THE EFFECTS OF EPICUTICULAR WAX ON THE RATE OF WATER LOSS OF SORGHUM BICOLOR (L.) MOENCH

By

MIJITABA HAMISSOU

Bachelor of Science in Agriculture

Oklahoma State University

Stillwater, Oklahoma

1985

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfilment of the
requirements for
the Degree of
MASTER OF SCIENCE
May, 1987

THE EFFECTS OF EPICUTICULAR WAX ON THE RATE OF WATER LOSS OF SORGHUM BICOLOR (L.) MOENCH

Thesis Approved:

Dale E. Weibel
Thesis Adviser

Paul E. Richardson

Richard Johnson

Norman N. Durham
Dean of the Graduate College

PREFACE

Inherent drought is a sad reality prevailing in most sub-saharan countries of Africa. The shortage of food combined with poor sanitation have created a high rate of human mortality, not only in Africa, but also in other parts of the World. The Food and Agricultural Organization (F.A.O.) and the World Health Organization (W.H.O.) have predicted that even worse conditions will prevail in the years to come, unless agricultural policies change in these countries. Not only the ever unpredictable weather of Africa causes a slow agricultural development, but also the ever unchanged prehistoric cultivation practices have contributed to the African drought problem. There is a need for modern agricultural technology in the areas of plant breeding, plant physiology, crop management and production, and soil sciences to meet the needs of the hungry people of these regions.

In 1975, the Government of Niger started a national program of agricultural development with the creation of a National Research Institute for Agronomic Research in Niger (INRAN). The government undertook a vast training program for agricultural scientists, to be involved with research, teaching, and extension. As part of this effort, I was sent to Oklahoma State University for a degree in Agronomy.

I am deeply grateful to my major advisor Dr. Dale E. Weibel for his support, sound guidance, and constant suggestions during the course of this study. Dr. Weibel has been helpful in the completion of this study by the effort, interest, and care he provided to me and my family in making us feel at home far from home.

I wish also to express my sincere appreciation to Dr. Paul E. Richardson and Dr. Richard C. Johnson for their advice, the use of laboratory equipment, and for agreeing to serve on my graduate advisory committee.

I wish to thank all the people who have contributed in one way or another to my education:

To the Government of Niger for selecting me for this training, the patience, and the hope they have shown in me,

To the brothers of the Masjid Al Siddiq for their friendship and the financial assistance provided to me and my family,

To the Oklahoma Sate University Agronomy Department for providing the facilities and materials to conduct this study,

To Gary Strickland and the sorghum crew of Oklahoma State University Agronomy Department,

To Keteme Belete who, despite his class work, has willingly helped and advised in putting this manuscript together,

To David M. Ferris for the help he provided in the Wheat Stress Physiology Laboratory, in the field with the instruments, and who willingly agreed to help print

this manuscript,

To Dr. Ronald W. McNew for analyzing the data,

To Steve D. Eckroat for the help he provided in the Microtechnique Laboratory.

I would also like to thank Dr. Soumana Amadou, Director General of INRAN, and Mahamadou Issaka Maga Maiga, DRA/INRAN, for their moral support.

I wish to express my special gratitude to my lovely wife Nana Hadiza Anna, to my son Abdoul Karim, to my daughters Ramatoulaye Lulu, and Salamatou Sally for their moral support and their patience,

To my parents-in-law Brah Maman and Absatou Zaki and their children for the support they provided to me and my wife,

To my parents Malam Hamissou and Fatou, and to my brothers and sisters back in Niger whose patience developed a self confidence in me, I am grateful.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Sorghum is normally grown in regions that are considered unsuitable for other crops such as wheat, corn, or barley. It is grown in the African sub-saharan tropics, and in the semi-arid regions of India where scanty rainfall is predominant, and the soil conditions are too harsh for other food crops. Grain sorghum is, however, the most important source of carbohydrate in the third world. In the United States, sorghum production is centered in the semi-arid portions of the Great Plains States. In either of these regions, the evaporative demand greatly exceeds transpiration. Where no irrigation water is available, sorghum production depends to a significant extent on the plant's drought resistance.

Sorghum leaves are normally covered with a powdery wax. This waxy cover ranges from a heavy covering termed "Bloom", to a light covering known as "Sparse-bloom". Another condition exists where there is no wax covering, and it is known as "Bloomless". This waxy cover is thought to play an important role in the plant's drought resistance mechanism.

The development of drought resistant sorghum has drawn considerable research attention; but due to the complexity of drought resistance, no single factor has been identified as responsible for the sorghum's drought resistance. There

is no single physiological response to one given environmental stimulus, but rather a complex chain of responses characterized by the metabolism of the organism. The objectives of this study are:

1. To determine the difference in diffusive conductance among three types of sorghum: the bloom, the sparse-bloom, and the bloomless,

2. To determine the relationship among diffusive conductance, transpiration rate, wax cover, and stomatal density of each type of sorghum,

3. To determine the relationship between the four factors above (diffusive conductance, transpiration, degree of wax cover, and stomatal density) and the water potential of each type of sorghum,

4. And to compare the rate of water loss between the flag leaf and the third leaf down, and between the upper surface and the lower surface of the leaves.

CHAPTER II

LITERATURE REVIEW

Sorghum plants [*Sorghum bicolor* (L.) Moench] exude an epicuticular wax known as bloom. The amount of wax ranges from a heavy covering on bloom to a light covering on sparse-bloom. In 1941, while working with the world collection of sorghum in Coimbatore, India, Ayyangar and Ponnaiya (4) discovered that an African variety from Tanganyika by the name of Vigage (M.B.S. no A.S.4572) from the group *Sorghum elegans* did not have the wax exudate. They described this condition as bloomless, and determined that the bloom formation on sorghum leaves is controlled by a single dominant gene *BmBm*. The bloomless recessive allele is denoted by *bmbm*, and the spase-bloom gene by *hh*. Peterson, *et al.* (30) reported the occurrence of two independent genes controlling bloomlessness and three independent genes controlling sparse-bloom. The greenbug [*Schizaphis graminum* (Rondani)] has been shown to exhibit nonpreference for bloomless sorghum (29).

Cummins and Dobson (9) concluded that bloomless sorghum varieties were 22% higher in digestibility than the bloom varieties. However, Ross (32) using Combine Kafir-60 isogenics, found that the bloomless lines yielded less than the normal bloom lines.

Waxes are an important class of organic substances of plants that are often used commercially. Waxes occur as a protective coating on the epidermis, but may be deposited within cells, as for example, in the pericarp of certain seeds (16). Most plants contain too little wax to be valuable for commercial use except the wax palm tree *Copernicia cerifera* which yields carnauba wax and the jojoba *Simmondsia chinensis* which yields liquid wax (12). Chemically, waxes are fatty acid esters of monohydric alcohols found most commonly on the protective surface tissues. In a biological sense, waxes represent a variety of lipid classes ranging from nonpolar hydrophobic hydrocarbons to more polar semi-hydrophilic sterols (12).

Wax appears in combination with cutin and suberin, all of which are organic compound combinations found in the cell wall (12). Cutin is found in the epidermis and suberin is found in the protective tissue of the phloem. The cuticle which represents an incrustation of the outer cell wall is commonly covered with wax (12). This cover of epicuticular wax can either be in a smooth flat-lying form, or rods of filaments growing outward from the surface (4). Cutin and wax are synthesized in the living protoplast and migrate to the cell wall. There is no agreement as to whether these materials pass through the cell wall or through some other channels (teichodes). As the waxes migrate to the surface, they inpregnate the wall to some extent forming a protective tissue (12). This tissue may play an important role in the plant-insect relationship, and is an important barrier to

water loss (6,8,9,12,23,29,32,42).

