SOME PHYSIOLOGICAL PROCESSES IN WINTER WHEAT (*TRITICUM AESTIVUM* L.) AND COWPEAS (*VIGNA UNGUICULATA* L.) AS AFFECTED BY DROUGHT STRESS

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CHAPTER I

INTRODUCTION

This dissertation is composed of four main chapters. Each chapter is prepared as a separate paper for publication in a professional journal.

Wheat and cowpeas are grown as major sources of calories and protein throughout the world. As a consequence, both crops are important in increasing world food production. While it is imperative to increase food production to accommodate population growth, this increase will have to come about on lands that are less productive. One of the main limiting factors to production is moisture availability. As is noted by Raheja (1966),[1] over a third of the earth's land is classified as arid or semiarid (i.e. receiving between 130 and 760 mm of rainfall per annum). He further noted that the problem is compounded when drought occurs temporarily in the other areas not classified as arid, resulting in starvation.

Increasing yield is always the basic goal in crop physiology, breeding, management and production systems.

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[1] Raheja, P. C. In Salinity and Aridity: New Approaches to Old Problems. Ed. Boykoed W. Jenks, (Netherlands, 1966), pp. 10-42.

Since survival of a crop or plant is a prelude to production, genotypes will have to be developed that are tolerant to drought stress conditions. Since both wheat and cowpeas are grown in areas where severe and often frequent moisture stress might occur, there has been great interest in genotypes that will survive adverse drought stress and yet are high producers. It is recognized that drought does play an important role in physiological processes; however, understanding of these processes is quite limited. Due to this limited knowledge of stress on physiological processes, much attention and research is being directed in this area.

The researcher interested in plant response to stress whether climatic or nutritional is always faced with the problem of implementation of his research. In order to further understand the complex problem of drought stress, the researcher might look at photosynthesis and nitrate assimilation and reduction. The differences among cultivars could be correlated to yield under various climatic conditions. In order to achieve the goal of sustained increased yield, physiologists and biochemists will have to work out various mechanisms which confer some degree of drought resistance to the plant. Plant breeders would therefore be able to develop plants with higher yields under adverse conditions.

Nitrogen is a constituent of enzymes and nucleoproteins which play an important role in plant growth and development. Cowpeas, as a legume, is capable of utilizing atmospheric nitrogen through nitrogen fixation, while wheat obtains its

nitrogen from the soil. Nitrate reductase activity in the plant governs the rate and the amount of nitrate utilization from the soil. Nitrate reductase activity is often reduced under nitrogen and moisture stress. It is imperative that plants are geared to maintain high nitrate reductase activity since this is associated with high grain yields and high protein.

The major objective of this research was to identify the relationship between drought resistance and some physiological responses in winter wheat and cowpeas.

CHAPTER II

CARBON DIOXIDE EXCHANGE RATES, STOMATAL DIFFUSIVE RESISTANCE AND TRANSPIRATION IN WINTER WHEAT UNDER FIELD CONDITIONS

Abstract

Careful management of a limited moisture supply would require that periods with reduced moisture coincide with growth stages which influence grain yield the least. This research was conducted under field conditions to study carbon dioxide exchange rates (CER), stomatal diffusive resistance (Rs) and transpiration from flag leaf emergence (stage 4) to head enlargement and grain filling (stage 8). Six winter wheat (Triticum aestivum L.) cultivars were evaluated in this study. In addition to CER, Rs and transpiration, the yield and yield components were also measured. Eight sampling dates were obtained throughout these stages of development and yield and yield component data were taken 236 days after planting.

The results showed a linear relationship between stomatal diffusive resistance and transpiration, with an increase in one resulting in a decrease of the other. There was a

negative correlation between CER and Rs; the data showed a decline in CER with an increase in Rs. Carbon dioxide exchange rate at the same time was positively correlated with yield and yield components.

In this study soil moisture was not a factor in wheat production since there was available moisture throughout the growing season and sampling periods. There were no comparisons available to study the effects of moisture stress under similar field conditions. However, under adequate moisture, 'Baca' outperformed the other cultivars in its water use efficiency. It is therefore possible to increase water use efficiency through selecting for higher CER combined with stomatal behavior.

Introduction and Literature Review

Plant mechanisms for dealing with drought were divided into four categories according to Kozlowski (1968); drought escape, drought evasion, drought endurance and drought resistance. Other workers have categorized drought responses differently. Arnon (1972) defined drought resistance as the ability of plants to survive under drought conditions, endure drought without injury and be efficient in their use of water. Levitt (1972) simply divided drought resistance into either drought avoidance or drought tolerance. He explained that the drought avoiding plants maintain a high internal water potential in spite of the demands placed upon them by the atmosphere. Drought tolerance refers to the ability of

a plant to survive at low tissue moisture content.

Drought stress occurs in the plant whenever transpiration exceeds absorption. This is the result of the inability of the soil to supply the amount of moisture required by the plant. Drought stress therefore occurs as a result of low precipitation, high temperature and/or high wind speed. Drought cannot be considered as a uniform concept, but is a reaction of the plant to environmental conditions, and its effect is dependent upon the stage of plant development at which drought occurred. This led Singh (1981) to conclude that, in relation to wheat grain yields if moisture is limited, deficits should be spread evenly over the growth and critical stages. When soil moisture is limited, growth and yield are affected through a number of physiological processes including photosynthetic activity and stomatal action. Drought stress does not have a uniform effect when it occurs during different stages of growth and development. Denmead and Shaw (1960) observed that stress in corn during silking was more harmful to grain yields than stress during any other growth stage. Gardener et al. (1981) found a tendency for plants subjected to moisture stress during the grain filling stage to mature faster than plants fully irrigated during that period.

Yield is the result of several interrelated physiological processes and is therefore the final stage in the plant survival mechanism. Since drought will affect different physiological processes, photosynthesis can be

directly or indirectly affected by stress. It can reduce growth, which provides the tissue for photosynthesis or it will affect reproduction, which provides the sink for the storage of photosynthesis (Denmead and Shaw, 1960).

The photosynthetic activity of a plant is mainly determined by its total leaf area and the rate of activity of each unit of leaf (Imai and Murata, 1976; Sionit et al., 1980). Since organic matter production through CO_2 fixation by photosynthesis accounts for most of the plant dry matter accumulation, then factors affecting photosynthesis will affect total dry matter and consequently grain production by crops (Sionit et al., 1981). Neales and Nicholls (1978) have observed that an increase in net assimilation rate in wheat due to CO_2 enrichment was dependent upon the plant age.

In cereals, growth that occurs after anthesis is characterized by the photosynthetic products that move to the grain rather than that going towards vegetative development. Estimates of the contribution to grain dry weight from photosynthesis above the flag leaf node range from 60 percent (Archibold, 1942) to 80-85 percent (Enyi, 1962). Thorne (1969) stated that flag leaf area had great importance on the grain yield after anthesis. Hsu and Walton (1971) supported Thorne's position on the relationship between photosynthesis area above the flag leaf node and cereal grain yield. Photosynthesis can be drastically modified by internal as well as external moisture stress. Baker and Musgrave (1964) have shown how soil and

atmospheric stresses can produce additive effects in reducing photosynthesis. Ashton (1956) stated that leaf area is more affected by water stress than net assimilation rate (NAR), but since NAR is directly affected by leaf area it will ultimately be affected proportionally.

Stomates play a significant role in controlling the transpiration of water from the plant and the diffusion of CO_2 into the plant. Stomatal behavior can therefore be interpreted as an indicator of water use as well as a measure of photosynthesis. Fully understanding the stomatal diffusive resistance to water vapor and CO_2 and the overall stomatal physiology has eluded researchers for a long time. Although stomates have been recognized as important in the overall dry matter production and water loss schemes, the status of the stomates in the dynamics of the plant is not constant and changes are based on environmental as well as endogenous factors. Kanemasu (1975), noted that stomatal behavior may vary drastically even within species.

Hsiao (1973), in summarizing the observed plant responses to water stress, noted that several factors are responsible for stomatal opening and closure. Not only was water stress a direct cause of stomatal closure but light intensity, CO_2, relative humidity, age of leaf, temperature, plant mineral nutrition and disease all affected stomatal opening. Stomatal opening would therefore have a direct effect upon the amount of CO_2 fixed by the plant. Brown and Rosenberg (1970) reported that diffusive

resistance to CO_2 exchange increases due to stomatal closure under moisture stress, which would result in reduced photosynthesis and consequently, yield. Water loss is relative and many different methods are used to characterize it. Furthermore some cultivars are most efficient in their water use per unit dry matter produced.

A number of empirical tests have been undertaken which differentiate among drought resistant cereal plants. Although considerable data is available on stomatal resistance, photosynthesis and transpiration, the physiological basis for drought resistance, is still not fully understood. The purpose of this study is to determine whether some cultivars have a higher carbon exchange rate in relation to water availability and use than others.

Materials and Methods

This study was performed in 1982-1983 growing season using six winter wheat cultivars ('Baca,' 'Blue Jacket,' 'KanKing,' 'Ponca,' 'Red Chief' and 'Sturdy') planted in a randomized complete block design with eight replications on the Agronomy Research Station, Stillwater, Oklahoma. The soil type was a Kirkland silt loam (Udertic Paleustoll) (Gray and Roozitalab, 1976). Plots were planted on October 19, 1982, in four rows 3.1 m long with 23 cm between rows using a seeding rate of 67 kg/ha to ensure adequate stands. Nitrogen was supplied in early spring at 120 kg/ha.

Net carbon dioxide exchange (NCE), stomatal diffusive resistance (Rs) and transpiration measurements were initiated at growth stage 4 (flag leaf emergence) (Bruns and Croy, 1983) and ended at stage 8 (grain filling). On each sampling date, samplings were started at 1100 (CST). The last fully expanded leaf in stage 4 and the flag leaf after stage 5, from each plot were selected at random and sampled. Apparent photosynthesis was determined by the rate NCE per unit leaf area using infrared gas analysis. The instrument used in this study was Beckman Model 865 Infrared Gas Analyzer with a Beckman Model 1005, 10 inch chart recorder attached.

The syringe method used by Clegg et al. (1978), and modified by Bruns (1981) was used to obtain samples. This method employed a portable, airtight, plexiglass chamber which was 6.4 cm long by 2.9 cm in diameter. The plexiglass chamber was affixed about midway on the sampled leaf and the chamber covered to prevent photosynthesis. A gas sample of about 6 cc was drawn from the chamber using a 10 cc hypodermic syringe. The cover of the chamber was then lifted and the leaf was illuminated for 15 seconds after which a second sample was taken. Syringes containing gas samples were brought to the laboratory and 5 cc of each sample was injected into the infrared gas analyzer via a stream of lamp grade nitrogen (1 litre.min^{-1}) to determine CO_2 concentration. The concentration of CO_2 was then compared to a standard of 300 ppm. A nondestructive

method was used to determine the leaf segment area used for photosynthesis.

AREA = L*W*C

L = Length of leaf chamber

W = Width of flag leaf

C = Correction factor (0.97) to correct for the flag leaf segment shape (Osmanzai, 1982)

Stomatal diffusive resistance and transpiration were measured by using an autoporometer model LI-65 and diffusive resistance sensor LI-205, Lambda Equipment Corporation, Lincoln, Nebraska. After calibration of the equipment, measurements were taken from the upper surface of the flag leaf. Illumination during the measurements ranged between 1800 and 2200 $\mu E.m^{-2}.sec^{-1}$. Leaf area of five fully expanded flag leaves was measured using a LI-COR LI-3000 portable leaf area meter. The average of these five readings was recorded for each plot.

Kernels per spike were determined based on the number of kernels averaged over 10 spikes sampled. Kernel weight was determined by averaging the weight of 100 kernels. The data for transpiration, diffusive resistance and carbon exchange rate were analyzed by pooling the results of each for the cultivars. Simple as well as multiple regression was carried out on the data in order to ascertain the relationship of physiological and agronomic characters to relative drought tolerance of cultivars.

Results and Discussion

Carbon dioxide exchange rates (CER) with respect to stomatal diffusive resistance (Rs) and transpiration were observed in the field on six winter wheat cultivars: Baca, Blue Jacket, KanKing, Ponca, Red Chief and Sturdy. From these observations relationships were determined among certain physiological characters and yield components. The experiment was originally initiated to subject the six cultivars to moisture stress, however, the meteorological data (Table I) show that there was greater than normal precipitation during the sampling period beginning 4 May 1983. There was a 7.14 cm deviation above normal during the sampling period and the data further show that moisture available to the crop throughout the growing period was 3.39 cm below normal. The other important aspect of moisture availability is its distribution just prior to anthesis, during anthesis and the grain filling period. During this period there was 28.24 cm of precipitation distributed over 17 rainy days.

The CER, leaf temperature, Rs, and transpiration of six winter wheat cultivars during the sampling period are reported in Table II. There were significant differences in the rates of carbon dioxide fixed, ranging from 9.6 to 16.0 mg $CO_2.dm^{-2}.hr^{-1}$ throughout the sampling period. While there was a relatively high coefficient of variability (36%) for CER there was little variability in leaf

temperature (5%). While there were significant differences among sampling dates, the daily mean temperatures also fluctuated as is noted in the meteorological data. Stomatal diffusive resistance generally declined as the plants matured, ranging from 0.91 $sec.cm^{-1}$ on 4 May to 0.65 $sec.cm^{-1}$ on 24 May. Transpiration followed a curvilinear pattern peaking at 31.8 $\mu g.cm^{-2}.sec^{-1}$ during stage 7 on May 23 and 24 then declining to 26.5 $\mu g.cm^{-2}.sec^{-1}$ at stage 8.

This pattern of water loss over the sampling period is shown in Figure 1. It should be noted that all the cultivars followed a similar pattern of water loss throughout the growing season. While water loss remained relatively high for all the cultivars, Sturdy on 23 May showed significant moisture loss compared to the other cultivars. This increased transpiration could be due to two factors: (1) the increased amount of moisture available through increased precipitation or (2) the growth stage of the plant (the plant still has much of its foliage remaining). While it must be realized that increased soil moisture played a significant role in the cycle of transpiration, stages 4 through 7 are actively transpiring stages in the plant's development. However, when plants approached stage 8, where foliage started to senesce, transpiration was reduced.

The physiological characteristics of six winter wheat cultivars are reported in Table III. There was no

significant difference in CER, Rs or transpiration among the cultivars. However, Baca had the highest rate of CER with KanKing the lowest, readings being 15.4 and 13.7 $mg.dm^{-2}.hr^{-1}$, respectively. Sturdy had the highest rate of transpiration (28.6 $\mu g.cm^{-2}.sec^{-1}$) while Ponca was lowest with 25.9 $\mu g.cm^{-2}.sec^{-1}$. As was pointed out earlier, there was adequate moisture, apparently ruling out moisture as a limiting factor in physiological activity. Under severe stress it would be expected that Rs would increase significantly with a concurrent fall in both CER and transpiration, however, during the sampling period transpiration and CER remained high with Rs being very low.

The ratio between transpiration and carbon dioxide exchange rate was used as a measure of water use efficiency (WUE). WUE was calculated for each cultivar based on its CER and transpiration levels expressed as $mg.m^{-2}.sec^{-1}$ and is presented in Table IV. The data show that Baca was the most efficient and Sturdy the least efficient. Sturdy transpired 724 $mg.m^{-2}.sec^{-1}$ of moisture for each $mg.m^{-2}.sec^{-1}$ of CO_2 fixed. This would have a major impact on areas where water supply is limited and crops are often subjected to soil moisture stress. Since photosynthetic CO_2 fixation is the source of plant dry matter production, the rate at which fixation occurs is critical to crop development and yield. Where moisture is a limitation over the growing season a high ratio of transpiration to carbon fixed could result in reduced production due to lack of

moisture in the reproductive stage.

Stomatal diffusive resistance Vs. transpiration (Figure 2) showed a general linear function for all six cultivars. At high transpiration all cultivars tended to show reduced stomatal resistance. That high stomatal resistance is associated with low transpiration is important under moisture stress conditions. However, since carbon dioxide fixation is important in dry matter production changes in stomatal conductance must be achieved so that a balance is achieved between carbon fixation and transpiration.

Yield component data on kernel weight, grain yield, kernels per spike and weight per spike showed significant differences among the six cultivars (Table V). Sturdy, the earliest maturing of the six cultivars, had lowest yield and yield components, while Baca and Red Chief were high. While there were small differences among the cultivars in the number of kernels per spike there were significant differences in weight per spike. Since yield components are related to each other and moisture stress was not a factor in yield, then differences observed between cultivars could be attributed to genetic capabilities.

The simple correlation coefficients for eight plant characters for the six winter wheat cultivars are presented in Table VI. Kernel weight was positively correlated with grain yield ($r = 0.91$) but negatively correlated with leaf area segment. CER and grain yield were positively correlated

but the correlation was not significant. This would suggest that by improving the CER of breeding material there is a good chance of improving grain yield. There was also a negative correlation between transpiration and stomatal diffusive resistance. Since CER is very important in crop productivity, it is necessary to balance the factors that will reduce CER. Although the correlation between CER and Rs was not significant, it should be noted that by reducing stomatal opening we are reducing the level of CER. The negative relationship between CER and leaf area might be suggesting that the sampling technique used might be unstable after a certain time and for a particular leaf size. By using a large leaf segment we run the risk of using up the CO_2 more rapidly than a smaller leaf resulting in lower readings over a longer exposure time.

Summary

The results of this study indicate that CER was negatively correlated with Rs and positively correlated with yield and yield components. Since vegetative growth and therefore yield is a result of carbon assimilation then the negative relationship between CER and stomatal resistance would suggest that the selection of any one of these traits will result in the reduction of the other. It was also noted that there was a positive relationship between transpiration and CER. In areas where moisture is the limiting factor to production then the cultivars that are capable of

producing with lower levels of transpiration will be desirable.

It can be concluded from this study that it is possible to increase water use efficiency of winter wheat through higher CER coupled with stomatal behavior. Since stomatal behavior controls transpiration then cultivars can be screened by means of selecting for high yields with reduced transpiration.

