ULTRASTRUCTURAL AND BEHAVIORAL STUDY OF GREENBUG FEEDING ON SORGHUM PLANTS UNDER DROUGHT STRESS; MICROTUBULE DISTRIBUTION IN SORGHUM CALLUS

BY

MIJITABA HAMISSOU

Bachelor of Science in Agriculture
Oklahoma State University
Stillwater, Oklahoma
1985

Master of Science in Agriculture
Oklahoma State University
Stillwater, Oklahoma
1987

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
May, 1992

ULTRASTRUCTURAL AND BEHAVIORAL STUDY OF GREENBUG FEEDING ON SORGHUM PLANTS UNDER DROUGHT STRESS; MICROTUBULE DISTRIBUTION IN SORGHUM CALLUS

Thesis Approved:

Paul D. Richardson
Thesis Advisor

Becky Johnson

Glenn A. Todd

James A. Webster

Thomas C. Collins
Dean of the Graduate College

PREFACE

Grain sorghum has long been a vital source of carbohydrates. In some countries, especially in Africa, it is often the principal staple food. Increasing world population and adverse weather conditions have led to an increase in demands for high yielding crop varieties resistant to environmental and biological stresses.

My Master's degree research in the Department of Agronomy at Oklahoma State University was concerned with how these stresses limit crop productivity. It was concerned with the effects of epicuticular wax on the rate of water loss in sorghum. The emphasis of the current research is on the effects of environmental stress and greenbug feeding on the sorghum ultrastructure.

I wish to express my sincere gratitude to my major advisor Dr. Paul E. Richardson for his support, sound guidance, and patience throughout the course of this study. Sincere appreciation is offered to the Department of Botany for the financial support which was vital to the completion of the study. I wish also to express deep appreciation to Dr. James A. Webster, United States Department of Agriculture/Agricultural Research Service, (USDA/ARS), Plant Science Research Laboratory, for agreeing to serve in my advisory committee, for his constant support, and

criticisms, and for the use of laboratory and greenhouse facilities without which the completion of this study would have been very difficult.

Sincere appreciation is offered to my other advisory committee members Dr. Glenn W. Todd and Dr. Becky Johnson for their helpful suggestions and criticisms in the preparation of this manuscript.

I also wish to thank Keith Mirkles, USDA/ARS, for the help in collecting and analyzing the data.

Finally I wish to express special thanks to my parents and my family for their constant moral support. Special appreciation is extended to my wife Nana Hadiza, my son Karim, my daughters Lulu and Sally for their moral support, understanding and patience throughout my research endeavor. To all those who have supported me during the period of my research, and whose support helped build my self-confidence, I am grateful.

TABLE OF CONTENTS

Chapter		Page
I.	INTRODUCTION	1
II.	LITERATURE REVIEW	5
	Insect/Host Plant Interaction	6
	Plants Cells Cytoskeleton	9
III.	MATERIALS AND METHODS	16
	Plant Materials and Experimental Design	16
	Electronic Feeding Monitor	17
	Biological Study of ROKY62 Bloom and ROKY62 Bloomless Sorghum Plants Using Callus Culture and Immunofluorescence	19
IV.	RESULTS AND DISCUSSION	22
	Plant Responses to Drought Stress	22
	Electronically Monitored Greenbug E Feeding on Bloom and Bloomless Sorghum Plants	25
	Ultrastructural Investigation of Drought Greenbug Damaged Sorghum Tissue	46
	Immunofluorescence Assay of Sorghum Callus	82
V.	CONCLUSIONS	88
LITERATURE CITED		91

LIST OF TABLES

Table		Page
I.	Change in Relative Water Content of ROKY62 Bloom and ROKY62 Bloomless Sorghum Lines Over 7 Days of Drought Stress, Experiment One.	24
II.	Frequencies of Biotype E Greenbug Feeding Activities Over Six Hours on ROKY62 Bloom and ROKY62 Bloomless Sorghum Lines Under Well-Watered and Droughted Conditions, Experiment One.	29
III.	Total Duration (in Min.) Spent By Biotype E Greenbug in Feeding Activities Over Six Hours on ROKY62 Bloom and ROKY62 Bloomless Sorghum Lines Under Well-watered and Droughted Conditions, Experiment One.	31
IV.	Average Duration (in Min.) Spent by Biotype E Greenbug on Feeding Activities Over Six Hours on ROKY62 Bloom and ROKY62 Bloomless Sorghum Lines Under Well-Watered and Droughted Conditions, Experiment One.	32
V.	Frequencies of Biotype E Greenbug Feeding Activities Over Eight Hours on ROKY62 Bloom and ROKY62 Bloomless Sorghum Lines Under Well-Watered and Droughted Conditions, Experiment 2)	34
VI.	Total Duration (in Min.) Spent By Biotype E Greenbugs in Feeding Activities Over Eight Hours on ROKY62 Bloom and ROKY62 Bloomless Sorghum Lines Under Well-watered and Droughted Conditions, Experiment Two.	35

Table Page

VII. Frequencies of Biotype E Greenbug Feeding Activities Over Eight Hours on ROKY62 Bloom and ROKY62 Bloomless Sorghum Lines Under Well-Watered and Droughted Conditions, Experiment Three. 37

VIII. Total Duration (in Min.) Spent By Biotype E Greenbug in Feeding Activities Over Eight Hours on ROKY62 Bloom and ROKY62 Bloomless Sorghum Lines Under Well-watered and Droughted Conditions, Experiment Three. 39

IX. Frequencies of Biotype E Greenbug Feeding Activities on ROKY62 Bloom and ROKY62 Bloomless Sorghum Lines Under Well-Watered and Droughted Conditions, Experiments One, Two, and Three combined. 40

X. Average Duration (in Min.) Spent by Biotype E Greenbug on Feeding Activites on ROKY62 Bloom and ROKY62 Bloomless Sorghum Lines Under Well-Watered and Droughted Conditions, Experiments One, Two, and Three combined. 42

XI. Total Duration (in Min.) Spent by Biotype E Greenbug on Feeding Activities on ROKY62 Bloom and ROKY62 Bloomless Sorghum Lines Under Well-watered and Droughted Conditions (Experiments One, Two, and Three combined) 43

LIST OF FIGURES

Figure — Page

1. Electrical Wave Form Patterns of Biotype E Greenbug Monitored While Feeding on ROKY62 Bloom Sorghum Plant, Under Drought Conditions. 26

2. Electrical Wave Form Patterns of Biotype E Greenbug Monitored While Feeding on ROKY62 Bloom Sorghum Plant, Under Well-Watered Conditions 26

3. Electrical Wave Form Patterns of Biotype E Greenbug Monitored While Feeding on ROKY62 Bloomless Sorghum Plant, Under Drought Conditions. 28

4. Electrical Wave Form Patterns of Biotype E Greenbug Monitored While Feeding on ROKY62 Bloomless Sorghum Plant, Under Well-Watered Conditions 28

5. Micrograph of Bundle Sheath Cells of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 3,600X. Experiment One. 49

6. Micrograph of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Drought Conditions, 5,800X. Experiment One 49

7. Micrograph of ROKY62 Bloomless Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 3,600X. Experiment One 52

8. Micrograph of ROKY62 Bloomless Sorghum Plant, Leaf Tissue Grown Under Drought Conditions, 3,600X. Experiment One 52

9. Micrograph of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 2,900X. 55

Figure Page

10. Micrograph of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, Showing Boundary Between Bundle Sheath and Vascular Bundles, 10,000X 55

11. Micrograph of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 3 Days After Biotype E Greenbug Feeding, 2,900X. 57

12. Micrograph of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 3 Days After Biotype E Greenbug Feeding, 19,000X 57

13. Micrograph of Phloem and Xylem Cells of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 3 Days After Biotype E Greenbug Feeding, 19,000X 60

14. Micrograph of Single Phloem Parenchyma Cell of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 3 Days After Biotype E Greenbug Feeding, 10,000X. 62

15. Micrograph of Companion Cell of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 3 Days After Biotype E Greenbug Feeding, 5,800X. 62

16. Single Bundle Sheath Cell of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Drought Conditions, 5,800X. 65

17. Micrograph of Bundle Sheath Cell Chloroplast From ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Drought Conditions, 29,000X 65

18. Micrograph of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 3 Days After Biotype E Greenbug Feeding, 2,900X 67

19. Plasmolyzed Epidermal Cell of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Drought Conditions, 3 Days After Biotype E Greenbug Feeding, 5,800X. 67

Figure Page

20. Micrograph of Damaged Cell of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Drought Conditions, 3 Days After Biotype E Greenbug Feeding, 10,000X 69

21. Micrograph of Mesophyll Cells of ROKY62 Bloomless Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 5,800X. 72

22. Micrograph of Mesophyll Cell of ROKY62 Bloomless Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 3 Days After Biotype E Greenbug Feeding, 1,900X 74

23. Micrograph of Mesophyll Cell of ROKY62 Bloomless Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 3 Days After Biotype E Greenbug Feeding, 5,800X 76

24. Single Chloroplast From Figure 23, Showing the Detailed Contents of the Chloroplast 78

25. Micrograph of ROKY62 Bloomless Sorghum Plant, Leaf Tissue Grown Under Drought Conditions, 2,900X. 81

26. Tangential Section of ROKY62 Bloomless Sorghum Plant, Leaf Tissue Grown Under Drought Stress, 3 Days After Biotype E Greenbug Feeding, 1,900X. 81

27. Photomicrography of ROKY62 Bloom Sorghum Callus, Using Bright Transmitted Light, 10X 84

Fluorescence Photomicrography of ROKY62 Bloom Sorghum Callus, Using Blue Light and BG-2 Filter 84

29. Bright Light Photomicrography of ROKY62 Bloomless Sorghum Callus, 100X. 87

30. Fluorescence Photomicrography of ROKY62 Bloomless Sorghum Callus, 100X. 87

NOMENCLATURE

B	Baseline
BS	Bundle Sheath
Chl	Chloroplast
CM	Cell Membrane
CW	Cell wall
EC	Epidermal Cell
G	Grana
ER	Endoplasmic Reticulum
ICS	Intercellular spaces
LP	Leaf penetration
M	Mesophyll
Mit	Mitochondria
Mt	Microtubule Arrays
N	Nucleus
Np	Non-Phloem Ingestion
Pg	Plastoglobuli
Ph	Phloem
Pi	Phloem Ingestion
S	Salivation
SL	Stroma Lamellae
St	Starch grains
T	Tracheid

Nomenclature

V	Vacuole
VB	Vascular Bundle
X	X-wave
Xy	Xylem

CHAPTER I

INTRODUCTION

Grain sorghum is one of the world's most drought-tolerant crops. It is grown in many third world countries, in Africa, India, Indonesia, China, and the Phillipines for food, and in the United States as forage. Sorghum production is centered in the semi-arid portions of the world, where scanty rainfall and potential insect predation are constant problems. In the United States, sorghum production is centered in the Great Plains states.

Insect predation constitutes a major obstacle to food production. It is estimated that losses due to insects in the United States alone amount to $3.8 billion annually (Anonymous, USDA/ARS, Agriculture Handbook No. 291) and that agricultural pests cause up to $1.8 billion in losses (Kramer, 1967). Chemical insecticides are most often used to control insect predation. Chemical controls are costly and there is a constant danger of health hazard for humans, non-target terrestrial organisms, and aquatic communities. In the United States alone, the cost of chemical control of agricultural insects amounts to $250 million annually (Kramer, 1967). With increased concern over health hazards caused by the use of insecticides, the chemical residues

that may be incorporated into the food chain, and the abilities of some insect species to overcome chemical controls, scientists have turned to using crop plants' own resistance mechanisms.

Plant insect resistance may be defined from the standpoint of the host plant, the insect pest, or an interaction between the plant and the insect. These are, by definition, a collective group of plant resistance factors that adversely affect an insect's survival while maintaining a level of crop productivity higher than that of a comparable variety under the same insect infestation. There are 3 mechanisms of resistance as defined by Painter (1951):

1. Antixenosis or non-preference/preference is a group of plant characteristics which lead to or away from the use of a plant variety for food or shelter.

2. Antibiosis denotes the plant's ability to prevent, injure, or destroy the life of insects which attempt to use that plant for food or shelter.

3. Tolerance is a type of resistance mechanism which involves the ability of a crop plant to grow and reproduce despite supporting a population of insects that could damage some susceptible varieties.

Sorghum plants are infested every year by chewing or sucking insects (Teetes et al., 1975). Chewing insects are herbivores, which destroy plants or parts of plants by ingesting them. Sucking insects include some aphids which induce chlorosis, necrosis, and eventual death of the plants due to injected materials and toxic agents which alter the plant's metabolic activities. This is in addition to

removal of biomass, a severe problem in itself.

One of the most important aphid pests of sorghum is the greenbug aphid, Schizaphis graminum (Rondani). There are several greenbug biotypes. Biotypes are morphologically indistinguishable aphid individuals usually recognized on the basis of biological differences such as insecticide resistance or their host-damaging abilities. They are the result of selection pressure imposed by genetic resistance factors. Seven greenbug biotypes are recognized: biotypes B, C, E, F, G, H (Puterka et al., 1988) and I (unpublished information, T. L. Harvey, Hays, KS).

Drought stress is another factor which extremely limits food crop production. Similar approaches of using a plant's own resistance mechanisms have been adopted to deal with this stress since supplemental irrigation can be as costly as insect chemical control. Scientists are using plant genetic factors for drought tolerance and incorporating these factors into cultivated crop species. A sorghum factor found to be related to drought tolerance involves the plant's surface wax. Sorghum plants are normally covered with a powdery waxy secretion termed "bloom." Another condition exists where there is no visible wax cover and this is termed "bloomless." Wax cover was shown to decrease the plant's transpirational water loss. Certain insects, including the greenbug, exhibit antixenosis to bloomless sorghum.

The objectives of this study are:

1. to determine the effects of drought and greenbug feeding activities on relative water content (RWC) as related to the ultrastructure of sorghum separately and when taken together.
2. To determine the effects of drought stress on the feeding activities of biotype E greenbugs.
3. To investigate the tubulin-containing organelles in sorghum callus.

CHAPTER II

LITERATURE REVIEW

Sorghum bicolor (L.) Moench is one of the world's most drought-tolerant crops. It is grown as food in several parts the third world. Sorghum production in the United States is centered in the Great Plains states. In these areas, sorghum production is noticeably hampered by drought and insect damage. One of the most important insect pests in sorghum is the greenbug, Schizaphis graminum (Rondani). Several biotypes have been identified (Puterka et al., 1988). Sorghum leaves are normally covered with a waxy secretion known as "bloom." The condition where there is no wax cover is known as "bloomless." Greenbugs exhibit a non-preference for bloomless sorghum (Peiretti et al., 1980; Weibel et al., 1972). Damage caused by the aphids is characterized by local chlorosis and necrosis of the infested plants, with severe damage to chloroplasts.

Conventional screening methods have been used to select sorghum strains resistant to greenbugs. The methods consist of growing crop plant cultivars and allowing insect infestation to occur. The surviving crop genotypes are then selected and put in an insect resistance breeding program. There have been several reports of greenbug

resistance in sorghum (DePew and Witt, 1979; Peiretti et al., 1980; Schuster and Starks, 1973; Teetes et al., 1975, Weibel et al., 1972). The authors of these studies interpreted resistance to greenbugs in sorghum as being the result of antibiosis, or antixenosis.

Observations on relationships between plant wax content and insect predation have been reported in other crops. Lowe et al. (1985) determined that waxy condition of wheat leaves was correlated with resistance to the aphid Sitobion avenae (Fabrieius) because of the diketone constituents in the normal wax of the wheat plant. They concluded that the glossy condition of a wheat variety may result in visual deterrence of winged insects, and that resistance in waxy wheat plants could be positively correlated with the amount of diketone wax on the plant surfaces. Greenbugs exhibit changes in feeding preference and behavior on their host plants with changes in the plants' wax cover and age. It is therefore important to understand the nature of insect damage and how to prevent physical and physiological injuries to the crop plants.

Insect/Host Plant Interaction

A new approach to the study of aphid feeding behavior began with McLean and Kinsey's (1964) electronic aphid feeding monitor device. Electrical wave patterns were used to distinguish among various feeding activities of aphids. In 1965, these authors identified specific recorded electrical wave patterns as indicating the features they

associated with salivation and phloem feeding. These studies were interpreted as indicating that the stylets of the pea aphid, Acyrthosiphon pisum (Harris), penetrated Vicia faba L. tissue intercellularly for the greater part of any feeding path. It was pointed out that there may have been some physical or biological barrier associated with the intercellular penetration of the stylet. This barrier was later considered to be related to the degree of methylation of the middle lamella (Dryer and Campbell, 1984; Campbell and Dryer, 1985). Since these studies, aphid probing activities have become the subject of intensive work, with major emphases on insect feeding studies in wheat, sorghum, and several other crops (Brzezina et al., 1986; Campbell et al., 1982; Campbell and Dryer, 1985; Dorschner et al., 1986; Kindler and Staples, 1981; Ryan et al., 1987; Schuster and Starks, 1973).

There is some agreement on the nature of the pathways of the aphid stylets through the plant mesophyll tissue. Some researchers have documented the penetration of leaves as being intercellular, intracellular, or a combination of the two. Most, however, agree that the majority of the damage is to the mesophyll tissue and the phloem. Evert et al. (1973) studied the penetration of barley leaves by the corn leaf aphid Rhopalosiphum maidis (Fitch) in an attempt to determine the practicability of an electron microscopic investigation of insect damage to plants. Using both light and electron microscopes, they found that the aphid stylets penetrated the epidermis and the mesophyll tissues

intercellularly, becoming intracellular at the level of vascular bundles and veins. Their investigations also demonstrated that the salivary sheath of the aphid stylets pushed aside the protoplasts of penetrated cells without perturbing them. Brzezina et al. (1986) observed several types of damage to the leaves of wheat plants subjected to greenbug probing and feeding activities and divided them into three categories:

1. Damaged cell contents, but intact cell wall and tonoplast, with chloroplasts having disorganized grana.
2. Ruptured tonoplast but intact cell wall with damaged contents.
3. Damaged cell contents with ruptured cell wall and disappearance of tonoplast and plasmalemma.

A detailed analysis of stylet pathways of feeding aphids is a useful tool in understanding the method of phloem feeding and the biological aspect of the host plant resistance. There are indications that the exact stylets pathways of an aphid's feeding are related to the plant cytochemical responses to feeding and that resistance to greenbugs may result from physiological and biochemical factors such as cellular pH and carbohydrate concentration (Dryer and Campbell, 1984; Campbell and Dryer, 1985).