It has been recently recognized that wax deposition on plants varies with water stress, drought resistance (4,11), photoperiod and temperature (15), and age (5,6, 13,17).

Recent ultrastructural and analytical investigations have revealed the chemical composition and structural features of plant wax (11,20,42).

In 1981, Wilkinson and Cummins (42) extracted waxes from ten leaf samples of the bloom, and the bloomless Redbine-60 near-isogenic lines. Then they separated them into classes via thin-layer chromatography (TLC), and quantitatively analyzed them via gas-liquid chromatography (GLC), utilizing both polar and nonpolar columns. They found the epicuticular wax composition to be 7% fatty acid, 31% fatty alcohol, and 61% alcanes, based on percent of the fresh weight. They also found that the bloomless sorghum genotypes had 57% less fatty acid + fatty alcohol + alcanes than the bloom genotypes, and that the bloomless lines had more accumulation of fatty alcohol, but no alcane synthesis. They concluded that quantitative differences in epicuticular wax may explain the resistance of sorghum to water loss.

Ebercon *et al.* (11) used the colorimetric method to quantitatively analyze wax on sorghum leaves. The method is based on the color change produced due to the reaction of wax with acidic $K_2Cr_2O_7$ reagent. They found that the amount of wax on the bloom genotypes ranged from 1.14 to 1.99 ($\pm$0.006) mg dm^{-2} of leaf.

Johnson *et al.* (20) overcame some limitations of the two

previous methods by using a method known as wide-line proton magnetic resonance (PMR) on wheat plants to determine the amount of wax. Their results are consistent with those obtained previously by Wilkinson and Cummins (42), and Ebercon *et al.* (11).

Blum (8) observed the characteristics of bloom and bloomless genotypes of sorghum leaves. He found that wax of the bloom type was deposited on the lower leaf surface. He also found that the epicuticular wax layer in the bloomless sorghum was thinner than that of the normal.

There is growing interest in wax content in plants or plant parts, and the role of wax in some important physiological processes of plants. It is agreed that wax plays an important role in the prevention of water loss in plants by interfering with the CO_2-water exchange through the stomatal openings (11,20,32,42).

Aspects of Drought and Drought Resistance Mechanisms of Plants

Drought can be defined as a period of time with insufficient rainfall, that reduces or disturbs the genetic growth potential of the organism. Another term that is often used to describe drought is water deficit. Water deficit is when plant metabolism is adversly affected by lack of water.

Drought resistance is the ability of the plant to either totally or partially avoid or tolerate the drought. In 1972, Levitt (25) defined drought resistance to include tolerance, escape, and avoidance components. In 1981 Jones

et al. (21) modified Levitt's definition of drought to include only escape and tolerance. Jones' philosophy is that once a plant is well established, it can no longer avoid drought.

Drought tolerance is when the drought enters the tissue and the plant still has a measure of resistance. The plant tissue approaches the energy equilibrium with the drought and damage is minimal. Drought escape is the resistance by escaping the period of drought. It involves the completion of the plant life cycle before the drought occurs. Drought avoidance on the other hand is a resistance by avoiding the energy equilibrium with the drought.

Most seed plants growing in the semi-arid environment must efficiently maximize their water use for a successful completion of their life cycle. The greater drought resistance of sorghum (26) is due to the plants ability to avoid severe wilting by retarding severe water loss primarily by producing a heavier waxy cuticle and more secondary roots.

Effects of Epicuticular Wax Deposition on Water Loss

Wax helps prevent desiccation, minimizes mechanical damage, and protects against excess ultra-violet radiation (7). The contribution of wax to the prevention of water loss was manifested in a reduction of solar energy load on the plant through increased reflectance, an avoidance of a reduced water potential, and maintenance of a more complete

stomatal control over transpiration.

Sanchez-Diaz *et al.* (33) proposed that wax filaments would also thicken the boundary layer, therefore increasing the diffusive resistance to gas exchange. The epicuticular wax deposited on the lamella is thick and amorphous and is covered by flakes of wax.

Working with leaf samples collected from bloom, sparse-bloom, and bloomless isogenic lines of sorghum, Jordan *et al.* (22) noticed that transpiration increased as epicuticular wax decreased over the range of 0.1 to 0.03 g cm^{-2}. Their data suggested that epicuticular wax in amounts greater than 0.067 g cm^{-2} constituted an effective barrier to water loss. The diffusive conductance to water vapor is controlled by a rather complex morphological, anatomical, and biochemical set of features. According to Schonherr (34), conductance is not directly related to the thickness of the cuticle, although high temperatures and water stress promote their thickness. Not only does the epicuticular wax play a role, but also the anatomy of the leaf is a factor in determining water loss of the plant.

Stomatal Control of Water Loss

Stomatal guard cells are known to play an active role in the physiological responses of a plant to its environment. They influence CO_2 exchange by controlling the stomatal aperture, and consequently affecting the rate of water loss of the plant. Changes in the stomatal aperture is not only a CO_2 flow regulating process; it is also an indication of

the moisture need, and moisture content of the plant. When moisture decreases, the stomata tend to give up their CO_2 regulating function to preserve water (24). Sullivan and Blum (36) mentioned that the stomata of sorghum remained slightly opened all day even during severe drought. This finding was supported by Turner (39) who indicated that maximum leaf conductance was maintained in sorghum until zero turgor potential is reached, at which point stomata close. Stomatal diffusive resistance is controlled by turgor-operated guard cells and adjacent epidermal cells. Stomatal diffusive conductance is a means of studying plant responses to drought. Several studies have been done to attempt to determine the utility of stomatal aperture as an indicator of plant drought resistance (14,19,24,27,31, 38,41). It is commonly known that leaf water stress induces partial stomatal closure. In many species, however, sorghum, for example, stomata are unaffected by leaf water status until the leaf water potential is reduced beyond a threshold level (1,28,38). This threshold level in sorghum is from - 1.5 to - 2.6 MPa.

CHAPTER III

MATERIALS AND METHODS

During the summer of 1984, field experiments were conducted at the Oklahoma State University Agronomy Research Station near Perkins, Oklahoma to determine the difference in the rate of water loss among three near-isogenic sorghum lines, ROKY62 bloom, ROKY62 sparse-bloom, and ROKY62 bloomless. After planting and emergence, visual observations on these single row plots of the dates of maximum wax deposition were made. Measurements of water loss factors were taken using a steady state porometer Li-Cor 1600. Two series of measurements at 1 hour intervals were taken every other day over a period of 2 weeks, beginning at boot stage and continuing through the grain filling stage.

The Li-Cor 1600 steady state porometer is a portable instrument designed for biological and industrial applications for the measurement of water loss and diffusive resistance. It is composed of the readout control console and the sensor head (Figure 1, Appendix). The readout console is equipped with several switches whose functions will be briefly described.

The leaf temperature switch displays the temperature of the thermocouple in contact with the leaf.

The relative humidity switch activates the percent

relative humidity inside the cuvette.

The diffusive resistance is displayed when the "Dif. Res." switch is activated.

The transpiration rate can be displayed when the corresponding switch is activated.

The quantum sensor switch displays the photosynthetically active rate (PAR) in μmol $m^{-2}s^{-1}$.