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TABLE I

METEOROLOGICAL DATA FOR JULY 1982 - JUNE 1983 AT THE AGRONOMY RESEARCH STATION, STILLWATER, OKLAHOMA

Sampling Date	Temperature (C) Min.	Max.	Month	Precip. (cm)	Dev. from Avg. (cm)
04 May 1983	4.4	21.7	July	5.03	-3.94
09 May 1983	7.2	21.7	August	3.51	-4.65
10 May 1983	10.6	23.3	September	5.84	-2.74
16 May 1983	5.0	18.3	October	2.46	-4.60
17 May 1983	7.8	21.1	November	7.04	2.34
23 May 1983	10.6	27.2	December	5.92	2.52
24 May 1983	12.2	25.6	January	0.84	-2.11
27 May 1983	17.2	28.9	February	7.65	4.22
			March	7.77	3.05
			April	4.14	-3.12
			May	18.87	7.14
			June	9.27	-1.50
			Total	78.34	-3.39

TABLE II

CARBON DIOXIDE EXCHANGE RATE (CER), LEAF TEMPERATURE (LT), STOMATAL DIFFUSIVE RESISTANCE (Rs) AND TRANSPIRATION AT SAMPLING DATES

Date	CER $mgCO_2.dm^{-2}.hr^{-1}$	LT C	Rs $sec.cm^{-1}$	Transpiration $\mu g.cm^{-2}.sec^{-1}$
04 May	15.7	21.7	0.91	22.4
09 May	14.3	22.4	0.86	23.5
10 May	14.3	24.0	0.91	22.2
16 May	16.0	23.5	0.72	28.5
17 May	15.0	23.5	0.82	25.5
23 May	14.0	28.1	0.65	31.8
24 May	14.8	28.1	0.65	31.8
27 May	9.6	33.5	0.80	26.5
$LSD_{.05}$	2.1	0.5	0.06	2.2
CV%	36	5	20	20

TABLE III

SOME PHYSIOLOGICAL CHARACTERS OF SIX WINTER WHEAT CULTIVARS

Cultivar	CER $mg.dm^{-2}.hr^{-1}$	Transpiration $\mu g.cm^{-2}.sec^{-1}$	Rs $sec.cm^{-1}$	Leaf Area (cm^2)
Baca	15.4 a*	26.2 a	0.83 a	15.6 e
Blue Jacket	15.2 a	26.5 a	0.75 a	17.2 cd
Sturdy	14.2 a	28.6 a	0.78 a	17.8 bc
Ponca	14.1 a	25.9 a	0.76 a	18.4 ab
Red Chief	14.0 a	25.9 a	0.82 a	16.6 de
KanKing	13.7 a	26.2 a	0.78 a	19.3 a
P>F	0.47	0.39	0.18	0.0001
CV%	14	10	9	6

Means with the same letters are not significantly different at the 5% level using the Duncan's Multiple Range Test.

TABLE IV

WATER USE EFFICIENCY OF SIX WHEAT CULTIVARS

Cultivars	CER ……mg.m^{-2}.sec^{-1}…….	Transpiration	Ratio Trans:CER
Baca	0.43	262	612
Blue Jacket	0.42	265	626
Sturdy	0.39	286	724
Ponca	0.39	259	662
Red Chief	0.39	259	662
KanKing	0.38	262	688

TABLE V

MEANS OF KERNEL WEIGHT, GRAIN WEIGHT, KERNEL PER SPIKE AND WEIGHT PER SPIKE IN WINTER WHEAT CULTIVARS

Cultivars	Kernel Wt. (mg)	Grain Yld. ($Q.ha^{-1}$)	Kernel/ Spike	Weight/ Spike(g)
Red Chief	41.6 a	31.6 a	27 b	1.13 a
Baca	40.2 ab	30.9 a	27 b	1.10 a
Blue Jacket	38.4 b	32.0 a	30 a	1.13 a
KanKing	37.8 b	28.8 b	27 b	1.02 b
Ponca	37.4 b	27.8 b	27 b	0.99 b
Sturdy	28.2 c	14.7 c	19 c	0.52 c
P>F	0.0001	0.0001	0.0001	0.0001
CV%	7	8	3	11

TABLE VI

SIMPLE CORRELATION COEFFICIENTS FOR EIGHT PLANT CHARACTERS

Plant Characters	2	3	4	5	6	7	8
1. CER	0.11	0.16	0.16	0.14	0.14	-0.14	-0.24
2. Kernel Weight		0.91	-0.16	0.74	0.93	0.18	-0.28
3. Grain Yield			-0.16	0.93	0.99	0.02	-0.25
4. Transpiration				-0.18	-0.17	-0.27	0.02
5. Kernel/Spike					0.93	-0.11	-0.25
6. Weight/Spike						0.04	-0.25
7. Stomatal Resistance							-0.31
8. Leaf Area							

Values from |0.27| are significant at the 5% and |0.360| at the 1% level. (N = 48)

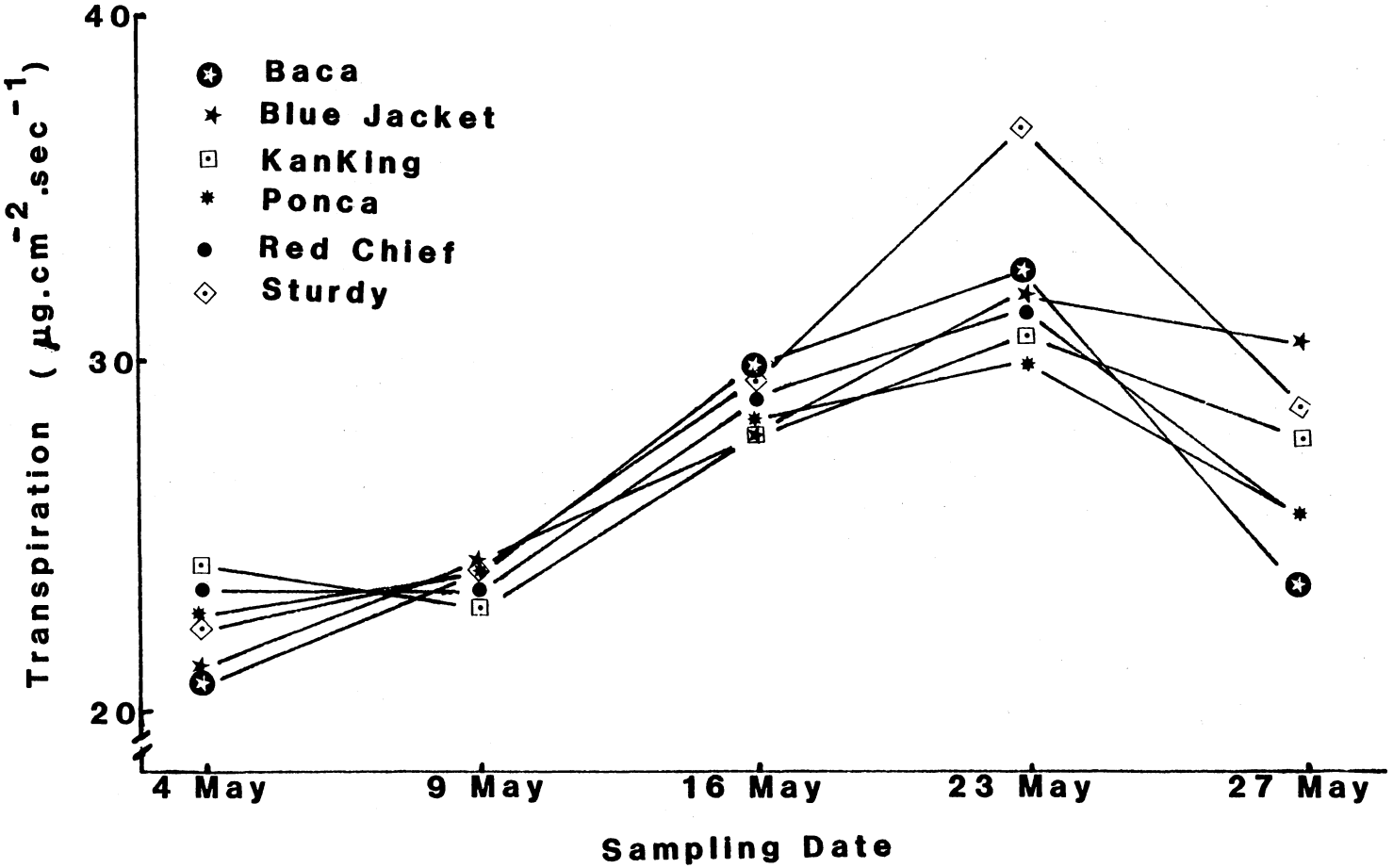

Fig 1. Changes in Transpiration During Stages 4-8 in Six Winter Wheat Cultivars

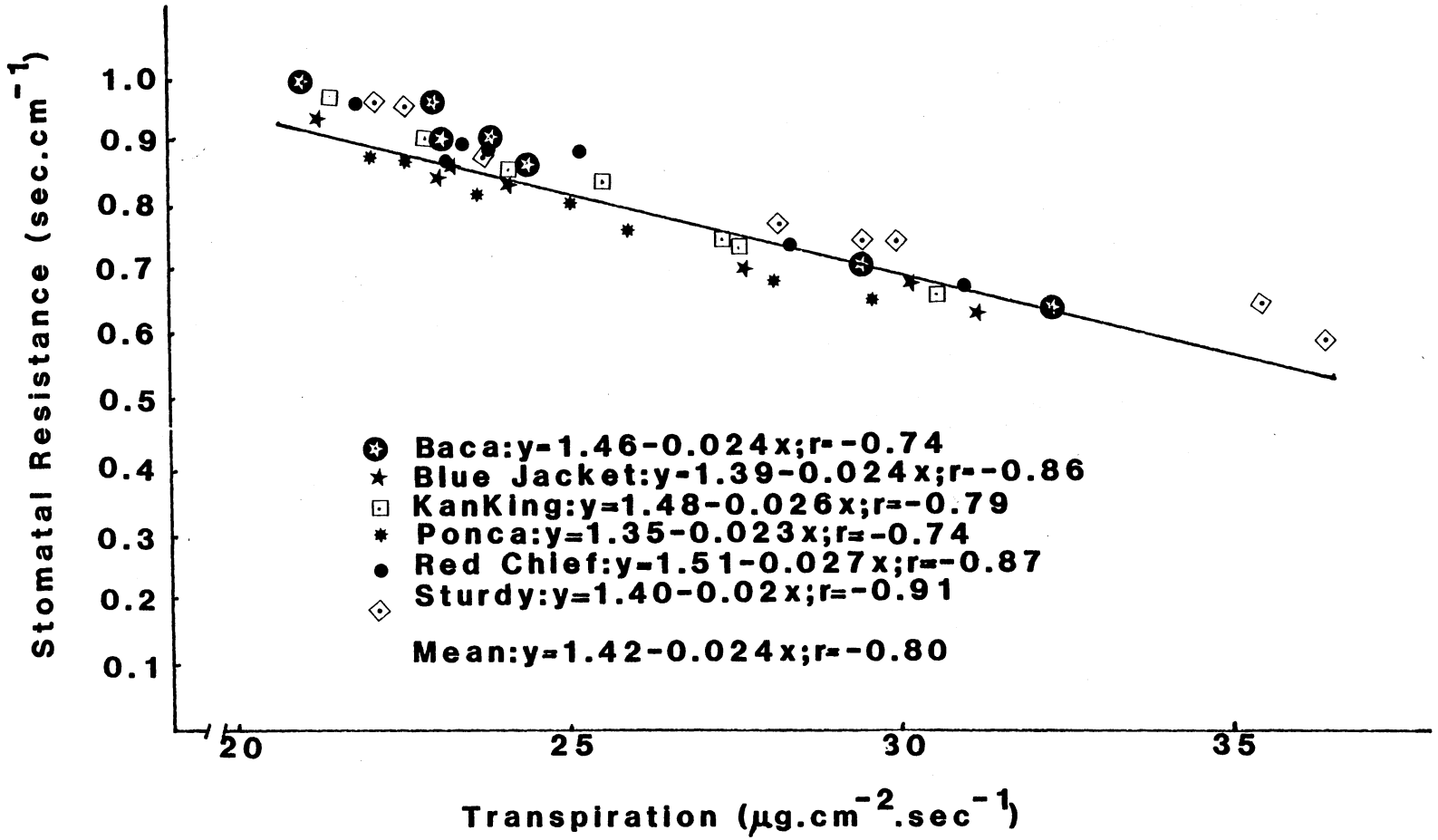

Fig 2. Stomatal Resistance Vs. Transpiration For Six Winter Wheat Cultivars.

CHAPTER III

DIURNAL VARIATIONS IN NITRATE REDUCTASE ACTIVITY OF WINTER WHEAT (*TRITICUM AESTIVUM* L.)

Abstract

Light, moisture and high temperature influence nitrate reductase activity and photosynthesis of winter wheat (*Triticum aestivum* L.). This research was conducted under field conditions to determine the nitrate reductase (NR) activity of winter wheat cultivars from flag leaf emergence to grain filling (stages 4-8). In addition to partial seasonal duration of NR, diurnal variation of NR activity in four winter wheat cultivars was also studied. The study was conducted in the 1982-1983 growing season on the Agronomy Research Station, Stillwater, Oklahoma.

Nitrate reductase activity levels (μmoles per gram fresh weight per hour) for the six winter wheat cultivars were highest in the flag leaf emergence stage and gradually declined to the grain filling stage. There were significant differences in the level of NR of the six cultivars with Blue Jacket having the highest and Sturdy the lowest. In the diurnal study the data indicate significant differences in the NR activity throughout the day. Rates of NR increased

gradually in the early morning then remained steady throughout the day.

It can be concluded from this study that under adequate moisture and illumination winter wheat cultivars can be sampled in the late morning to early afternoon without significant differences in readings.

Introduction and Literature Review

Plant growth, development and yield are the result of a series of biochemical reactions occurring in the plant, each of which is governed by specific enzymes (Hageman, et al., 1968). To aid in the development of cultivars with superior traits, isolating an enzyme system and its control mechanism are important. The nitrate reductase (NR) system is an example of a physiological process which might be limiting yield under various or specific environmental and internal conditions. Induction of NR was achieved by the addition of a nitrate substrate and the enzyme activity was further increased as the concentration of nitrate was further increased in the media (Hageman and Flesher, 1960; Rhoden, 1984). High NR activity is a factor associated with high grain and protein yield (Croy and Hageman, 1970).

Nitrate is considered the primary source of nitrogen available from the soil. The reduction of nitrate to nitrite through the NR system is well documented (Beevers and Hageman, 1969). This system can be manipulated through physiological research and plant breeding to enhance

productivity. Aslam et al. (1976), have shown that NR is related to a metabolic nitrogen pool as well as to the total nitrogen accumulated in the plant. They further pointed out that light promoted the movement of NO_3^- from the storage pool into the metabolic pool. This enzyme is also associated with increased formation and decreased nitrate content (Hageman and Flesher, 1960); and linearly related to grain protein production within a genotype (Croy and Hageman, 1970). Sionit et al. (1981) have shown that wheat responds favorably to carbon dioxide enrichment, while Aslam et al. (1979) have presented evidence showing that the early products of photosynthetic CO_2 fixation can double the rate of NR in barley seedlings. Harper and Paulsen (1967), working with winter wheat, obtained a positive correlation coefficient of 0.856 between NR and water soluble protein (WSP) content. Their work was in agreement with that of Hatam (1980), showing that NR activity decreased with maturity.

Higher NR activity and WSP content would appear to be associated with grain protein and yield production (Croy and Hageman, 1970). They further pointed out that increasing NR levels whether by improved genotypes, by increased plant nutrition, or by improved crop production management should result in increased yield and protein production. While Hageman and Flesher (1960) noted a positive correlation on a diurnal basis between NR and WSP content they also observed a negative relationship between NR and nitrate

content. On the other hand, Johnson et al. (1968) stated that high grain protein was not associated with differential nitrogen uptake or nitrogen accumulation in the plant. Working with soybeans, Hatam (1980) reported an increase in NR activity in early to late afternoon depending on the nitrogen treatment given to the plants.

While it is recognized that a reduced level of NR activity will result in lowered protein levels (Eilrich and Hageman, 1973), the relationship between NR and plant water status has received little attention. Beevers and Hageman (1969) suggested that NR and drought might be related. Huffaker et al. (1970) and Mattas and Pauli (1965) observed significant reduction in NR activity when barley and corn were exposed to moisture stress. Reduction in NR levels under moisture stress might be caused by inactivation of the enzyme due partially to denaturation of the enzyme (Plaut, 1973). Plaut (1974) also pointed out that a fraction of NR may be present in the cytoplasm and activity during stress might be reduced because of inhibition of protein synthesis.

The objectives of this research were: (a) to study the NR activity among wheat cultivars during growth stages 4 (flag leaf emergence) through 8 (grain filling) (Bruns and Croy, 1983); and (b) to investigate the diurnal variation in NR activity during stage 5 (anthesis).

Materials and Methods

This research was initiated in the 1982-1983 season on the Agronomy Research Station, Stillwater, Oklahoma. This experiment was designed to subject the wheat cultivars to moisture stress, however no stress was applied since moisture was adequate during the spring (Table I).

Field plots were arranged in a randomized complete block design with eight replications containing six winter wheat cultivars: 'Baca,' 'Blue Jacket,' 'KanKing,' 'Ponca,' 'Red Chief' and 'Sturdy.' These cultivars were used because they are considered to have different levels of drought resistance. The soil type was a Kirkland Silt loam (Udertic Paleustoll) (Gray and Roozitalab, 1976). Cultivars were seeded into the plots on October 19, 1982, at the rate of 67 kg/ha. The plots were 3.1 m long with rows 23 cm apart. 'TAM W-101' was used as guard rows in each block to reduce border effects. Plots were supplied with nitrogen in early spring at 120 kg/ha.

Flag leaf samples were collected between 1000 and 1200 CST throughout the sampling period (stage 4 through stage 8). For each sampling date, all plots were sampled the same day in order to reduce variability. Six flag leaves were randomly removed from each plot on each sampling date and taken to the laboratory. These samples were then cut into small sections discarding tips and bases.