Drought stress is another factor affecting the plant's physiological processes. When drought stress and greenbug infestation are combined, complex effects on the overall plant responses are produced which may be difficult to separate. Kindler and Staples (1981) found that greenbug

feeding more adversely affects grain sorghum when the plants are weakened by drought stress. Dorschner et al. (1986) investigated the effects of greenbug feeding on physiological plant responses associated with drought stress. After measuring several factors including aphid fecundity, plant water potential, and membrane and cell wall stability, they concluded that greenbug feeding caused membrane injury in winter wheat. They also concluded that when plants were subjected to greenbug feeding and drought stress, greenbugs caused increased (less negative) plant water potential as compared to drought stress alone, indicating an increase in solute production as a result of feeding. Ryan et al. (1987) reported that the major effect of drought stress on greenbug feeding behavior was to reduce the time needed for the stylets to reach the phloem. Campbell et al. (1982) studied the probing behavior of greenbug biotype C on resistant and susceptible varieties of sorghum. They demonstrated that physical differences not associated with phloem tissue may contribute to resistance to greenbug in sorghum. They also found that brief probing attempts on resistant varieties by greenbugs may be related to epicuticular wax differences among the varieties. This hypothesis is supported by the works of Klingauf et al. (1988) who found that removal of epicuticular wax deterred probing by the pea aphid on Vicia faba L.

Plant Cell Cytoskeleton and Drought Stress

The cytoskeleton is the supporting framework of eukaryotic cells. It is composed of 3 types of fibrillar systems: microtubules, actin filaments, and intermediate filaments. The cytoskeleton controls several biological processes of the cells, such as contraction, movement of organelles, cell elongation, and expansion. This fibrillar system network is found inside the cell and performs specific functions. The system builds up over time and is dynamic. Parts of it may break down to amino acids and polypeptides in the cytoplasm and the system is continually reassembling. The frequency of assembly and disassembly of these proteinaceous organelles may vary with the endogenous cell physiological state or may be environmentally induced. The concept of a cytoskeletal fibrillar system in plants is not new (Ledbetter and Porter, 1963), but knowledge of it is still embryonic. Many factors affecting the dynamics of the cytoskeletal system have yet to be investigated (Lloyd, 1986).

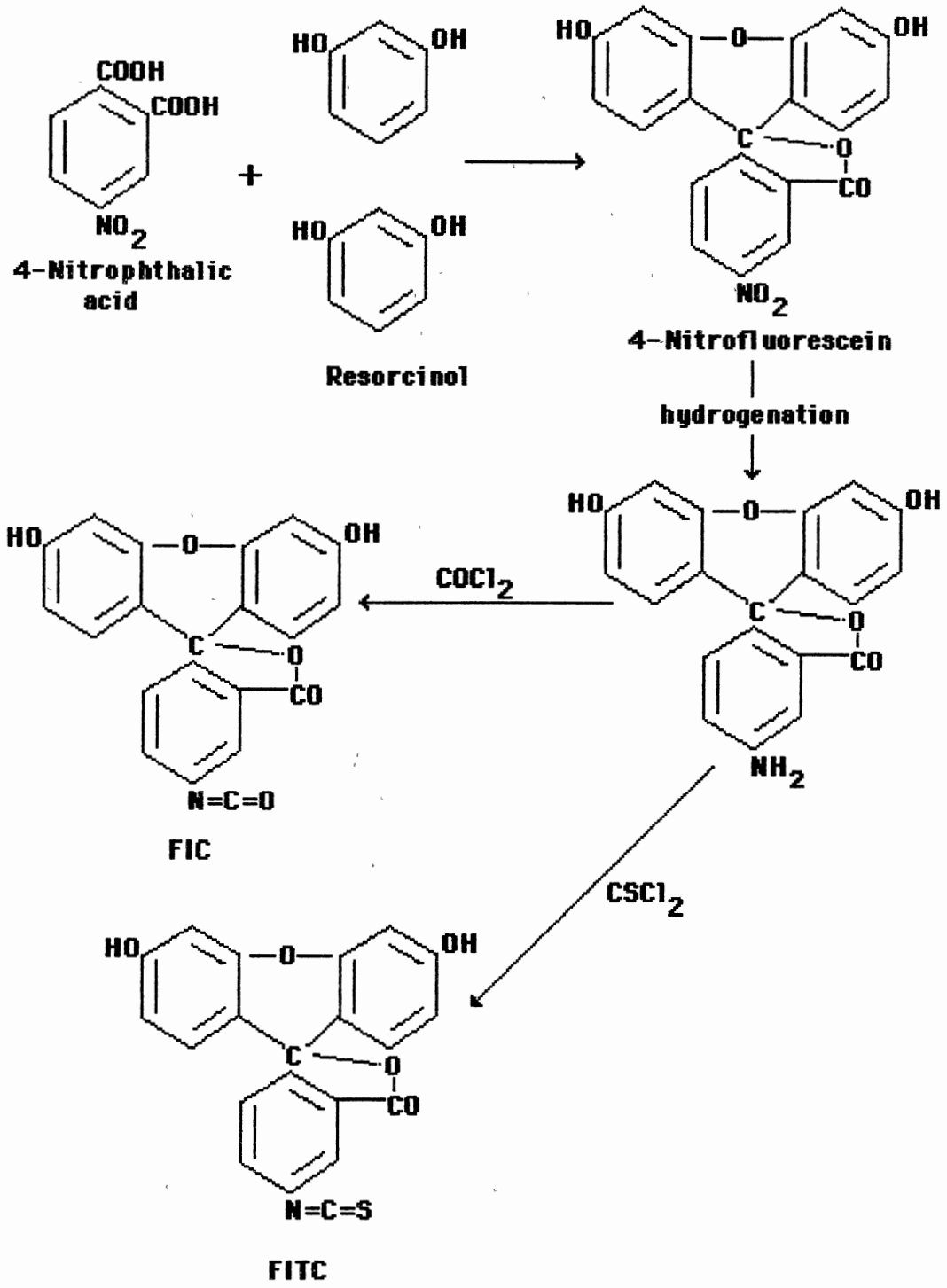
The microtubules are one of the major components of the cytoskeleton (Dustin, 1984). They are proteinaceous structures present in nearly all eukaryotic cells. They are made of subunits of tubulin molecules assembled into a tubular structure with an average interior diameter of 24 nanometers (Dustin, 1984) capable of changing their length by assembly or disassembly of their subunits. The system of microtubules is composed of four distinct arrays of microtubules: interphase cortical microtubules which polymerize from the nuclear envelope, the prophase band of

microtubules which are predictive of the cell division plane, the mitotic spindle fibers which move the chromosomes during cell division, and the phragmoplast, a system composed of a mixture of endoplasmic reticulum fragments and cortical microtubules that form the cell plate.

In plant cells, microtubules participate in regulating cell shape (Wick et al., 1981; Galatis, 1980; Lloyd, 1986; Pickett-Heaps, 1967), repartitioning of intracellular organelles (Hepler and Newcomb, 1964), and deposition and orientation of cell wall materials (Volfova et al., 1977; Falconnier and Seagull, 1987; Pickett-Heaps, 1967; Ledbetter and Porter, 1963). Microtubules affect the shape of cells in particular, and plant morphology in general. In most elongated cells, microtubules are oriented with their longitudinal axis parallel to the long axis of the cells. LaClaire II (1987) observed that in green algae, wounding induced reticulation and separation of the cytoplasm into numerous spheres involving a fasciation of microtubules within regions of the cytoplasm.

The study of intracellular fine structure was made possible with the discovery of the fixative glutaraldehyde and its application in electron microscopic staining techniques (Ledbetter and Potter, 1963). Most electron microscopic analyses using thin sections to study the microtubules encounter problems of image contrast. New techniques of labelling microtubules for viewing with light microscopy evolved based on the principle of antibody/antigen interaction. Many of these are based on

the method originally developed by Coons (1958) which makes it possible to label proteins with fluorescent dye molecules. The techniques have been further developed and the field of investigation is known as immunofluorescence microscopy (IFM) using polyclonal or monoclonal antibodies. Immunofluorescence microscopy is a biological method of labelling proteinaceous cellular molecules using antibodies produced in a particular host animal against a specific biological molecule which in turn is the antigen. The entire antigen antibody complex may then be reacted with fluorescein isothiocyanate (FITC), forming a conjugate . FITC may be synthesized by reacting 4 - nitrophthalic acid with resorcinol to produce 4 - nitrofluorescein, reducing this to amino-fluorescein by catalytic hydrogenation and finally converting the amine to isothiocyanate with gaseous phosgene, as shown on the following diagram.


COOH
COOH
NO2
4-Nitrophthalic acid
HO
OH
HO
OH
Resorcinol
HO
O
OH
C
O
CO
NO2
4-Nitrofluorescein
hydrogenation
HO
O
OH
C
O
CO
NH2
COCl2
HO
O
OH
C
O
CO
N=C=O
FIC
CSCl2
HO
O
OH
C
O
CO
N=C=S
FITC

Labelling with FITC is accomplished by reacting the alkaline buffered microtubules with the anti-tubulin antibody, then exposing them to the dye over a period of 45 minutes at room temperature. The technique offers the possibility of labeling a cell without killing it, and also offers the advantage of looking at the cell in two or three dimensions.

Fluorescence microscopy has found numerous applications in biological research and diagnostic procedures. A very important application of antibody techniques which is of great potential use is the identification of antigens that are unique to a particular type of tissue. There are clear advantages in being able to identify cell types at very early stages of development, for example cells in a culture which are capable of becoming embryogenic (Roberts et al., 1985). Another potential use of the antibody labeling techniques is the early detection of cells or tissues which are capable of becoming cancerous. Use of IFM has been extended to plant cells (Wick et al., 1981; Wick, 1985; Falconnier and Seagull, 1987; Lloyd, 1986; Lloyd, 1987; Roberts et al., 1985). These and several other researchers recognized the power of IFM methods and were able to develop techniques of labelling the cytoskeleton in plant cells. One of the major problems encountered in applying fluorescent dye techniques in plant cells, besides permeabilizing them is the interference from the autofluorescence of chlorophyll molecules. Due to this and other autofluorescing features of plant cells, most studies

in this area have utilized tissue culture cells or shoot apical meristematic tissue which lack some of the interfering components.

Plant tissue cultures can be used in studying the dynamics of the plant cytoskeleton. The technique has been used in selection for stress tolerance. Several stresses have been selected against using tissue culture: salt tolerance (Barlass and Skene, 1981; Nabors et al., 1974; Orton, 1979), herbicide tolerance (Chaleff and Parson, 1978), drought tolerance (Bhaskaran et al., 1985; Smith et al., 1982), and resistance to Helminthosporium toxins in corn (Gengenbach et al., 1977). The use of tissue culture in screening procedures has been well established for environmental and biological stresses.

CHAPTER III

MATERIALS AND METHODS

Plant Materials and Experimental Design

Two near-isogenic sorghum lines, ROKY62 bloom (ROKY62 Bm) and ROKY62 bloomless (ROKY62 bm) were grown and maintained in the greenhouse. ROKY62 Bm is a waxy, drought-tolerant line, while ROKY62 bm is a non-waxy line susceptible to drought (Weibel et al., 1972). The experiments were conducted in the summer of 1988, fall of 1990 and summer of 1991. All plants were maintained in a well-watered condition for three weeks in potting soil and the light was supplemented with fluorescent tubes. After three weeks of growth, drought stress was imposed on experimental bloom and bloomless plants for a period of seven days by withholding water.

Relative water content, or RWC, (Todd et al., 1962) was measured by excising one leaf from plants of each sorghum line under drought and non-drought conditions, and the fresh weights (W_f) were recorded. The leaves were then sliced in five small pieces each to fit into petri dishes and floated for 24 hours in distilled water at room temperature to bring them to full turgidity. The turgid weights (W_t) were recorded after blotting the leaf segments. The leaves were

then dried in a 55°C oven for 48 hours and the dry weight (W_d) recorded. The relative water content was calculated as:

$$RWC = \frac{W_f - W_d}{W_t - W_d} \times 100$$

Electronic Feeding Monitor

Greenbug feeding activities were followed on ROKY62 Bm and ROKY62 bm droughted and well-watered plants by means of an electronic feeding monitor (EFM). The EFM is a device consisting of an amplifier connected to a strip chart recorder and two electrodes. One of the electrodes is used to include the aphid in the circuit, by means of thin gold wire glued with colloidal silver to the back of the insect. The second electrode is used to complete the circuit by inserting it into the pot containing the plant. The greenbug was then placed on the adaxial leaf surface of the plant and allowed to feed six hours during the first experiment and eight hours for Experiments Two and Three. The recorded electrical impulses are translated into actual times and frequencies of the various feeding activities of the aphid: baseline, leaf penetration, X - wave, phloem ingestion, and non-phloem ingestion.

Baseline occurs when there is no feeding but the aphid is in contact with the leaf. Leaf penetration occurs when the aphid's stylet penetrates the epidermis of the leaf.

Salivation occurs when the aphid's stylet reaches any of the mesophyll cells. X - wave occurs when the aphid's stylets penetrate the vascular bundle cells. Phloem ingestion is said to have occured when the aphid's stylet is located inside the phloem for feeding. Non-phloem ingestion occurs when feeding is taking place in any non-phloem cells of the vascular bundle.

One plant from each treatment-ROKY62 Bm droughted, ROKY62 bm droughted, ROKY62 Bm well-watered, and ROKY62 bm well-watered-was monitored for six hours of biotype E greenbug feeding in Experiment One and eight hours in Experiments Two and Three. This was replicated three times. An OSU-ARS SAS computer program was used to convert the measurements in centimeters from the strip chart recorders to feeding activity time in minutes. The data were then analyzed using the SAS general linear models procedure. Where significant differences in variables occurred, means were separated using Duncan's multiple range test at the 5% probability level (SAS Institute, 1985).

To evaluate the ultrastructural changes induced by drought, greenbug feeding, or a combination of both, leaf tissue samples were taken before feeding and processed for routine transmission electron microscopy. Another set of leaf samples was taken 72 hours after the feeding monitor tests, at the exact location of feeding sites. All samples were fixed in Karnovsky's (1965) fixative. After dehydration in a graded alcohol series, the leaf samples were infiltrated for three days and embedded in Spurr's

(1969) firm formulation hard resin. The embedded tissues were allowed to polymerize at 60°C for three days. Thick sections were cut with glass knives to determine an appropriate area for thin sectioning. Thin sections were cut with glass and diamond knives, stained with 5% uranyl acetate and counterstained in 0.3% lead citrate (Venable and Coggeshall, 1965). The grids were viewed and photographed with a JEOL 100CX II electron microscope.

Biological Study of ROKY62 Bloom and ROKY62 Bloomless Sorghum Lines Using Immunofluorescence Microscopy

Undifferentiated callus tissue was initiated from dry seeds of ROKY62 Bm and ROKY62 bm on Murashige and Skoog's (1962) medium. Mature sorghum seeds were surface sterilized as described by MacKinnon et al. (1987) with a brief (thirty seconds) rinse with 95% ethanol, followed by a twenty-minute wash with stirring in 20% clorox solution plus 2 to 3 drops of Tween 20 and rinsed 6 times with triple-distilled sterile water. The sterilized seeds were placed directly in petri dishes on medium containing the major and minor minerals of Murashige and Skoog (1962), Gamborg's B-5 vitamins, 30 g/liter sucrose, 5 mg/liter 2,4-dichlorophenoxyacetic acid at pH 5.5, and with 7 g/liter agar (Johnson and Worthington, 1987; MacKinnon et al., 1987). The medium was sterilized by autoclaving. Ten seeds per petri dish were plated with the embryo in contact with the medium. Five days after plating,

the seeds were transferred to petri dishes containing fresh medium. Subsequent transfers were made only when contamination or anthocyanin accumulation occurred.

When callus tissue was well established, it was processed for immunofluorescence microscopic study of microtubules. The technique involves labelling the microtubules with an anti-tubulin antibody, then counterstaining with a fluorescent dye, fluorescein isothiocyanate (FITC). The procedure consists of two major phases: slide preparation and tissue preparation.

I. Slide preparation

1. Select slides of appropriate thickness (1.0-1.2 mm), 50X75mm
2. Wash slides and cover slips with detergent, rinse with methanol and distilled water
3. Air dry slides and cover slips
4. Coat slides and cover slips with polylysine for 15 minutes
5. Rinse in distilled water

II. Tissue preparation

1. Layer cells onto cover slips by making a smear, or sectioning with a cryostat microtome
2. Let settle for 15 minutes
3. Fix cells on cover slips in 4% paraformaldehyde in microtubule stabilizing buffer (MSB), #1, pH 6.9 (van der Valk et al., 1980) for one hour
4. Rinse cover slips with MSB-1 three times, three minutes each
5. Optional: cells may be treated with 0.1-1.0% cellulase in MSB-1 for three to fifteen minutes followed by three washes of three minutes each in MSB-1
6. Extract cells in 1% Triton-X 100 in MSB-2, pH 6.9.

7. Wash three times, three minutes each in MSB-2

8. Fix in 1% glutaraldehyde in phosphate-buffered saline (PBS), pH 7.0, for ten minutes

9. Wash three times in 0.1% $NaBH_4$ in PBS, three minutes each

10. Rinse three times in PBS, three minutes each

11. Incubate the cells at room temperature in a drop of diluted anti-tubulin antibody (1:20 in PBS) in a moist chamber for 45 minutes

12. Rinse three times in PBS, three minutes each

13. Incubate at room temperature in a drop of Fluorescein isothyocyanate (FITC) labelled IgG (1:16 in PBS) in a moist chamber, in the dark for 45 minutes

14. Rinse thoroughly, three times, five minutes each, with PBS

15. Mount cover slips in 9:1 glycerol/PBS, allow the PBS to evaporate and ring with fingernail polish

Labelling efficiency can be significantly increased by increasing the alkalinity of the buffers. After tissue fixation and preparation, the slides were viewed with a fluorescence microscope and photographed using Kodak Tri-X film or 400 ASA Kodacolor film.

CHAPTER IV

RESULTS AND DISCUSSION

Plants Responses to Drought Stress

In all three experiments, 50% or more of the seeds germinated four days after planting. Visual observations of greenhouse-grown plants indicated that wax deposition is more apparent on the leaf sheaths of bloom sorghum than on other parts of the plant body. A substantial wax deposition was also observed on the lower surface of leaf blades. As pointed out by Atkin and Hamilton (1982), it was observed that wax deposition on bloom sorghum increased with increasing drought stress. The bloomless ROKY62 plants had smooth and wax-free leaf sheaths and leaf blades. No morphological difference was detected under control conditions other than the difference in surface wax.

One observed morphological reaction of the plants to drought stress was leaf rolling. All plants subjected to seven days of water stress responded by rolling their leaves along the midrib axis, with the upper surface of the blade inside so as to expose the lower surface. This response occurs in some plants under water stress and reduces the transpiring surface. It may also be a drought-resistance

mechanism in the bloom ROKY62 lines resulting in deflection of light since only the waxy lower surface of the leaf blades is exposed. For bloomless, on the other hand, it may be the only means of reducing water loss. In bloomless plants, the leaf tip and edges burn, resulting in less viable tissue. These plant responses involving structural modifications may impede normal mesophyll cell function, because intercellular spaces are necessary for gas exchange. Most of the plants under water stress had one to two fewer leaves than their counterparts under controlled, well-watered, conditions.

After seven days of water stress, the difference in the degree of wilting between the bloom and bloomless plants was obvious. The bloomless plants were smaller in size, partly desiccated, while the bloom plants were just wilted.