Making the measurements consisted of clamping a leaf sample, either attached to the plant or freshly cut, into the sensor head so that the leaf comes in contact with the thermocouple. The desired parameters can then be displayed on the control console.

Leaf temperature, PAR, transpiration rate, and diffusive resistance on both upper and lower leaf surfaces of the flag leaf and of the third leaf down, and the internal and the external surfaces of the leaf sheaths were measured.

The resistances of the lower surface of the leaf (r_{sb}) and that of the upper surface (r_{st}) were measured separately on adjacent portions of the leaf and the total leaf resistance (r_l) was calculated assuming that the two leaf surfaces acted as parallel resistors:

$$1/R_l = 1/R_{st} + 1/R_{sb}$$

Leaf conductance (g) was derived as the reciprocal of the leaf resistance ($1/R_l$), then converted to molar units (moles $m^{-2}s^{-1}$).

Similar experiments were conducted in the greenhouse during the fall of 1984, and the spring of 1985 using the

same three near-isogenic lines ROKY62 bloom, ROKY62 sparse-bloom, and ROKY62 bloomless, and 3 near-isogenic hybrids in a randomized complete block with three replications. The same parameters were again measured.

In the summer of 1985, the experiments were repeated at Perkins under dryland conditions and supplemental irrigation. Where no supplemental irrigation was provided, nine near-isogenic lines were used: ROKY62 bloom, ROKY62 sparse-bloom, ROKY62 bloomless, ROKY47 bloom, ROKY47 sparse-bloom, ROKY47 bloomless, ROKY78 bloom, ROKY78 sparse-bloom, and ROKY78 bloomless with no replications. Measurements of water loss factors were taken every other day on the flag leaf only, beginning at the boot stage and extending over a period of two weeks, using a photosynthesis system Li-Cor 6000. The system is designed to measure the CO_2 and water vapor exchange rate of plants or plant leaves. It is composed of a system console, a 40 key terminal and a leaf chamber to which is attached a sensor head (Figure 2, Appendix). The sensor head comprises the leaf temperature thermocouple, and a fan. The system principle is based on the water vapor concentration gradient and the saturation vapor concentration of the leaf.

Operating the instrument consists of clamping a leaf into the leaf chamber, and waiting for a few seconds for logging to be completed. The same water loss parameters were measured and in addition, the photosynthetic rate, and the leaf internal CO_2 content were recorded.

Where supplemental irrigation was applied, six near-

isogenic lines: ROKY62 bloom, ROKY62 sparse-bloom, ROKY62 bloomless, ROKY78 bloom, ROKY78 sparse-bloom, and ROKY78 bloomless were used, in a randomized complete block with three replications.

Using a modified technique of Sinclair and Dunn (35), and of Withman *et al.* (43), leaf peals were made in the summer of 1984 using finger nail polish as a plastic solution, to determine the stomatal density.

The technique consists of applying a thin layer of finger nail polish onto the leaf surface, let dry for two to three minutes. Then, using forceps, the finger nail polish is gently peeled to avoid distortion of the stomata. The peels are then placed on a clean microscopic slide and covered with a microscopic slide cover. The mounted slide can either be observed immediately or sealed with histoclad and stored for later study. The stomata can then be counted using a light microscope. Replicated measurements were recorded by moving the field of view from one area of the specimen to another.

In the summer of 1985, leaf water potential was measured using a leaf cutter thermocouple psychrometer. Collecting the samples consisted of placing a leaf over a hand held teflon block. Discs were cut by applying pressure with a twisting motion to the cutting edge of the psychrometer and the leaf. Eighteen samples from each near-isogenic line were taken. The samples were then sealed and placed in 30 C water bath for 2 h for the psychrometer to reach temperature and vapor equilibrium. The samples were then read using a

Wescor HP-115 water potential data system.

The data were analyzed, in a randomized complete block design, using the days of sampling as blocks in both field and greenhouse experiments.

CHAPTER IV

RESULTS AND DISCUSSION

Wax Deposition and Transpirational Loss

As pointed out by Atkin and Hamilton (3), Chatterton *et al.* (10), and several other workers, it was observed that wax deposition on sorghum leaves reaches its maximum 25 to 30 days after emergence. Some researchers (3,18,23) have also mentioned that wax deposition changes with the degree of drought stress. Based on observations of sorghum plants grown under rainfed conditions at Perkins in 1984, wax deposition increased drastically not only with plant age, but also with increasing drought. The leaves of sorghum also presented another morphological and/or physiological reaction to drought. This reaction was characterized by a rolling of leaves with the upper surface inside so that the lower surface was more exposed to solar radiation, and to other environmental factors. It is important to mention here that wax on sorghum plants is more heavily deposited on the lower surface of the leaves than on the upper surface. This rolling was observed to continue from as early as 1000 h until late in the afternoon, depending on the amount of moisture in the soil, humidity in the air, wind, the degree of cloudiness, and the increasing drought. Under these

conditions of constant stress, measurements of diffusive conductance among the bloom, the sparse-bloom, and the bloomless sorghum lines did not show significant differences. However, the data tended to verify the literature (10,32) in which the bloomless sorghum lines were shown to have higher rates of water loss than the bloom lines.

The measurements of diffusive conductance and transpiration rate of the flag leaf blade are shown in Table I.

TABLE I

DIFFUSIVE CONDUCTANCE AND TRANSPIRATION RATE OF THE FLAG LEAF BLADE, PERKINS, SUMMER 1984

Cultivar	Isogenic Lines	Conductance			Transpiration Rate
		Upper Surface	Lower Surface	Total	
		-----moles $m^{-2}s^{-1}$-----			mmoles $m^{-2}s^{-1}$
ROKY62	Bloom	0.060a	0.065a	0.125a	4.13a
ROKY62	Sparse-bloom	0.076a	0.068a	0.144a	4.72a
ROKY62	Bloomless	0.059a	0.073a	0.132a	3.09b

Means followed by the same letter do not differ significantly at 5% probability level.

There was however a signigicant difference in the transpiration rate among the bloom, the sparse-bloom, and the bloomless lines. The sparse-bloom line transpired at a rate which was 12% higher than the bloom line. It should be mentioned here that for statistical analysis purposes, days of measurements were considered as blocks. It was found

that a statistical difference existed among the days of sampling, at 5% probability level. This can be explained by the meteorological changes occurring during the growing season.

There were no statistical differences among the three near-isogenic lines for the total diffusive conductances of the flag leaf blade. However, the bloomless line was 6% higher than the bloom line. The data also showed that there was an interaction between the days of measurements and the degree of wax deposition. This suggested that during cloudy and cool days, or during hot and dry days, the three near-isogenic lines behave differently with regard to water loss. The results observed were similar to those of Muchow *et al.* (28), Akerson *et al.* (1), and Turner (38) who pointed out that up to a certain point in increasing drought, the bloom and the bloomless lines responded differently to water loss. But when the drought reaches a certain threshold level, no differences can be detected between the two lines. The lower surface of the blade was also observed, in two of three cases, to be 8 and 19% higher in diffusive conductance than the upper surface of the bloom and the bloomless lines, respectively. Similar results were obtained by Teare and Kanemasu (37).

Further investigations were conducted at the flag leaf sheath level. The results are presented in Table II. For total diffusive conductance, the sparse-bloom line was statistically higher (35 and 24%) than the bloom and the bloomless lines, respectively. The bloomless line was 14% higher

than the bloom line, but it was not a significant difference.