Nitrate reductase activity was measured using the in

vitro method adapted by Hageman and Flesher (1960), and modified by Croy and Hageman (1970). One gram of the cut leaf samples was ground for 30 seconds with a Brinkmann Polytron ST 20 homogenizer at 3/4 speed with the samples in 7 mL of grinding medium (25 mM K_2HPO_4, 5.0 mM EDTA, 10 mM cysteine, adjusted with KOH to a pH of 9.0). The homogenate was then passed through cheese cloth and the extract centrifuged at 13,000 rpm (20,000g) for 15 minutes. Throughout the extraction process samples were kept in an ice bath. Aliquots (0.2 mL) of plant extracts were placed in a buffered solution (pH 7.5) which contained the substrate, nitrate and a reducing agent ($NADH_2$). During the incubation period, the temperature was maintained at 30C which is the optimum for the enzyme. After 15 minutes, the reaction was terminated using a 'stop reaction-color solution' (1% sulfanilamide reagent in 3N HCl and 0.02% N-[1-napthyl] ethylene diamine HCl combined in a 1:1 ratio). Contents of the tube were then mixed and allowed to stand for 15 minutes, after which tubes were centrifuged for 10 minutes at 2,000 rpm using an IEC HN-S11 centrifuge. Absorbance was read after 20 minutes at 540 nm on a Bausch and Lomb spectronic 2000 spectrophotometer.

For diurnal variation in NR activity, one block was used to obtain leaf area, leaf surface temperature, transpiration rate and stomatal diffusive resistance. Samples for NR activity were randomly taken from the other seven blocks. In order to reduce the variability among the six

cultivars, the two cultivars having the highest and the lowest NR activity were not used to measure the diurnal variation. The four winter wheat cultivars used in measuring diurnal variation were: Baca, Blue Jacket, Ponca and Red Chief. Measurements were taken on an hourly basis throughout the day using the flag leaves and sampling was terminated at 1800 CST. Light levels during the study were fairly constant on days of sampling. At the onset of measurements (0600 CST) light levels were approximately 500 $\mu E.m^{-2}.sec^{-1}$. By 0700 CST the levels were up to 1,400 $\mu E.m^{-2}.sec^{-1}$ and by 0800 CST 2,000 $\mu E.m^{-2}.sec^{-1}$ were obtained and remained fairly constant until 1700 CST. A decline to 1,000 $\mu E.m^{-2}.sec^{-1}$ was observed at 1800 CST. Measurements were taken when plants were in Stage 5. Light levels, leaf surface temperature and stomatal diffusive resistance were monitored during the sampling period using a LI-COR quantum meter equipped with a sensor.

Results and Discussion

Moisture was not a major factor throughout the sampling period as is noted in Table I. Nitrate reductase activity for various sampling dates differed significantly (Table II). NR activity was highest at the initial sampling date and then gradually declined (Fig. 1). This is widely accepted and was reported by Harper and Hageman (1972) and Hatam (1980). In order to reduce the variation in sampling, the cultivars with the highest and the lowest NR activity

were determined by the first two samplings after flag leaf emergence.

The NR activity of the various cultivars over the growing season is illustrated in Figure 2. From the first two sampling dates it was decided to remove KanKing and Sturdy. KanKing had the highest level of NR activity while Sturdy had the lowest. While this trend remained true for Sturdy throughout the sampling period, there were some overlapping between KanKing and Blue Jacket. As is shown in Table III, Blue Jacket and not KanKing should have been eliminated from the diurnal study. This was not a serious error in judgment since both cultivars were not significantly different in their NR activities. By removing KanKing and Sturdy we were able to reduce the variability from 27% (Table III) to 15% (Table IV). In the diurnal study Blue Jacket still had the highest NR levels with a mean of 20.3 μmoles.gfw^{-1}.hr^{-1} and Red Chief the lowest with a mean of 16.0 μmoles.gfw^{-1}.hr^{-1}. Although there were significant differences among the cultivars during the sampling period there were no significant differences among the four cultivars selected to be used in the diurnal study (Table III).

There were significant differences occurring in the diurnal NR activity (Fig. 3). Average NR activity was lower in the mornings, then gradually increased to late morning, and remained steady the rest of the day. This is consistent with work done by Hatam (1980), who showed that the lowest rate of NR activity occurred in the early morning and the

highest in the afternoon. Although there were fluctuations in NR activity after 0800 CST, there were only small differences from one hour to the next. This stability in NR activity parallels the levels of light observed throughout the day. Harper and Hageman (1972) found a fourfold increase in NR activity of corn seedlings grown in solution containing NO_3^- when shifted from the dark to light. Hatam (1980) pointed out that plants under nutritional stress might not be able to reduce nitrogen as rapidly as when supplied adequate nutrients, and, since moisture and nutrition were high in this study then the sustained levels of NR could probably be linked with illumination levels throughout the day.

Summary

Methods for testing the time of day best suited for obtaining samples for NR are always sought. Some experiments have determined the NR activity throughout the light and dark periods and showed higher and sustained level throughout the illumination period. Other methods have looked at long periods without giving any consideration to increases or decreases in light levels throughout the sampling period. A study of the NR activity of six winter wheat cultivars from flag leaf emergence through grain filling was conducted. Four cultivars were also used to study the diurnal variations in NR activity during anthesis. The study for diurnal variation was started at 0600 CST and was

stopped at 1800 CST.

Rates of NR activity increased significantly from first light to 0800 CST. There was a sustained level of NR activity throughout the day until sampling was terminated. This was consistent with Hatam (1980) and Harper and Hageman (1972) showing that NR activity increased from 0700 CST and peaked between 1300 and 1800 CST and declined throughout the dark period.

From this study it can be concluded that sampling for NR activity can be measured without any appreciable decrease from 0900 to 1800 CST.

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TABLE I

METEOROLOGICAL DATA FOR JULY 1982 - JUNE 1983
AT THE AGRONOMY RESEARCH STATION,
STILLWATER, OKLAHOMA

Sampling Date	Temperature (C) Max.	Min.	Month	Precip. (cm)	Dev. from Avg. (cm)
26 April 1983	25.0	10.6	July	5.03	-3.94
03 May 1983	18.3	6.1	August	3.51	-4.65
10 May 1983	23.3	10.6	September	5.84	-2.74
18 May 1983	21.1	11.1	October	2.46	-4.60
25 May 1983	28.3	16.7	November	7.04	2.34
			December	5.92	2.52
			January	0.84	-2.11
			February	7.65	4.22
			March	7.77	3.05
			April	4.14	-3.12
			May	18.87	7.14
			June	9.27	-1.50
			Total	78.34	-3.39

TABLE II

NITRATE REDUCTASE ACTIVITY LEVELS FROM STAGE 4 TO STAGE 8 OF SIX WINTER WHEAT CULTIVARS

Sampling Date	Growth Stages	NR $\mu moles.gfw^{-1}.hr^{-1}$
26 April	4	21.6
03 May	5	17.9
10 May	6	17.8
18 May	7	17.4
25 May	8	16.6
lsd .05		2.3

TABLE III

MEANS OF NITRATE REDUCTASE (NR) ACTIVITY
AND WATER SOLUBLE PROTEIN (WSP)
OF SIX WINTER WHEAT CULTIVARS

Cultivars	NR $\mu moles.gfw^{-1}.hr^{-1}$	WSP $mg.gfw^{-1}$
Blue Jacket	18.9 a*	17.56 a
KanKing	18.8 a	17.33 ab
Red Chief	18.6 a	16.40 c
Ponca	18.3 a	16.87 bc
Baca	17.9 ab	16.38 cd
Sturdy	17.1 b	16.13 d
CV %	27	8

* Means with the same letters are not significantly different at the 5% level using Duncan's Multiple Range Test.

TABLE IV

NITRATE REDUCTASE ACTIVITY OF FOUR WINTER WHEAT CULTIVARS

Cultivars	NR $\mu moles.gfw^{-1}.hr^{-1}$
Blue Jacket	20.3 a*
Ponca	17.9 b
Baca	17.3 b
Red Chief	16.0 c
CV%	15

* Means with the same letters are not significantly different at the 5% level using the Duncan's Multiple Range Test.

TABLE V

DIURNAL VARIATION IN NITRATE REDUCTASE ACTIVITY OF WINTER WHEAT

Time (CST)	NR $\mu moles.gfw^{-1}.hr^{-1}$ #	Standard Dev.
0600	15.6	6.9
0700	15.4	2.2
0800	17.9	3.1
0900	17.1	2.8
1000	18.9	4.3
1100	15.8	2.7
1200	19.3	3.3
1300	18.6	3.5
1400	17.6	3.0
1500	19.3	3.1
1600	17.6	2.8
1700	19.6	3.1
1800	20.0	3.9
lsd .05	2.3	
CV%	15	

Means of Four Cultivars and Three Replications.

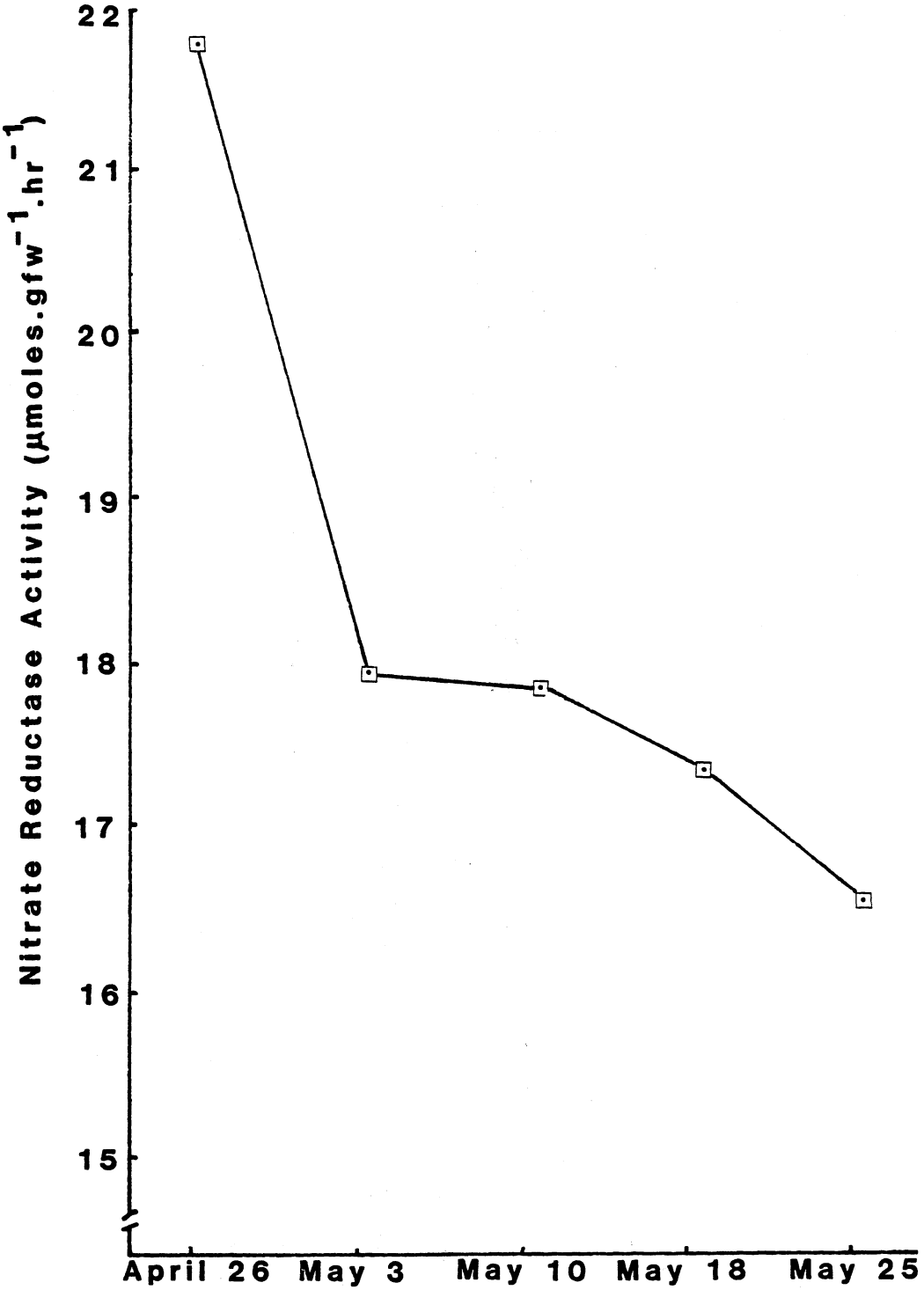

Fig 1. Nitrate Reductase Activity During Sampling Period.

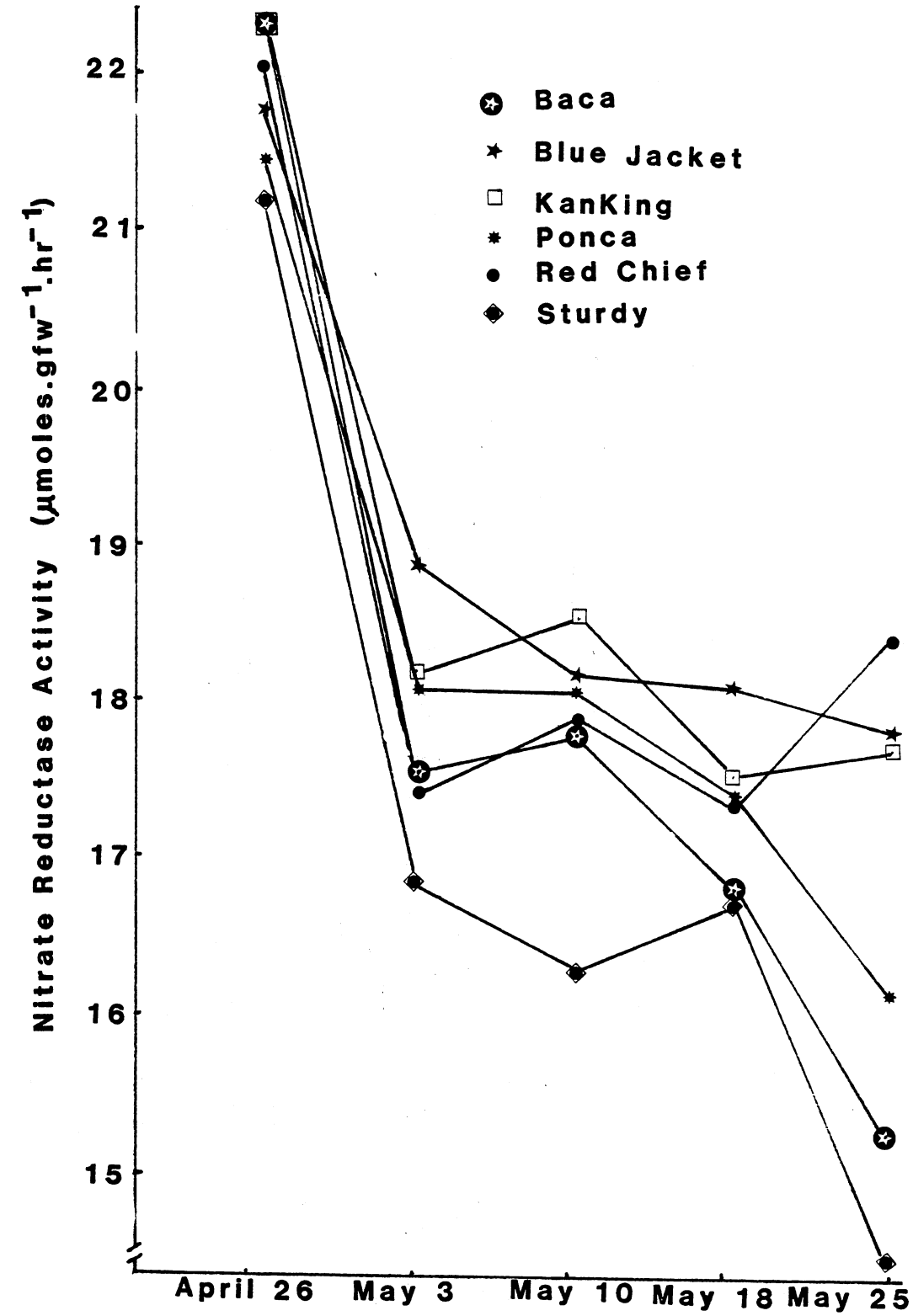

Fig 2. Nitrate Reductase Activity of Six Winter Wheat Cultivars at Five Sampling Dates.

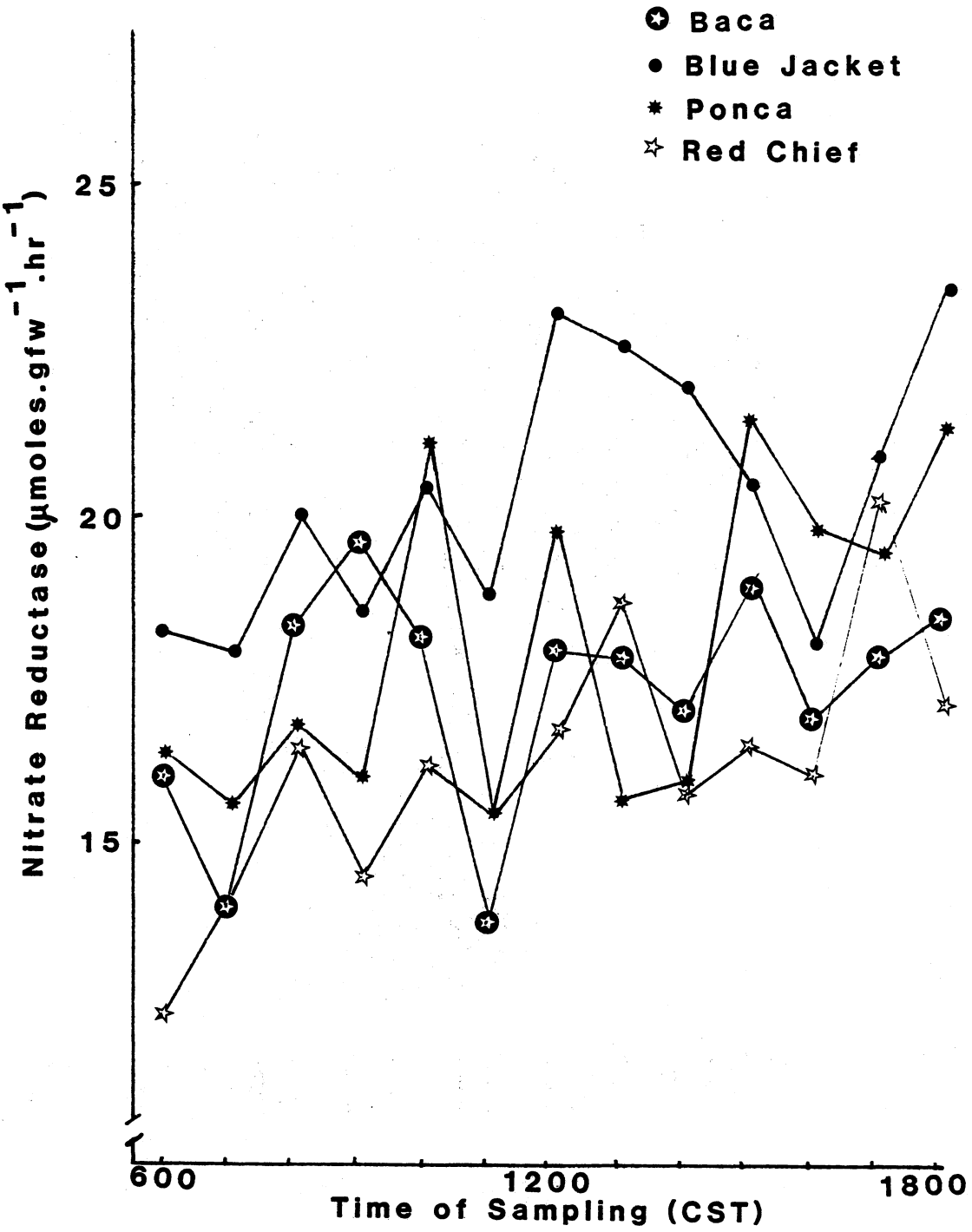

Fig 3. Diurnal Variation in Nitrate Reductase of Winter Wheat.

