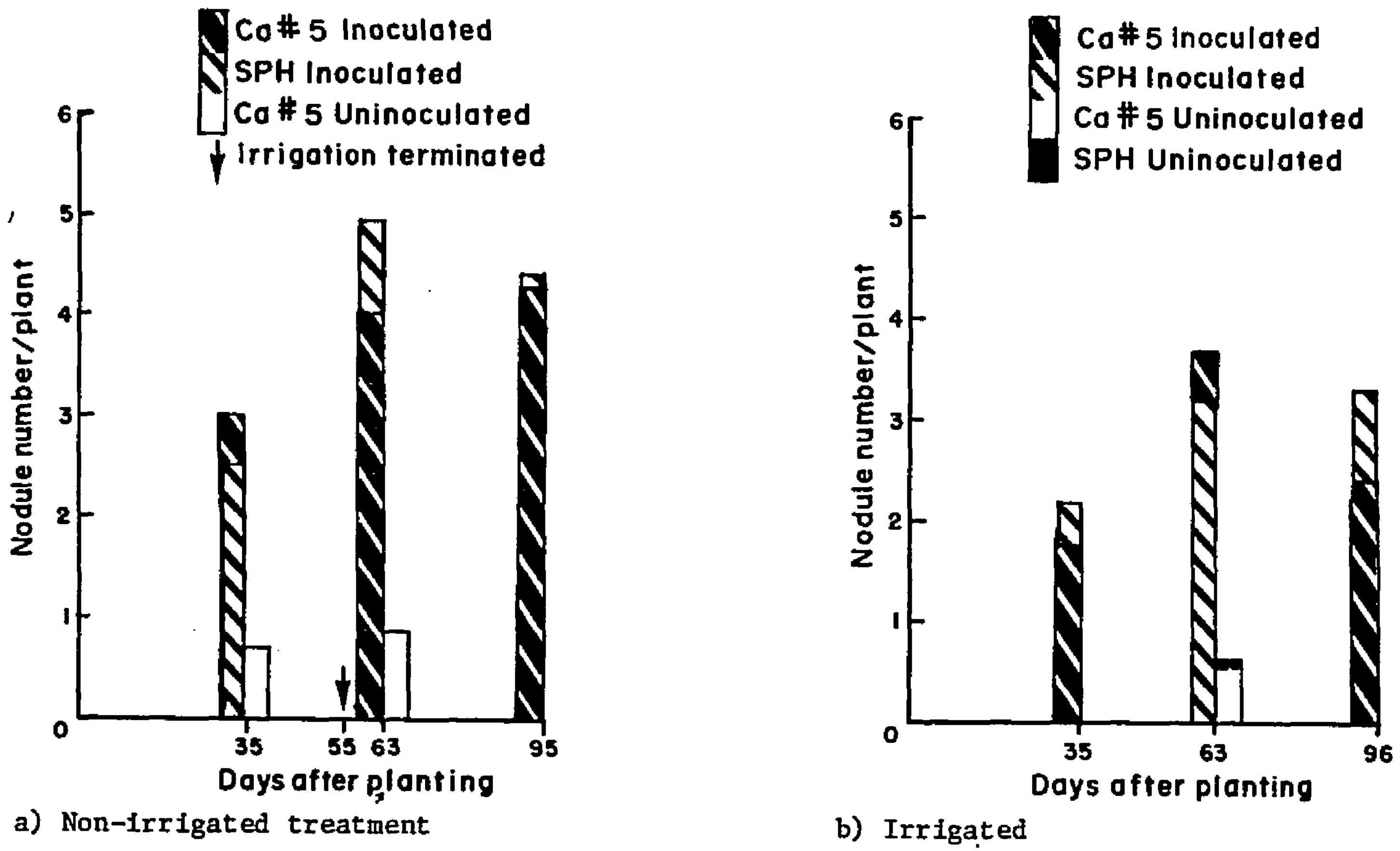


Figure 4. Mean nodule numbers of inoculated and uninoculated cowpeas over the growing season.

Table 2a. Analysis of variance of nodule number of cowpea cultivars California #5 and Speckled Purple Hull as affected by drought.

Source of Variation	DF	Mean Square
Whole Plot		
Inoculated	1	154.49976**
Cultivar	1	0.41704
Inoculation X Cultivar	1	1.55766
Error A	9	2.30142
Subplot		
Date	2	3.17718
Date X Inoculation	2	4.44739
Date X Cultivar	2	0.70842
Date X Inoculation X Cultivar	2	0.93564
Error B	24	1.44395

Table 2b. Comparison between the means of nodule number of cowpea cultivars California #5 (Ca#5) and Speckled Purple Hull (SPH) in the dry treatment.