TABLE II

DIFFUSIVE CONDUCTANCE OF THE FLAG LEAF SHEATH, PERKINS, SUMMER 1984

Cultivar	Isogenic Lines	External Surface	Internal Surface	Total
		-------- moles $m^{-2}s^{-1}$ --------		
ROKY62	Bloom	0.060a	0.066a	0.126a
ROKY62	Sparse-bloom	0.104b	0.089a	0.193b
ROKY62	Bloomless	0.086a	0.061a	0.147a

Means followed by the same letter do not differ significantly at 5% probability level.

The sheath is an envelop overlapping 75 to 100% of the stem. There was a continuous condensation on the internal surface. Free water was found in all cases. The measurements were taken after the surface had been blotted to remove the free water, while causing little or no damage to the structural features of the sheath. It was found that the external sheath surface was 15 and 30% higher in diffusive conductance than the internal surface of the sparse-bloom and the bloomless lines, respectively.

Similar measurements were recorded on the blade of the third leaf down and its sheath. The results on the third leaf blade presented in Table III demonstrated that the sparse-bloom line was 30% higher in transpiration rate than

the bloomless line, but the bloomless line was 22% lower than the bloom line. The diffusive conductance followed the same pattern as the transpiration rate in the sense that the sparse-bloom was 26% higher than the bloom line, and 25% higher than the bloomless line.

TABLE III

DIFFUSIVE CONDUCTANCE AND TRANSPIRATION RATE OF THE THIRD LEAF BLADE, PERKINS, SUMMER 1984

Cultivar	Isogenic Lines	Conductance Upper Surface	Conductance Lower Surface	Conductance Total	Transpiration Rate
		-----moles $m^{-2}s^{-1}$-----			mmoles $m^{-2}s^{-1}$
ROKY62	Bloom	0.066a	0.070a	0.135b	4.10ab
ROKY62	Sparse-bloom	0.089a	0.095a	0.183a	4.60a
ROKY62	Bloomless	0.061a	0.077a	0.138b	3.20b

Means followed by the same letter do not differ significantly at 5% probability level.

When the upper and the lower surfaces of the blade were compared, the lower surfaces were 6, 6, and 22% higher in diffusive conductance than the upper surfaces of the bloom, the sparse-bloom, and the bloomless lines, respectively.

No statistical differences were found at the third leaf sheath (Table IV), but there was a consistent trend similar to that observed for the flag leaf sheath, with the bloomless line having 11 and 1% higher diffusive total conductance than the sparse-bloom and the bloom lines,

respectively. The internal surface showed a reversed order of expectance by being higher in diffusive conductance than the external surface.

TABLE IV

DIFFUSIVE CONDUCTANCE OF THE SHEATH OF THE THIRD LEAF, PERKINS, SUMMER 1984

Cultivar	Isogenic Lines	External Surface	Internal Surface	Total
		-------moles $m^{-2}s^{-1}$-------		
ROKY62	Bloom	0.072a	0.083a	0.155a
ROKY62	Sparse-bloom	0.067a	0.072a	0.139a
ROKY62	Bloomless	0.076a	0.081a	0.157a

Means followed by the same letter do not differ significantly at 5% probability level.

Physiological Role of the Third Leaf Down in Water Loss

To compare the rate of water loss between the flag leaf and the third leaf down, a summary of all the data related to water loss was compiled in Table V. Based on the observations, the third leaves down were 8, 11, and 4% higher in diffusive conductance than the flag leaves of the bloom, the sparse-bloom, and the bloomless lines, respectively. The transpiration rates of the third leaves on the other hand were similar to those of the flag leaves. A preliminary conclusion can be drawn here based on these

comparisons. The third leaf down was involved in the study because it is in most cases the largest leaf. The flag leaf on the other hand plays an important physiological role in providing photosynthates to the plant's reproductive organs.

TABLE V

COMPARISON OF DIFFUSIVE CONDUCTANCE AND TRANSPIRATION RATE OF THE FLAG LEAF AND THIRD LEAF BLADES OF ROKY62, PERKINS, SUMMER 1984

Leaf	Isogenic Lines	Diffusive Conductance	Transpiration Rate
		moles $m^{-2}s^{-1}$	mmoles $m^{-2}s^{-1}$
Flag	Bloom	0.125	4.13
	Sparse-bloom	0.163	4.72
	Bloomless	0.132	3.09
Third	Bloom	0.135	4.10
	Sparse-bloom	0.183	4.60
	Bloomless	0.138	3.20

No statistical analyses were involved between leaves. The analyses within leaves and among isogenic lines were performed and presented in previous tables.

Teare and Kanemasu (37) observed that in the afternoon, there was an increased stomatal closure and a reduced stomatal conductance of the upper leaves as compared to the lower leaves in the plant canopy. The results in this study also showed a tendency for a decreased diffusive conductance in the upper leaves. Morgan (27) found that plants under

drought maintained open stomata but had a low transpiration rate because of the reduced leaf area. Morgan's findings were confirmed by comparing the field and the greenhouse results where the magnitude of the data from the greenhouse was smaller than the magnitude of the data from the field. The larger values of diffusive conductance of the third leaf down may be due to its importance to the plant's photosynthesis. The flag leaf could be an important storage site of the carbohydrates to the point that water is more limiting than photosynthesis. Therefore, stomatal closure on the flag leaf blade is more frequent than on the third leaf. The flag leaf is nearer to the panicle than the third leaf down, and more exposed to solar radiation. One might expect it to loose more water (therefore ensuring high transpiration rate) than the third leaf down, or other supporting leaves.

In all cases, it was observed that the stomata responded to continuous drought conditions as if they had been conditioned by preexisting days of drought and that their normal functions no longer depended on the amount of wax cover, but on the need for carbohydrate production as observed by Wong *et al.* (44).

Questions have been raised about the role of the sheath in the rate of water loss even though there is constantly free water in its internal surface. For this reason, results were summarized for the diffusive conductances of both flag leaf and third leaf down, and of their sheaths in Table VI. The third leaf sheath was 19 and 7% higher than

the flag leaf sheath of the bloom, and the bloomless lines, respectively, as observed previously on blades of the leaves. The data in Table VI also showed that there is greater diffusive conductance on the leaf sheath than on the leaf blade. The leaf sheath has a relatively higher wax cover than the leaf blade and still the average diffusive conductance of the sheath was 12% higher than the blade. The sheath always has free water internally, and it may be that its water status is satisfactory enough to maintain normal physiological activities. Further investigations may bring detailed information on the physiological importance of the sheath of the plant under drought conditions.

TABLE VI

COMPARISON OF DIFFUSIVE CONDUCTANCE OF THE SHEATHS OF THE FLAG LEAF AND THE THIRD LEAF OF ROKY62, PERKINS, SUMMER 1984

Leaf	Isogenic Lines	Blade	Sheath
		-----moles $m^{-2}s^{-1}$-----	
Flag	Bloom	0.125	0.126
	Sparse-bloom	0.163	0.193
	Bloomless	0.132	0.147
Third	Bloom	0.135	0.155
	Sparse-bloom	0.183	0.139
	Bloomless	0.138	0.157

No statistical analyses were involved between leaves. The analyses within leaves and among isogenic lines were performed and presented in previous tables

Stomatal Density, a Factor in Water Loss

Stomatal density was determined on the three near-isogenic lines to obtain further information related to the rate of water loss. The results presented in Table VII showed that the ratio of upper to lower stomatal density was statistically different for the flag leaf blade among the bloom, the sparse-bloom, and the bloomless lines. The ratio of stomata between the upper and the lower surfaces may help explain the consistently higher total diffusive conductance observed on the lower surfaces of the leaf blades. Sixty-seven percent of the stomata were on the lower surface of the leaf, confirming results obtained by Teare and Kanemasu (37).