CHAPTER IV

NONDESTRUCTIVE MEASUREMENT OF LEAF AREA AND ITS RELATIONSHIPS TO DRY MATTER IN COWPEAS (*VIGNA UNGUICULATA* L. WALP)

Abstract

Rapid, nondestructive and precise methods for measuring leaf area are required in agronomic and physiological studies. Several mathematical formulae have been observed for evaluating and estimating leaf areas of several crops, however, information on cowpeas is limited. The objective of these experiments, therefore, was to develop prediction equations for estimating leaflet areas which could be used to obtain total leaf area of the cowpea plant. The relationship between leaf area and dry matter production was also established, with the primary aim of using dry matter to estimate leaf area.

Five cowpea cultivars were grown in a controlled environment using a Kirkland silt loam soil (Udertic Paleustoll). Four experiments were conducted to develop leaf area prediction equations and dry matter relationships. Statistical analysis on experiments 1 and 2 were done comparing the predictive abilities of seven regression equations, each

involving length, width or products of both. In experiments 3 and 4, plants were cut at ground level for leaf area and dry matter determinations.

With the use of independent variables involving measurements of length and width, regression analyses were performed. In general, data indicate that a single regression equation could be used to predict leaf area for all cultivars studied. LW, L^2 and W^2 gave as good predictive values as more complicated models. Leaflet areas of the five cowpea cultivars studied can be estimated by the following equations: $A = 0.34 + 0.67\ LW$ ($R^2 = 0.998$); $A = -0.37 + 0.41\ L^2$ ($R^2 = 0.939$); $A = 3.93 + 0.98\ W^2$ ($R^2 = 0.943$).

Leaf area and leaf dry matter were closely correlated ($R^2 = 0.90$ to 0.95) for the five cultivars. Leaf area vs. plant dry matter correlated highly with each other ($R^2 = 0.91$ to 0.96). In modeling studies where leaf area indices are required, leaf dry matter could be substituted for leaf area.

Introduction and Literature Review

Measurements of leaf area and leaf dry matter are often necessary for physiological and agronomic studies. They serve as indices of growth and are inputs in the evapotranspiration and photosynthesis models. In a comprehensive review of leaf measurement techniques, Marshall (1968) categorized them as destructive, nondestructive, direct or indirect. The method that the investigator uses is dependent upon the crop and conditions under which the crop is grown.

Kemp (1960) suggested that grass leaves were difficult to measure by planimeter due to the narrowness of these leaves. However, direct measurements using an electronic area meter as suggested by Hatfield et al. (1976) improved accuracy and rate of measurement over the photoelectric planimeter.

One of the most frequently used nondestructive and indirect methods is that of estimating leaf areas from mathematical formulae utilizing linear measurements of the leaf or leaflet. Wiersma and Bailey (1975), along with Kemp (1960), studied the accuracy of linear measurements and concluded that they gave valid estimates of leaf areas. In using the linear measurements, the length (L), width (W) and area (A) of a set of leaf samples are obtained, then several correlation coefficients, regression coefficients, or leaf factors are derived ($K = LW/A$ or $K = A/LW$) (Wiersma and Bailey, 1975); $A = K*LW$ (Osmanzai, 1982) for predicting areas of subsequent samples.

In obtaining leaf area, several workers have noted the correlation between leaf fresh or dry weight and area. Shibles and Weber (1965) working with soybeans noted a positive relationship between leaf area and dry matter production. Watson (1937) working with wheat showed that leaf fresh and dry weights were well fitted by a linear regression equation. Leaf area and leaf dry matter were found to be closely related ($R^2 = 0.951$) as was leaf area and plant dry matter in winter wheat (Aase, 1978).

Agronomic performance is an important criteria in

selecting crops. Shading of lower leaves can be detrimental to production especially in legumes since the lower leaves provide the carbon source for root nodules (Pate, 1966). Duncan (1971) quantitatively evaluated the rate of photosynthesis that occurred in the canopy of corn. He noted that as leaf area increased there was a corresponding increase in photosynthesis, however, photosynthesis decreased in the lower part of the canopy. Sakamoto and Shaw (1967) and Wien and Wallace (1973) noted the orientation of field bean and soybean canopies. They observed that increased radiation made plant leaves more angled and therefore light was distributed throughout the lower profile more uniformly which contributed to higher productivity. However, Wien (1982) noted that cowpea cultivars with narrow leaflets did not have greater productivity than the broad leaflet species.

Several mathematical formulae have been developed to estimate leaf area of various crops, but very little information is available on cowpeas. Wiersma and Bailey (1975), working with soybeans, showed that a single regression equation could be used for leaflet, trifoliate and total leaf area of the cultivars studied. Splinter and Beeman (1968) calculated a regression equation estimating leaflet areas of soybean, however, this equation was obtained from leaves of unspecified internodes and genotypes.

The total leaf area of a cowpea plant could be derived by summing the individual leaflets, although some workers

might not require individual measurements. Investigators measuring carbon dioxide exchange rates (CER) may require individual measurements. With this in mind the following objectives were pursued: (1) to develop separate prediction equations for estimating leaflet areas of cowpea plants; (2) to determine whether prediction equations derived from measurements of length*width were superior to those utilizing measurements of length or width only; (3) to determine the relationship between leaf dry matter and leaf area of cowpea cultivars.

Materials and Methods

Experiments were conducted at the Controlled Environment Research Laboratory (CERL), Oklahoma State University, Stillwater, using a Kirkland silt loam soil (Udertic Paleustoll) (Gray and Roozitalab, 1976). The soil was mixed with peat moss at a 3:1 ratio. Pots containing 2.7 kg of soil were inoculated with Rhizobium and six seeds were planted to each pot. After germination, the seedlings were thinned to four plants per pot. Plants were grown at 27/21C (following the recommendations of Dart and Mercer, 1965) day/night temperature with a photoperiod of 13 hours (0800-2100 CDT) of light from white fluorescent and incandescent lamps. The plants obtained a flux density of approximately 700 $\mu E.m^{-2}.sec^{-1}$. Relative humidity was kept at 75%.

Source of Leaflet

Leaflets used in these experiments were obtained from five cultivars of cowpeas (Vigna unguiculata L. Walp.), 'Pinkeye Purple Hull' (PPH), 'Mississippi Silver' (MS), 'Freezegreen' (FG), 'Minnesota 13' (MN 13) and 'Minnesota 139' (MN 139). Experiment 1 was planted in May and experiment 2 in October, 1982. Plants were grown in a randomized complete block design with three replications. Plants were watered daily and once weekly were supplied with 200 mL of 0.1 strength Hoaglands solution. Measurements were taken for all five cultivars 30 to 34 days after emergence (DAE). All trifoliate leaves were removed from the plant and separated based on leaflet orientation (left, terminal and right). No unifoliate leaflets were used since some had senesced. Each leaflet was then separated at the junction of the blade with the petiole. The leaf was then placed between two grided plastic sheets so that the leaflet's midrib was on a straight line to allow for easier measurement of the leaflet.

Leaflet Measurements

Maximum lengths and widths of leaflets were measured to the nearest millimeter. The area of each leaflet was then measured using a leaf area meter (LI-COR model LI-3000 Portable Area Meter LiCor Corp., Lincoln, Nebr.).

Plant Growth Experiments

Leaf area development and dry matter production of five cowpea cultivars were studied in two experiments planted in March and September 1982 (here designated experiments 3 and 4). Growth conditions for both experiments were similar to experiments 1 and 2. Beginning 14 days after emergence (DAE) and on alternate days one plant was removed from each pot and total leaf area taken. Dry matter of leaves and stems was determined as the weight after drying to constant weight in an oven set at 70C.

The units associated with subsequent regression equations are centimeters for lengths (L) and widths (W), and square centimeters for area (A), and grams for weights. In order for the prediction equations to be of benefit, these equations would have to hold their predictiveness over a wide range of leaflet sizes and shapes. Seven prediction equations were selected in order to determine which linear models gave the best estimate of leaf area. For leaf dry matter and total dry matter only one independent variable was utilized in the equation. The general linear model was used to obtain an equation for estimating leaf area. The corrected R^2 was calculated as described by Draper and Smith (1981).

Results and Discussion

The cultivars used in this study varied in maturity as

well as growth habits. Normal classifications would have FG, MS and PPH as early maturing cultivars since they take between 67 to 71 days to harvest. However, compared to the other two cultivars (MN 13 and MN 139) which take about 45 days to harvest (Table 1), they could be classified as intermediate. The growth habits of these cultivars differ only slightly. Means of leaflet area, length and width are reported in Table II. Tests of significance showed that terminal leaflet differed significantly from right and left leaflets in length, width and area. Generally, terminal leaflets were longer, wider, and had greater leaf area than either left or right leaflets for all cultivars.

Regression Analyses

In formulating prediction equations in regression analyses, the independent variable(s) has/have to be considered. Therefore, several prediction equations, involving different independent variables or combinations, were used in estimating left, terminal and right leaflet areas. The following regression equations were used in the study:

(1) $Y_i = B_0 + B_1LW + B_2L^2 + B_3W^2 + E_i$;

(2) $Y_i = B_0 + B_1L + B_2W + B_3LW + E_i$

(3) $Y_i = B_0 + B_1LW + B_2W^2 + E_i$;

(4) $Y_i = B_0 + B_1LW + B_2L^2 + E_i$;

(5) $Y_i = B_0 + B_1LW + E_i$;

(6) $Y_i = B_0 + B_1L^2 + E_i$;

(7) $Y_i = B_0 + B_1W^2 + E_i$.

Using the prediction equation 1, $Y_i = B_0 + B_1LW + B_2L^2 + B_3W^2 + E_i$, (Table III), the analyses show that the percentage variation accounted for was very high and did not vary among cultivars or leaflets. The R^2 ranked from 0.98 for the left leaflet of MN 139 to 0.997 for the right leaflet of FG. The coefficients of variability (CV) of these equations were fairly low, ranging from 2.41 to 4.60. The coefficients of regression for this equation are also reported in Table III. Although there were high R^2 values most of these coefficients were not statistically significant. However, the coefficients for LW of the right leaflets for FG and MN 13 and terminal leaflets of MS were significant. The coefficient for L^2 of terminal leaflet of MN 139 was also significant as well as the coefficient of W^2 for the terminal leaflets of MN 13 and MS. When the squared products of the length and the width from the regression equation were dropped and the length and the width retained (equation 2), it was noted that L was significant for terminal and left leaflets of MN 13 and right leaflets of PPH (Table IV). Width (W) was also significant for the terminal leaflets of both MN 13 and PPH. However, it should be observed that LW was significant for all cultivars at all leaflet positions ($P<0.01$). The R^2 (0.981 to 0.997) or CV (2.43 to 4.80) did not change drastically.

It would therefore appear that LW is very important in prediction equations in obtaining leaf areas. This was further borne out in equations 3 and 4. Using only LW and

W^2 in the equation (Table V), significance was observed for W^2 in FG (terminal), MN 13 (right) and MS (terminal and right). Substituting L^2 for W^2 resulted in significance for the same cultivars and leaflet positions (Table VI). The R^2 and CV values did not vary considerably for the various leaflet positions for the five cowpea cultivars.

In a preliminary analysis, using a regression equation that included all the variables, it was shown that L and W did not contribute significantly to the equation. Therefore, three prediction equations involving separate independent variables (LW, L^2 and W^2) were used for estimating the areas of left, terminal and right leaflets. Results of these analyses are presented in Tables VII, VIII and IX. The data show that the percentage variation accounted for by the regression is large and different for both cultivars and leaflet position. The R^2 values associated with LW are higher than either L^2 or W^2. In the case of MN 139 the right leaflets had R^2s of 0.982 (LW), 0.770 (L^2) and 0.834 (W^2). Standard error of regression had a low of 0.006 (FG) and a high of 0.016 (MN 139(. For the independent variable L^2, these values were 0.009 to 0.027 while W^2 had relatively high values ranging from 0.024 to 0.066. Terminal leaflets had the same general trend as did right and left leaflets although the standard error of regression was generally lower than the other two leaflets.

When cultivars were compared, FG and PPH had higher R^2s over all leaflet positions for all three independent

variables. For these two cultivars, the use of LW, L^2 or W^2 as independent variables all provided very good predictive abilities. However, since either using L^2 or W^2 would entail making only one measurement it might be more advantageous to use the equations with only these variables because of the time saved.

The results from all samplings of the leaf area versus leaf dry matter are presented in Fig. 1. The linear regression equation accounted for between 90 to 95% of the variance in the leaf area of the five cowpea cultivars studied. There was a relationship established between leaf area and total dry matter of the cowpea cultivars from 14 DAE to anthesis. The unifoliate leaves were not included in the study since some of these leaves had senesced before anthesis. Linear relationships between leaf area and total dry matter were very good (R^2 = 0.91-0.96, Fig. 2), with a slope two-thirds that of Fig. 1.

Observing the relationships between leaf dry matter, plant dry matter and leaf area throughout the growing periods would suggest that there are positive correlations between these variables and growing period. For both leaf dry matter and plant dry matter there was no difference between the two growing periods. In both experiments the plant and leaf dry matter parallel each other (Figs. 3 and 4). For leaf area there were differences between the two growing periods (Fig. 5). In general, experiment 4 had greater leaf area than experiment 3 when plants were the

same age. Leaf area per plant varied from 50 to 412 cm^2.

Observing the five cowpea cultivars showed that there was a negative regression of leaf area: leaf dry weight over the growing period (Fig. 4). Watson (1937) also noted this negative relationship in several field crops and concluded a priori that leaf area:leaf weight ratio was constant over the useful life of winter wheat plants. While MN 13 started out with a high ratio between leaf area and leaf dry matter and dropped significantly following the pattern that Watson (1937) suggested, other cultivars such as MS were constant over the growing season as was seen by Aase (1978).

Summary

On the basis of the results for these five cowpea cultivars, it was shown that the relationship between leaf area and LW was linear. The data also indicate that LW, L^2 and W^2 are important variables in the prediction of leaf area. Although LW was by far the most accurate variable in predicting leaflet area, considerable savings of time, with minute loss in predictive ability, could be possible by measuring only the length or the width. An increase in predictive ability of two to six percent (as is indicated by the R^2 in Tables VII, VIII and IX) may not be necessary or warrant doubling the number of measurements. In general, high predictive power can be obtained for left, terminal and right leaflets with an overall equation of: Area =

$0.34 + 0.67LW$ ($R^2 = 0.988$).

Studies in plant growth, physiology and evapotranspiration models may require growth parameters such as leaf area or leaf area indices. Leaf dry matter or plant dry matter may serve equally well and may be substituted for leaf area saving time, personnel and funds. Where leaf area indices are explicitly required, they can be obtained from the following equations for cowpeas: Area = 41.84 + 190.41 LDM or Area = 42.70 + 127.12 TDM.

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TABLE I

CHARACTERS OF COWPEA CULTIVARS
USED IN THIS STUDY

Cultivars	Origin	Relative Maturity	Plant Growth Type
Freezegreen (FG)	Alabama	Intermediate (71#)	Low bushy
Minnesota 13 (MN 13)	Minnesota	Early (45##)	Erect
Minnesota 139 (MN 139)	Minnesota	Early (47##)	Erect
Mississippi Silver (MS)	Mississippi	Intermediate (69#)	Nonvining
Pinkeye Purple Hull (PPH)	Mississippi	Intermediate (67#)	Nonvining

#, ## Days to harvest under Auburn, AL., and Stillwater, OK. growing conditions respectively (Chambliss, 1979)

TABLE II

MEANS FOR LEAFLET LENGTH, WIDTH AND AREA OF COWPEA CULTIVARS

Cultivar	Leaflet	Length cm	Width cm	Area cm^2
FG	Left	9.70 ± 1.2[a]	5.98 ± 0.9	41.83 ± 10.5
	Terminal	10.16 ± 1.2	6.56 ± 1.1	45.54 ± 11.6
	Right	9.75 ± 1.2	6.01 ± 0.9	42.32 ± 10.7
MN 13	Left	6.81 ± 0.8	4.30 ± 0.4	20.64 ± 3.8
	Terminal	7.26 ± 0.7	4.77 ± 0.4	23.45 ± 4.1
	Right	6.74 ± 0.8	4.32 ± 0.4	20.79 ± 3.8
MN 139	Left	8.36 ± 0.6	4.96 ± 0.3	28.77 ± 3.6
	Terminal	8.51 ± 0.7	5.37 ± 0.4	30.46 ± 4.0
	Right	8.27 ± 0.5	4.89 ± 0.4	28.02 ± 2.9
MS	Left	9.22 ± 0.8	5.17 ± 0.5	32.37 ± 5.9
	Terminal	10.35 ± 0.9	5.95 ± 0.7	39.54 ± 7.6
	Right	9.29 ± 0.8	5.15 ± 0.6	33.65 ± 7.2
PPH	Left	9.73 ± 1.3	5.81 ± 0.8	40.90 ± 10.4
	Terminal	10.15 ± 1.4	5.79 ± 0.8	40.71 ± 10.4
	Right	9.63 ± 1.3	5.73 ± 0.8	40.06 ± 10.2

a Means ± standard deviation.
N: FG = 58, MN 13 = 39, MN 139 = 40, MN = 32, PPH = 60.