The plants' relative water content (RWC) was measured over the seven days of drought, and the results presented in Table I. No statistical analyses were performed on the RWC.

TABLE I

CHANGE IN RELATIVE WATER CONTENT OF ROKY62 BLOOM AND BLOOMLESS SORGHUM LINES OVER 7 DAYS OF DROUGHT STRESS, EXPERIMENT 3

Days of drought	ROKY62 Bloom		ROKY62 Bloomless	
	Drought	Control	Drought	Control
1	91.5	95.0	90.0	90.6
2	87.7	91.0	86.2	93.3
3	83.8	89.7	69.0	86.6
4	75.2	84.1	67.5	88.6
5	75.3	94.0	67.0	90.1
6	62.2	90.5	64.4	91.4
7	27.0	96.8	23.3	97.0

The results indicated that the relative water content of bloomless sorghum plants decreased at a rate faster than that of the bloom plants as drought progressed. Four days after withholding water, the bloomless plants lost 23.1% of their relative water content while the bloom plants only lost 19.8%. Seven days after the onset of the drought, no difference in RWC could be detected between the bloom and the bloomless plants. Under well-watered conditions, bloom and bloomless plants had an average daily RWC of 91.6% and 91.1% respectively, while under drought conditions, the average daily RWC of the bloom and bloomless plants were 71.8% and 66.8% respectively.

Electronically Monitored Feeding of Biotype E Greenbug On Bloom And Bloomless Sorghum Plants

The wave forms generated by biotype E greenbug feeding on the bloom plants under controlled and droughted conditions are shown in Figures 1 and 2. The time needed to achieve committed phloem feeding was shorter on well-watered plants while more leaf penerations and exits were observed in plants under drought conditions. Figures 3 and 4 represent the wave forms generated by greenbug E feeding on bloomless plants under well-watered and droughted conditions. Despite the antixenotic effects of bloomless sorghum plants on biotype E greenbugs, the aphid will sucessfully feed on bloomless plants if a more suitable host is not available. It was observed that drought is unfavorable to greenbug feeding on bloomless sorghum plants.

Figure 1. Electrical Wave Form Patterns of Biotype E Greenbug Monitored While Feeding on ROKY62 Bloom Sorghum Plant, Under Drought Conditions.

Figure 2. Electrical Wave Form Patterns of Biotype E Greenbug Monitored While Feeding on ROKY62 Bloom Sorghum Plant, Under Well-Watered Conditions.

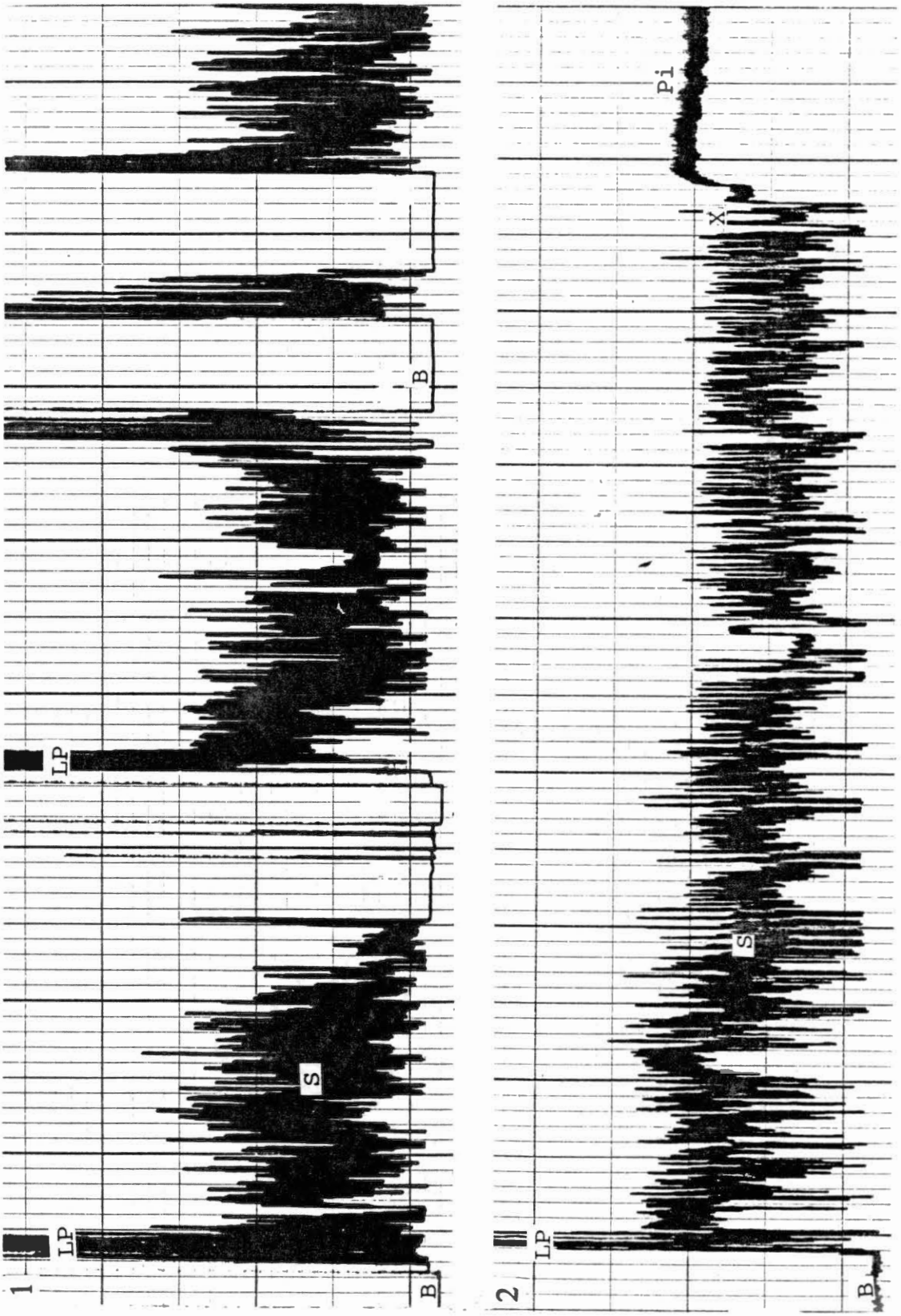
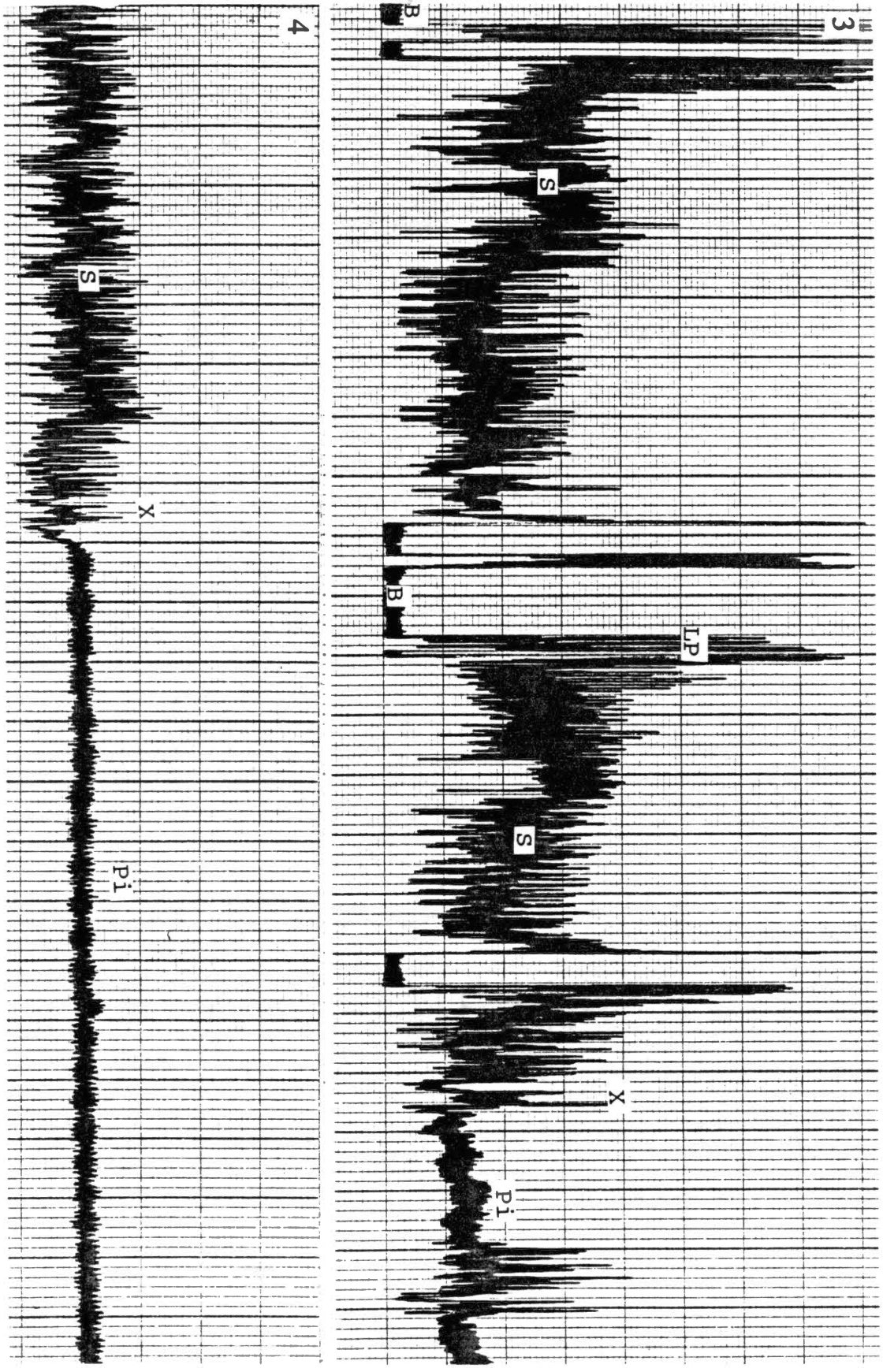

1
LP
S
B
LP
B
2
LP
S
Pi
X
B

Figure 3. Electrical Wave Form Patterns of Biotype E Greenbug Monitored While Feeding on ROKY62 Bloomless Sorghum Plant, Under Drought Conditions.

Figure 4. Electrical Wave Form Patterns of Biotype E Greenbug Monitored While Feeding on ROKY62 Bloom Sorghum Plant, Under Well-Watered Conditions.


3
B
B
S
B
B
LP
S
X
Pi
4
S
X
Pi

The greenbug feeding activity frequencies from Experiment One are shown in Table II.

TABLE II

FREQUENCIES** OF BIOTYPE E GREENBUG FEEDING ACTIVITIES OVER SIX HOURS ON ROKY62 BLOOM AND ROKY62 BLOOMLESS SORGHUM LINES UNDER WELL-WATERED AND DROUGHTED CONDITIONS, EXPERIMENT ONE

Activities	ROKY62 Bloom		ROKY62 Bloomless	
	Well-watered (96.8)*	Drought (41.2)*	Well-watered (97.0)*	Drought (33.3)*
Baseline	12.3	4.5	8.5	5.8
Leaf penetration	12.3	4.5	8.5	5.8
Salivation	15.5b	5.0a	5.8a	7.8a
X-Wave	7.8b	1.3a	2.5a	3.0a
Phloem ingestion	7.8b	1.3a	2.5a	3.0a
Non-Phloem ingest.	1.5	1.0	0.0	1.0

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

*Plant's relative water content at the intiation of the monitored feeding

**Number of times behavior occurred over six hours

There were no statistical differences in the frequencies at which baseline and leaf penetration in either bloom or bloomless sorghum plants. In most cases, frequencies of feeding activities occured were higher in ROKY62 bloom in well-watered conditions than in droughted conditions. For ROKY62 bloomless, only the frequencies of baseline and leaf

penetrations were higher under well-watered than under droughted conditions. The frequencies of phloem ingestion for ROKY62 bloom and ROKY62 bloomless were significantly higher in well-watered plants.

Feeding tolerance in sorghum, as in other crops, is commonly measured by the degree of seedling survival after aphid infestation. With the introduction of McLean and Kinsey's (1964) electronic feeding monitor, it is possible to assess the degree of efficiency of aphid feeding on crop plants by comparing wave forms generated by the monitored aphid. This feeding efficiency is expressed by the time it takes the aphid to attain committed phloem ingestion and the total time spent in other feeding activities. The total duration of feeding activities by biotype E greenbugs during the first experiment is presented in Table III.

TABLE III

TOTAL DURATION (IN MIN.) SPENT BY BIOTYPE E GREENBUGS IN FEEDING ACTIVITIES OVER SIX HOURS ON ROKY62 BLOOM AND ROKY62 BLOOMLESS SORGHUM LINES UNDER WELL-WATERED AND DROUGHTED CONDITIONS, EXPERIMENT ONE

Activities	ROKY62 Bloom		ROKY62 Bloomless	
	Well-watered (96.8)*	Drought (41.2)*	Well-watered (97.0)*	Drought (33.3)*
Baseline	27.6	28.9	23.7	16.5
Leaf penetration	8.2	3.4	6.3	2.7
Salivation	225.7a	70.0b	72.3b	82.4b
X-Wave	4.5a	0.6b	1.1b	1.6b
Phloem ingestion	86.7b	256.7a	172.6ab	256.4a
Non-phloem ingest.	5.9	0.3	0.0	0.5
Ratio Phlm/Saliv.	0.4	3.7	2.4	3.1

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

*Plant's relative water content at the intiation of the feeding monitor

The results presented in Tables II and III demonstrate that with the bloom plants under well-watered condition, most of the feeding time was spent salivating, while under drought condition, the majority of the time was spent in phloem ingestion. The ratio of the times of phloem feeding to salivation in bloom plants under control and drought conditions were 0.4 and 3.7 respectively. In the case of bloomless plants more time was spent in phloem feeding than salivation at a ratio of 2.4 and 3.1 for well-watered and droughted conditions respectively. Statistically significant differences were observed during salivation,

X-wave, and phloem feeding. A statistically significant increase in committed phloem feeding was also observed in both bloom and bloomless lines under drought conditions.

One possible mechanism of a plant's drought stress tolerance involves the accumulation of solutes at the cellular level to allow osmotic adjustment to unfavorable environmental conditions. Because greenbugs are aphids that feed in phloem tissue, it is possible that with greater solute concentrations, greenbugs are better able to engage in phloem feeding. A similar pattern is also observed with the average time spent on each activity, shown in Table IV.

TABLE IV

AVERAGE DURATION (IN MIN.) SPENT BY BIOTYPE E GREENBUG ON FEEDING ACTIVITIES OVER SIX HOURS ON ROKY62 BLOOM AND ROKY62 BLOOMLESS SORGHUM LINES UNDER WELL-WATERED AND DROUGHTED CONDITIONS, EXPERIMENT ONE

Activities	ROKY62 Bloom		ROKY62 Bloomless	
	Well-watered (96.8)*	Drought (41.2)*	Well-watered (97.0)*	Drought (33.3)*
Baseline	2.4a	7.7b	2.1a	2.4a
Leaf penetration	0.7	0.9	0.5	0.5
Salivation	15.3	16.3	11.4	10.0
X-Wave	0.5	0.5	0.3	0.5
Phloem ingestion	12.7b	228.7a	92.8ab	194.3a
Non-Phloem ingest.	2.0	0.3	0.0	0.5

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

*Plant's relative water content at the intiation of the feeding monitor

It can be concluded from Table IV that drought stress is a factor which interferes with the abilities of biotype E greenbugs to successfully commit to phloem ingestion. But when phloem feeding occurred under drought stress, the low relative water content of the plants appeared to be related to longer-lasting feeding. In the first experiment, longer-lasting phloem feeding was observed under drought stress. The first experiment provided an indication that drought stress may be a factor involved in interference with the degree of success or failure of aphids in locating suitable feeding sites on host plants. The experiment was therefore repeated two more times and the observed feeding behavior was analyzed in a similar manner. The frequencies of feeding activities from Experiment Two are presented in Table V.

TABLE V

FREQUENCIES** OF BIOTYPE E GREENBUG FEEDING ACTIVITIES OVER EIGHT HOURS ON ROKY62 BLOOM AND ROKY62 BLOOMLESS SORGHM LINES UNDER WELL-WATERED AND DROUGHTED CONDITIONS, EXPERIMENT TWO

Activities	ROKY62 Bloom		ROKY62 Bloomless	
	Well-watered	Drought	Well-watered	Drought
Baseline	5.0b	27.5a	17.0a	18.5a
Leaf Penetration	5.0b	27.5a	17.0a	18.5a
Salivation	6.0b	24.5a	19.5a	30.5a
X-Wave	2.5	4.0	3.5	7.5
Phloem ingestion	2.5	4.0	3.5	7.5
Non-Phloem ingest.	1.0	1.0	2.5	9.6

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

**Number of times behavior occurred over six hours

The results enumerated in this table indicate the degree of difficulty of greenbug feeding on sorghum plants under drought conditions. The frequencies of leaf penetration and salivation were statistically higher when the plants are under drought stress than under well-watered conditions for the bloom and bloomless plants. The higher the frequencies of different activities, the more difficult it is for the aphid to achieve committed feeding. Although no significant difference existed in bloomless plants under the two conditions, I observed greater frequencies in feeding activities of aphids under drought-stressed plants than under well-watered conditions. In this experiment it

was found that under drought conditions, a large amount of non-phloem ingestion was observed with the bloomless plants.

The damage caused by greenbugs to their host plants is related to the removal of phloem sap from the leaf tissue and the injection of salivary enzymes which facilitates tissue entry. Measured frequencies of salivation, committed phloem feeding, as well as time spent in these activities, are good indicators of the type of resistance mechanism a host plant carries. The total time spent by greenbugs feeding during Experiment Two is shown in Table VI.

TABLE VI

TOTAL DURATION (IN MIN.) SPENT OVER EIGHT HOURS BY BIOTYPE E GREENBUGS IN FEEDING ACTIVITIES OVER EIGHT HOURS ON ROKY62 BLOOM AND ROKY62 BLOOMLESS SORGHUM GROWN UNDER DROUGHTED CONDITIONS, EXPERIMENT TWO

Activities	ROKY62 Bloom		ROKY62 Bloomless	
	Well-watered	Drought	Well-watered	Drought
Baseline	13.4	46.8	44.6	49.9
Leaf penetration	1.8	13.3	7.8	10.0
Salivation	85.2a	144.7a	178.8b	205.7b
X-Wave	1.2	2.4	1.9	2.7
Phloem ingestion	316.8a	196.2ab	172.6b	35.6b
Non-Phloem ingest.	1.6a	16.6a	14.4a	116.1b

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

A great amount of greenbug damage is interpreted as being due to the ability of the aphid to induce severe chlorosis in areas surrounding the feeding sites. When feeding was intensive and prolonged, the chlorotic tissue later became necrotic followed by generalized browning of infested areas. Salivation and phloem ingestion are two major causes of tissue disturbance that can affect normal mesophyll photosynthetic functioning and phloem transport. The total time of salivation tended to increase as drought stress increased while phloem feeding time in the bloom and bloomless plants was greater under well-watered conditions. Several factors, plant or aphid, may be associated with the duration of feeding.