TABLE VII

COMPARISON OF STOMATAL DENSITY BETWEEN THE FLAG LEAF AND THIRD LEAF BLADES OF ROKY62, PERKINS,SUMMER 1984

Leaf	Isogenic Lines	Upper Surface	Lower Surface	Total	Ratio	% Stomata on Lower Surface
		-----Stomata cm^{-2}-----				
Flag	Bloom	6897	20115	27012	1:2.7b	74
	Sparse-bloom	10115	17816	27931	1:1.7a	64
	Bloomless	9196	22977	32173	1:2.5b	71
Third	Bloom	9885	15977	25862	1:1.5a	62
	Sparse-bloom	5977	13448	19425	1:2.2a	69
	bloomless	10920	17701	28621	1:1.6a	62

Means followed by the same letter do not differ significantly at 5% probability level.

Environmental Effects on the Rate of Water Loss

Muchow *et al.* (28) pointed out that when investigating physiological processes of a plant, the place where the experiment was conducted may influence the data. These authors also showed that studies under field conditions give more accurate and realistic results. Nevertheless, experiments were conducted in the greenhouse in the fall of 1984. The results presented in Table VIII, support those obtained in the summer of 1984 under field conditions. Perhaps because of the more controlled environment in the greenhouse, the magnitude of the data on the diffusive conductance and transpiration rate was smaller.

TABLE VIII

COMPARISON OF DIFFUSIVE CONDUCTANCE OF THE FLAG LEAF AND THE THIRD LEAF BLADES OF ROKY62, GREENHOUSE, FALL 1984

Leaf	Isogenic Lines	Upper Surface	Lower Surface	Total
		moles $m^{-2}s^{-1}$		
Flag	Bloom	0.032	0.052	0.084a
	Sparse-bloom	0.035	0.037	0.072a
	Bloomless	0.027	0.039	0.067a
Third	Bloom	0.034	0.037	0.071a
	Sparse-bloom	0.050	0.038	0.088a
	Bloomless	0.026	0.030	0.056b

Means followed by the same letter do not differ significantly at 5% probability level.

The experiment was repeated in the spring of 1985 in the greenhouse. Again similar data of smaller magnitude for diffusive conductance and transpiration rate were recorded as shown in Table IX. Sparse-bloom ROKY62 had a significantly higher rate of transpiration compared to bloom and bloomless. Significant differences were not found in diffusive conductance among the three near-isogenic lines in either greenhouse study. The results confirmed those obtained in field conditions where the lower surfaces were higher in diffusive conductance than the upper surfaces.

TABLE IX

COMPARISON OF DIFFUSIVE CONDUCTANCE AND TRANSPIRATION RATE, GREENHOUSE, SPRING 1985

Cultivars	Isogenic Lines	Conductance Upper Surface	Conductance Lower Surface	Conductance Total	Transpiration Rate
		---moles $m^{-2}s^{-1}$----			mmoles $m^{-2}s^{-1}$
ROKY62	Bloom	0.030	0.049	0.079a	1.61a
ROKY62	Sparse-bloom	0.039	0.065	0.104a	4.03b
ROKY62	Bloomless	0.042	0.085	0.127a	1.68a
AOK11xROKY62	Bloom	0.054	0.076	0.130a	2.18a
AOK11xROKY62	Sparse-bloom	0.040	0.063	0.103a	2.04a
AOK11xROKY62	Bloomless	0.034	0.068	0.101a	2.19a

Means followed by the same letter do not differ significantly at 5% probability level.

Similar experiments were repeated in the summer of 1985

under field conditions. Two varieties, ROKY62 and ROKY78, each having the bloom, the sparse-bloom, and the bloomless near-isogenic lines, were tested under supplemental irrigation. In addition, three varieties ROKY62, ROKY78, and ROKY47, each again having the bloom, the sparse-bloom, and the bloomless near-isogenic lines were tested under rainfed conditions. The results from the site where supplemental irrigation was available are shown in Table X. The statistical analysis suggested large fluctuations between the days of measurements. These observed differences may be attributed, as mentioned above, to the daily changing environmental conditions. There were statistical differences between the varieties ROKY62 and ROKY78, in transpiration rate and diffusive conductance. Since statistical differences existed between the two varieties, the reaction of each variety to water loss was investigated separately. No statistical differences exited among the bloom, the sparse-bloom, and the bloomless lines of either variety. However, the bloomless ROKY62 was slightly higher in diffusive conductance than its bloom and sparse-bloom, counterparts, respectively. And the sparse-bloom was slightly higher than the bloom line. The investigations on ROKY62 confirmed the linear relationship observed between the transpiration rate and the diffusive conductance observed by Aston (2). ROKY78 followed the same pattern in the sense that the sparse-bloom line exceeded the bloom line by 8% in diffusive conductance. A trend similar to that of diffusive conductance was again observed with the

transpiration rate.

TABLE X

MEANS OF DIFFUSIVE CONDUCTANCE AND TRANSPIRATION RATE, PERKINS, SUMMER 1985 SUPPLEMENTAL IRRIGATION

Cultivars	Isogenic Lines	Diffusive Conductance	Transpiration Rate
		moles $m^{-2}s^{-1}$	mmoles $m^{-2}s^{-1}$
ROKY62	Bloom	0.180a	5.24a
ROKY62	Sparse-bloom	0.183a	5.41a
ROKY62	Bloomless	0.184a	5.42a
ROKY78	Bloom	0.194a	5.68a
ROKY78	Sparse-bloom	0.211a	5.90a
ROKY78	Bloomless	0.184a	5.52a

Means followed by the same letter do not differ significantly at 5% probability level.

The near-isogenic lines of ROKY62, ROKY78, and ROKY47 were investigated under rainfed conditions. The results are presented in Table XI. It is important to mention here that extreme leaf rolling was observed at this site. Since wax is mainly thought to be important in areas where scanty rainfall is predominant, and where no supplemental irrigation is available, particular attention was given to this location. No statistical differences were observed among varieties, or among the near-isogenic lines. However, the bloomless ROKY62 was 37 and 18% higher in diffusive conductance than the sparse-bloom and the bloom,

respectively. The transpiration rate of the bloomless was 31 and 17% higher than that of the sparse-bloom and the bloom lines, respectively. ROKY78 showed similar patterns in diffusive conductance and transpiration rate, with the sparse-bloom being 32 and 39% above the bloom and the bloomless lines. ROKY47 on the other hand showed a totally reversed order of magnitude with the bloom line being higher than the sparse-bloom and the bloomless lines in diffusive conductance and transpiration rate.

TABLE XI

MEANS OF DIFFUSIVE CONDUCTANCE AND TRANSPIRATION RATE, PERKINS, SUMMER 1985
RAINFED CONDITIONS

Cultivars	Isogenic Lines	Diffusive Conductance	Transpiration Rate
		moles $m^{-2}s^{-1}$	mmoles $m^{-2}s^{-1}$
ROKY62	Bloom	0.101a	4.60a
ROKY62	Sparse-bloom	0.080a	3.87a
ROKY62	Bloomless	0.127a	5.59a
ROKY78	Bloom	0.087a	4.32a
ROKY78	Sparse-bloom	0.128a	4.88a
ROKY78	Bloomless	0.079a	4.39a
ROKY47	Bloom	0.122a	4.73a
ROKY47	Sparse-bloom	0.093a	4.20a
ROKY47	Bloomless	0.071a	3.34a

Means followed by the same letter do not differ significantly at 5% probability level.