TABLE III

COEFFICIENTS OF REGRESSION FOR LEAFLET AREA OF FIVE COWPEA CULTIVARS (Equation 1)

Cultivars		Parameter				R^2	CV%
		Intercept	LW	L^2	W^2		
FG	Left	-0.82	0.56	0.05	0.09	0.996	2.95
	Terminal	0.00	0.50	0.08	0.04	0.995	3.30
	Right	-0.65	-1.04*	-0.09	-0.31	0.999	2.41
MN 13	Left	-0.65	1.32*	-0.18	-0.50	0.987	3.81
	Terminal	-0.16	0.42	0.08	0.19	0.987	3.68
	Right	-0.20	-1.50**	-0.28	0.55	0.987	3.77
MN 139	Left	-0.86	-0.33	0.29	0.91	0.981	3.10
	Terminal	-0.98	-0.50	0.41*	0.82*	0.983	3.06
	Right	0.20	-0.34	0.30	0.86*	0.984	2.43
MS	Left	1.06*	0.07	0.15	0.55	0.986	3.96
	Terminal	2.01*	2.19**	-0.43	-1.16*	0.985	4.31
	Right	0.80	0.75	-0.10	0.14	0.986	4.61
PPH	Left	-0.98*	1.72	-0.30	-0.86	0.994	3.55
	Terminal	-0.45	1.57	-0.24	-0.85	0.990	4.48
	Right	-0.81	-1.53	0.63	1.95	0.993	3.77

* significant at 0.05
** significant at 0.01

TABLE IV

COEFFICIENTS OF REGRESSION FOR LEAFLET AREA OF FIVE COWPEA CULTIVARS (EQUATION 2)

Cultivars	Leaflet	Parameter				R^2	CV%
		Intercept	L	W	LW		
FG	Left	1.40	-0.02	-0.74	0.74**	0.996	2.93
	Terminal	2.12	0.48	-1.43	0.68**	0.995	3.26
	Right	0.29	0.14	-0.59	0.72**	0.997	2.43
MN 13	Left	0.41	-0.16	-0.28	0.75**	0.987	3.85
	Terminal	-13.15*	1.79*	3.01*	0.26	0.989	3.39
	Right	5.28	-1.40*	-0.59	0.92**	0.986	3.90
MN 139	Left	-1.35	-0.10	0.36	0.69**	0.981	3.14
	Terminal	-4.02	0.81	0.51	0.54**	0.981	3.28
	Right	-1.96	0.20	0.62	0.62**	0.982	2.52
MS	Left	4.79	-0.67	-0.15	0.71**	0.986	3.95
	Terminal	13.49	-0.36	-2.95	0.75**	0.984	4.44
	Right	1.65	-1.30	1.85	0.68**	0.985	4.80
PPH	Left	3.64	-0.31	-0.25	0.80**	0.994	3.50
	Terminal	5.08	-0.05	-2.02*	0.77**	0.991	4.40
	Right	5.05	-1.16*	-0.31	0.82**	0.994	3.69

* significant at 0.05
** significant at 0.01

TABLE V

COEFFICIENTS OF REGRESSION FOR LEAFLET AREA OF FIVE COWPEA CULTIVARS (EQUATION 3)

Cultivar	Leaflet	Parameter			R^2	CV%
		Intercept	LW	W^2		
FG	Left	-0.73	0.73**	-0.05	0.996	2.93
	Terminal	0.15	0.75**	-0.15**	0.995	3.28
	Right	-0.80*	0.75**	-0.08	0.997	2.40
MN 13	Left	-0.67	0.71**	0.0	0.987	3.81
	Terminal	-0.15	0.66**	0.01	0.987	3.64
	Right	-0.32	0.59**	0.19**	0.986	3.90
MN 139	Left	-0.85	0.68**	0.05	0.981	3.09
	Terminal	0.18	0.72**	-0.10	0.980	3.27
	Right	0.04	0.67**	0.02	0.982	2.50
MS	Left	1.14	0.59**	0.09	0.986	3.91
	Terminal	1.98	0.71**	-0.19*	0.984	4.47
	Right	0.76	0.40**	0.44**	0.986	4.54
PPH	Left	-1.09**	0.72**	-0.01	0.994	3.55
	Terminal	-0.51	0.74**	-0.13	0.990	4.46
	Right	-0.86	0.61**	0.15	0.993	3.77

* significant at 0.05
** significant at 0.01

TABLE VI

COEFFICIENTS OF REGRESSION FOR LEAFLET AREA OF FIVE COWPEA CULTIVARS (EQUATION 4)

Cultivar	Leaflet	Parameter			R^2	CV%
		Intercept	LW	L^2		
FG	Left	-0.78	0.67**	0.02	0.996	2.92
	Terminal	0.02	0.55**	0.07**	0.995	3.27
	Right	-0.81*	0.66**	0.03	0.997	2.41
MN 13	Left	-0.68	0.72**	0.0	0.987	3.81
	Terminal	-0.14	0.67**	0.0	0.987	3.64
	Right	-0.32	0.83**	0.08**	0.986	3.82
MN 139	Left	-0.85	0.73**	0.02	0.981	3.10
	Terminal	-0.02	0.58**	0.05*	0.981	3.22
	Right	0.02	0.69**	0.0	0.982	2.50
MS	Left	1.14	0.69**	0.03	0.986	3.91
	Terminal	2.08*	0.49**	0.06*	0.983	4.54
	Right	0.81	0.91**	-0.14**	0.986	4.53
PPH	Left	-1.08	0.70**	0.0	0.994	3.55
	Terminal	-0.52	0.60**	0.04	0.990	4.46
	Right	-0.87	0.79**	0.05	0.993	3.77

* significant at 0.05
** significant at 0.01

TABLE VII

REGRESSION ANALYSIS INVOLVING THREE INDEPENDENT VARIABLES FOR LEFT LEAFLETS

Cultivar	X	$\bar{X}$	$S_{\bar{b}}$	B	R^2	CV%
FG	LW	41.83	0.006	0.70	0.996	2.93
	L^2	41.83	0.015	0.48	0.948	10.07
	W^2	41.83	0.025	0.97	0.964	8.44
MN 13	LW	20.64	0.014	0.71	0.987	3.75
	L^2	20.64	0.021	0.37	0.893	10.62
	W^2	20.64	0.066	1.09	0.879	11.28
MN 139	LW	28.77	0.016	0.71	0.981	3.07
	L^2	28.77	0.027	0.39	0.842	8.76
	W^2	28.77	0.057	1.01	0.893	7.19
MS	LW	32.37	0.014	0.65	0.986	3.90
	L^2	32.37	0.022	0.37	0.900	10.25
	W^2	32.37	0.046	1.00	0.940	7.95
PPH	LW	40.90	0.007	0.71	0.994	3.52
	L^2	40.90	0.009	0.42	0.972	7.39
	W^2	40.90	0.024	1.13	0.974	7.21

TABLE VIII

REGRESSION ANALYSIS INVOLVING THREE INDEPENDENT VARIABLES FOR TERMINAL LEAFLETS

Cultivar	X	$\bar{X}$	$S_{\bar{b}}$	B	R^2	CV%
FG	LW	45.54	0.007	0.63	0.994	3.47
	L^2	45.54	0.012	0.48	0.963	8.61
	W^2	45.54	0.021	0.81	0.965	8.40
MN 13	LW	23.45	0.013	0.67	0.987	3.59
	L^2	23.45	0.022	0.40	0.901	9.76
	W^2	23.45	0.049	0.93	0.905	9.57
MN 139	LW	30.46	0.016	0.65	0.979	3.36
	L^2	30.46	0.026	0.40	0.862	8.59
	W^2	30.46	0.060	0.81	0.827	9.61
MS	LW	39.54	0.015	0.58	0.981	4.81
	L^2	39.54	0.022	0.36	0.898	10.98
	W^2	39.54	0.049	0.78	0.894	11.24
PPH	LW	40.71	0.009	0.66	0.990	4.48
	L^2	40.71	0.008	0.38	0.972	7.57
	W^2	40.71	0.028	1.11	0.964	8.54

TABLE IX

REGRESSION ANALYSIS INVOLVING THREE INDEPENDENT VARIABLES FOR RIGHT LEAFLETS

Cultivar	X	$\bar{X}$	$S_{\bar{b}}$	B	R^2	CV%
FG	LW	42.32	0.005	0.70	0.997	2.44
	L^2	42.32	0.013	0.48	0.963	8.55
	W^2	42.32	0.023	0.97	0.970	7.68
MN 13	LW	20.79	0.015	0.70	0.982	4.30
	L^2	20.79	0.025	0.35	0.848	12.62
	W^2	20.79	0.059	1.08	0.900	10.21
MN 139	LW	28.02	0.015	0.69	0.982	2.47
	L^2	28.02	0.031	0.35	0.770	8.88
	W^2	28.02	0.064	0.88	0.834	7.54
MS	LW	33.65	0.017	0.68	0.981	5.26
	L^2	33.65	0.024	0.41	0.904	11.82
	W^2	33.65	0.031	1.05	0.976	5.96
PPH	LW	40.06	0.008	0.71	0.993	3.80
	L^2	40.06	0.009	0.42	0.973	7.44
	W^2	40.06	0.020	1.14	0.982	5.98

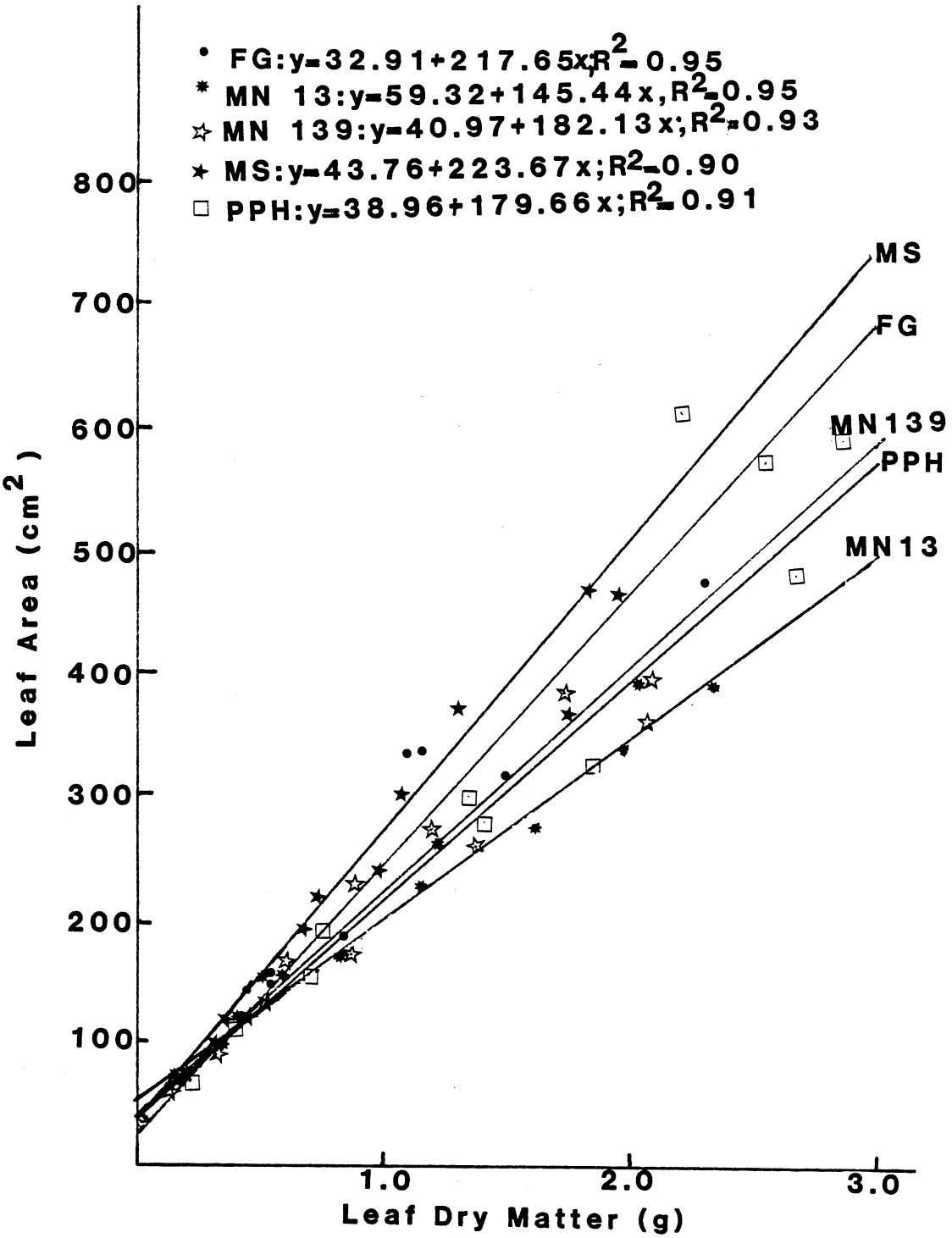

Fig 1 Leaf Area Vs. Leaf Dry Matter For Five Cowpea Cultivars.

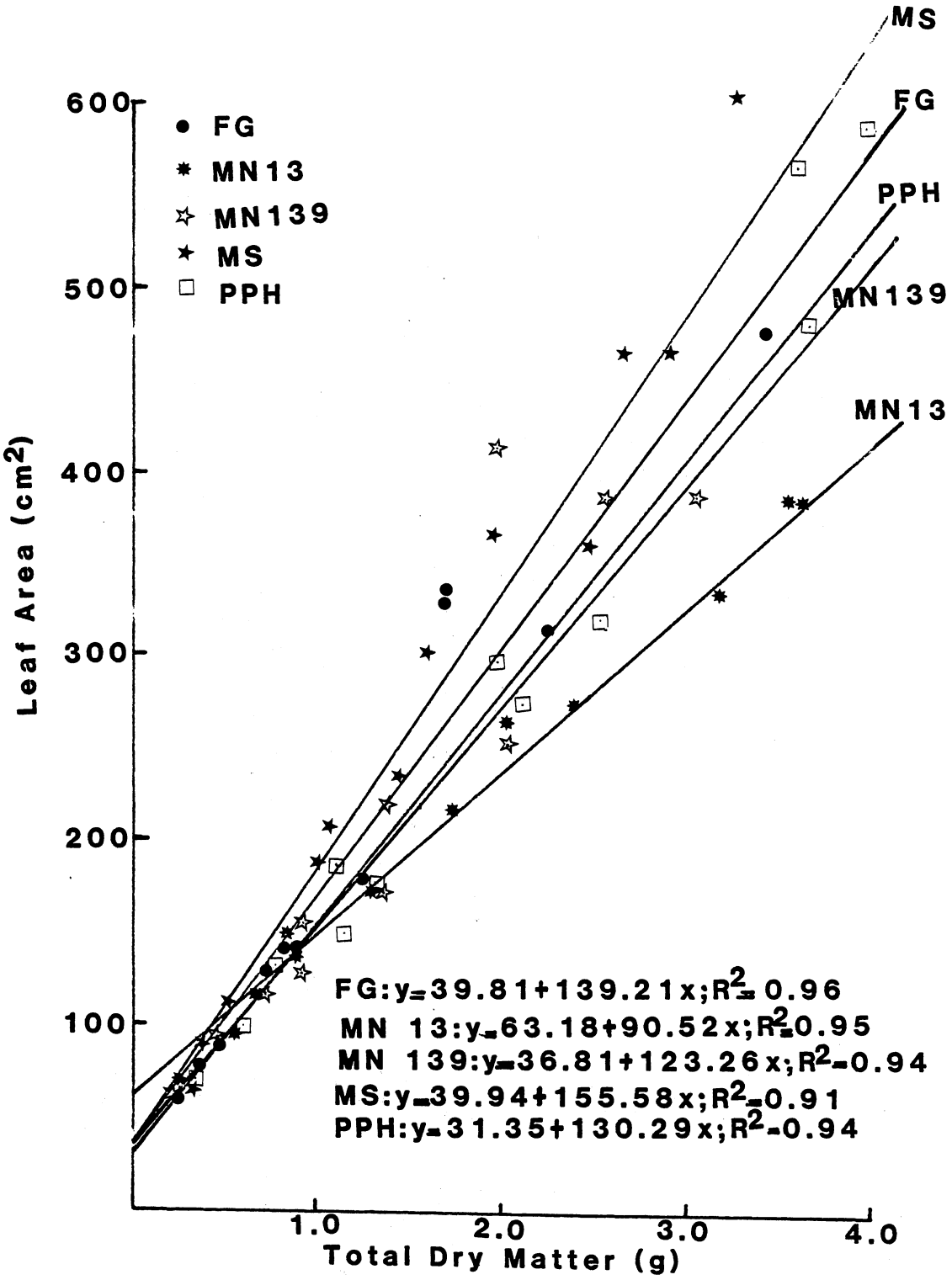

Fig 2. Leaf Area Vs. Plant Dry Matter For Five Cowpea Cultivars.