A third experiment was conducted under similar conditions to provide a better understanding of the trend observed in the effects of drought on the abilities of the greenbug to feed on bloom and bloomless sorghum plants. The results of the frequencies of different feeding activities of Experiment Three are shown in Table VII.

TABLE VII

FREQUENCIES** OF BIOTYPE E GREENBUG FEEDING ACTIVITIES OVER EIGHT HOURS ON ROKY62 BLOOM AND ROKY62 BLOOMLESS SORGHUM LINES UNDER WELL-WATERED AND DROUGHTED CONDITIONS, EXPERIMENT THREE

Activities	ROKY62 Bloom		ROKY62 Bloomless	
	Well-watered (96.8)*	Drought (27.0)*	Well-watered (97.0)*	Drought (23.3)*
Baseline	12.0	15.3	10.7	15.0
Leaf penetration	12.00	15.33	10.6	15.0
Salivation	9.7	16.7	11.7	17.7
X-Wave	3.3	4.7	3.0	6.3
Phloem ingestion	3.3	4.7	3.0	6.3
Non-Phloem ingest.	1.0	1.0	1.3	0.0

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

*Plant's relative water content at the intiation of the feeding monitor

**Number of times behavior occurred over six hours

Although no significant differences were detected among the frequencies of activities and between the two sorghum lines, it can be seen that several attempts at feeding occured more often under drought than under well-watered conditions in both the bloom and bloomless plants. This reflects the difficulty in achieving committed feeding on host plants affected by drought. It was mentioned earlier that several reasons may be associated with the inconsistency of feeding on plants under drought stress. One possible explanation is that the drought stressed plants have been hardened by reduced relative water content. It may also reflect increased antixenotic effects of the plants, by leaf rolling and partial desiccation. Greenbugs perform test probes before committed feeding (Olonju-Dixon et al., 1990). Under drought conditions, the number of test probes was observed earlier to be higher than under well-watered conditions. The total times spent in feeding during Experiment Three are presented in Table VIII.

TABLE VIII

TOTAL DURATION (IN MIN.) SPENT BY BIOTYPE E GREENBUG IN FEEDING ACTIVITIES OVER EIGHT HOURS ON ROKY62 BLOOM AND ROKY62 BLOOMLESS SORGHUM LINES UNDER WELL-WATERED AND DROUGHTED CONDITIONS, EXPERIMENT THREE

Activities	ROKY62 Bloom		ROKY62 Bloomless	
	Well-watered (96.8)*	Drought (27.0)*	Well-watered (97.0)*	Drought (23.3)*
Baseline	34.8	96.7	60.0	26.3
Leaf penetration	5.1	6.9	55.7	10.1
Salivation	106.5	150.7	90.1	162.5
X-Wave	1.7	2.4	1.6	3.3
Phloem ingestion	247.3	151.1	195.1	217.8
Non-Phloem ingest.	26.5	18.3	17.4	0.0

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

*Plant's relative water content at the intiation of the feeding monitor

The results shown in Table VIII tend to indicate as was the case in the prior experiments that drought stress is a factor interferring with the resistance and the time to committed phloem feeding of biotype E greenbug. Similar to the data presented in Table VII, the data of Table VIII also show that most feeding activities have higher frequencies than under well-watered conditions, with the exception of phloem ingestion in bloom line. I also observed in Experiment Three more non-phloem ingestion time under well-watered conditions than in Experiments One and Two.

To summarize the study, the feeding activities from all

three experiments were combined and statistically analyzed as one experiment with nine replications. Because Experiment One was a six-hour test, only the first six hours of Experiments Two and Three were included in the combined analysis of variance. The results of the frequencies are presented in Table IX.

TABLE IX

FREQUENCIES** OF BIOTYPE E GREENBUG FEEDING ACTIVITIES ON ROKY62 BLOOM AND ROKY62 BLOOMLESS SORGHUM LINES UNDER WELL-WATERED AND DROUGHT CONDITIONS, EXPERIMENTS ONE, TWO, AND THREE COMBINED

Activities	ROKY62 Bloom		ROKY62 Bloomless	
	Well-watered	Drought	Well-watered	Drought
Baseline	6.9b	16.4a	11.4ab	9.3a
Leaf penetration	6.9b	16.3a	11.4ab	9.2b
Salivation	6.3b	17.6a	11.0ab	14.1a
X-Wave	1.6a	2.6a	1.7a	3.8a
Phloem ingestion	2.0b	5.7a	2.9ab	4.8ab
Non-Phloem ingest.	0.8a	3.8a	1.9a	3.0ab

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

**Number of times behavior occurred over six hours

The frequencies of baseline were significantly higher in the droughted bloom line than in the well-watered bloom

plants, while in bloomless plants, higher frequencies were observed under well-watered conditions. Salivation frequencies were significantly higher in the droughted than in the well-watered bloom line. A similar trend was noted in the bloomless line except that the difference was not statistically significant at the 5% probability level. The frequencies of phloem ingestion were significantly higher in the droughted than in the well-watered bloom line, while no significant difference existed between the well-watered and droughted bloomless line. No significant difference was observed in non-phloem ingestion although it occurred at greater magnitude in droughted bloom or bloomless lines. It can be concluded from this table that drought stress negatively affects the success of biotype E greenbugs in achieving committed phloem feeding in ROKY62 bloom sorghum plants, while in bloomless plants, only the phloem ingestion was negatively affected by drought, but was not significantly different.

The results of the mean time spent on feeding activities are presented in Table X.

TABLE X

AVERAGE DURATION (IN MIN.) SPENT BY BIOTYPE E GREENBUG ON FEEDING ACTIVITIES ON ROKY62 BLOOM AND ROKY62 BLOOMLESS SORGHUM LINES UNDER WELL-WATERED AND DROUGHTED CONDITIONS, EXPERIMENTS ONE, TWO AND THREE COMBINED

Activities	ROKY62 Bloom		ROKY62 Bloomless	
	Well-watered	Drought	Well-watered	Drought
Baseline	4.9a	3.5a	3.6a	2.4a
Leaf Penetration	0.7a	0.6a	1.1a	0.6a
Salivation	17.8a	11.1ab	9.1b	9.6ab
X-Wave	0.4a	1.1a	0.5a	0.3a
Phloem ingestion	60.1a	17.8a	37.3a	22.1a
Non-Phloem Ingest.	110.2a	13.4a	90.1ab	42.3ab

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

The results indicated that the average duration of salivation was significantly higher in the well-watered bloom than in the bloomless sorghum line. Salivation in the well-watered bloom line was also of longer duration than that in the well-watered bloomless line as well as in droughted bloom and bloomless lines although these differences were not significant. No significant differences were found in the phloem feeding. However, the average duration of time spent in phloem feeding in well-watered bloom and bloomless lines was greater than in droughted lines. The average duration of phloem feeding in the bloomless well-watered line was also greater than that

in the droughted bloom line. The average duration of non-phloem ingestion in the well-watered bloom line was also significantly greater than in the droughted bloom line. A similar trend was observed between the well-watered and droughted bloomless plants. the value for the well-watered bloomless was over two times that of droughted bloomless even though it was not significantly different. It can be concluded from the table that drought stress is a factor that may interfere with the mean duration of aphid feeding activities.

The total duration in various feeding activities are presented in Table XI.

TABLE XI

TOTAL DURATION (IN MIN.) SPENT BY BIOTYPE E GREENBUG ON FEEDING ACTIVITIES OVER SIX HOURS ON ROKY62 BLOOM AND ROKY62 BLOOMLESS SORGHUM LINES UNDER WELL-WATERED AND DROUGHTED CONDITIONS, EXPERIMENTS ONE, TWO, AND THREE COMBINED

Activities	ROKY62 Bloom		ROKY62 Bloomless	
	Well-watered	Drought	Well-watered	Drought
Baseline	27.0a	51.7a	35.6a	26.9a
Leaf Penetration	3.56a	8.7a	14.6a	7.8a
Salivation	80.1b	179.6a	100.3b	123.1ab
X-Wave	0.9a	3.8a	1.1a	1.7a
Phloem ingestion	125.8a	69.0a	88.3a	67.7a
Non-Phloem Ingest.	122.7a	47.2a	120.1a	95.3a

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

The duration of salivation was significantly greater in droughted bloom than in well-watered bloom and bloomless sorghum plants. In addition, salivation was also somewhat greater in drought-stressed bloomless plants than in the corresponding well-watered plants. This observation confirmed trends observed in Experiments One, Two and Three except for well-watered bloom line, as stated in the discussion of Experiment Three. It is possible that the droughted plants have been hardened by reduced relative water content, making it difficult for the aphids to commit to phloem feeding, thus spending more time salivating. Significant differences were not observed in phloem ingestion between the two lines, or between the well-watered and the droughted plants, but the total time spent in phloem feeding in well-watered bloom plants was greater than in droughted plants. Similar trends were observed between the well-watered and droughted bloomless lines. The more time an aphid spends salivating, the less time it will spend in phloem or non-phloem feeding. The time spent in non-phloem feeding was greater than that observed in the individual experiments. Although no significant differences were found in non-phloem feeding time, I observed more time spent in well-watered bloom and bloomless than in droughted plants with a coefficient of variation of 79.5%. This may be explained by the plant's low relative water content. It was mentioned earlier that sorghum plants respond to water stress by concentrating solutes for osmotic adjustment. It

may be possible that the solute concentration in the mesophyll cells of these droughted plants is sufficient for the aphids to feed without going into the phloem tissue.

The electronic feeding monitor tests provided preliminary information on the effects of drought stress on the feeding behavior of biotype E greenbugs on sorghum plants. Because of variations, more replications may be necessary in future tests to adequately assess greenbug damage to crop plants. The degree of greenbug damage to host plants depends largely on how much of the leaf area has been test probed, and how much of the salivary enzyme has been injected to facilitate tissue entry. I did, however, find that drought stress increased the degree of restlessness during feeding of biotype E greenbugs on sorghum plants. These results confirm Ryan et al. (1987) who have reported that the major effect of drought stress on greenbug feeding behavior is to reduce the time needed to reach the phloem tissue. These experiments can be interpreted as indicative that frequency of and the duration of time to committed phloem feeding increased under drought conditions.

Dorschner et al. (1986) stated that greenbug feeding activities caused membrane injury in winter wheat, resulting in solute leakage and a more negative plant water potential. As previously indicated in a study of aphid damage to host plants, Brzezina et al. (1986) classified three categories of cellular damage by greenbugs to their host plants.

It appeared that the later conclusion of Dorschner et.

al. (1986), is related to Brzezina's category three. Damage category three was characterized by damaged cell contents with cell wall rupture and disappearance of tonoplasts and plasmalemma.

ULTRASTRUCTUAL INVESTIGATION OF DROUGHT AND GREENBUG DAMAGE TO SORGHUM TISSUE

I conducted an ultrastructural investigation of the greenbug feeding sites, three days after the feeding monitor tests, by marking the leaves with a water-proof ink pen. The tissue processing procedures were outlined in Chapter Three.

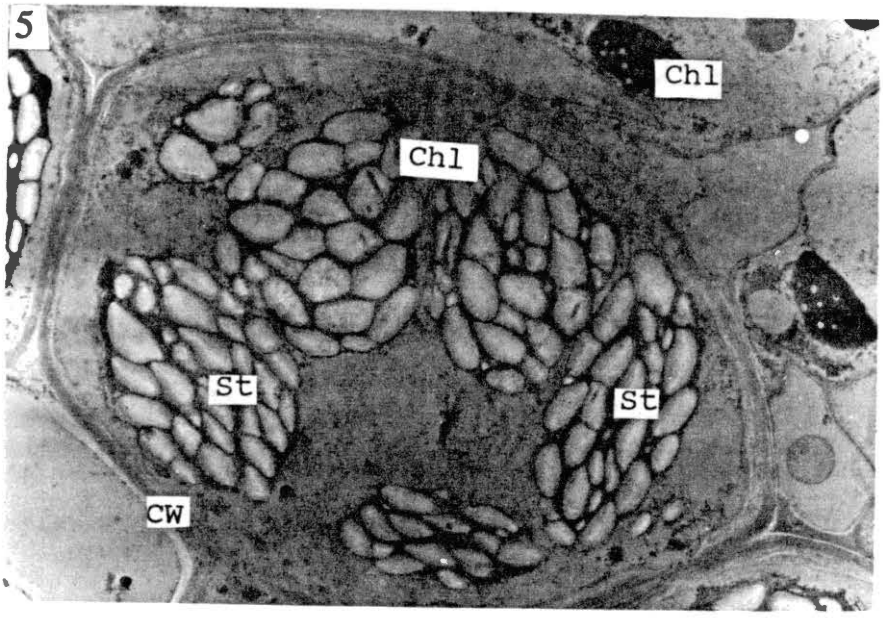
The results of the electron microscopic observations of Experiment One are presented in Figures 5 through 8. Under well-watered conditions, the bloom ROKY62 plants had well fixed and preserved tissues. The epidermal cells had large vacuoles and the mesophyll cell layer had many intercellular spaces. The vascular tissue was surrounded by a layer of parenchymatous bundle sheath cells. The mesophyll cells had chloroplasts with normal structure. Higher magnification revealed normal ultrastructure in these chloroplasts with stroma lamellae, thylakoid membranes, grana, and dense stroma. The bundle sheath chloroplasts on the other hand were surrounded by a double membrane and had stroma lamellae not differentiated into grana. This C_4 plant mesophyll structure has been described by Esau (1977). The bundle sheath cell chloroplasts were packed with starch grains,

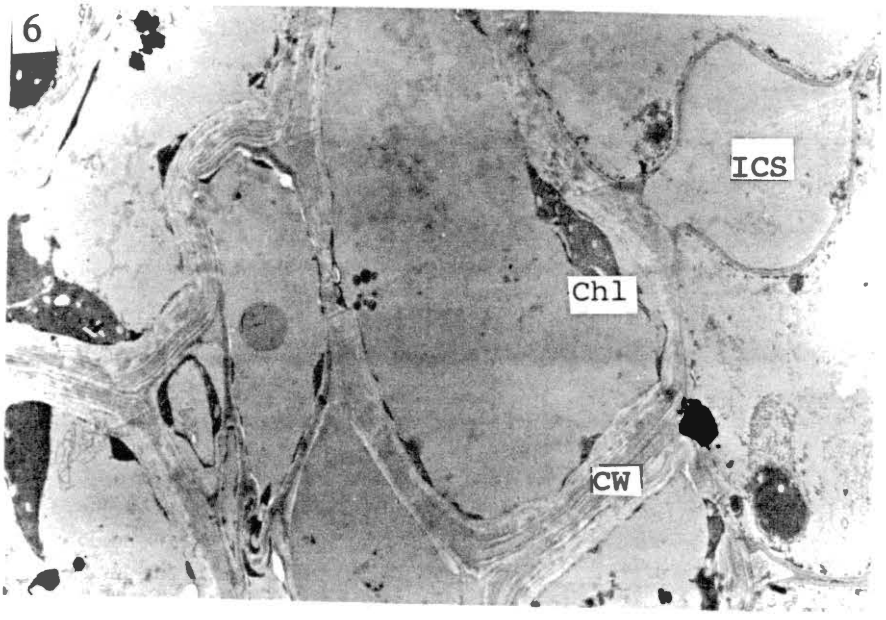
occupying almost the entire volume of the chloroplasts

Figure 5.

Figure 5. Micrograph of Bundle Sheath Cells of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 3,600X. Experiment One.

Figure 6. Micrograph of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Drought Conditions, 5,800X. Experiment One.


5
Chl
Chl
St
St
CW

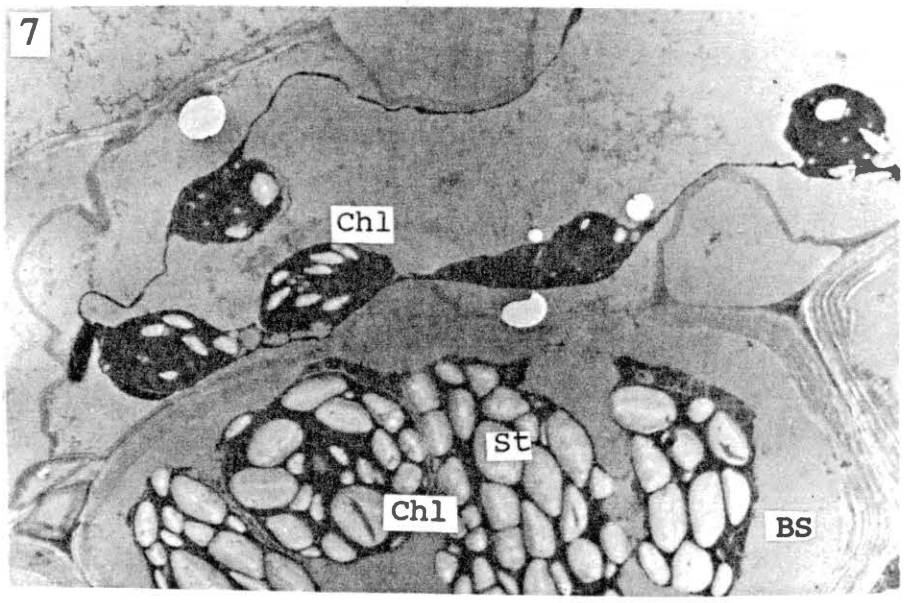

6
ICS
Chl
CW

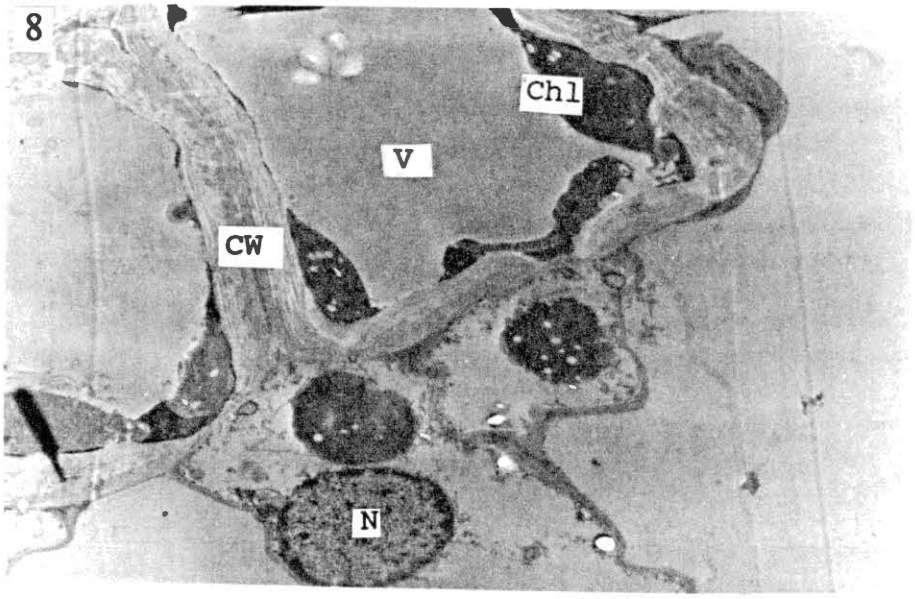
An average of 26 starch grains per chloroplast and six chloroplasts per cell in transverse section were observed. Similar observations were made in ROKY62 bloomless, Figure 7. In both cases, the mesophyll chloroplasts contained sparse, small starch grains.