Water Potential and Wax Deposition

To better understand the phenomenon relating transpiration rate and diffusive conductance to wax deposition, measurements of water potential at both sites were conducted (summer 1985). Only one site (under supplemental irrigation) was analyzed. The results are presented in Table XII. No statistical differences were observed between varieties. However, there were statistical differences among the bloom, the sparse-bloom, and the bloomless lines of both varieties. ROKY62 bloomless was 10 and 16% more negative than the sparse-bloom and the bloom, respectively. The sparse-bloom in turn was 6% more negative than the bloom line. Similar results were observed on ROKY78, where the bloomless was 14 and 16% more negative than the sparse-bloom and the bloom line, respectively. The sparse-bloom was again 2% less negative than the bloom line.

TABLE XII

MEANS OF WATER POTENTIAL, PERKINS, SUMMER 1985
SUPPLEMENTAL IRRIGATION

Isogenic Lines	Cultivars ROKY62	ROKY78
	----------MPa----------	
Bloom	-1.43a	-1.42a
Sparse-bloom	-1.53a	-1.45a
Bloomless	-1.70b	-1.69b

Means followed by the same letter do not differ significantly at 5% probability level.

Throughout the course of this study, the sparse-bloom lines were in most cases relatively higher in diffusive conductance and transpiration rate than the bloom or the bloomless lines. These differences could not be traced to the stomatal density or to the water potential. Nor do the observations of the visible wax deposition on the leaves, explain the highest diffusive conductances and transpiration rates of the line.

Photosynthesis Related to Wax Deposition

A separate study on the rate of photosynthesis is reported in Table XIII.

TABLE XIII

MEANS OF PHOTOSYNTHETIC RATE, WITH AND WITHOUT SUPPLEMENTAL IRRIGATION, PERKINS, SUMMER 1985

Cultivars	Isogenic Lines	Photosynthetic Rate With Irrigation	Photosynthetic Rate Without Irrigation
		μmoles $m^{-2}s^{-1}$	μmoles $m^{-2}s^{-1}$
ROKY62	Bloom	19.81a	14.05a
ROKY62	Sparse-bloom	21.28b	10.54a
ROKY62	Bloomless	18.94a	15.06a
ROKY78	Bloom	22.07b	12.24a
ROKY78	Sparse-bloom	22.45b	14.73a
ROKY78	Bloomless	19.06a	10.89a
ROKY47	Bloom		15.61a
ROKY47	Sparse-bloom		14.04a
ROKY47	Bloomless		11.29a

Means followed by the same letter do not differ significantly at 5% probability level.

It was found that, where supplemental irrigation was applied, the sparse-bloom was statistically higher than the bloom and the bloomless lines for ROKY62 and ROKY78. Under rainfed conditions, on the other hand, no statistical differences were obtained. However, the bloomless ROKY78 and the bloomless ROKY47 were 11 and 28% lower than their bloom counterparts. A similar trend with the variety ROKY62 was not detected.

Some questions still need to be answered in defining the effects of the epicuticular wax on the rate of water loss. It is well documented that wax helps reduce the radiation load on the plants, and helps prevent water loss. The epicuticular wax deposition does not seem to be the sole factor to consider when determining the rate of water loss of the sorghum plant, although the bloomless lines showed a trend for higher rates of transpiration and diffusive conductance than the bloom lines.

To what extent can wax prevent water from escaping from the sub-stomatal cavity, may depend on other physiological and/or anatomical factors of the plant. As Kanemasu (24) pointed out, the status of stomata in a plant is dynamic and changes in response to many environmental factors. Various researchers (1,24,40) have indicated that selection solely based on stomatal responses to water stress has limitations. This is probably due partly to characteristic changes due to the stage of development (age), or to mild stress preconditioning.

CHAPTER V

SUMMARY AND CONCLUSIONS

Two years of experiments were conducted to determine the rate of water loss of three near-isogenic lines of sorghum, the bloom, the sparse-bloom, and the bloomless. In 1984, single row plots of three near-isogenic lines of ROKY62 were tested at the Oklahoma State University Agronomy Research Station near Perkins. Two levels in the crop canopy were sampled for the study, the flag leaf and the third leaf down. Measurements of transpiration rate and diffusive conductance were recorded for the upper and the lower surfaces of the blades, and the internal and the external surfaces of the sheaths. Two measurements at 1-hour intervals, were recorded every other day, beginning from boot stage until the grain filling stage, constituting 6 days of measurements. The days of sampling were used as blocks for statistical analysis. Stomatal density on both surfaces of the leaf blades were also determined by a modified plastic solution method.

During the fall of 1984, and the spring of 1985, similar experiments were conducted in the Oklahoma State University Agronomy Department sorghum greenhouse to support the observations under field conditions.

In the summer of 1985, the experiments were repeated at

two places on the Oklahoma State University Agronomy Research Station near Perkins. At one site, two varieties (ROKY62 and ROKY78) both having the bloom, the sparse-bloom, and the bloomless near-isogenic lines were tested in a randomized complete block with three replications under supplemental irrigation. At the second site, representing the rainfed condition, three varieties (ROKY62, ROKY78, and ROKY47), all having the bloom, the sparse-bloom, and the bloomless near-isogenic lines were tested. In addition to diffusive conductance and transpiration rate, measurements of photosynthetic rate and water potential were recorded.

It was observed that the wax deposition on the sorghum leaves reached its maximum 25 to 30 days after emergence. This wax cover was also observed to be enhanced by the drought stress level. When the drought stress reaches a certain point, the sorghum plant tends to reduce its leaf area through rolling, and exposure of the lower surface to the solar radiation. The rolling was thought to be motivated by a need of the plant to reduce the transpiring surface and conserve water and to reflect more solar radiation thereby reducing the energy load on the plant. This water budget seems to be more crucial in C_3 plants than C_4 plants like sorghum. Sorghum being a C_4 plant has the capability to recycle the internal CO_2 to ensure photosynthesis. A C_3 plant on the other hand, has to maintain a detrimental stomatal opening even under severe drought conditions, to maintain a normal level of internal CO_2 and photosynthesis. Wax cover by itself can be regarded as a

mechanism to reduce the transpiration from the surface of the plants.

Statistical differences were observed in the rate of transpiration among the bloom, the sparse-bloom, and the bloomless lines, while no differences were observed in the diffusive conductance. In most cases, higher diffusive conductances persisted in the sparse-bloom lines. Under field conditions, the days of sampling were statistically different. This may be due to the changing climatic conditions from day to day during the growing season. In the greenhouse, varietal differences were detected. The difference between varieties suggested that the diffusive conductance or transpiration rate may be used as a selection tool for drought resistance. Breeding for drought resistance so far has consisted of growing genotypes in an experimental plot, and allowing the natural occurrence of the drought to act on the genotypes. The genotypes that survived the natural or imposed drought are selected and put into a breeding program. The problem associated with this conventional drought resistance breeding method is that there are differences associated with genetic differences. With the use of isogenic lines, this problem has been solved. Drought resistance selection can either be within bloomless lines or within bloom lines by studying their diffusive conductances or transpiration rates. Selection within the bloom lines might identify heavier than normal wax as proposed by Ross (32). Breeding for "Super Wax" for use in dryland agriculture could be a means of increasing

yield by maintaining higher plant water content, and consequently maintaining higher rates of photosynthesis.