Treatment	Cultivar	Mean Nodule Number 35	63	96
		Days after planting		
Inoculated	Ca#5	10.00 a	17.00 a	21.00 a
Uninoculated	Ca#5	1.25 b	1.00 b	0.00 b
Inoculated	SPH	7.25 a	33.00 a	22.00 a
Uninoculated	SPH	0.00 b	0.00 b	0.00 b

Means followed by the same letter within a column are not significantly different at the 5% level using Least Significant Differences.

Table 3a. Analysis of variance of nodule number of cowpea cultivars California #5 and Speckled Purple Hull as affected by irrigation.

Source of Variation	DF	Mean Square
<u>Whole Plot</u>		
Inoculated	1	80.06712**
Cultivar	1	0.21062
Inoculation X Cultivar	1	0.16606
Error A	9	0.84381
<u>Subplot</u>		
Date	2	4.14438**
Date X Inoculation	2	0.91747
Date X Cultivar	2	0.40801
Date X Inoculation X Cultivar	2	0.47169
Error B	24	0.47897

**Significant at 0.01 level.

Table 3b. Comparison between the means of nodule number of cowpea cultivars California #5 (Ca#5) and Speckled Purple Hull (SPH) in the irrigated plots.

Treatment	Cultivar	Mean Nodule Number		
		35	63	96
		Days after planting		
Inoculated	Ca#5	3.50 a	14.50 a	6.75 a
Uninoculated	Ca#5	0.00 b	1.25 b	0.00 b
Inoculated	SPH	5.00 a	11.00 a	11.75 a
Uninoculated	SPH	0.00 b	0.75 b	0.00 b

Means followed by the same letter within a column are not significantly different at the 5% level using Least Significant Differences.

Nodule number data for the non-irrigated regime (Table 2b) revealed significant differences between inoculated and uninoculated treatments of both cultivars. Inoculated SPH at the flowering period (Table 2b) had the highest mean nodule number of 33 with a range of 7 to 97 nodules/plant. However, uninoculated SPH were not infected by the indigenous rhizobia strains as were uninoculated Ca#5 (Fig. 4a). There were no significant differences between the two-way interactions (Table 2a).

In the wet treatment the mean nodule number of inoculated Ca#5 at the flowering stage was 14 with a range of 6 to 24 nodules/plant (Table 3b). Although nodule number of Ca#5 was greater than of SPH, there were no significant differences between inoculated and uninoculated treatments of both cultivars (Tables 3a and 3b).

Wilson (1931) reported that nodules are sloughed off under water stress (drought) conditions. Comparisons between nodule numbers in the dry and wet treatments in this field experiment did not support this observation, rainfall during the growing season (Fig. 1) may have been responsible for this effect.