Under drought conditions, the chloroplasts of the bloom sorghum, Figure 6, did not seem to be surrounded by a membrane. The amount of the starch in the chloroplasts of the bloomless line, (Figure 8) was lower than for the bloom line under similar water stress. In well-watered plants, cell walls appeared thicker and to be composed of two distinct layers, while in droughted plants, the wall appeared as a single thinner layer which was amorphous and stained more densely. Based on several micrographs observed in plant tissue from Experiment One, I noticed that well-watered plants of both bloom and bloomless lines had a large number of starch grains occupying most of the volume of the bundle sheath cloroplasts. Under drought conditions, the cell walls collapsed and the vacuole size was reduced.

Figure 7. Micrograph of ROKY62 Bloomless Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 3,600X. Experiment One.

Figure 8. Micrograph of ROKY62 Bloomless Sorghum Plant, Leaf Tissue Grown Under Drought Conditions, 3,600X. Experiment One.


7
Chl
St
Chl
BS

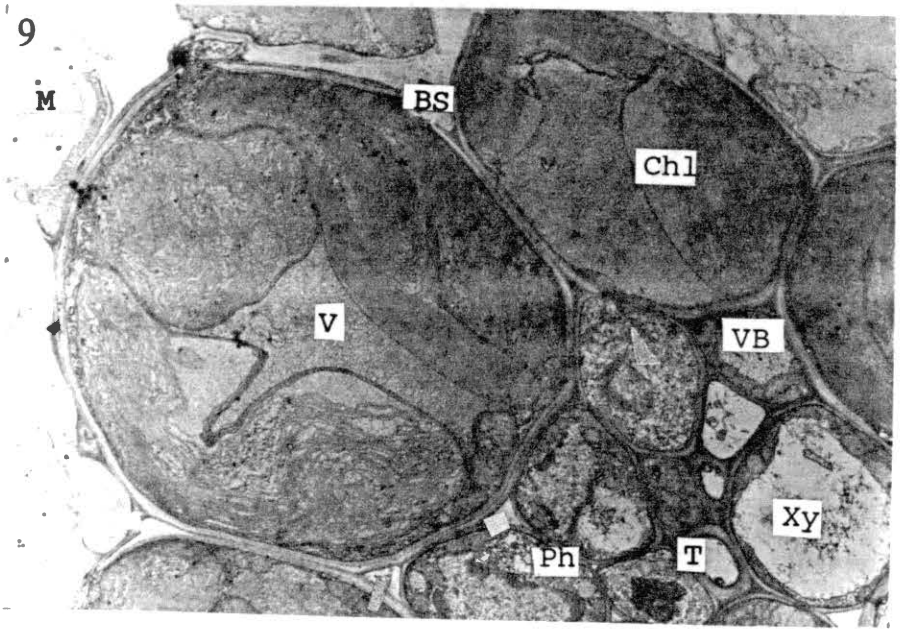

8
Chl
V
CW
N

The micrographs made from tissue from plants of Experiment Two are presented in Figures 9 through 26. These micrographs are representative of the ultrastructural observations of both lines under well-watered and drought conditions, with or without greenbug feeding. The ultrastructure of well-watered bloom ROKY62 before and after greenbug feeding is shown in Figure 9 though Figure 15. Before greenbug feeding, I observed cellular characteristics similar to those observed in Experiment One. The vascular cells were easily distinguisable with distinct phloem, xylem, and companion cells (Figure 9). The bundle sheath cells have an average of two to three large chloroplasts per cell, occupying more than two thirds of the cells' volume. The double membranes of the chloroplasts were visible and the stroma lamellae were running parallel to the longitudinal axis of the chloroplasts. The bundle sheath cell vacuoles were relatively small. Mesophyll cells were highly vacuolated with smaller chloroplasts. At higher magnification (Figure 10), the cytoplasm appeared dense and rich in several cellular organelles, mitochondria and endoplasmic reticulum. In both mesophyll and bundle sheath cells, the cell walls were composed of two distinct layers and stained densely (Figure 11).

Following greenbug feeding, there were changes in the ultrastructure. All the cell layers were still distinguishable, but most of the bundle sheath cell volume was occupied by starch grains (Figure 11 and 12).

Figure 9. Micrograph of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 2,900X.

Figure 10. Micrograph of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, Showing Boundary Between Bundle Sheath and Vascular Bundles, 10,000X.


9
M
BS
Chl
V
VB
Xy
Ph
T

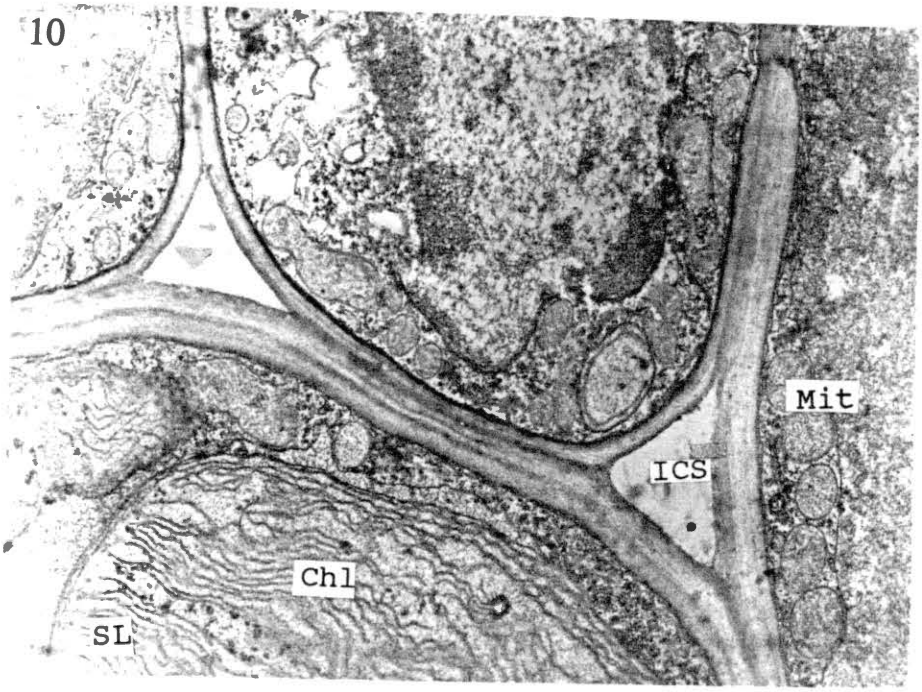
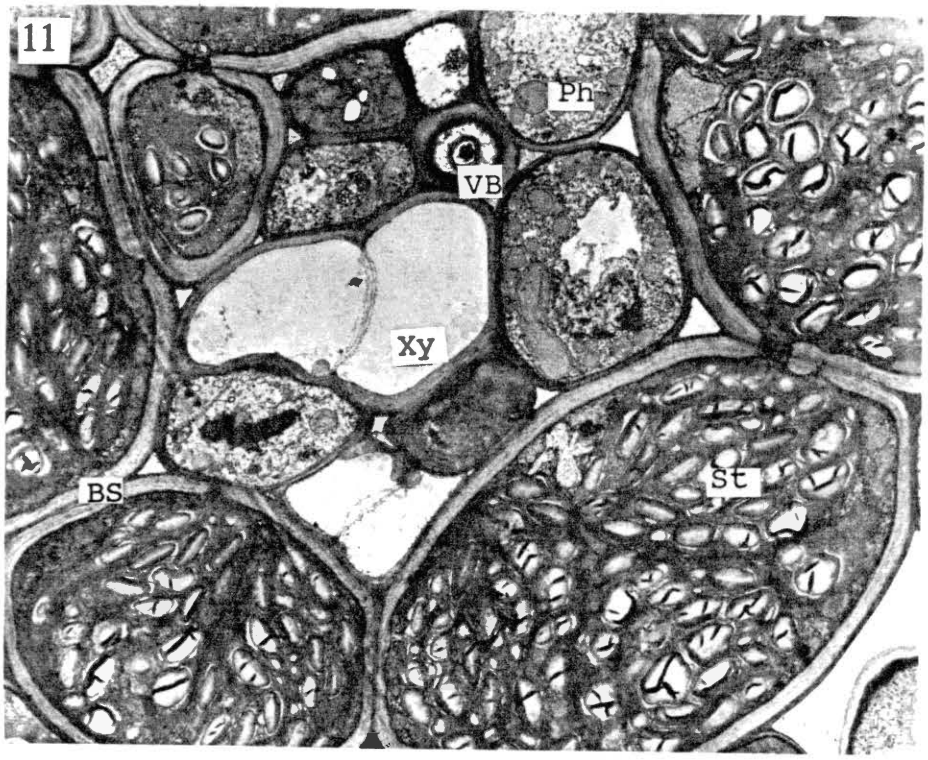
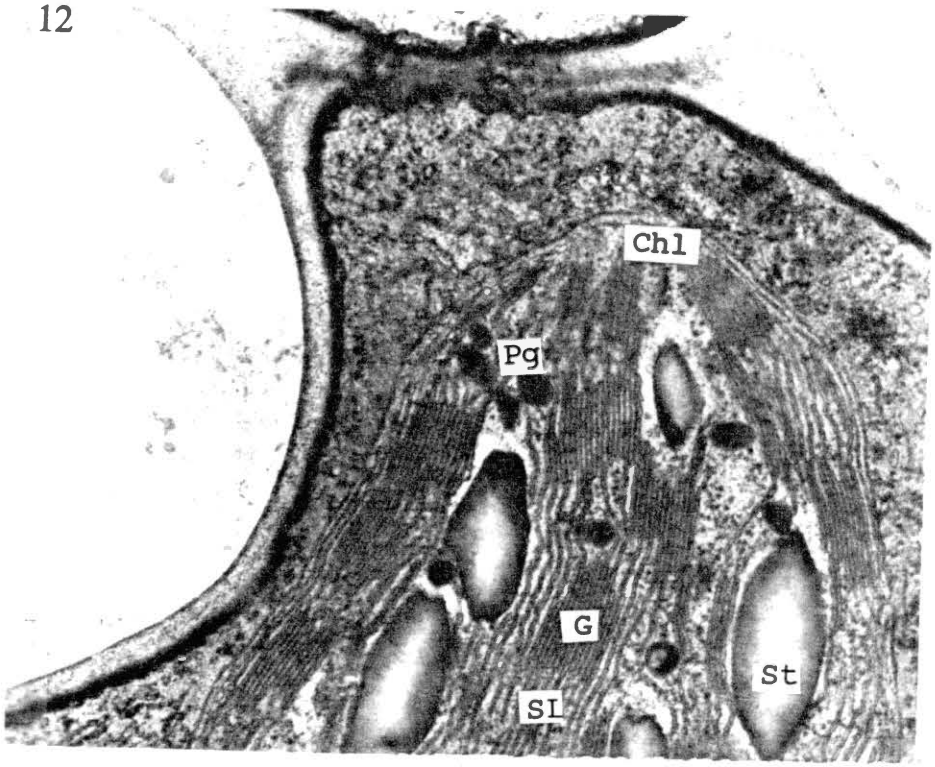

10
Mit
ICS
Chl
SL

Figure 11. Micrograph of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 3 Days After Biotype E Greenbug Feeding, 2,900X.

Figure 12. Micrograph of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 3 Days After Biotype E Greenbug Feeding, 19,000X.


11
Ph
VB
Xy
BS
St


12
Chl
Pg
G
St
Sl

It was previously stated that more than two-thirds of the cell volume was occupied by starch in chloroplasts of plants grown under well-watered conditions. Similar observations were made for well-watered plants subjected to the greenbug feeding.

Another characteristic observed in ROKY62 bloom following greenbug feeding was an increasing number of cell plasmodesmata from the bundle sheath to the vascular bundles shown in Figure 11. The number of starch grains in the mesophyll cells has increased. Some plastoglobuli formed following the aphid feeding. Despite the increased amount of starch, the chloroplasts had distinct grana and visible stroma lamellae. It seemed that following greenbug feeding, sucrose transport might have been blocked leading to starch accumulation. In Figures 13, 14, and 15, I observed a greater number of mitochondria, a relatively denser cytoplasm and greater amount of plasmodesmata than in similar cells of bloom ROKY62 before greenbug feeding. At higher magnification, the phloem parenchyma cells shown in Figure 14, appear to have large nucleii. The companion cells appear to have similar features as shown in Figure 15.

Figure 13. Micrograph of Phloem and Xylem Cells of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 3 Days After Biotype E Greenbug Feeding, 19,000X.

13

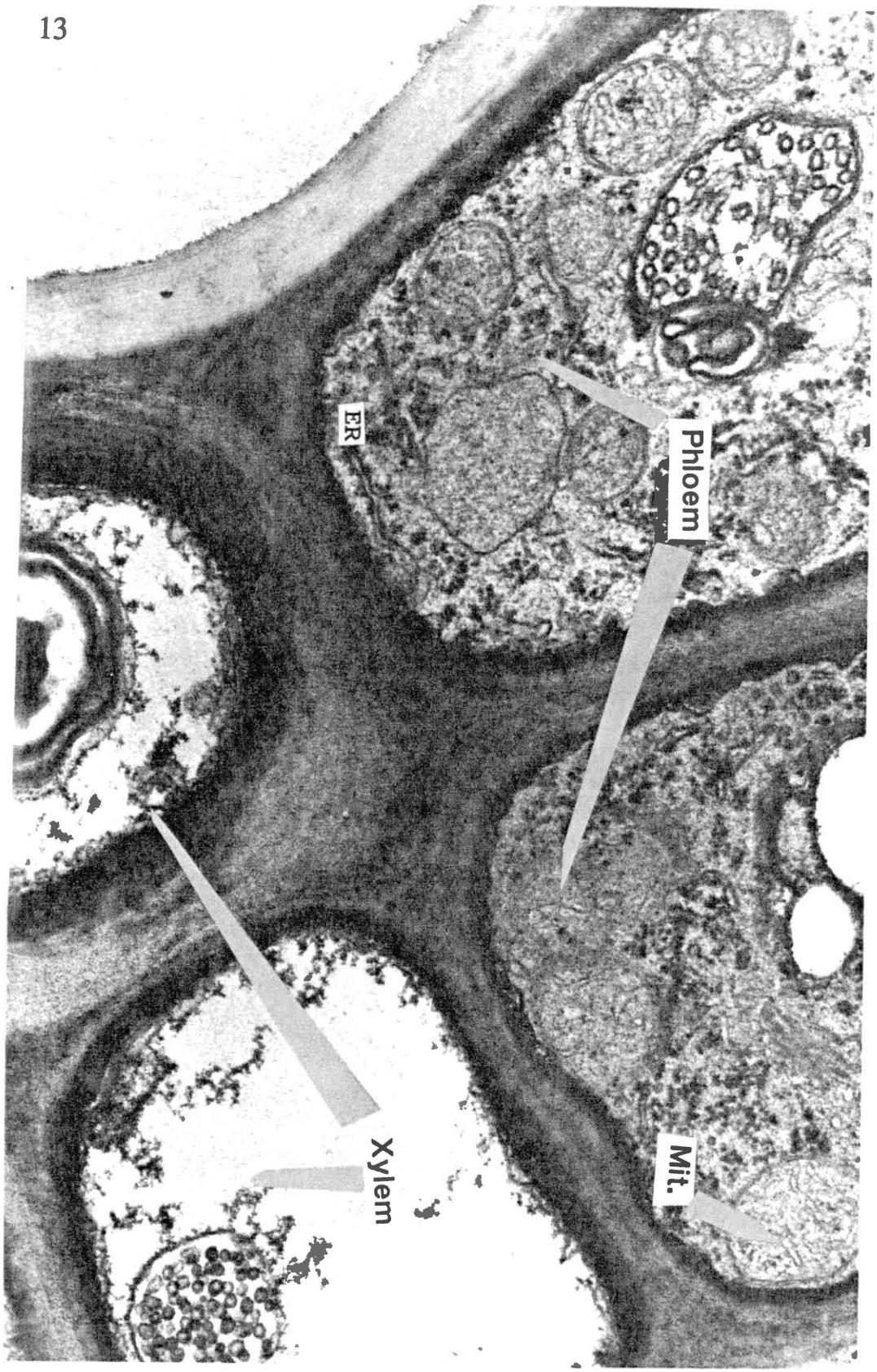
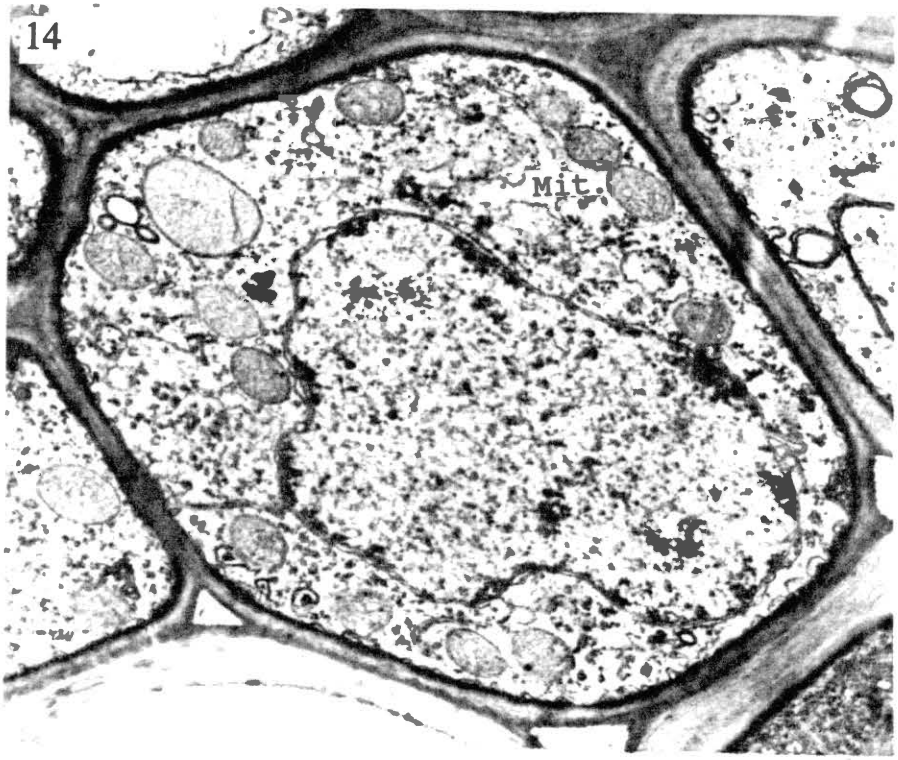
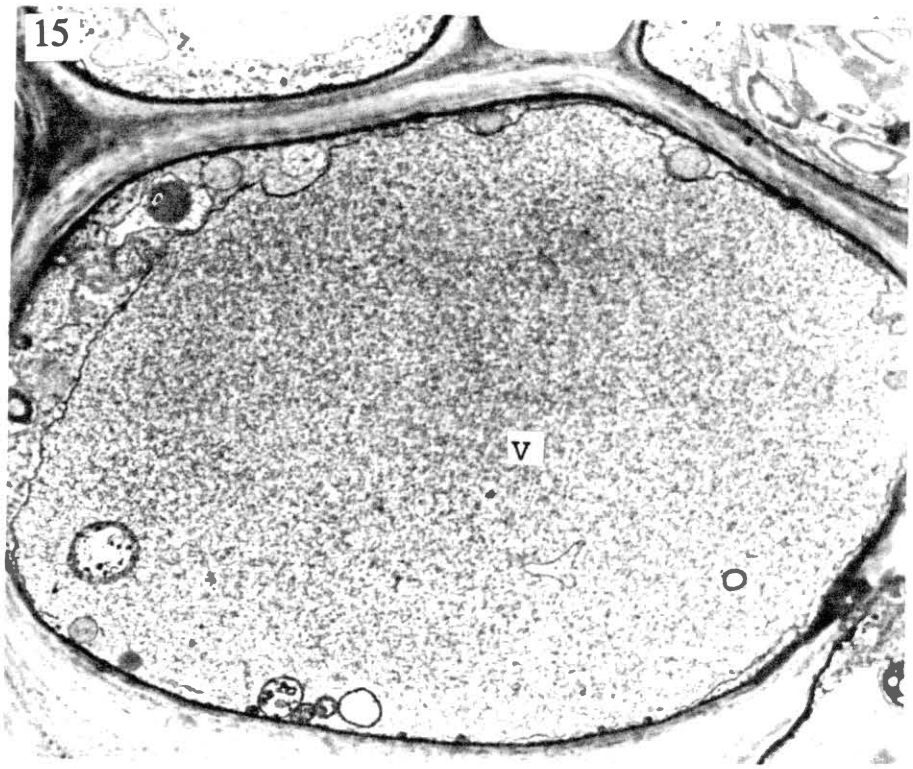


Figure 14. Micrograph of Single Phloem Parenchyma Cell of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 3 Days After Biotype E Greenbug Feeding, 10,000X.