In both field and greenhouse experiments, the lower surfaces of the leaves showed higher diffusive conductances than the upper surfaces. In the field experiments as well as in the greenhouse experiments a greater rate of water loss from the third leaf down than from the flag leaf blades was observed. These findings also held true for the sheaths. When the leaf blades and the leaf sheaths were compared, the sheaths were shown to have higher diffusive conductance than the blades.

The rates of photosynthesis of the bloomless lines were in most cases found to be lower than that of the bloom and the sparse-bloom lines. Wax deposition undoubtedly has a function in the plant drought resistance mechanism, but the major factor determining the water loss of a plant is the number of stomata per unit of leaf area. The investigation of the stomatal density showed that the lower surfaces of either flag leaf or third leaf down had significantly more stomata than the upper surfaces. Water potential of the bloomless lines was statistically more negative (indicating more loss of water) than that of the sparse-bloom, or the bloom lines.

Several factors have been investigated, along with wax deposition to determine the rate of water loss among near-isogenic lines of sorghum. The contribution of the wax deposition to water loss from the sorghum leaves and sheaths has been studied here. However, the study raised additional

questions. Further study on the biochemical nature, physiological effects, and/or cuticular characteristics of the sorghum wax will probably bring about definitive answers to the role of the epicuticular wax on the rate of water loss of sorghum.

LITERATURE CITED

1. Akerson, R. C., D. R. Krieg, and F. J. M. Sung. 1980. Leaf conductance and osmoregulation of field-grown sorghum genotypes. Crop Sci. 20:10-14.

2. Aston, M. J. 1967. The relationship between transpiration and water uptake of sunflower (*Helianthus annuus*, cultivar Advance) in relation to some environmental factors. Ph. D. Thesis. University of California, Davis.

3. Atkin, D. S. J., and R. J. Hamilton. 1982. The changes with age in the epicuticular wax of *Sorghum bicolor*. J. Nat. Products. 45:697-703.

4. Ayyangar, G. N. R., and B. W. X. Ponnaiya. 1941. The occurence and inheritance of a bloomless sorghum. Current Sci. 10:408-409.

5. Bengtson, C., S. Larson, and C. Lifjenberg. 1979. p.269. *In* L.A. Appelquist and C. Lifjenberg (ed) A.D.V. Biochemistry and Physiology of Plant Lipids.

6. Bianchi, G., and M. Corbellini. 1977. Epicuticular wax of *Triticum aestivum* Demar 4. Phytochemistry. 8:943.

7. Blum, A. 1975a. Effect of the *Bm* gene on epicuticular wax and water relations of *Sorghum bicolor*. (L.) Moench. Isr. J. Bot. 24:50-51.

8. Blum, A. 1975b. Effect of the Bm gene on epicuticular wax deposition and the spectral characteristics of sorghum leaves. Sabrao J. 7:45-52.

9. Cummins, D. G., and J. W. Dobson, Jr. 1972. Digestibility of bloom and bloomless sorghum leaves determined by a modified in vitro technique. Agron. J. 64:682-683.

10. Chatterton, N. J., W. W. Hanna, J. B. Powel, and D. R. Lee. 1975. Photosynthesis and transpiration of bloom and bloomless sorghum.. Can. J. Plant Sci. 55:641-643.

11. Ebercon, A., A. Blum, and W. R. Jordan. 1977. A rapid colorimetric method for epicuticular wax content of sorghum leaves. Crop Sci. 17:179-180.

12. Essau, K. 1977. Anatomy of seed plants. 2nd ed. John Wiley and Sons Pub. Inc., New York, NY.

13. Faboya, O. O. P., J. I. Okogun, and D. Goddard. 1980. Dependence of the hydrocarbon constituents of the leaf wax of *Khoya* species on leaf age. Phytochemistry. 19:1226-1227.

14. Garrity, D. P., C. Y. Sullivan, and D. G. Watts. 1984. Changes in grain sorghum stomatal and photosynthetic response to moisture stress across growth stages. Crop Sci. 24:441-446.

15. Gneise, B. N. 1975. Effects of light and temperature on epicuticular wax of barley leaves. Phytochemistry. 14:921.

16. Hadley, N. F. 1980. Surface wax and intertegumentary permeability. Am. Scientist. 68:546-553.

17. Hamilton, R. J., and D. M. Power. 1969. The chemical composition of the surface wax of *Lolium perenne*. Phytochemistry. 8:1771-1775.

18. Henzel, R. G., K. J. McGree, C. H. M. VanBaval, and K. F. Shertz. 1975. Method for screening sorghum genotypes for stomatal sensitivity to water deficits. Crop Sci. 15:516-518.

19. Johnson, C. B. 1981. p.110-199. *In* Butterworth (ed). Physiological Processes limiting plant productivity. Butterworths, London, England.

20. Johnson, D. A., M. L. Tonnet, and R. A. Richards. 1984. Estimation of epicuticular wax amount in wheat using wide-line proton magnetic resonnance. Crop Sci. 24:679-682.

21. Jones, M. M., N. C. Turner, and C. B. Osmond. 1981. Mechanism of drought resistance. p.15-37. *In* T. G. Paleg and D. Aspinall. (ed). The physiology and biochemistry of drought resistance in plants. Academic Press, New York, NY.

22. Jordan, W. R., R. L. Monk, F. R. Miller, D. T. Rosenow, L. E. Clark, and P. J. Shouse. 1983. Environmental physiology of sorghum. I. Environmental and genetic control of epicuticular wax load. Crop Sci. 23:552-558.

23. Jordan, W. R., P. J. Shouse, A. Blum, F. R. Miller, and R. L. Monk. 1984. Environmental physiology of sorghum. II. Epicuticular wax load and cuticular transpiration. Crop Sci. 24:1168-1173.

24. Kanemasu, T. E. 1975. Measurement of stomatal aperture and diffusive resistance. College of Agri. Res. Center. Washington State University Bull. 809:1-2.

25. Levitt, J. 1972. Responses of plants to environmental stresses. Academic Press, New York, NY.

26. Martin, J. H. 1930. The comparative drought resistance of sorghum and corn. Agron. J. 22:993-1003.

27. Morgan, J. M. 1977. Changes in diffusive conductance and water potential of wheat plants before and after anthesis. Austr. J. Plant Physiol. 4:75-86.

28. Muchow, R. C., M. J. Fisher, M. M. Ludlow, and J. K. Myers. 1980. Stomatal behavior of kenaf and sorghum in a semi-arid environment. II. During the day. Austr. J. Plant Physiol. 7:621-628.

29. Peiretti, R. A., Iraj Amini, D. E. Weibel, K. F. Starks, and R. W. McNew. 1980. Relationship of "bloomless" (*bmbm*) sorghum to greenbug resistance. Crop Sci. 20:173-176.

30. Peterson, G. C., K. Suksayretrup, and D. E. Weibel. 1982. Inheritance of some bloomless and sparse bloom mutants in sorghum. Crop Sci. 22:63-67.

31. Raschke, K, and U. Kuhl. 1969. Stomatal responses to changes in atmospheric humidity and water supply: experiment with leaf sections of *Zea mays* in CO2 free air. Planta. 87:36-48.

32. Ross, W. M. 1972. Effect of bloomless (*blbl*) on yield in Combine Kafir-60. Sorghum Newsletter. 15:121.

33. Sanchez-Diaz, M. E., J. D. Hesketh, and F. J. Kramer. 1972. Wax filaments on sorghum leaves as seen with scanning electron microscope. J. Ariz. Acad. Sci. 7:6-7.