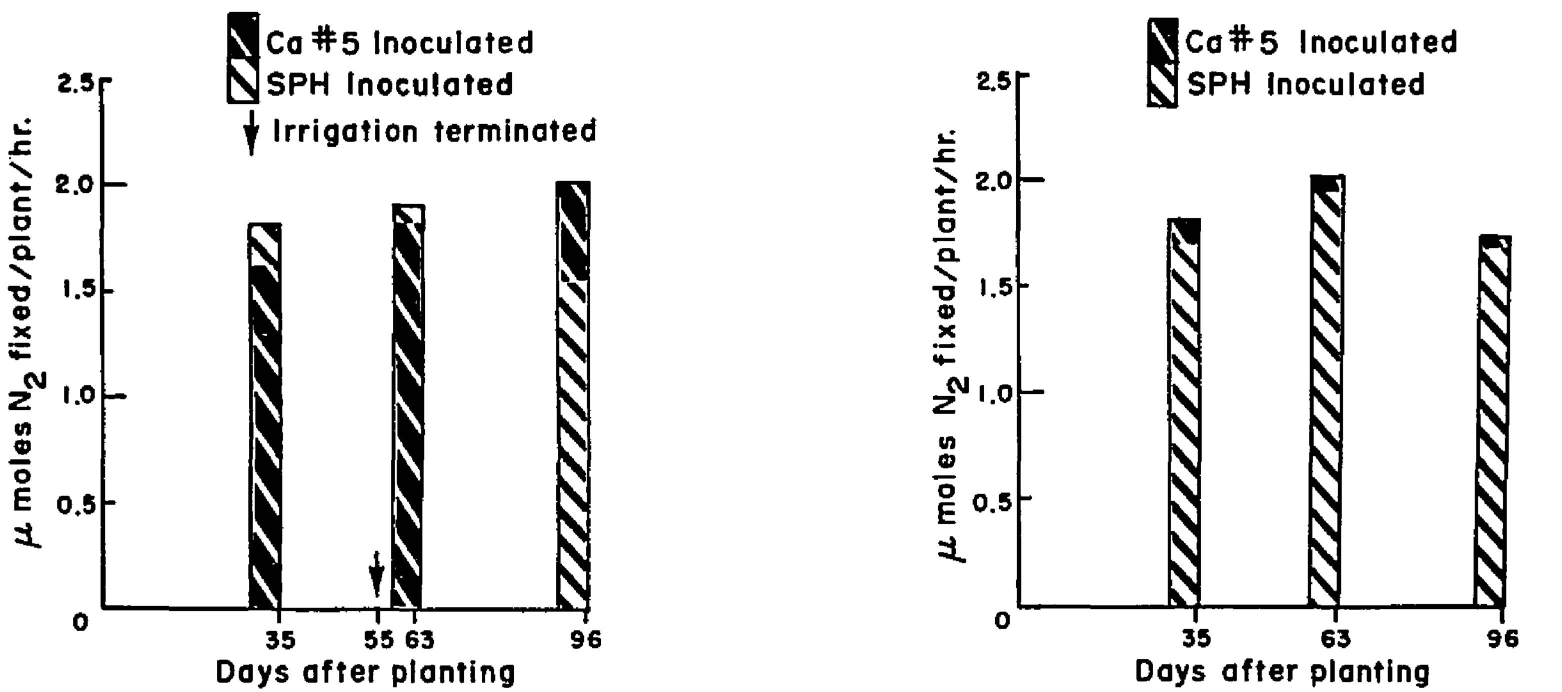
The two cultivars responded differently to the favorable and unfavorable conditions. The observed differences due to the life cycle of each cultivar (Table 1). Also, variability among cultivars for nodulation could have accounted for differences seen. However, variability could have some effect on nitrogenase activity of both cultivars.

Nitrogenase activity of water stressed cowpeas was minimal during the vegetative growth period, with mean total nitrogenase activity being 1.6 and 1.8 (μ moles/plant/hour) for Ca#5 and SPH, respectively. Beyond the developmental stage, there was an increase of total nitrogenase activity for Ca#5, while decreased in the pod stage for SPH (Fig. 5a). There was no significant difference between cultivars, however the two-way interaction harvest time (D) X treatment (T) was significantly different (Table 4).

Nitrogenase activity for irrigated cowpeas was maximum during the flowering period with a mean nitrogenase activity of 2.0 and 1.95 (μ moles/plant/hr.) for Ca#5 and SPH, respectively. As plants mature beyond this growth stage, there was a highly significant decline to 1.7 and 1.65 μ moles for Ca#5 and SPH, respectively (Fig. 5b). The drop in nitrogenase activity was probably due to the competition for photosynthate between seed set and nodule activity. Herridge and Pate (1977) reported that little photosynthate is available to support nodule activity since most of it goes to the reproductive tissues at this stage of development. Although there was no significant difference in the main plot, significant differences were established between harvest times (Table 5).

The observation that maximum total nitrogenase activity occurs between flowering and pod fill stages is similar to those made on greenhouse-grown cowpeas (Herridge and Pate, 1977) and field grown soybean (Hardy et al., 1968; Ayanaba and Lawson, 1977).

Comparisons of nitrogenase activity in the wet and the dry treatments (Figs. 5a and 5b) indicate that under long periods of drought with occasional rain, approximately 3.00 inches, non-irrigated nodulated plants maintained a low but sustained nitrogenase



a) Non-irrigated treatment

b) Irrigated

Figure 5. Nitrogenase activity of inoculated cowpeas over the growing season.

Table 4. Analysis of variance of nitrogen fixed per plant of cowpea cultivars California #5 and Speckled Purple Hull as affected by drought.