Figure 15. Micrograph of Companion Cell of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 3 Days After Biotype E Greenbug Feeding, 5,800X.


14
Mit.


15
V

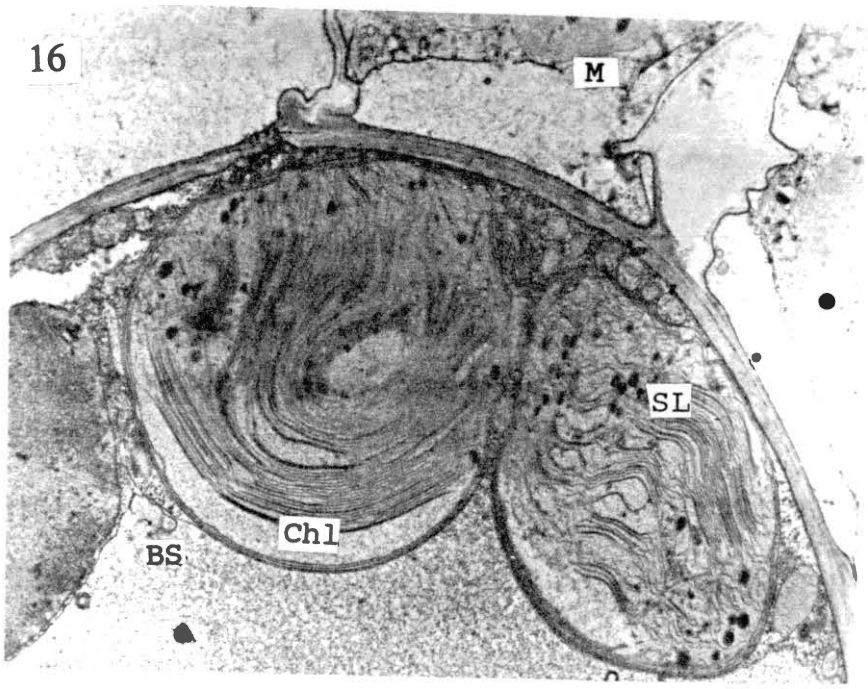
When ROKY62 bloom plants grown under drought conditions were examined, it was found that the bundle sheath chloroplasts, Figures 16 and 17, and the mesophyll chloroplasts have intact outer envelopes but disarranged granal and intergranal lamellae. No starch accumulation was observed. Most cells of the mesophyll and bundle sheath layers had reduced amounts of cytoplasm.

The effects of combined drought stress and greenbug on the structure of bloom plants are presented in Figures 18 through 20. The cells' organelles seemed to have been damaged, as seen in Figure 18. The cell walls stained more densely and appeared to have extra material on the cytoplasmic side. The size of the intercellular spaces was reduced. When examined at higher magnification, Figure 19, most cells of drought stressed plants were plasmolyzed, with disrupted cell membranes. Because of the disrupted cell membranes, several remnants of cellular organelles were found scattered in a mixture of vacuole and cytoplasm. Where distinguishable, chloroplasts seem not to have a continuous outer membrane and the stroma lamellae may be discontinuous and shows breaks, Figure 20.

Examination of several micrographs from water stressed, greenbug-infested bloom plants showed a greater number of mitochondria. Cell walls were osmophilic and seemed to have some extra material on the cytoplasmic side.

Figure 16. Single Bundle Sheath Cell of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Drought Conditions, 5,800X.

Figure 17. Micrograph of Bundle Sheath Cell Chloroplast From ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Drought Conditions, 29,000X.


16
M
SL
Chl
BS

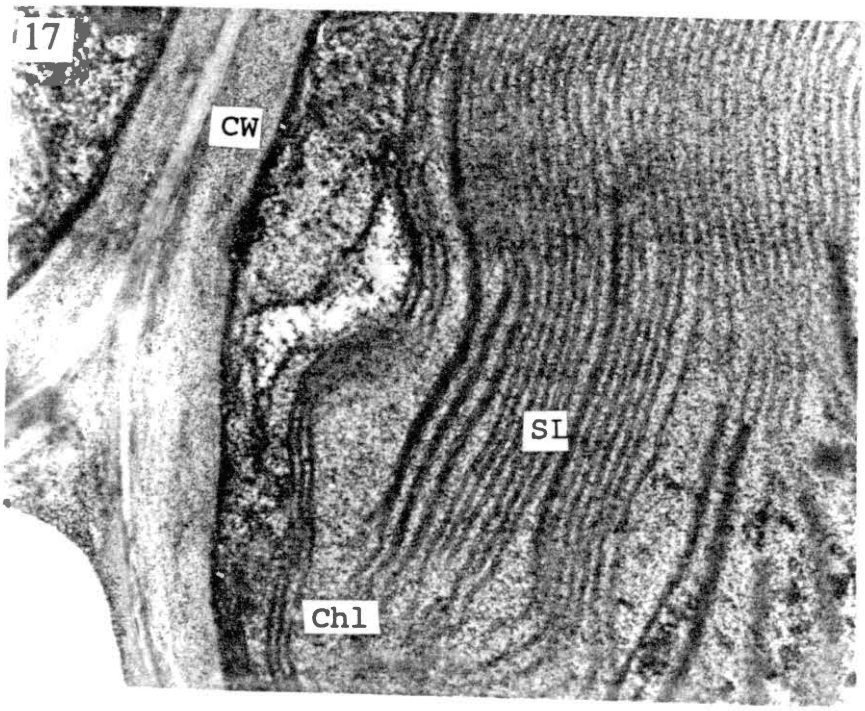
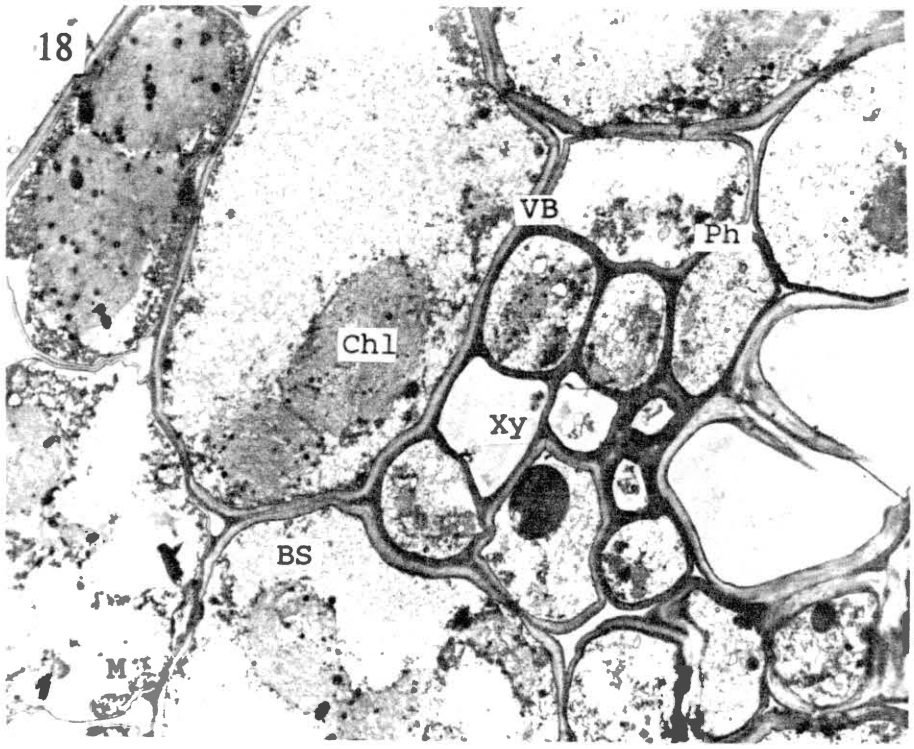

17
CW
SL
Chl

Figure 18. Micrograph of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 3 Days After Biotype E Greenbug Feeding, 2,900X. Note that the bundle sheath chloroplast still maintained some fragments of membrane.

Figure 19. Plasmolyzed Epidermal Cell of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Drought Conditions, 3 Days After Biotype E Greenbug Feeding, 5,800X. Note the reticulation of the cell wall and the fragmentation of the cell membrane.


18
VB
Ph
Chl
Xy
BS
M

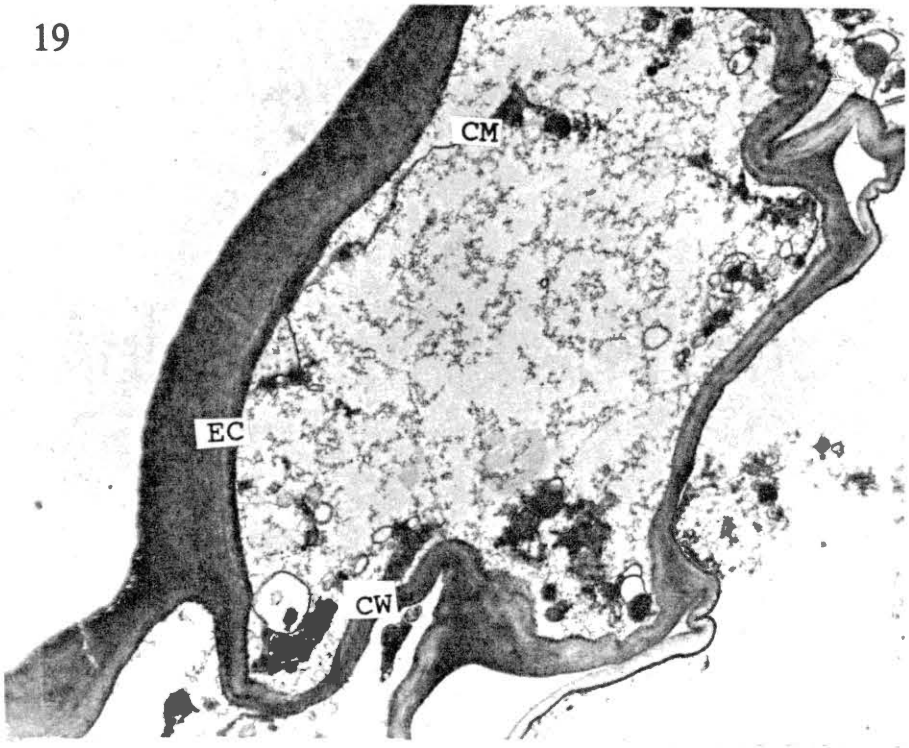
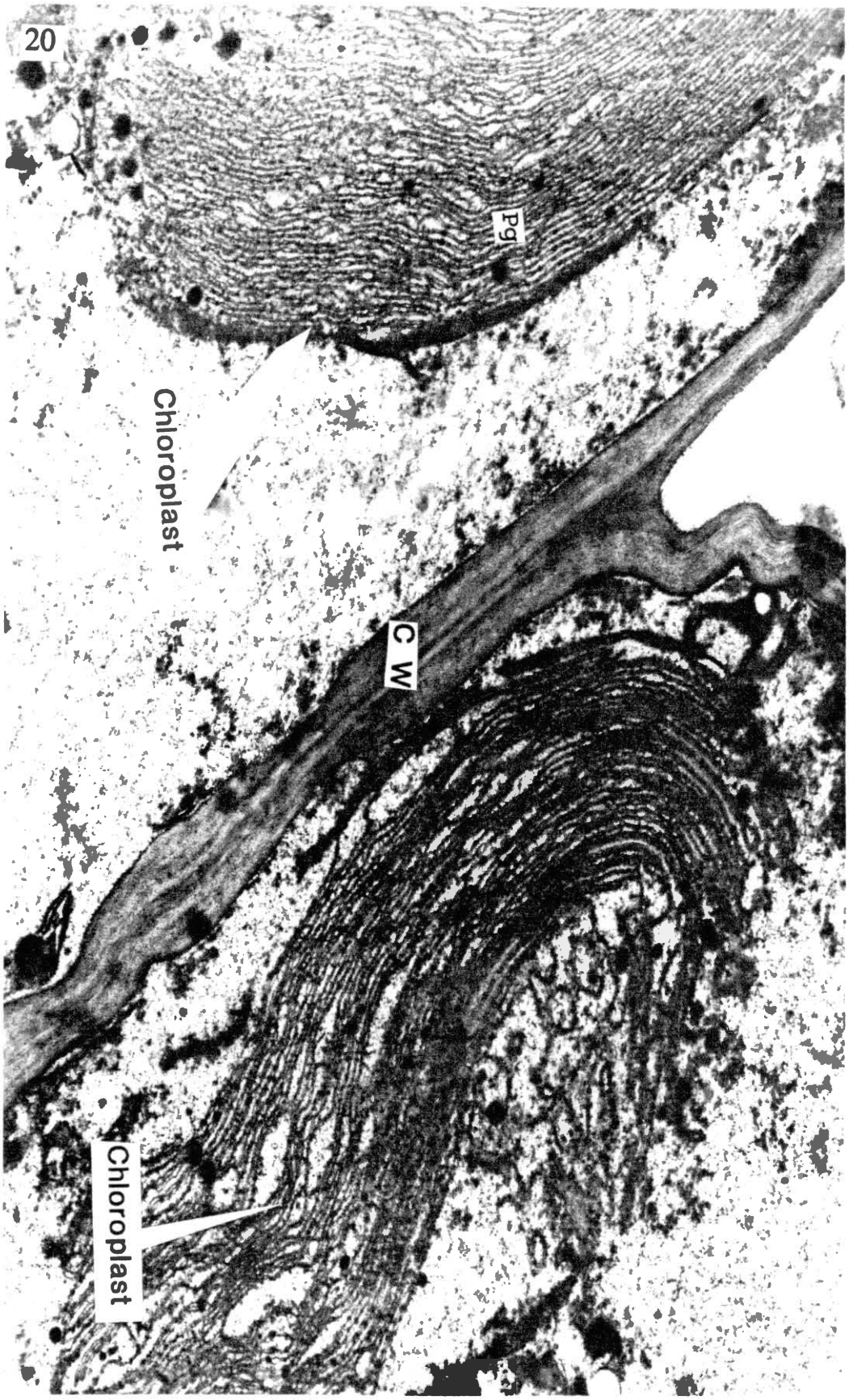

19
CM
EC
CW

Figure 20. Micrograph of Damaged Cell of ROKY62 Bloom Sorghum Plant, Leaf Tissue Grown Under Drought Conditions, 3 Days After Biotype E Greenbug Feeding, 10,000X. Note the disintegrating Chloroplast (Chl).


20
Pg
Chloroplast
C W
Chloroplast

Well-watered bloomless ROKY62 plants had large intact epidermal cells, and their mesophyll cells had few starch grains. Chloroplasts were intact and the outer mesophyll cells had thin cell walls and large intercellular spaces (Figure 21). Following greenbug feeding, the intercellular spaces remained approximately the same as seen in Figure 22. The chloroplasts, as observed earlier, were intact but occupied by starch grains. The grana were still distinct. At higher magnification (Figures 23 and 24), the large amount of starch became evident. Besides starch accumulation, I also observed the appearance of plastoglobuli in the chloroplasts (Figure 24). Cell walls did not appear different in size from those observed before greenbug feeding but seemed to have some extra material on the cytoplasmic side. The cytoplasm itself was denser than before greenbug feeding, with a greater number of mitochondria and greater amount of endoplasmic reticulum.

Figure 21. Micrograph of Mesophyll Cells of ROKY62 Bloomless Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 5,800X.