34. Shronherr, J. 1976. Water permeability of cuticular membrane. *In* O. L. Kappe, and D. E. Schulze (ed). Water and plant life. Springer-Verlag, New York, NY.

35. Sinclair, C. B., and D. B. Dunn. 1961. Surface printing of plant leaves for phylogenic studies. Stain Technol. 3:299-304.

36. Sullivan, C. Y., and A. Blum. 1970. Drought and heat resistance of sorghum and corn. Proc. Annu. Corn and Sorghum Res. Conf. 25:55-66.

37. Teare, I. D., and T. E. Kanemasu. 1972. Stomatal diffusion resistance and water potential of soybean and sorghum leaves. New Phytol. 71:805-810.

38. Turner, N. C. 1974a. Stomatal behavior and water status of maize, sorghum, and tobacco under field conditions. II. At low water potential. Plant Physiol. 53:336-365.

39. Turner, N. C. 1974b. Stomatal response to light and water under field conditions. R. Soc. N. Z. Bull. 12:423-432.

40. Turner, N. C., J. E. Begg, H. M. Rawson, S. D. English, and A. B. Hearn. 1978. Agronomic and physiological responses of soybean and sorghum crops to water deficits. III. Components of leaf water potential, and adaptation to water deficits. Austr. J. Plant Physiol. 5:178-194.

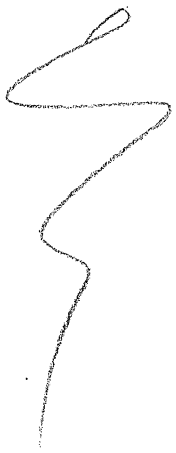
41. Turner, N. C., J. E. Begg, and M. L. Tonnet. 1978. Osmotic adjustment of sorghum and sunflower crops in response to water deficits and its influence on the water potential at which stomata close. Austr. J. Plant Physiol. 5:597-608.

42. Wilkinson, R. E., and D. G. Cummins. 1981. Epicuticular fatty acid, fatty alcohol, and alcane content of sorghum 'Redbine 60' leaves. Crop Sci. 21:397-400.

43. Withman, F. M., D. F. Blaydes, and R. M. Devlin. 1971. Stomates. p.103-107. *In* Experiments in plant physiology. Van Nostrand Reinhold Co., New York, NY.

44. Wong, S. C., I. R. Cowan, and G. D. Farquhar. 1979. Stomatal conductance correlates with photosynthetic capacity. Nature. 282:424-426.

APPENDIX



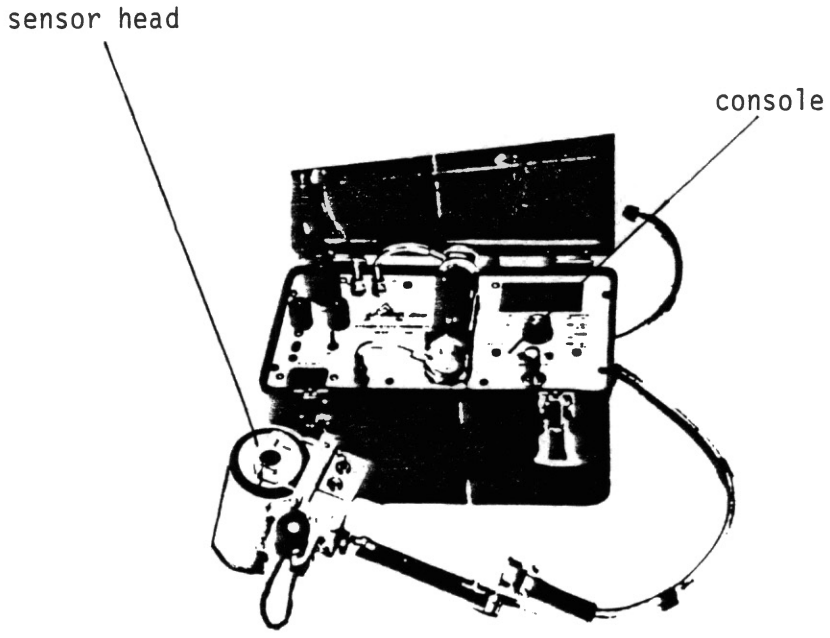


Fig. 1. Discriptive Figure of the Steady State Porometer Li-1600, Showing the Readout Control Console, and the Sensor Head

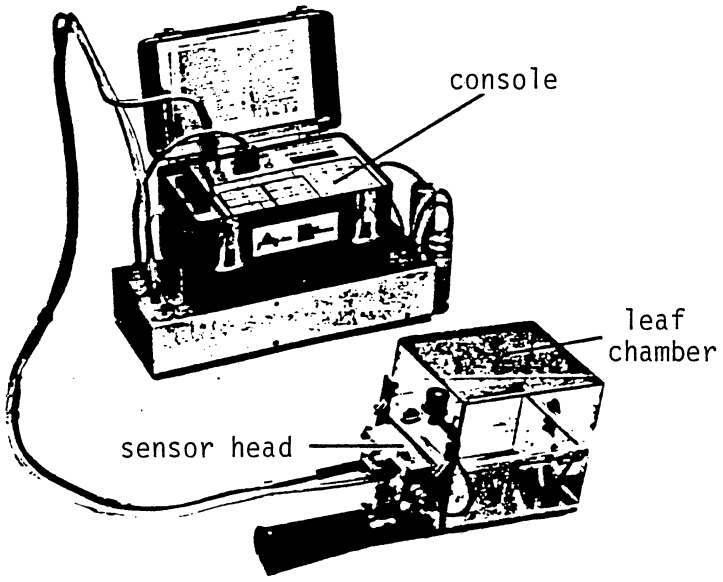


Fig. 2. Descriptive Figure of the Portable Photosynthesis System Li-6000, Showing the Readout Control Console, the Leaf Chamber, and the Sensor Head

VITA

Mijitaba Hamissou

Candidate for the Degree of

Master of Science

Thesis: THE EFFECTS OF EPICUTICULAR WAX ON THE RATE OF WATER LOSS OF SORGHUM BICOLOR (L.) MOENCH

Major Field: Agronomy

Biographical:

Personal Data: Born in Bande (Magaria), Republic of Niger on September 7, 1952, the son of Malam Hamissou Alkali Souley and Fatouma Liman Moumouni.

Education: Graduated from the Lycee National du Niger with the Baccalaureat Degree Mathematics,Physics and Natural Sciences in June 1973; completed two years at the University of Niger, Niamey 1973-1975; received the Bachelor of Science Degree in Agronomy from Oklahoma State University in May 1985; completed the requirements for the Master of Science Degree at Oklahoma State University in May, 1987.

Professional Experience: Trainee in Sorghum and Millet Breeding at the International Crops Research Institute for the Semi-Arid Tropics, ICRISAT, India for 10 months. Sorghum and Millet breeder at the Institut National des Recherches Agronomiques du Niger, INRAN from 1976 to 1981. Director of the Agricultural Experiment Station INRAN, Kolo, 1976-1977. Technical training Officer, Centre National des Recherches Agronomiques, CNRA-INRAN, Tarna, 1977-1978. Director of the Sugar Cane Research Station, INRAN, Tillabery, 1978-1980. Joint Head of Rice Research Laboratory, INRAN, Niamey, 1980-1981.