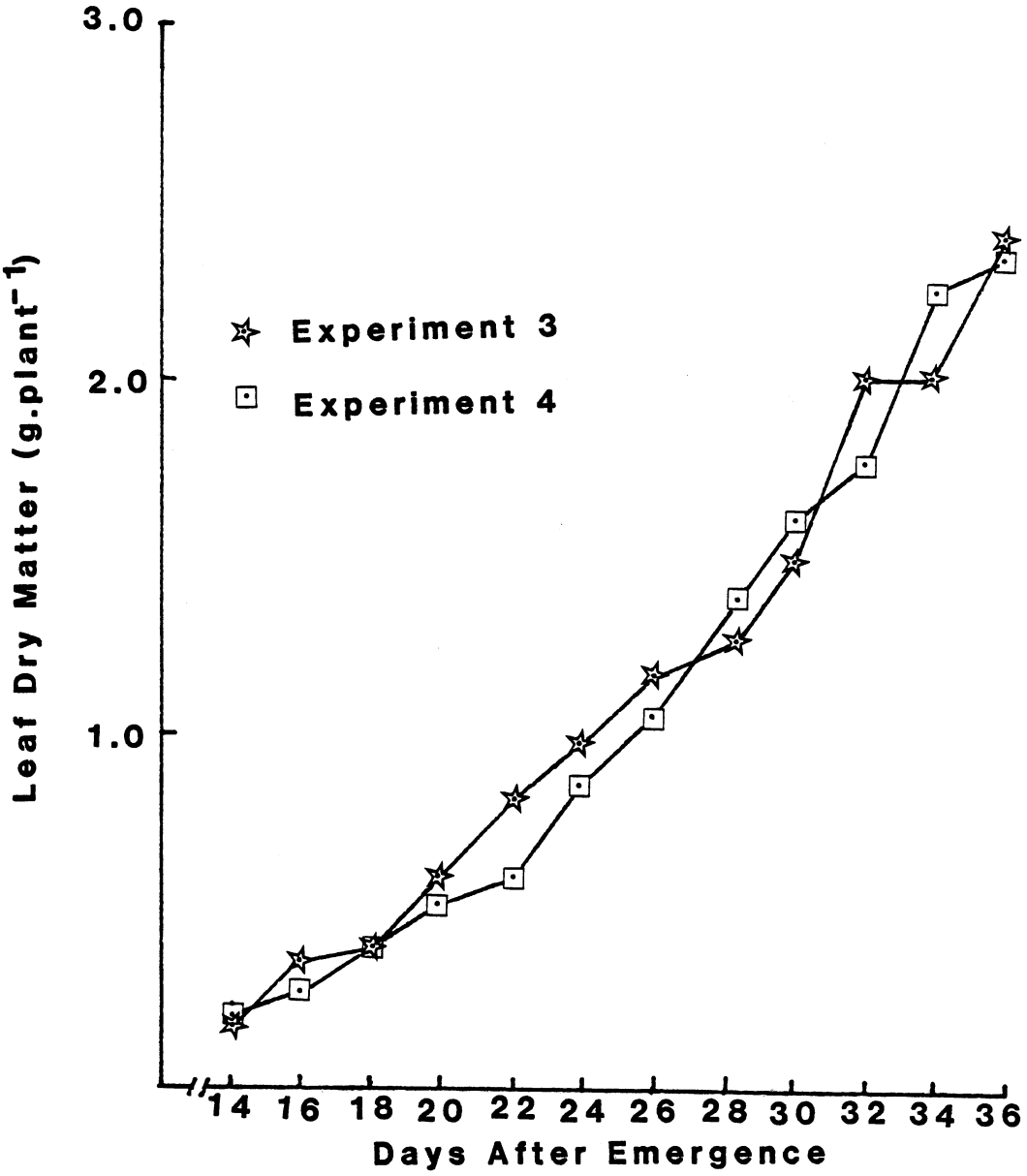

Fig 3. Relationship Between Leaf Dry Matter Over Two Growing Periods.

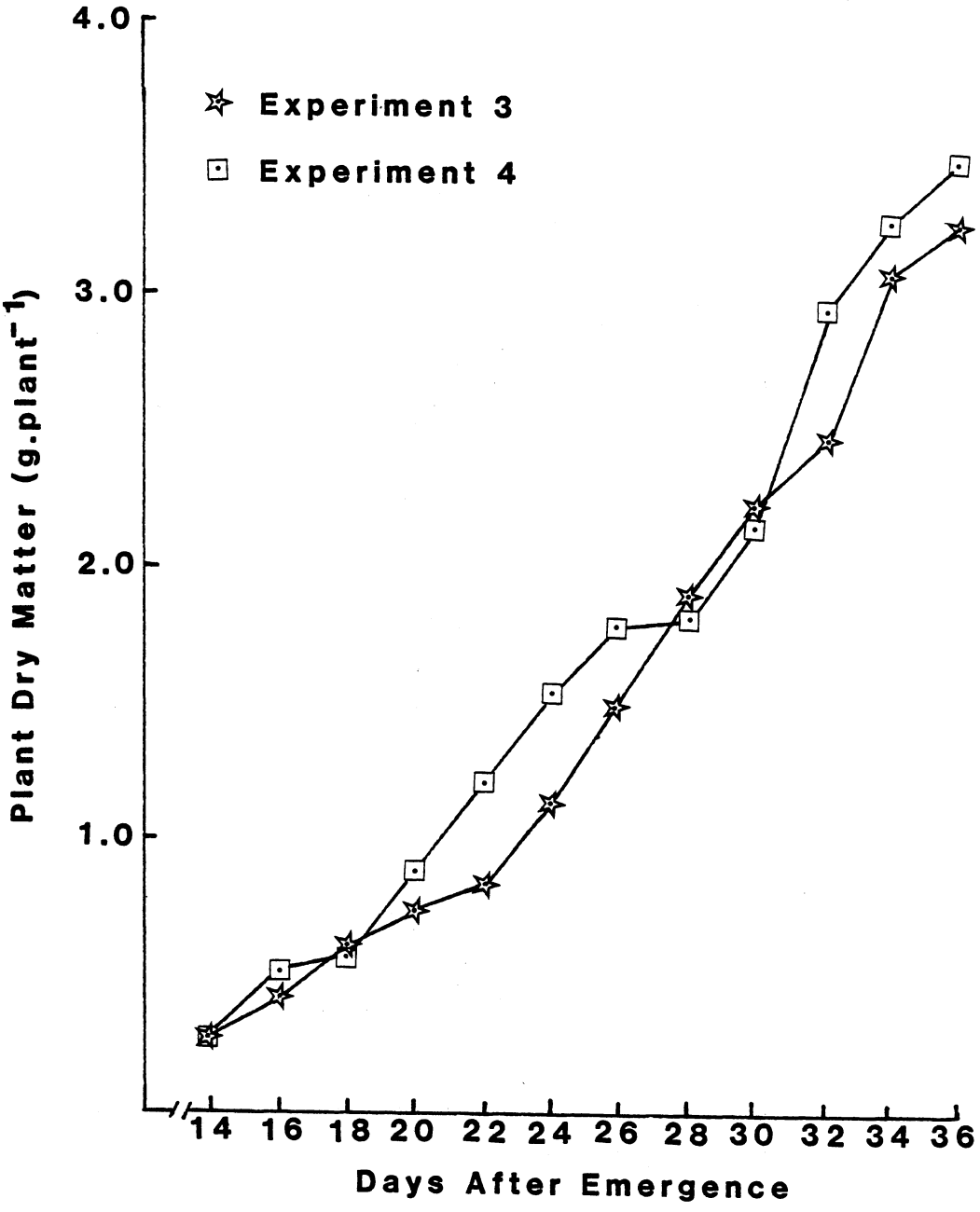

Fig 4. Relationship Between Cowpea Plant Dry Matter Over Two Growing Periods.

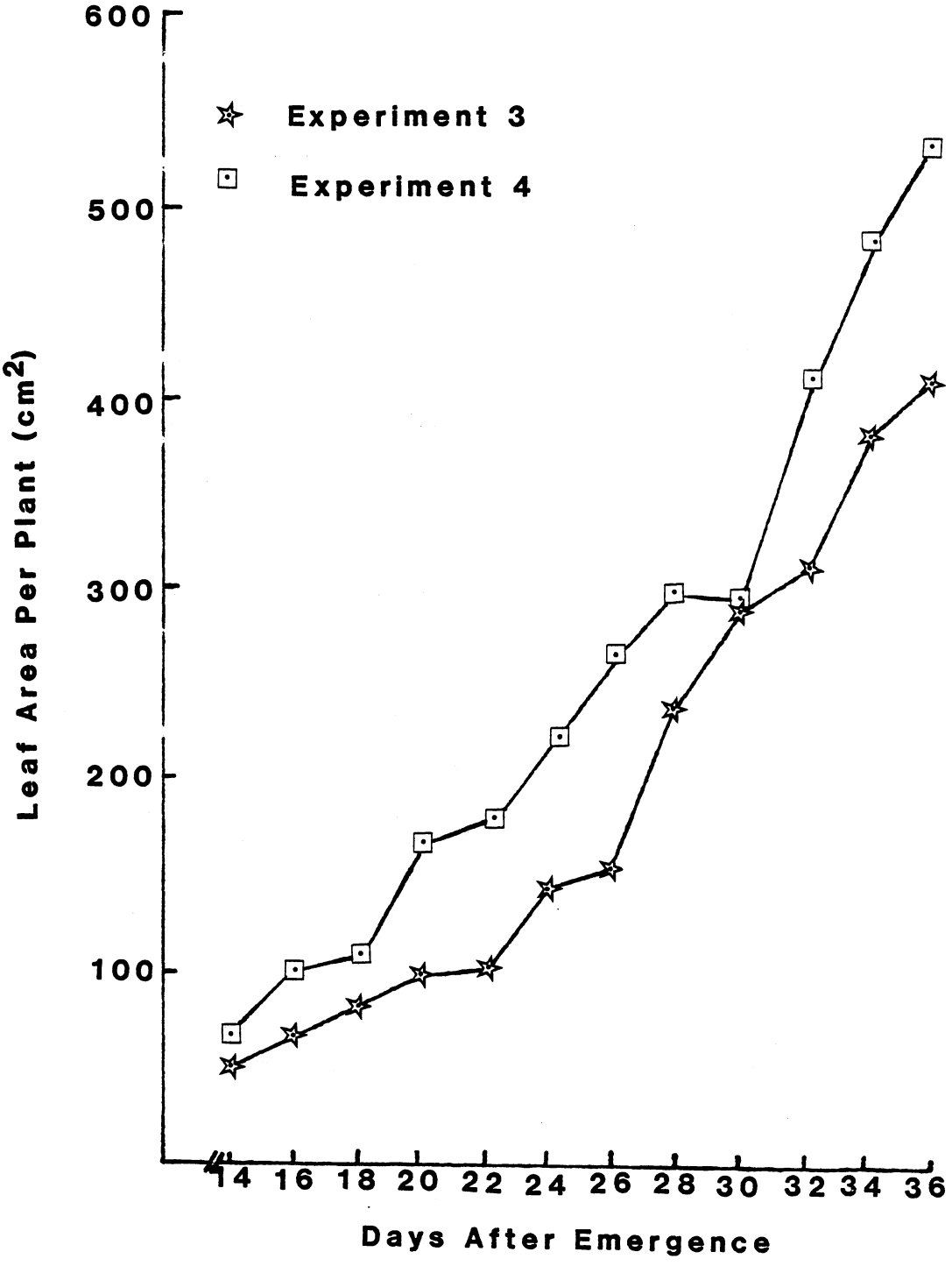

Fig 5. Relationship Between Plant Leaf Area Over Two Growing Periods.

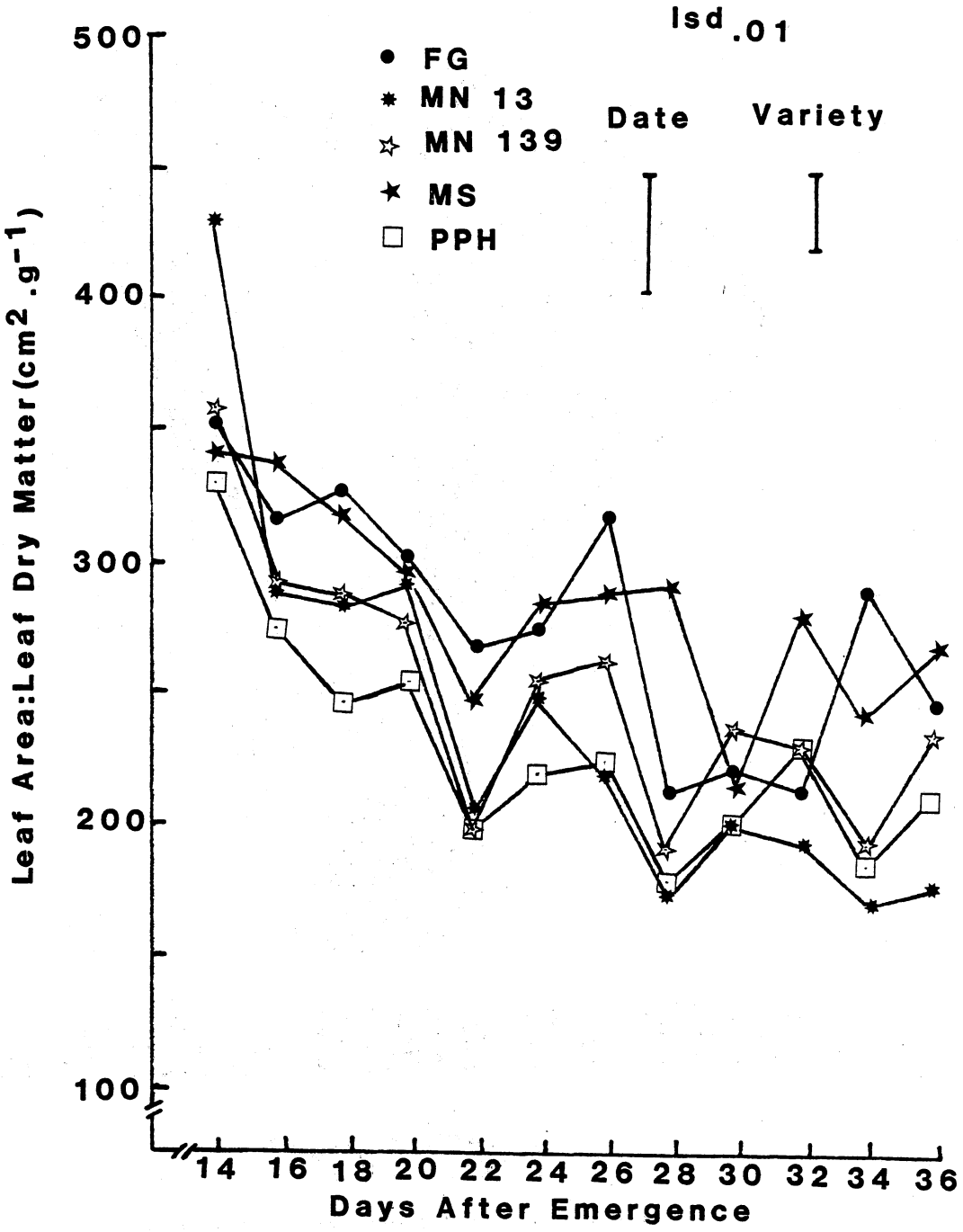

Fig 6. The Relationship Between Leaf Area:Leaf Dry Matter During The Growing Season.

CHAPTER V

RESPONSE OF NITRATE REDUCTASE TO APPLIED NITROGEN IN COWPEAS (*VIGNA UNGUICULATA* L. WALP.)

Abstract

Cowpeas (*Vigna unguiculata*) and other legumes do not respond by yield increases to soil or applied nitrogen as do other plant families. The utilization of applied nitrogen, its effects on nitrate reductase (NR) and water soluble protein (WSP) vary from one legume cultivar to another. Nitrate reductase activity and leaf water soluble protein of five cowpea cultivars were evaluated in pot experiments in a growth chamber from seedling up to anthesis. Mean activity per gram fresh weight per hour was highest at the seedling stage and gradually decreased. Water soluble protein increased up to 10 days after nitrogen application but decreased thereafter. Nitrate reductase activity was highly correlated to nitrogen application. Plants supplied with high levels of nitrogen had a significant increase in nitrate reductase activity; there was however, a concurrent reduction in nodulation.

Nitrate reductase activity of cowpea cultivars was not

significantly different over the growing season; however, 'Freezegreen' consistently had the highest activity. When the cultivars were evaluated at anthesis, there were significant differences among the cultivars and nitrogen levels. While Freezegreen had the highest nitrate reductase activity, the short growing season cultivars ('MN13' and 'MN 139') had higher water soluble protein content. Application of 5 mg N.plant^{-1} increased nodulation but additional applied nitrogen above this level resulted in depressed nodulation. Nitrogen level was positively correlated with nitrate reductase, water soluble protein and leaf area while negatively correlated with nodule number and nodule weight.

Introduction and Literature Review

Cowpea cultivars (Vigna unguiculata L. Walp.) are capable of utilizing two principal sources of nitrogen in its nutrition: soil nitrate and atmospheric nitrogen (Brill, 1977, Demeterio et al., 1972; Ryle et al., 1979). Felix et al. (1981) observed that an early application of nitrogen promoted nitrogenase activity, while a post-flowering application enhanced nitrate reductase activity in Phaseolus vulgaris. Depending on the stage of development, leguminous plants take advantage of applied nitrogen (Miller et al., 1982). Halsey (1960) stated that nitrogen fertilization is important in the production of cowpeas and that nitrogen obtained from the atmosphere by the action of nitrogen-fixation might not be adequate for the plant's needs. Previous work

by Lorz et al. (1955) has shown that in the early stages of growth, nitrogen is needed to enhance seedling development before nitrogen-fixation has a chance to occur. However, Kahn and Kahn (1981) observed significant reduction in nodulation with an application of nitrate nitrogen.

Since some cultivars were developed under conditions with only a low level of soil nitrate present for assimilation, these cultivars would therefore tend to have greater nodule development and ultimately higher efficiency for nitrogen fixation (Israel, 1981). Rhoden and Allen (1982) also observed differences in the rate of nodulation and nitrogen fixation of cowpea cultivars. For improvement in biological nitrogen fixation of cowpeas, several inoculation trials have been undertaken, with variable results (Halsey, 1960; Zary et al., 1978; Keyser et al., 1979; Foulds, 1971). However, cowpeas as well as other nodulated plants make maximum benefit of their nitrogen source depending on their physiological stage of development. Franco et al. (1979) noted that nitrate utilization decreased as the plant matured. This is consistent with work done by Felix et al. (1981) showing that the common bean utilizes more nitrogen in the earlier stages of development. Some of these studies did not establish clearcut correlation among such variables as nitrate reductase activity, nodulation, leaf water soluble protein and nitrate levels.