Source of Variation	DF	Mean Square
<u>Whole Plot</u>		
Replication	3	0.12272
Treatment	1	0.00394
Error A	3	0.01863
<u>Subplot</u>		
Date	2	0.06295
Date X Treatment	2	0.23008*
Error B	12	0.03662

*Significant at 0.05 level

Treatment = Inoculation X Cultivar

Table 5. Analysis of variance of nitrogen fixed per plant of cowpea cultivars California #5 and Speckled Purple Hull as affected by irrigation.

Source of Variation	DF	Mean Square
Whole Plot		
Replication	3	0.02001
Treatment	1	0.00685
Error A	3	0.01472
Subplot		
Date	2	0.15987*
Date X Treatment	2	0.00222
Error B	11	0.02529

*Significant at 0.05 level

Treatment = Inoculation X Cultivar

activity. This may be due to a depression of nodule initiation or inability of nodules to recover significantly after prolonged stress to the level of non-stressed nodules depending on the severity and duration of the stress (Engin and Sprent, 1973).

Nodule dry weights closely parallel the nodule number data. Nodule dry weights were greater for non-irrigated inoculated plots than for the irrigated inoculated plots for both cultivars (Tables 6b and 7b). No significant differences between harvest times and its interactions were observed in the two levels of irrigation (Tables 6a and 7a). There were significant differences between inoculated cultivars in the drought regime (Table 6a).

During the vegetative growth period Ca#5 and SPH had a mean nodule dry weights of 0.8 and 0.3 (gm) respectively (Fig. 6a) in the dry treatment. While in the wet treatment at the same growth stage the mean nodule dry weights were recorded 0.5 and 0.35 for Ca#5 and SPH respectively (Fig. 6b).

Pod weights constituted a significant part of the total plant weight. Under drought, mean pod dry weights of 136 and 120 (gm) were recorded for uninoculated and inoculated Ca#5 respectively; 14 and 10 (gm) were found for inoculated and uninoculated SPH respectively (Table 10). There were no significant differences within cultivars (inoculated and uninoculated) however, significant differences existed between the pod dry weights of Ca#5 and SPH (Table 8) and this was probably reflected in the final seed yield of either cultivar.

Table 6a. Analysis of variance of nodule dry weight of cowpea cultivars California #5 and Speckled Purple Hull as affected by drought.

Source of Variation	DF	Mean Square
Whole Plot		
Replication	3	0.61891*
Treatment	1	0.69168*
Error A	3	0.03276
Subplot		
Date	2	0.03186
Date X Treatment	2	0.04774
Error B	12	0.09704

*Significant at 0.05 level

Treatment = Inoculation X Cultivar

Table 6b. Comparison between a mean nodule dry weight per plant of cowpea cultivars California #5 (Ca#5) and Speckled Purple Hull (SPH) in the dry treatment.

Treatment	Cultivar	Nodule Dry Weight (gm)		
		35	63	96
		Days after planting		
Inoculated	Ca#5	0.8193 a	0.7722 a	0.7163 a
Uninoculated	Ca#5	0.6939 a	*	*
Inoculated	SPH	0.3138 b	0.5723 a	0.4032 a
Uninoculated	SPH	*	*	*

Means followed by the same letter are not significantly different at 5% level using Least Significant Differences.

*Nodules are absent

Table 7a. Analysis of variance of nodule dry weight of cowpea cultivars California #5 and Speckled Purple Hull as affected by irrigation.

Source of Variation	DF	Mean Square
Whole Plot		
Replication	3	0.00406
Treatment	1	0.35500
Error A	3	0.05390
Subplot		
Date	2	0.01918
Date X Treatment	2	0.00870
Error B	12	0.03619