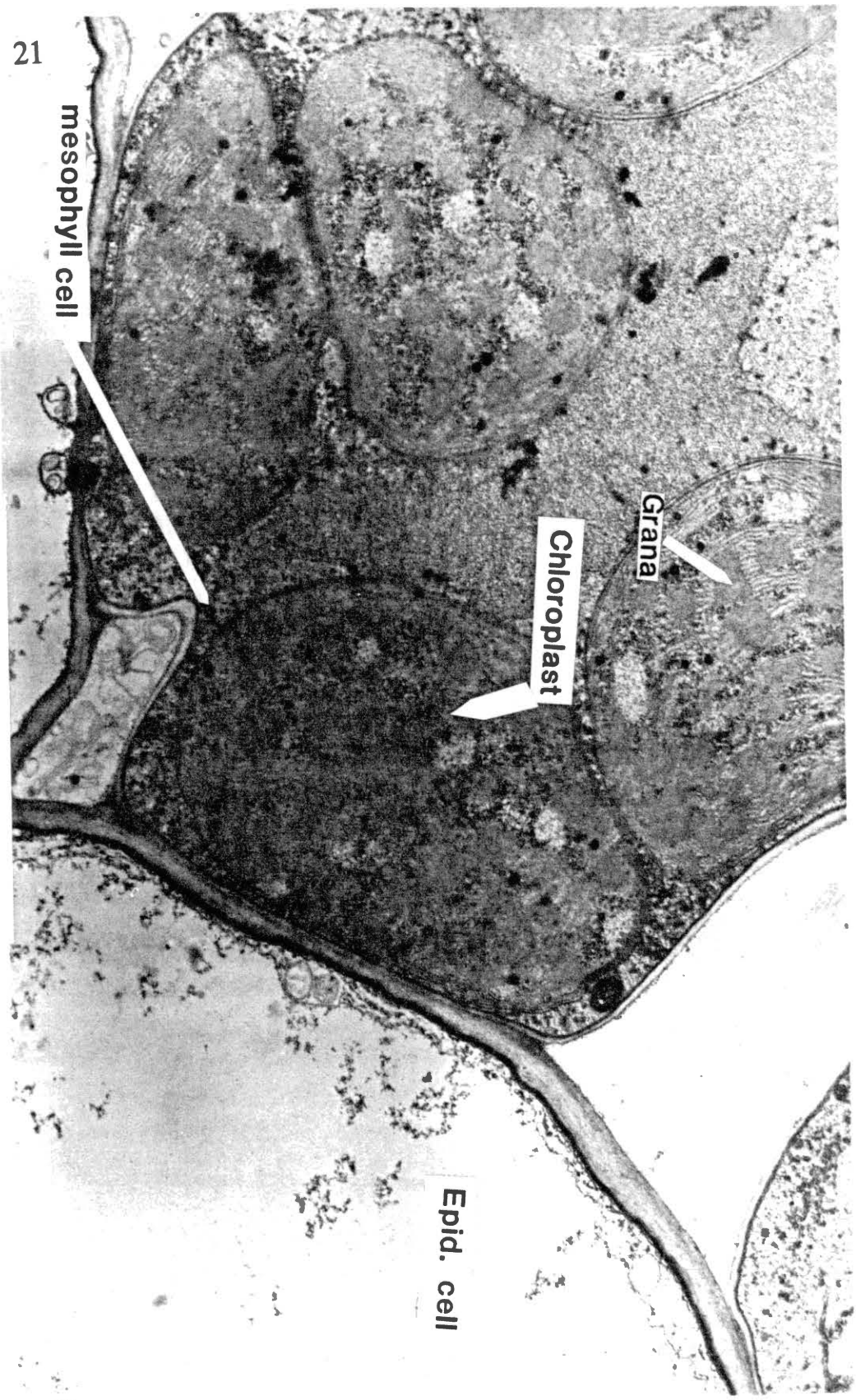

21
mesophyll cell
Grana
Chloroplast
Epid. cell

Figure 22. Micrograph of Mesophyll Cell of ROKY62 Bloomless Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 3 Days After Biotype E Greenbug Feeding, 1,900X. Note the amount of starch in the chloroplasts, and the amount of intercellular space (ICS).

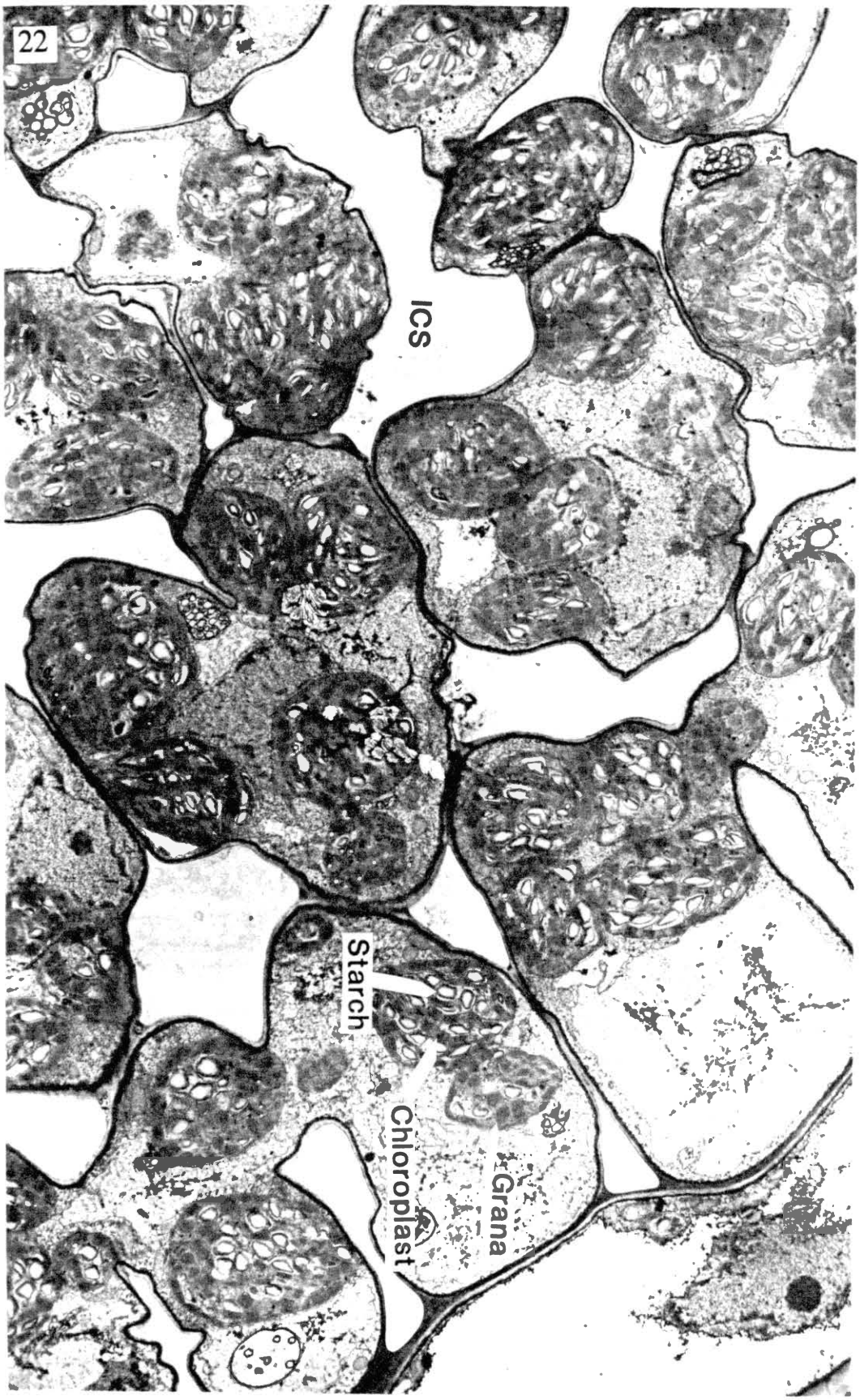

22
ICS
Starch
Chloroplast
Grana

Figure 23. Micrograph of Mesophyll Cell of ROKY62 Bloomless Sorghum Plant, Leaf Tissue Grown Under Well-Watered Conditions, 3 Days After Biotype E Greenbug Feeding, 5,800X.

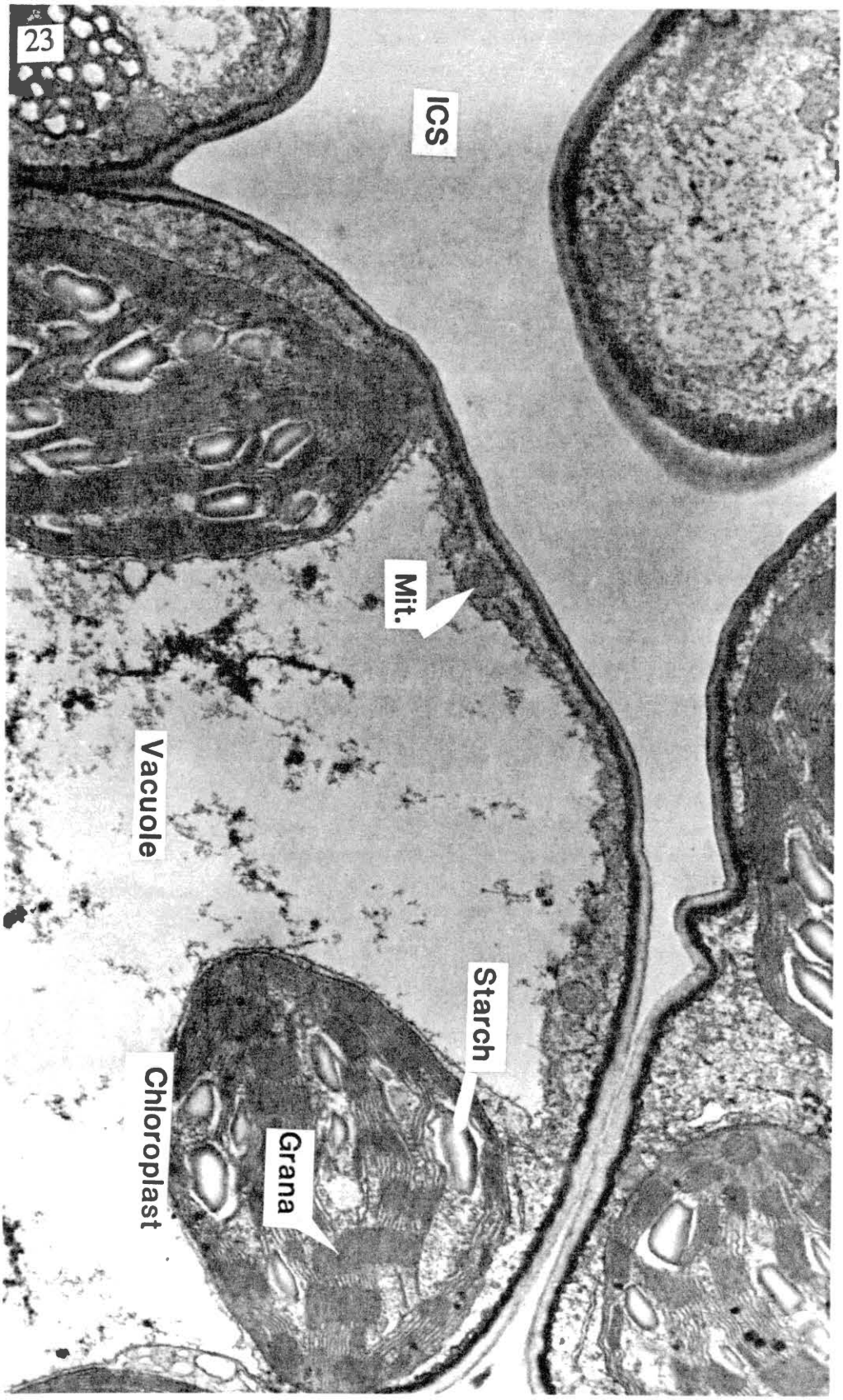
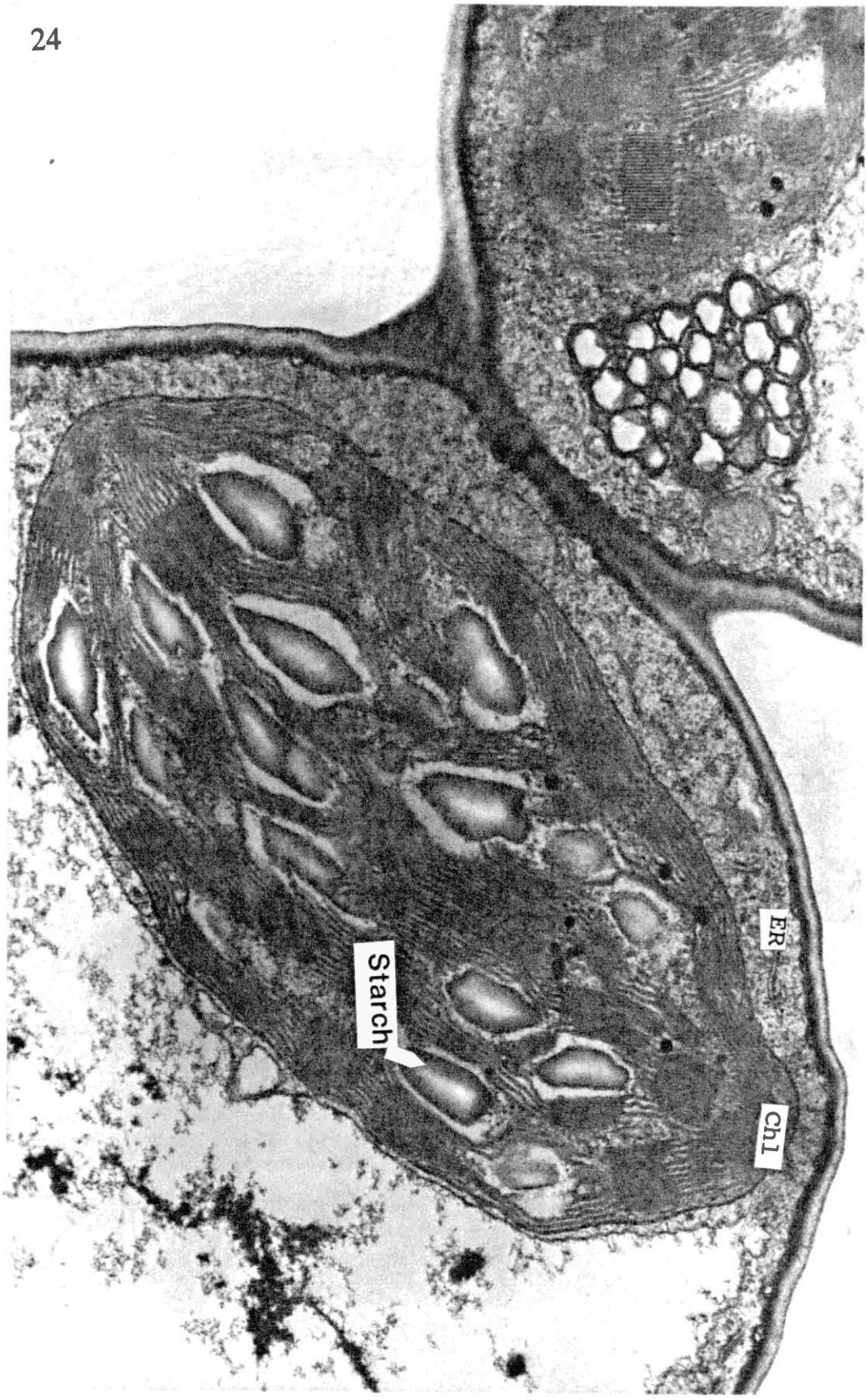

23
ICS
Mit.
Vacuole
Starch
Grana
Chloroplast

Figure 24. Single Chloroplast From Figure 23, Showing the Detailed Contents of the Chloroplast.

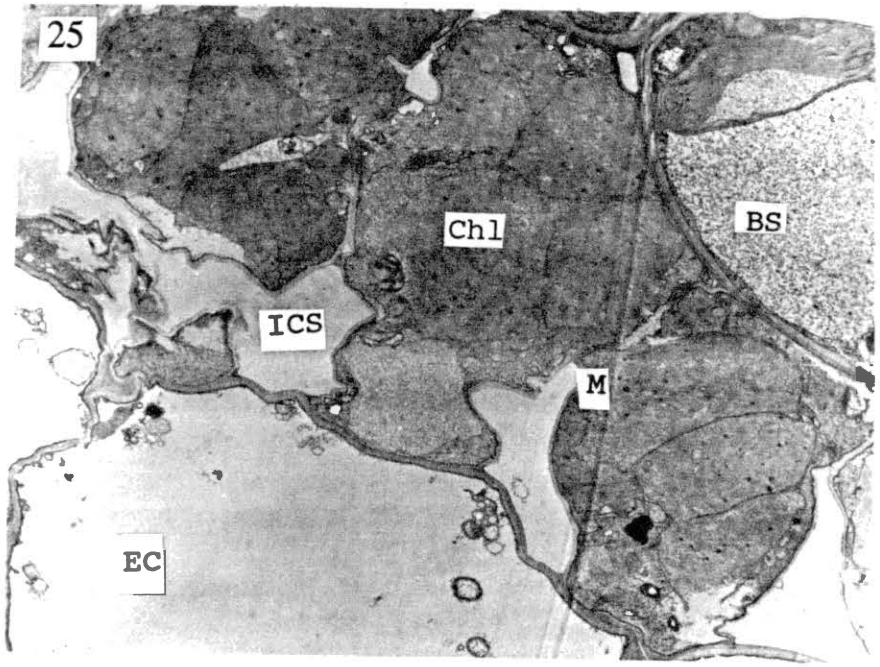
24

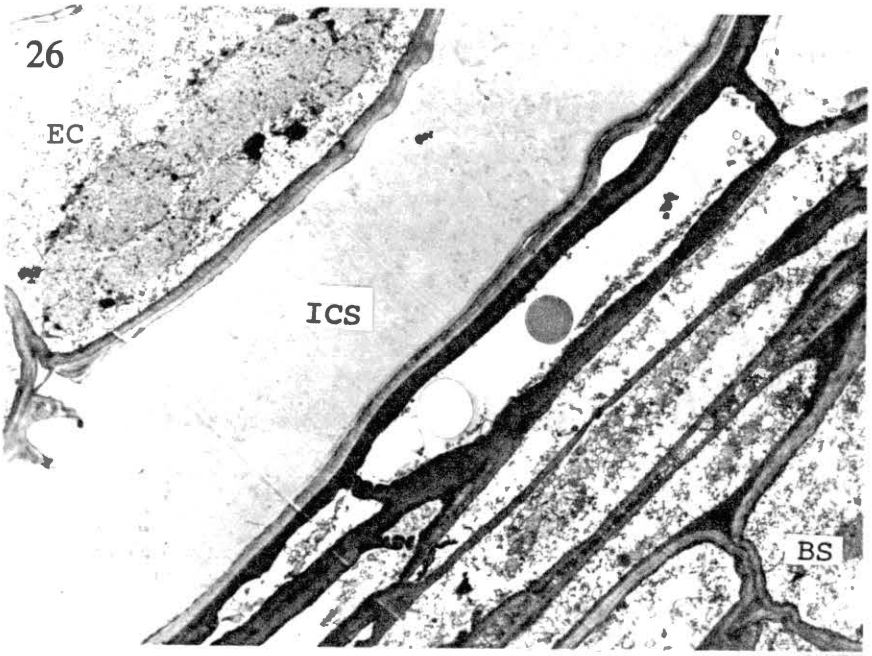


Under drought stress, bloomless sorghum leaf cells had walls stained more densely than under control conditions. The epidermal cells have collapsed cell walls. The volume of the epidermal cells decreased. There was little cytoplasmic content. The outer mesophyll cells had less intercellular space and had many chloroplasts, Figure 25. The characteristics of the mesophyll chloroplasts are similar in both lines. Starch accumulation was not observed. Following greenbug feeding, I observed the formation of plastoglobuli and a small accumulation of starch in the chloroplasts.

Figure 25. Micrograph of ROKY Bloomless Sorghum Plant, Leaf Tissue Grown Under Drought Conditions, 2,900X.

Figure 26. Tangential Section of ROKY62 Bloomless Sorghum Plant, Leaf Tissue Grown Under Drought Stress, 3 Days Following Biotype E Greenbug Feeding, 1,900X.