Nitrate reductase is an adaptive enzyme, its activity is affected by several internal as well as external factors

(Beevers and Hageman, 1969). Afridi and Hewitt (1969) have shown that nitrate reductase activity was readily induced by the addition of nitrate substrate, while Hageman and Flesher (1960) established that increased levels of nitrate in the nutrient solution also increased nitrate reductase activity. They further showed that prolonged darkness would result in greatly reduced activity. In studying the levels of nitrate reductase activity throughout the day, Harper and Hageman (1972) noted that the greatest activity occurred in the early afternoon in soybeans. Wallace and Pate (1965), studying field peas, also observed that maximum nitrate reductase activity occurred in the early afternoon.

Felix et al. (1981) found varying levels of nitrate reductase among bean cultivars of different geographic areas of origin. Nodulated and non-nodulated cowpeas fed NO_3-nitrogen had greater than 90 percent of their reductase activity in their leaves, making the shoots the major organs of nitrate reduction (Atkins et al., 1980). Nitrate is the primary source of the nitrogen available from the soil. Then the uptake of nitrogen and its subsequent reduction by nitrate reductase is the main pathway of soil nitrogen utilization. Harper and Hageman (1972) noted that there is variation in the rate that nitrate is reduced in soybeans throughout the growing season. They also observed that mean activity per gram fresh weight per hour was highest in the seedling stage while total activity (i.e., activity per

gram fresh weight per hour times the total leaf weight) reached a maximum when plants were in the full bloom to mid-pod stage. Hatam (1980) also found differences in nitrate reductase activity in soybeans having nitrogen supplied versus those without nitrogen applied. However, there was a gradual decline in nitrate reductase activity from seedling to maturity.

The present study was initiated to evaluate the relative ability of cowpea cultivars to utilize nitrogen and the seasonal pattern of nitrate reductase activity. The effect that varying levels of applied nitrogen had on nodule development, leaf area and water soluble protein was also evaluated.

Materials and Methods

Two pot experiments were conducted in a growth chamber at the Control Environment Research Laboratory (CERL), Oklahoma State University, Stillwater, Oklahoma. Experiment 1 began 16 May 1982 and experiment 2 on 20 May 1983. Five cowpea cultivars--'Pinkeye Purple Hull,' 'Mississippi Silver,' 'Freezegreen,' 'MN 13' and 'MN 139'--were used in the study. These cultivars were selected for their contrast in growth habits and dates of maturity.

The test soil was a Kirkland silt loam (Udertic Paleustoll) (Gray and Roozitalab, 1976) obtained from the Agronomy Research Station, Stillwater, Oklahoma. Plastic pots 13 cm in diameter were filled with (3:1) soil to peat

mixture. Each container had 2.7 kg of soil which had been sterilized in an autoclave. The soil was then inoculated with a commercial strain of Rhizobium 'Nitragin.' Six seeds were planted in each pot and plants were thinned to four plants per pot. One week after germination nitrogen was applied as NH_4NO_3 to the various treatments at 0, 5, 10 and 20 mg nitrogen per plant (0, 20, 40, 80 mg N per pot).

Plants received a photoperiod of 13 hours (0700-2000 DST) of light from white fluorescent and incandescent lamps. These lamps provided a flux density of 720 $\mu E.m^{-2}.s^{-1}$ at the plant level. The day/night temperature throughout the experimental period was 27/21C following the recommendations of Dart and Mercer (1965). Relative humidity was set at 75%. Plants were provided with a nutrient solution except nitrogen at weekly intervals.

Leaf samples for nitrate reductase and leaf water soluble protein were taken twice weekly during the two experiments. At each sampling the uppermost, fully expanded leaf was selected because this leaf had the highest nitrate reductase activity (Harper and Hageman, 1972). Random sampling of these leaves was always made after six hours of full light on each sampling date. Samples obtained were immediately placed in polyethylene bags, placed on ice and taken to the laboratory.

Nitrate Reductase

Nitrate reductase activity was measured using the in

vitro method adapted by Hageman and Flesher (1960) and modified by Croy and Hageman (1970). Leaf samples were cut into small pieces and one gram samples were homogenized for 30 seconds in a Brinkmann Polytron ST 20 homogenizer at 3/4 speed in 7 mL of grinding medium (25 mM K_2HPO_4, 5.0 mM EDTA, 10 mM cysteine, adjusted with KOH to a pH of 9.0). The homogenate was then passed through cheese cloth, the extract centrifuged at 13,000 rpm (20,000g) for 15 minutes and the supernatant saved for assay. Throughout the extraction process samples are kept in an ice bath. Aliquots (0.2 mL) of plant extracts were placed in a buffered solution (pH 7.5) which contained the subtrate, nitrate and a reducing agent ($NADH_2$). During the incubation period, the temperature was maintained at 30C which is the optimum for the enzyme. After 15 minutes, the reaction was terminated using a 'stop reaction-color solution' (1% sulfanilamide reagent in 3N NCl and 0.02% N-[1-napthyl] ethylene diamine HCl combined in a 1:1 ratio). Contents of the tube were mixed and allowed to stand for 15 minutes after which tubes were centrifuged at 2,000 rpm using an IEC HN-S11 centrifuge. Absorbance was read after 20 minutes at 540 nm using a spectronic 2000 spectrophotometer.

Water Soluble Protein

Reagents: Bio-rad Protein Dye Reagent Concentrate was obtained from Bio-rad Laboratories, Richmond, California, and was diluted (1 part concentrate to 4 parts deionized

water). Bovine gamma globulin diluted to 0.2 to 1/4 mg. mL^{-1} was used as the protein standard.

Water soluble protein determinations were measured using the method of Bradford (1976). Plant extracts were obtained as described for nitrate reductase. 250 µl of plant extract was placed in a test tube to which 5.0 mL of diluted reagent dye was added. The contents of the text tube were agitated and allowed to incubate for 15 minutes at room temperature. The absorbance at 595nm was obtained after the incubation period using water and the dye reagent as a blank. The protein standard was obtained as described by Bradford (1976). The protein standards were plotted against the corresponding absorbance to obtain a standard curve which was used to determine the protein in the unknown samples. All absorbances were read using a Baush and Lomb Spectronic 2000.

The experiment was terminated at anthesis (36 days after emergence), after which plants were removed from the pots and nodule number, nodule weight, root weight and leaf area were taken. Leaf area was determined by the use of a LI-COR Leaf Area Meter (Model LI-3000 A, Lambda Instrument Corp., Lincoln, Nebraska).

Results and Discussion

Nitrate Reductase Activity

Nitrate reductase activity (NR) for the seven sampling dates differed significantly (Table I). NR activity was

highest on the initial sampling date. This, however, gradually declined during the growing season up to anthesis. This pattern is widely accepted and was reported by Harper and Hageman (1972). The data show that NR activity declined from an average of 5.24 μmoles.gfw^{-1}.hr^{-1}, 14 days after emergence (DAE) to 0.80 μmoles.gfw^{-1}.hr^{-1} 35 DAE (Fig. 1).

There was a consistent increase in NR activity with increased level of nitrogen application throughout the growing season. Plants supplied with 20 mg N.plant^{-1} on the average had 22 percent higher activity over the control throughout the growing season. It should be noted that although there was a general decline in NR activity during the growing season, this reduction was 6:1 in the case of plants supplied with 20 mg N.plant^{-1} compared to a 2:1 decline in the control plants. This suggests that the rate of NO_3^- reduction and possibly absorption did not vary greatly during seedling to anthesis. This could also indicate that nitrogen fixing bacteria might be active in the control plants but suppressed in the high nitrogen application treatments. This is manifested in the plants receiving 20 mg N/plant since they had significantly lower number of nodules (Fig. 2).

The results of this study show that the NR differences among the five cultivars were not significant (Table II). Ranking of the cultivars show that Freezegreen (FG) had the highest rate of NR activity while Pinkeye Purple Hull (PPH) the lowest. The data further show that NR activity

had a relatively high coefficient of variability (CV), 36%.

Water Soluble Protein

The data (Table II) show that water soluble protein (WSP) varied significantly for the five cowpea cultivars. The two short season cultivars, MN 13 and MN 139, had significantly higher levels of WSP than the other three cultivars with longer growing seasons. Unlike NR, which had a gradual drop in activity as plants matured, WSP increased one week after application of nitrogen (Table III). While there was a significant decline in WSP as the plants matured, there was not a significant response to applied nitrogen. Although there was a general decline in WSP over the growing season, cultivars also differed in their protein content (Fig. 3). For all cultivars protein content increased from the first to the second sampling date but the two short season cultivars (MN 13 and MN 139) had a steady decline over the rest of the growing season. The other three cultivars, MS, PPH, and FG all had significant quartic relationships between sampling date and WSP content.

Nodulation

It is essential to note that soil or applied nitrogen has inhibitory effects on nodule growth and development suggesting reduced nitrogen fixation (Miller et al., 1982).

Means for nodule number, nodule weight, NR, WSP, root dry weight and leaf area (LA) taken at the onset of anthesis

are given in TAble IV. The data show that there were significant differences in all these measurements at different levels of applied nitrogen. Increasing nitrogen application to 5 mg N/plant resulted in significant increase in nodulation, both in terms of number and weight of nodules. Increasing the application above this level to 10 or 20 mg N/plant reduced nodulation drastically. This trend has been reported by Dart and Mercer (1965) and Miller et al. (1982).

Increasing nitrogen application is also responsible for increased rates of NR, WSP content and LA. However, root dry weight was not enhanced by increased nitrogen application. Although no differences were noted in root dry weights, when 5 mg $N.plant^{-1}$ was applied the largest root system was obtained. When the data were analyzed (Table V) for cultivar differences, it was noted that cultivars did not vary in nodule number or weight. NR activity ranged from 4.8 to 2.3 $\mu mole.gfw^{-1}.hr^{-1}$ for FG and MN 139 respectively. The five cultivars studied showed significant differences for WSP, root dry weight and LA. It was observed that coefficient of variability was very high for nodule number and nodule weight, 38.9% and 47.3% respectively. On the other hand the coefficient of variability for WSP was quite low being 12.4%. These results suggest that many environmental factors would have to be taken into account before predicting plant enzyme activity. In the case of NR, nitrogen levels as well as plant age would influence the

level of this enzyme activity.

Simple correlation coefficients for the seven characters of five cowpea cultivars under four nitrogen applications is presented in Table VI. Nitrogen level was significantly correlated with all other variables except root dry weight. While nitrogen level was negatively correlated with nodule number and weight it was positively correlated with NR, WSP and LA. Recent studies by Kahn and Kahn (1981) have shown that there was a reduction in nodulation with increased application of nitrate nitrogen.

NR and WSP were significant and positively correlated ($r = 0.36^{**}$). While it is important to note that these two traits followed a similar pattern throughout the growing season, Croy and Hageman (1970) found no numerical relationship between these two traits for winter wheat. These authors suggested that there should not be a relationship between these two traits since NR is subjected to fluctuations with environmental changes and declines with maturity but protein levels remain fairly constant.

Leaf area showed a relatively high positive correlation ($r = 0.65^{****}$) with increased nitrogen application. However, LA was negatively correlated with nodule number and weight. The data further show that NR was positively correlated with LA ($r = 0.46^{***}$). As reported by Hatam (1980), NR was enhanced by increased application of nitrogen. It is therefore reasonable to assume that applied nitrogen produced vegetative growth at the expense of root growth

and nodule development. The plant will therefore utilize soil nitrate at the expense of nitrogen fixation and a symbiotic relationship with Rhizobium.

Summary

The results indicate that NR activity as well as WSP content decreased throughout the growing season. Cowpea cultivars vary in their NR activity with FG consistently having the highest activity. At the same time the short season cultivars (MN 13 and MN 139) had higher WSP than the longer season cultivars. While an application of 5 mg N/plant was important to nodule development, increasing soil applied nitrogen above this level resulted in reduced nodulation. Concurrently, there was an increase in LA, NR activity and WSP content.

It has been long established that an application of nitrogen as a 'starter' was important to legume growth and development, however, developing the balance between nitrogen application and nodule development is not yet totally understood. Since higher levels of NR and WSP content are achieved with increased nitrogen, it is necessary that we find genotypes that will develop nodules when soil nitrate is still adequate so that these systems will not be curtailed when soil nitrate levels become critical. It was observed that NR was positively correlated with increased nitrogen levels and WSP content. On the other hand high nitrogen application resulted in reduced nodulation.

It can therefore be concluded from this study that cowpea cultivars can maintain high levels of NR with high nitrogen application. This would be achieved at the expense of nodulation and ultimately nitrogen fixation.

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TABLE I

SEASONAL VARIATION IN NITRATE REDUCTASE ACTIVITY OF COWPEA CULTIVARS

Date of Sampling	Days After Emergence	N Applied mg.plant^{-1}	NR activity µmoles.gfw^{-1}.hr^{-1}	Average
6-07	14	00	4.43	5.24
		05	4.84	
		10	5.19	
		20	6.41	
6-10	17	00	3.64	4.76
		05	4.54	
		10	4.47	
		20	6.25	
6-14	21	00	3.38	3.61
		05	3.61	
		10	3.83	
		20	3.61	
6-17	24	00	3.98	3.62
		05	3.37	
		10	2.99	
		20	4.08	
6-21	28	00	2.56	2.80
		05	2.97	
		10	2.31	
		20	3.31	
6-25	32	00	1.98	2.36
		05	2.05	
		10	2.42	
		20	2.96	
6-28	35	00	0.51	0.80
		05	0.92	
		10	0.63	
		20	1.17	
			1.29	0.74
$LSD_{.05}$				
CV%				36

TABLE II

NITRATE REDUCTASE (NR) AND WATER SOLUBLE PROTEIN (WSP) FOR FIVE COWPEA CULTIVARS

Cultivar	NR	WSP
	μmoles NO_2^-.gfw^{-1}.hr^{-1}	mg.gfw^{-1}
Freezegreen (FG)	3.68	15.89
Minnesota 13 (MN 13)	3.55	17.76
Minnesota 139 (MN 139)	3.46	18.73
Mississippi Silver (MS)	3.19	16.53
Pinkeye Purple Hull (PPH)	2.66	15.74
LSD	NS	1.05
CV%	36	13

NS = not significant at the 5% level.

TABLE III

SEASONAL VARIATION IN WATER SOLUBLE PROTEIN OF COWPEA CULTIVARS

Date of Sampling	Days after Emergence	N Applied mg.plant^{-1}	WSP ....mg.gfw^{-1}.....	Average
6-07	14	00	16.47	16.05
		05	15.62	
		10	15.89	
		20	16.18	
6-10	17	00	18.56	18.71
		05	19.68	
		10	18.05	
		20	18.54	
6-14	21	00	18.29	17.86
		05	17.55	
		10	18.24	
		20	17.43	
6-17	24	00	17.47	17.42
		05	18.65	
		10	17.00	
		20	16.57	
6-21	28	00	16.95	16.53
		05	17.07	
		10	17.08	
		20	15.13	
6-25	32	00	17.38	16.53
		05	17.24	
		10	16.18	
		20	16.23	
6-28	35	00	16.09	15.28
		05	15.31	
		10	15.29	
		20	14.48	
LSD .05			NS	0.79
CV%				13

NS = not significant at 5% level.

TABLE IV

NODULE NUMBER, NODULE WEIGHT, NITRATE REDUCTASE, WATER SOLUBLE PROTEIN, ROOT WEIGHT AND LEAF AREA AVERAGED OVER 5 CULTIVARS AND 4 NITROGEN LEVELS

Measurements	.....Nitrogen Levels..... 0	5	10	20	LSD .05	CV %
Nodule Number	30.6	39.4	21.7	21.5	8.2	38.9
Nodule Weight (mg)	150.3	191.2	92.6	80.5	45.0	47.3
Nitrate Reductase (μmole/gfw/hr)	2.9	2.7	3.1	4.5	0.7	30.1
Water Soluble Protein (mg/gfw)	16.5	16.5	17.1	18.0	0.6	12.4
Root Weight (mg)	580.4	612.1	595.9	545.1	NS	37.9
Leaf Area (cm^2)	568.1	706.3	889.1	1187.1	110.8	17.9

TABLE V

NODULE NUMBER, NODULE WEIGHT, NITRATE REDUCTASE, WATER SOLUBLE PROTEIN, ROOT WEIGHT AND LEAF AREA OF FIVE COWPEA CULTIVARS

Measurements	.....Cultivars..... FG	MN 13	MN 139	MS	PPH	.05	CV %
Nodule Number	29.7	30.1	27.9	28.9	25.0	NS	38.9
Nodule Weight	137.8	107.2	126.7	144.7	126.9	NS	47.3
NR (μmoles/gfw/hr)	4.8	2.8	2.3	3.3	3.2	0.8	30.1
WSP (mg/gfw)	18.1	16.1	17.3	16.7	16.9	0.7	12.4
Root Weight (mg)	347.7	591.2	821.1	554.0	603.0	180.0	37.9
Leaf Area (cm^2)	787.0	702.6	593.3	853.1	1252.3	123.9	17.9

TABLE VI

SIMPLE CORRELATION COEFFICIENTS FOR SEVEN CHARACTERS OF FIVE COWPEA CULTIVARS UNDER FOUR NITROGEN LEVELS

Variables	2	3	4	5	6	7
1. Nitrogen Level	-0.38**	-0.48**	0.34**	0.43**	-0.06	0.65****
2. Nodule Number		0.78****	-0.20	-0.12	0.30*	-0.34**
3. Nodule Weight			0.14	-0.09	0.27*	-0.27*
4. Nitrate Reductase				0.36**	-0.13	0.46***
5. Water Soluble Protein					-0.08	0.25
6. Root Weight						-0.01
7. Leaf Area						

*, **, ***, **** significant at the 5%, 1%, 0.1%, and 0.01% level (df = 59)