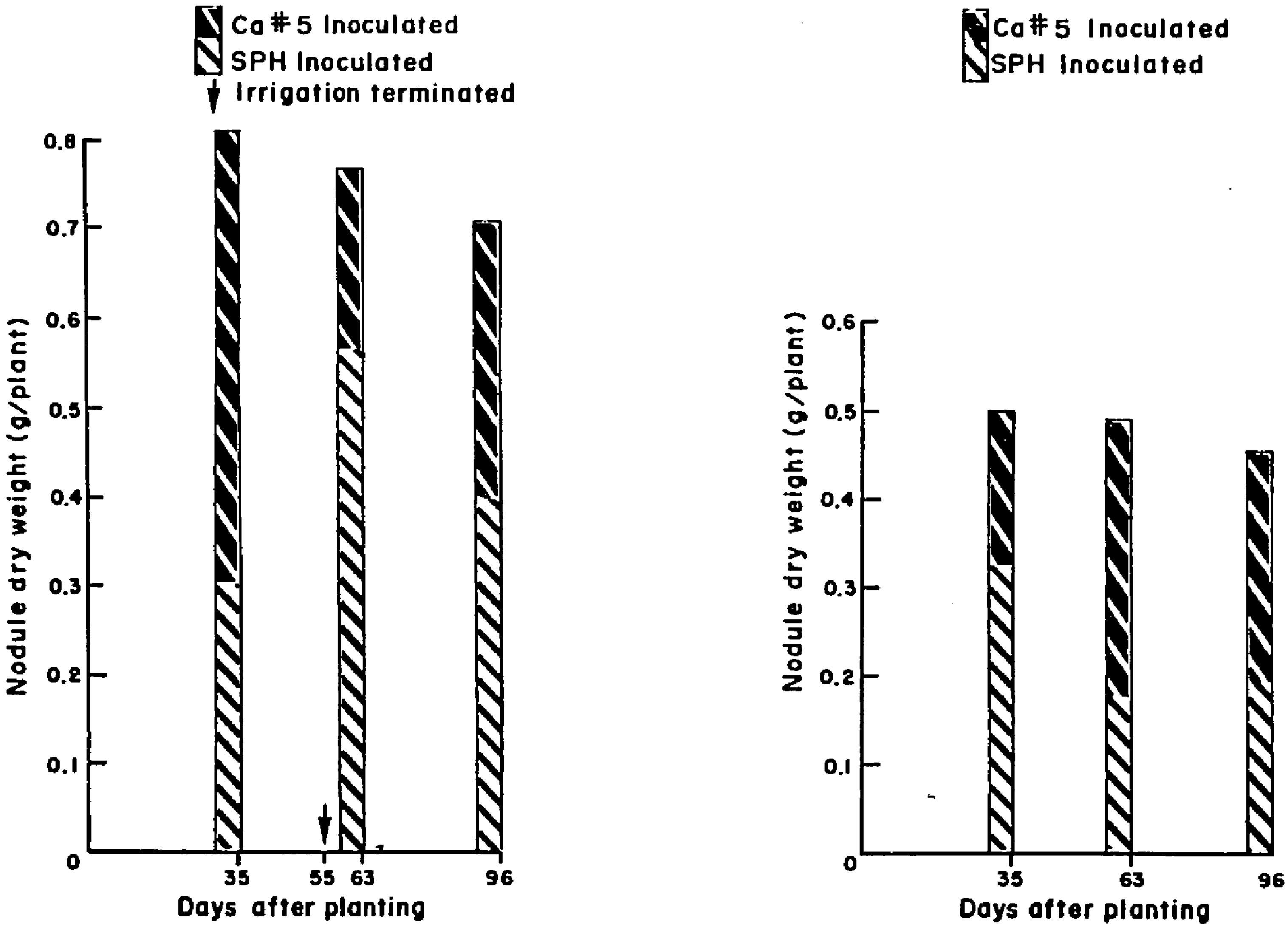
Treatment = Inoculation X Cultivar

Table 7b. Comparison between a mean nodule dry weight per plant of cowpea cultivars California #5 (Ca#5) and Speckled Purple Hull (SPH) in the irrigated plots.

Treatment	Cultivar	Nodule Dry Weight (gm)		
		35	63	96
		Days after planting		
Inoculated	Ca#5	0.5074 a	0.4907 a	0.4595 a
Uninoculated	Ca#5	*	0.3424 a	*
Inoculated	SPH*	0.3340 a	0.1862 a	0.2077 a
Uninoculated	SPH	*	0.2553 a	*

Means followed by the same letter are not significantly different at 5% level using Least Significant Differences

*Nodules are absent.



a) Non-irrigated treatment

b) Irrigated treatment

Figure 6. Mean nodule dry weight of inoculated cowpeas over the growing season.

Table 8. Analysis of variance of pod dry weight of cowpea cultivars California #5 and Speckled Purple Hull as affected by drought.

Source of Variation	DF	Mean Square
Whole Plot		
Inoculation	1	40.36511
Cultivar	1	59374.58000**
Inoculation X Cultivar	1	120.90125
Error A	9	555.07462
Subplot		
Date	1	13606.72561**
Date X Inoculation	1	98.21011
Date X Cultivar	1	7009.28000*
Date X Inoculation X Cultivar	1	349.80125
Error B	12	1244.81331

**Significant at 0.01 level

* Significant at 0.05 level

In the irrigated plots, pod dry weights were higher than the pod dry weights in the dry plots. Mean pod dry weights of 286 and 173 (gm) were reported for uninoculated and inoculated Ca#5, while 59 and 41 (gm) were recorded for inoculated and uninoculated SPH respectively (Table 11). Data shown in Table 9 suggest that differences in pod dry weights were highly significant between cultivars regardless of nodulation.

Turk et al. (1980) found that the yield component most sensitive to environmental stresses was the number of pods/m^2. It would appear that cowpeas response to stress in many circumstances involves an extreme reduction in pod number which insures that viable seeds are produced.

The effects of moisture availability on the final seed yield of cowpea cultivars Ca#5 and SPH are shown in Tables 12 and 13. Under the drought regime, final seed yield was significantly different between cultivars. However, there were no such differences within cultivars (Table 12). Mean final seed yield of inoculated and uninoculated Ca#5 were 764 and 1018 (kg/ha) and inoculated and uninoculated SPH 90 and 110 (kg/ha) respectively (Table 14).

However, the final seed yield of both cultivars was higher under irrigation than the final seed yield in the drought regime. The mean final seed yield of inoculated and uninoculated Ca#5 were 2020 and 1685 (kg/ha) respectively. While the mean final seed yield for inoculated and uninoculated SPH were 705 and 763 (kg/ha) respectively (Table 15).

Table 9. Analysis of variance of pod dry weight of cowpea cultivars California #5 and Speckled Purple Hull as affected by irrigation.

Source of Variation	DF	Mean Square
Whole Plot		
Inoculation	1	5725.57005
Cultivar	1	93493.52820**
Inoculation X Cultivar	1	10369.44005*
Error A	9	1391.28399
Subplot		
Date	1	119355.20820**
Date X Inoculation	1	3361.18005
Date X Cultivar	1	41064.04820*
Date X Inoculation X Cultivar	1	7079.31005
Error B	12	1663.66613

**Significant at 0.01 level

* Significant at 0.05 level

Table 10. Comparison between pod dry weight of cowpea cultivars California #5 (Ca#5) and Speckled Purple Hull (SPH) in the dry treatment.

Treatment	Cultivar	Mean Total Pod Dry Weight (gm) 96 Days After Planting
Uninoculated	Ca#5	136.2500 a
Inoculated	Ca#5	120.0000 a
Inoculated	SPH	14.7500 b
Uninoculated	SPH	10.0000 b

Means followed by the same letter are not significantly different at 0.05 level using Least Significant difference.

Table 11. Comparison between pod dry weight of cowpea cultivars California #5 (Ca#5) and Speckled Purple Hull (SPH) in the irrigated plots.

Treatment	Cultivar	Mean Total Dry Weight (gm) 96 Days After Planting
Uninoculated	Ca#5	286.7500 a
Inoculated	Ca#5	173.7500 b
Inoculated	SPH	59.7500 c
Uninoculated	SPH	41.2500 c

Means followed by the same letter are not significantly different at 0.05 level using Least Significant difference.

Table 13 shows the analysis of variance of final seed yield for Ca#5 and SPH as affected by the wet treatment. There was no significant difference within cultivars. However, differences in the final seed yield were highly significant between cultivars (Table 13).

Hanway et al. (1971) reported that major difference in final seed yields among soybean varieties and between years resulted primarily from differences in the time seeds increased in weight rather than from differences in the rates of dry matter accumulation. A similar trend could explain the major differences in the final seed yield of cowpeas in this experiment.

On the other hand, Mahler and Wollum (1981) found that significant yield differences of soybeans due to irrigation and Rhizobia japonicum strains were obtained. However, comparison of data in Tables 14 and 15 shows that a relationship clearly exists between the level of irrigation and the final seed yield. This relationship could not be established statistically since there were only two moisture levels used.

Data for seed protein content of cowpea cultivars Ca#5 and SPH in the dry and the wet treatments are shown in Tables 16 and 17 respectively. There were no significant differences between cultivars in the drought regime (Table 16). However, in the wet treatment the mean seed protein content of SPH was significantly different from that of Ca#5 (Table 17). Inoculated SPH showed the highest seed protein content 1.45 (gm) Table 18.

Table 12. Analysis of variance of final seed yield of cowpea cultivars California #5 and Speckled Purple Hull as affected by drought.