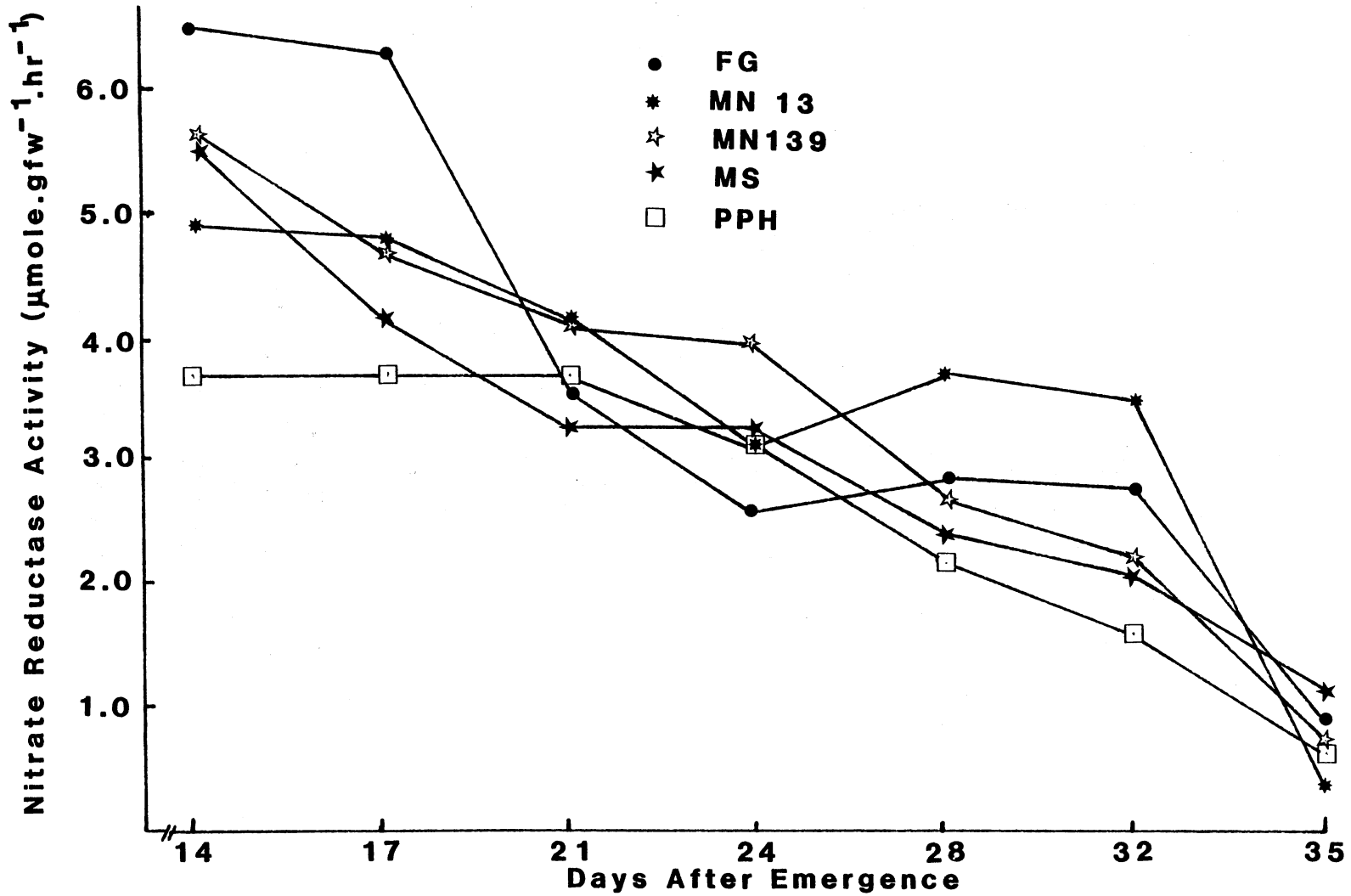

Fig 1. Seasonal Variation in Nitrate Reductase Activity of Cowpea Cultivars.

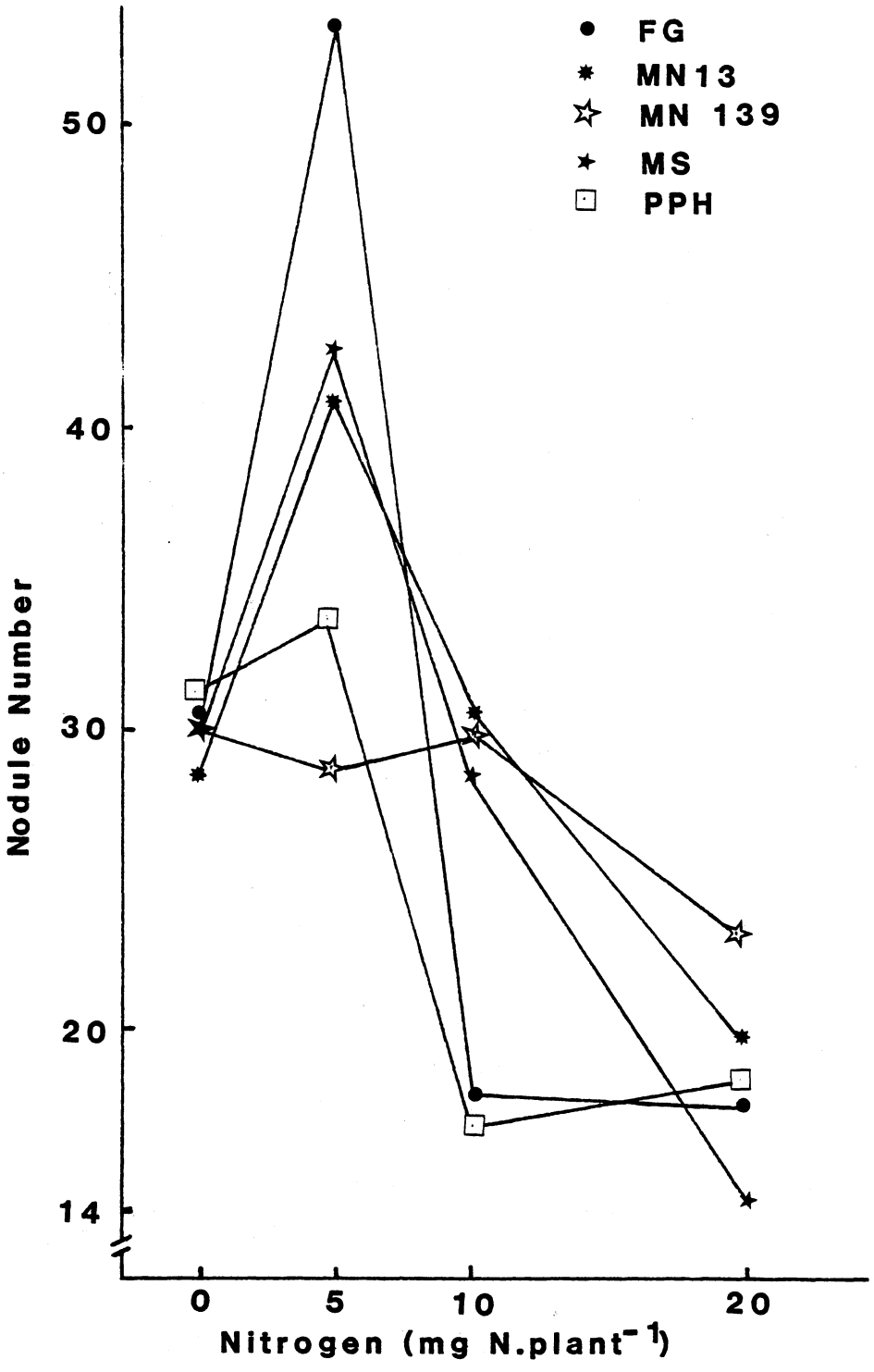

Fig 2. Effect of Applied Nitrogen on Nodulation of Five Cowpea Cultivars.

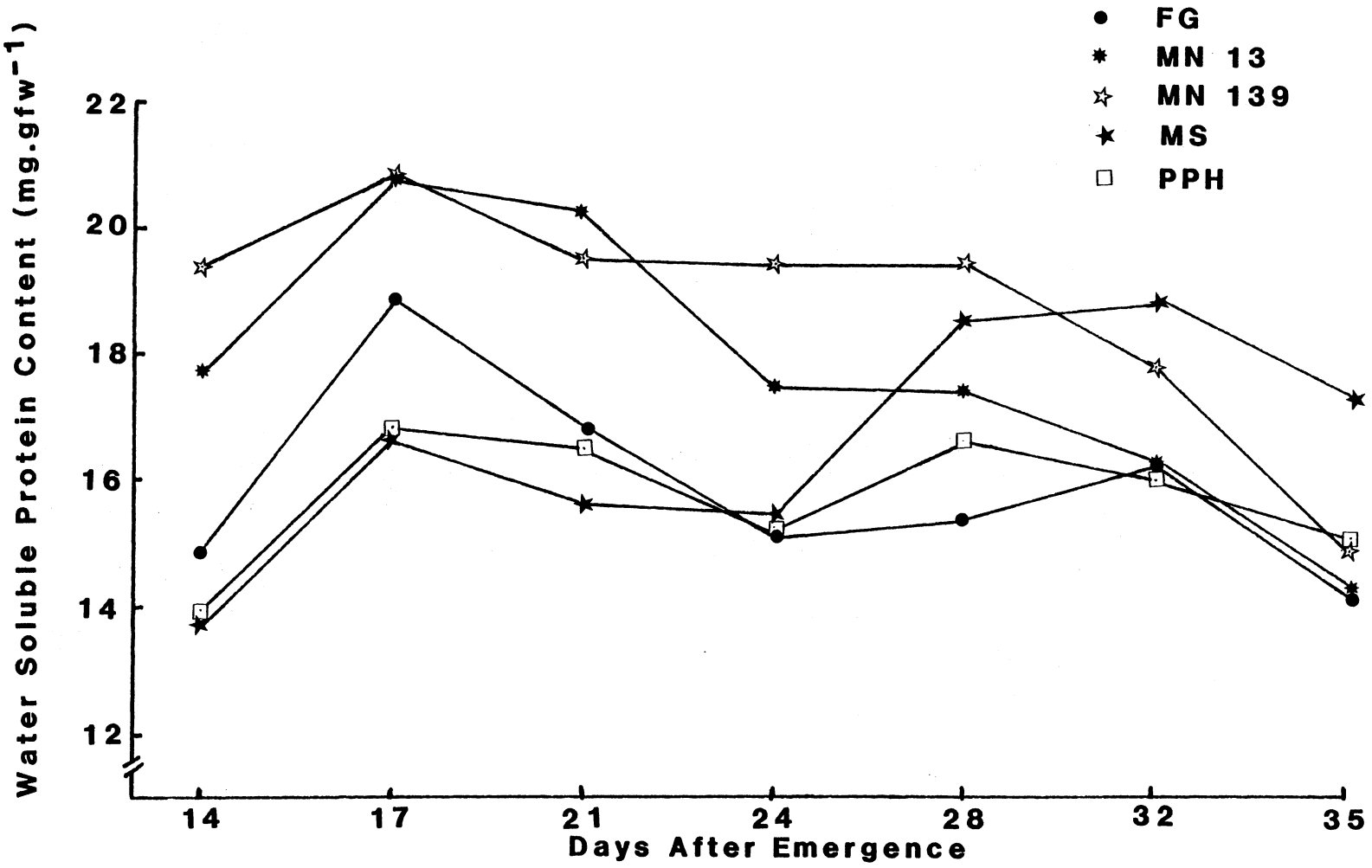

Fig 3. Seasonal Variation in Water Soluble Protein of Cowpea Cultivars.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Studies were conducted to evaluate possible cultivar differences in photosynthesis and nitrate reductase activity in winter wheat. A comparison of nitrate reductase and leaf area measurements was also made with various cowpea cultivars. The research was carried out by conducting different experiments under various environmental conditions. Some studies were conducted under field conditions while others were done in the growth chamber or greenhouse.

During the course of the study physiological characters such as carbon dioxide exchange rate (CER), nitrate reductase (NR) activity, stomatal diffusive resistance (Rs), leaf area (LA), leaf surface temperature, leaf area measurements, yield and yield components were evaluated. CER was determined on wheat cultivars grown in the field using infrared gas analysis, while NR was determined on both winter wheat and cowpea in the field and the growth chamber. Leaf area measurements were obtained in the growth chamber and greenhouse in order to measure CER of cowpea cultivars in the future.

The data obtained from this research indicated that there was a significant negative correlation between carbon

dioxide exchange rate (CER) and stomatal diffusive resistance (Rs). It was noted that as stomatal resistance increased there was a decrease in the rate of carbon dioxide fixed. The results also show that there was a negative relationship between diffusive resistance and transpiration. Such conclusion as can be drawn from this study would suggest that increasing transpiration would result in high rates of carbon dioxide exchange. At the same time it was noted that higher Rs would reduce CER and lower transpiration.

Nitrate reductase (NR) activity in wheat declined from the flag leaf emergence stage to the grain filling stage. There were significant differences in the level of activity of the six winter wheat cultivars. Diurnal variations in NR activity during this period showed that activity increased rapidly from sunrise to midmorning then remain fairly constant throughout the day. The data for diurnal variation in NR activity would suggest that under adequate moisture and illumination winter wheat can be sampled between midmorning and late afternoon without significant differences in readings.

In order to obtain methods which are rapid, non-destructive and precise in measuring leaf area of cowpea cultivars, several prediction equations were developed. The results show that there was a linear relationship between length*width and leaf area. When studies on growth, evapor-transpiration and other physiological studies on cowpea

cultivars are conducted and leaf area is needed the area can be obtained by using the equation: Area = 0.34 + 0.67LW with R^2 of 0.988. Although length*width was accurate in predicting leaflet area, considerable saving of time, with minute losses in predictive ability, could be obtained by only measuring the length or the width.

Nitrate reductase activity in cowpea cultivars was highest at the seedling stage and decreased toward maturity. Plants supplied with high levels of nitrogen had significantly higher levels of NR activity. At the same time increasing the nitrogen application depressed nodule weight and number. Therefore, in cowpea increased nitrate reductase activity through nitrogen application is achieved at the expense of nodulation.

APPENDIXES

TABLE I

ANALYSIS OF VARIANCE FOR CARBON DIOXIDE EXCHANGE RATE (CER), LEAF SURFACE TEMP (LT), STOMATAL DIFFUSIVE RESISTANCE (Rs) AND TRANSPIRATION OF WINTER WHEAT

Source	df	ANOVA SS	F
CER			
Block	3	23.36	0.29
Variety	5	191.12	1.44
Sampling Date	7	1333.35	7.19**
Variety X Sampling Date	35	708.98	0.76
LT			
Block	3	8.94	1.91
Variety	5	1.50	0.19
Sampling Date	7	5394.54	495.35**
Variety X Sampling Date	35	35.31	0.65
Rs			
Block	3	0.09	1.24
Variety	5	0.25	2.04
Sampling Date	7	3.73	21.54**
Variety X Sampling Date	35	0.70	0.81
TRANSPIRATION			
Block	3	47.36	0.55
Variety	5	334.29	2.31*
Sampling Date	7	5020.66	21.54**
Variety X Sampling Date	35	728.22	0.72

* Significant at .05
** Significant at .01

TABLE II

ANALYSIS OF VARIANCE FOR SEASONAL NITRATE REDUCTASE ACTIVITY OF WINTER WHEAT

Source	df	ANOVA SS	F
Block	3	158.08	1.59
Variety	5	590.95	3.57*
Sampling Date	4	4968.99	37.52**
Variety X Sampling Date	20	425.01	0.64

* Significant at .05
** Significant at .01

TABLE III

ANALYSIS OF VARIANCE FOR DIURNAL VARIATION OF NITRATE REDUCTASE ACTIVITY IN WINTER WHEAT

Source	df	ANOVA SS	F
Block	2	5.29	0.35
Variety	3	361.55	15.74**
Time	12	356.15	3.88**
Variety X Time	36	255.21	0.93

** Significant at .01

TABLE IV

ANALYSIS OF VARIANCE FOR NITRATE REDUCTASE ACTIVITY (NR) AND WATER SOLUBLE PROTEIN (WSP) OF COWPEAS

Source	df	ANOVA SS	F
NR			
Block	2	28.79	1.43
Variety (V)	4	55.78	1.39
Nitrogen Level (NL)	3	51.36	1.70
Variety X Nitrogen Level	12	87.38	0.73
Sampling Date (SD)	6	806.29	42.05**
Variety X Sampling Date	24	101.43	1.32
NL X SD	18	74.09	1.29
V X NL X SD	72	146.27	0.64
WSP			
Block	2	35.86	1.58
Variety (V)	4	555.72	12.23**
Nitrogen Level (NL)	3	66.95	1.96
Variety X Nitrogen Level	12	107.15	0.79
Sampling Date	6	479.41	16.39**
Variety X Sampling Date	24	601.68	5.14**
NL X SD	18	87.03	0.99
V X NL X SD	72	305.83	0.87

** Significant at .01

TABLE V

ANALYSIS OF VARIANCE FOR NODULE NUMBER (NN), NODULE WEIGHT (NW), ROOT WEIGHT (RW), AND LEAF AREA (LA) OF COWPEAS AT ANTHESIS

Source	df	ANOVA SS	F
NN			
Block	2	168.13	0.69
Variety	4	197.57	0.41
Nitrogen Level	3	3261.12	8.97**
Variety X Nitrogen Level	12	1937.63	1.33
NW			
Block	2	2445.41	0.33
Variety	4	9727.20	0.66
Nitrogen Level	3	120070.07	10.81**
Variety X Nitrogen Level	12	37331.98	0.84
RW			
Block	2	39.06	0.40
Variety	4	1360.30	6.97**
Nitrogen Level	3	36.87	0.25
Variety X Nitrogen Level	12	912.97	1.56
LA			
Block	2	31224.25	0.70
Variety	4	3031587.11	33.82**
Nitrogen Level	3	3220011.39	47.90**
Variety X Nitrogen Level	12	605040.30	2.25*

* Significant at .05
** Significant at .01

VITA 2

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Candidate for the Degree of

Doctor of Philosophy

Thesis: SOME PHYSIOLOGICAL PROCESSES IN WINTER WHEAT (TRITICUM AESTIVUM L.) AND COWPEAS (VIGNA UNGUICULATA L.) AS AFFECTED BY DROUGHT STRESS

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