Source of Variation	DF	Mean Square
Whole Plot		
Inoculated	1	0.02047
Cultivar	1	6.22961**
Inoculation X Cultivar	1	0.00381
Error A	9	0.20387

**Significant at 0.01 level.

Table 13. Analysis of variance of final seed yield of cowpea cultivars California #5 and Speckled Purple Hull as affected by irrigation.

Source of Variation	DF	Mean Square
Whole Plot		
Inoculated	1	0.00399
Cultivar	1	0.73765**
Inoculation X Cultivar	1	0.01100
Error A	9	0.01903

**Significant at 0.01 level.

Table 14. Comparison of the means of the final seed yield of cowpea cultivars California #5 (Ca#5) and Speckled Purple Hull (SPH) in the dry treatment.

Treatment	Cultivar	Mean of Final Seed Yield (kg/ha)
Uninoculated	Ca#5	1018 a
Inoculated	Ca#5	764 a
Uninoculated	SPH	110 b
Inoculated	SPH	90 b

Means followed by the same letter are not significantly different at 0.05 level using Least Significant Difference.

Table 15. Comparison of the means of final seed yield of cowpea cultivars California #5 (Ca#5) and Speckled Purple Hull (SPH) in the wet treatment.

Treatment	Cultivar	Mean of Final Seed Yield (kg/ha)
Inoculated	Ca#5	2020 a
Uninoculated	Ca#5	1685 a
Uninoculated	SPH	763 b
Inoculated	SPH	705 b

Means followed by the same letter are not significantly different at 0.05 level using Least Significant Difference.

Table 16. Analysis of variance of protein content of cowpea cultivars California #5 and Speckled Purple Hull as affected by drought.

Source of Variation	DF	Mean Square
Whole Plot		
Inoculated	1	0.00008
Cultivar	1	0.00326
Inoculation X Cultivar	1	0.00003
Error A	9	0.00095

Table 17. Analysis of variance of protein content of cowpea cultivars California #5 and Speckled Purple Hull as affected by irrigation.

Source of Variation	DF	Mean Square
Whole Plot		
Inoculated	1	0.00001
Cultivar	1	0.00206**
Inoculation X Cultivar	1	0.00000
Error A	9	0.00011

**Significant at 0.01 level

Table 18. Comparison between the means of protein content of cowpea cultivars California #5 (Ca#5) and Speckled Purple Hull (SPH) in the irrigated treatment.

Treatment	Cultivar	Mean of Protein Content (gm)
Inoculated	SPH	1.4519 a
Uninoculated	SPH	1.4514 a
Inoculated	Ca#5	1.4302 b
Uninoculated	Ca#5	1.4278 b

Means followed by the same letter are not significantly different at 0.05 level using Least Significant Difference

Analysis of variance of 100 seed weight for Ca#5 and Speckled Purple Hull is shown in Appendix A (Tables A-1 and A-2). There were no significant differences between cultivars and irrigation treatments. Also, no significant differences were observed between interactions. Tables A-3 and A-4 show the comparison between the means of 100 seed weight of cowpeas in the dry and the irrigated plots. The results confirmed that no significant differences were observed.

Shoot and root analysis of variance are presented in Appendix A (Tables A-5 and A-6). Differences were highly significant for stem, leaf and root over time for both levels of irrigation. However, the three-way interactions (Date X inoculation X cultivar) for the root was significantly different in the dry plots (Table A-5). There was no such difference for the same parameter in the irrigated plots (Table A-6).

SUMMARY AND CONCLUSIONS

Cowpea cultivars California #5 blackeye (Ca#5) and Speckled Purple Hull (SPH) inoculated with granular inoculum were grown at Campbell Ave. Farm (Tucson, Arizona) in summer 1981 to examine the extent by which symbiotic nitrogen fixation may be affected by optimal or sub-optimal irrigation.

Nodule number data revealed that there were no significant differences between inoculated cultivars in irrigated and non-irrigated treatments. However, nodule numbers were significantly different in both levels of irrigation depending on whether or not cowpeas were inoculated. Nodule dry weights were greater in nonirrigated-inoculated than irrigated-inoculated plots, although there were no significant differences between harvest dates and its interactions.

Maximum total nitrogenase activity was observed between flowering and pod-fill stages. Comparison between nitrogenase activity in irrigated and non-irrigated plots showed that water availability was important to insure high nitrogenase activity. Percent nodulation of cowpea cultivars over the growing season showed that differences existed between Ca#5 and SPH. However, Ca#5 had a higher percentage of nodulated plants at both irrigation levels throughout the season.

Partitioning of assimilates among the vegetative components, stem, leaf and root was affected by the level of irrigation. Assimilate partitioning was increased over time for both cultivars. Higher amounts of assimilates were observed in irrigated plots. However, there were no significant differences between cultivars in both levels of irrigation with respect to inoculation.

Higher pod dry weights were found in irrigated plots. Differences in pod dry weights between cultivar were probably reflected in the final seed yield of both cultivars. Final seed yield of cowpeas showed that in arid environments irrigation is essential for maximum productivity. Even though inoculation resulted in nodulation, biological nitrogen fixation was not correlated with increased seed yield in either cultivar. However, protein content of seeds was increased.

APPENDIX A

ADDITIONAL INFORMATION ON PLANT CULTIVARS

Table A-1. Analysis of variance of 100 seed weight of cowpea cultivars California #5 and Speckled Purple Hull as affected by drought.

Source of Variation	DF	Mean Square
Whole Plot		
Inoculated	1	18.06399
Cultivar	1	7.72520
Inoculation X Cultivar	1	1.56206
Error A	9	11.60592

Table A-2. Analysis of variance of 100 seed weight of cowpea cultivars California #5 and Speckled Purple Hull as affected by irrigation.

Source of Variation	DF	Mean Square
Whole Plot		
Inoculated	1	7.52967
Cultivar	1	3.09012
Inoculation X Cultivar	1	3.07853
Error A	9	13.38072

Table A-3. Comparison between the means of 100 seeds weight of cowpea cultivars California #5 (Ca#5) and Speckled Purple Hull (SPH) as affected by drought.

Treatment	Cultivar	Mean of 100 seeds weight (gm)
Inoculated	Ca#5	6.7737 a
Uninoculated	Ca#5	5.2735 a
Inoculated	SPH	6.0089 a
Uninoculated	SPH	3.2589 a

Means followed by the same letter are not significantly different at 0.05 level using Least Significant Difference.

Table A-4. Comparison between the means of 100 seeds weight of cowpea cultivars California (Ca#5) and Speckled Purple Hull (SPH) as affected by irrigation.

Treatment	Cultivar	Mean of 100 Seeds Weight (gm)
Uninoculated	Ca#5	5.7678 a
Inoculated	Ca#5	5.2731 a
Uninoculated	SPH	5.7662 a
Inoculated	SPH	3.5169 a

Means followed by the same letter are not significantly different at 0.05 level using Least Significant Difference.

Table A-5. Analysis of variance of stem, leaf, and root dry weight of cowpea cultivars California #5 and Speckled Purple Hull as affected by drought.

Source of Variation	DF	Mean Square Stem	Leaf	Root (gm)
Whole Plot				
Inoculated	1	0.00231	0.02721	0.00430
Cultivar	1	0.26202**	0.00953	0.00077
Inoculation X Cultivar	1	0.03263	0.05280	0.05682
Error A	9	0.01027	0.02474	0.01370
Subplot				
Date	2	5.53490**	0.47411**	1.32962**
Date X Inoculation	2	0.01889	0.00813	0.00293
Date X Cultivar	2	0.00863	0.01883	0.00076
Date X Inoculation X Cultivar	2	0.01595	0.00549	0.02108*
Error B	24	0.02129	0.00979	0.00518

**Significant at 0.01 level
* Significant at 0.05 level

Table A-6. Analysis of variance of stem, leaf, and root dry weight of cowpea cultivars California #5 and Speckled Purple Hull as affected by irrigation.

Source of Variation	DF	Mean Square		
		Stem	Leaf	Root (gm)
Whole Plot				
Inoculated	1	0.04014	0.01138	0.00029
Cultivar	1	0.33396**	0.23502**	0.00445
Inoculation X Cultivar	1	0.00005	0.03672	0.00008
Error A	9	0.02240	0.01757	0.00854
Subplot				
Date	2	8.34258**	1.15801**	1.34045**
Date X Inoculation	2	0.02194	0.00988	0.00615
Date X Cultivar	2	0.00519	0.02982	0.00384
Date X Inoculation X Cultivar	2	0.03628	0.05341*	0.00386
Error B	24	0.00928	0.01252	0.00512

**Significant at 0.01 level.
* Significant at 0.05 level.

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