25
Chl
BS
ICS
M
EC


26
EC
ICS
BS

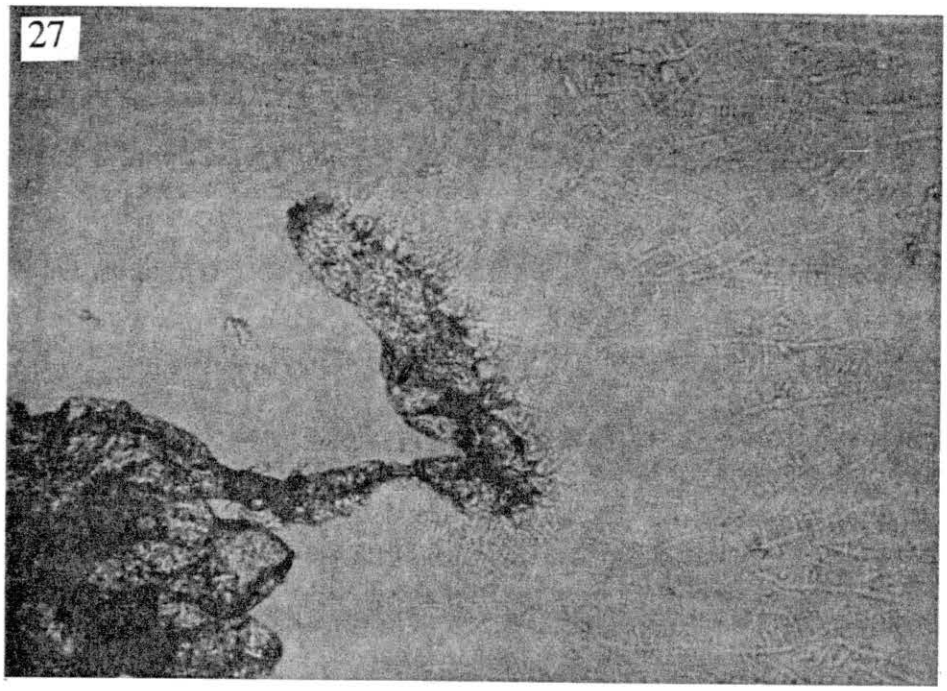
Immunofluorescence Assay of Sorghum Callus

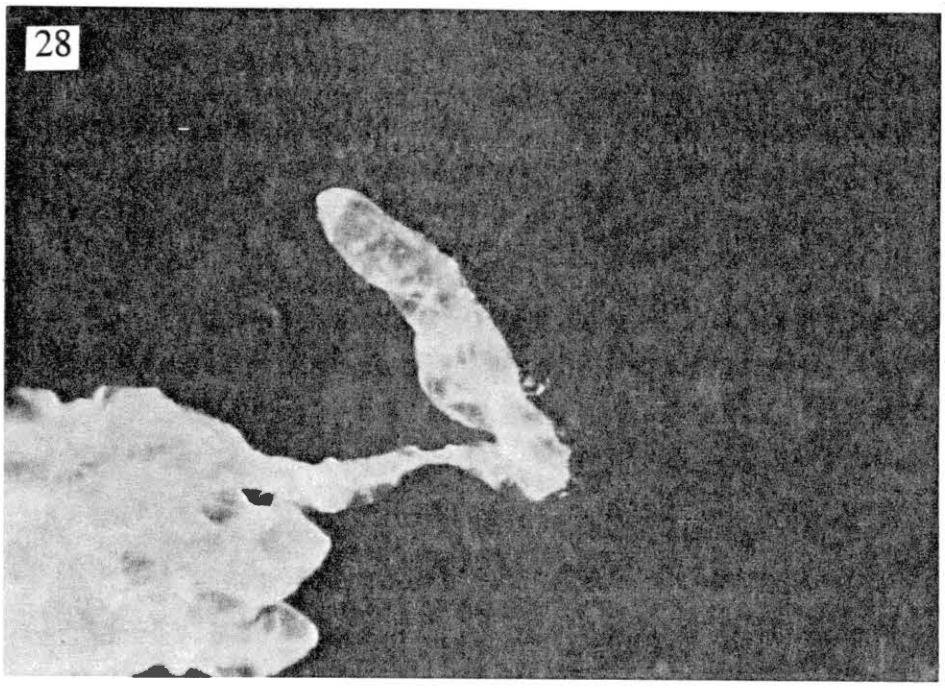
Calli were obtained from bloom and bloomless sorghum seeds in approximately three weeks following callus initiation. It was possible to maintain six plates of good transluscent calli from each line which were maintained for a period of 6 months by continuous transfer to fresh medium. Large amounts of anthocyanin pigment were produced by some portions of the growing calli and diffused in the medium. When this occurred, the segments of the callus tissue producing the pigment were excised under sterile conditions in a laminar flow hood, and transferred to fresh medium. No embryogenic callus was identified.

An immunofluorescence technique of labelling microtubules was applied to the non-embryogenic calli. Figures 27 and 28 represent a segment of callus tissue of ROKY62 bloom treated with anti-tubulin antibody and counter-stained with FITC. In Figure 27 photographed with white transmitted light, no structure could be distinguished. When the same slide was examined with blue light using a BG-2 filter, it was possible to distinguish structure inside the cells.

Figure 27. Photomicrography of ROKY62 Bloom Sorghum Callus, Using Bright Transmitted Light, 10X

Figure 28. Fluorescence Photomicrography of ROKY62 Bloom Sorghum Callus, Using Blue Light and BG-2 Filter. Note that at low magnification, some cellular components can be distinguished from the unstained components.

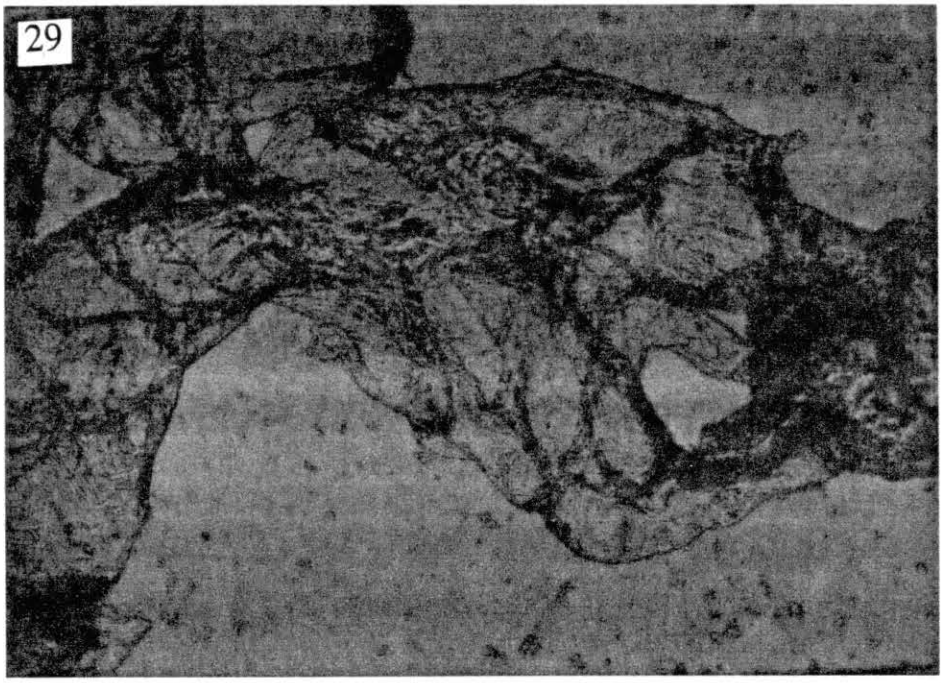

27

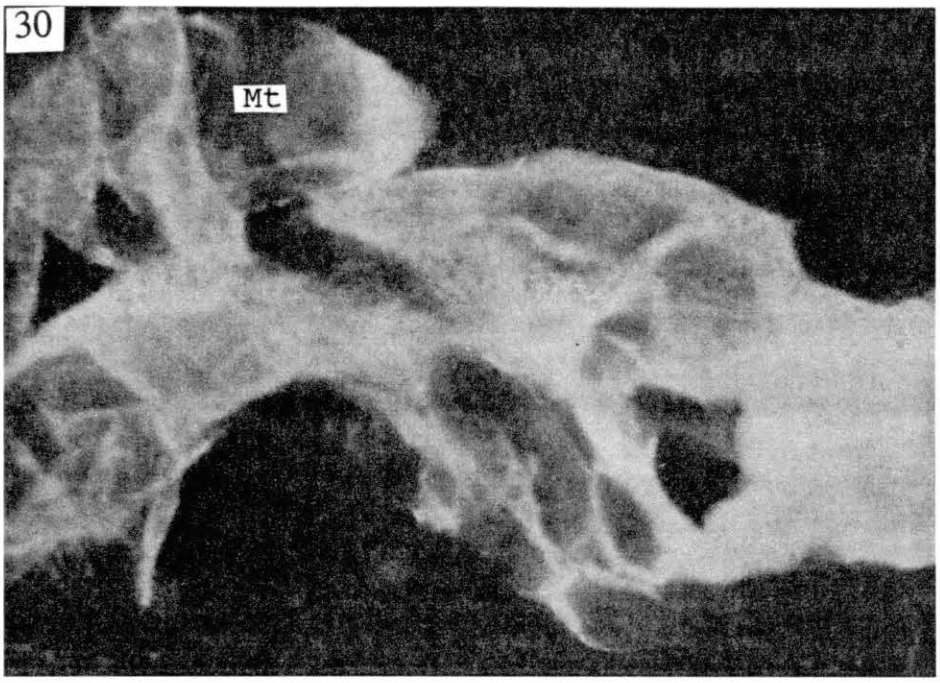

28

Figures 29 and 30 are photomicrographs of callus tissue of ROKY62 bloomless treated with anti-tubulin antibody and counter-stained with FITC. In Figure 29 where the source of illumination was white light, it was not possible to identify any cellular structure while in Figure 30 where the source of illumination was blue light, strands of white fine lines, possibly microtubules could be seen as fluorescent structures. No difference in the amount of fluorescence was detected between the two sorghum lines. In the electron microscopic investigation, it was observed that starch and plastoglobuli accumulated in the chloroplasts in response to drought stress and greenbug feeding on bloom and bloomless lines of sorghum. Immunofluorescent labelling techniques may be used to identify the mechanisms associated with plastoglobuli and starch accumulation in plants under drought or aphid feeding.

Figure 29. Bright Light Photomicrography of ROKY62 Bloomless Sorghum Callus, 100X. The tissue was stained with FITC Conjugate after incubation with anti-tubulin antibody.

Figure 30. Fluorescence Photomicrography of ROKY62 Bloomless Sorghum Callus, 100X. Note the arrays of tubulin containing cellular components in the cell on the upper left.


29


30
Mt

CHAPTER V

CONCLUSIONS

Visual observations of the plants revealed that wax deposition on the bloom plants is primarily on the leaf sheath and the abaxial leaf blade surface, and that this wax deposition increased with a plant's age and drought stress. Under drought condition, leaf rolling was observed in the bloomless and bloom plants. Beside leaf rolling, leaf tip and edge burning were also observed in the bloomless plants under drought stress. All these changes occurring under water stress reduce transpiring leaf areas.

The plant relative water content was shown to decrease sharply when water was withheld. The relative water content reduction of bloomless plants was greater than that of the bloom plants under similar water stress. Under well-watered conditions, there was no apparent difference in relative water content between the two lines over the seven days.

The electronic feeding monitor study showed that water stress on host plants has negative effects on greenbug feeding behavior. The frequencies of greenbug salivation and non-phloem ingestion increased under water stress. It was concluded that drought stress is a factor interferring with the ability of greenbugs to successfully commit to

phloem feeding, but when feeding occured, it lasted longer in drought-stressed than in non-drought stressed plants. Salivation frequencies and total duration of salivation in bloomless plants were higher than in bloom plants, under similar watering conditions. It can be concluded that drought stress increases the degree of restlessness in feeding aphids on sorghum plants.

Electron microscopic investigations indicated that the ultrastructures of ROKY62 bloom and ROKY62 bloomless plants were similar under similar watering conditions. Under well-watered condition, both lines accumulated large amounts of starch grains in the mesophyll cell and bundle sheath chloroplasts. There was no starch accumulation in either line under the drought conditions. However, following greenbug feeding, there was an accumulation of starch grains and plastoglobuli in the bundle sheath and mesophyll cells of both lines under drought condition. It was also found that drought stress induced cell plasmolysis, cell wall collapse, and an increase in organelles related to metabolic activities in both lines. I found that the cell walls of all plants stained more densely when under drought stress or following greenbug feeding than in plants under a higher moisture regime, and had extra wall materials on the cytoplasmic side. In addition, the size of the vacuoles, the cell cytoplasmic volume and the volume of the intercellular spaces were reduced in plants grown under drought stress. It was concluded that greenbug feeding induces a process that might affect sucrose translocation,

leading to starch accumulation in the chloroplasts of the mesophyll and bundle sheath cells of sorghum plants.

LITERATURE CITED

Anonymous. 1965. Losses in Agriculture. USDA/ARS. Agricultural Handbook No. 291. p. 40.

Atkin, D. S. J. and R. J. Hamilton. 1982. The Changes With Age In the Epicuticular Wax of Sorghum bicolor. J. Nat. Products. 10:697-703.

Barlass, M. and K. G. Skene. 1981. Relative NaCl Tolerant Grapevine Cultivars and Hybrids in Vitro. Z. Pflanzenphysiol. 102:147-156.

Bhaskaran, S., R. H. Smith, and R. J. Newton. 1985. Physiological Changes in Cultured Sorghum Cells in Response to Induced Drought Stress I. Free Proline. Physiol. Plant. 79:266-269.

Brzezina, A. S., N. J. Spiller, and M. Llewllyn. 1986. Mesophyll Cell Damage of Wheat Plants Caused By Probing of the Aphid Motopolophium dhirodum. Entomol. Exp. Appl. 42:195-200.

Campbell, B. C., D. L. McLean, M. G. Kinsey, K. C. Jones, and D. L. Dryer. 1982. Probing Behavior of the Greenbug (Schizaphis graminum, Biotype C) on Resistant and Susceptible Varieties of Sorghum. Entomol. Exp. Appl. 31:140-146.

Campbell, B. C. and D. L. Dryer. 1985. Host-Plant Resistance of Sorghum: Differential Hydrolysis of Sorghum Pectic Substances by Polysaccharides of Greenbug Biotypes (Schizaphis graminum, Homoptera: Aphididae). Arch. Ins. Biochem. Physiol. 2:203-215.

Chaleff, R. S. and M. F. Parson. 1978. Direct Selection In Vitro for Herbicide Resistant Mutant of Nicotiana tabacum. Proc. Natl. Acad. Sci. USA. 75:5104-5107.

Coons, A. H. 1958. Fluorescent Antibody Methods. In. General Cytochemistry. Vol. 1. pp 399-422. Academic Press, New York.

DePew, L. J. and M. D. Witt. 1979. Evaluation of Greenbug Resistant Hybrids. J. Econ. Entomol. 72:177-179

Dorschner, K. W., R. C. Johnson, R. D. Eikenbary, and J. D. Ryan. 1986. Insect-Plant Interactions: Greenbugs (Homoptera: Aphididae) Disrupt Acclimation of Winter Wheat to Drought Stress. Environ. Entomol. 15:118-121.

Dryer, D. L. and B. C. Campbell. 1984. Association of the Degree of Methylation of Intercellular Pectin With Plant Resistance to Aphids and With Induction of Aphid Biotypes. Experientia 40:224-226.

Dustin, P. 1984. Microtubules. 2nd Edition. Springer-Verlag. Berlin, Heidelburg, New York, Tokyo.

Esau, K. 1977. Anatomy of Seed Plants. 2nd Edition. John Wiley & Son, Inc. New York, USA.

Evert, R. F., W. Eschrich, S. E. Eichorn, and S. T. Limbach. 1973. Observation on Penetration of Barley Leaves By the Aphid Rhopalosiphum maidis (Fitch). Protoplasma 77:95-110.

Falconnier, M. M. and R. W. Seagull. 1987. Immunofluorescence And Calcofluor White Stainning of Developing Tracheary Element in Zinnia elegans L. Suspension Cells. Protoplasma 136:118-124.

Galatis, B. 1980. Microtubules And Guard Cell Morphogenesis in Zea mays L. J. Cell Sci. 45:211-244.

Gengenbach, B. G., C. E. Green, and M. C. Donovan. 1977. Inhereitance of Selected Pathotoxin Resistance in Maize Plants Regenerated From Cell Cultures. Proc. Natl. Acad. Sci. USA. 74:5113-5117.

Hepler, P. K. and E. H. Newcomb. 1964. Microtubules And Fibrils in the Cytoplasm of Coleus Cells Undergoing Secondary Wall Deposition. J. Cell Biol. 20:529-533.

Johnson, B. B. and M. Worthington. 1987. Establishment of Suspension Cultures from Seeds of Plains Bluestem (Bothriochloa ischaemum (L.) Keng.) and Regeneration of Plants via Somatic Embryogenesis. In Vitro Cel. Develop. Biol. 23:783-788.

Karnovsky, M. J. 1965. A Formaldehyde-Glutaraldehyde Fixative of High Osmolarity For Use in Electron Microscopy. J. Cell Biol. 27, 137.

Kindler, S. D. and R. Staples. 1981. Schizaphis graminum: Effect on Grain Sorghun Exposed to Severe Drought. Environ. Entomol. 10:247-248.

Klingauf, F. K. E. Noecker-Wenzel, and U. Roettger. 1988. Role of Cuticle Waxes in Insect Infestation Behavior. Z. Pflanzenkr. Pflanzenschtz. 85:228-237.

Kramer, H. H. 1967. Plant Protection and World Crop Production. In Pflanzenschutz-Nachrichten. Vol, 20, p. 22.

LaClaire II, J. W. 1987. Microtubules Cytoskeleton in Intact And Wounded Coenocytic Green Algae. Planta 171:30-42.

Ledbetter, M. C. and K. R. Porter. 1963. A "Microtubule" in Plant Cell Fine Structure. J. Cell Biol. 19:239-250.

Lloyd, C. W. 1986. Microtubules And Cellular Morphogenesis of Plants. In. Developmental Order: A Comprehensive Treatise. Vol. 2. The Cellular Basis of Morphogenesis. Morphogenesis. Ed. L. W. Browder. PP. 31-57. New York (London).

Lloyd, C. W. 1987. The Plant Cytoskeleton: The Impact of Fluorescence Microscopy. Ann. Rev. Plant. Physiol. 38:119-139.

Lowe, H. J., G. J. P. Murphy, and M. L. Parker. 1985. Non-Glaucousness, a Probable Aphid Resistance Character of Wheat. Ann. Appl. Biol. 106:555-560.

MacKinnon, C., G. Gunderson, and M. W. Nabors. 1987. High Efficiency Plant Regeneration by Somatic Embryogenesis from Callus of Mature Embryo Explants of Bread Wheat (Triticum gestivum) and Grain Sorghum (Sorghum bicolor). In Vitro Cel. Develop. Biol. 23:443-448.

McLean, D. L. and M. G. Kinsey. 1964. A Technique for Electronically Recording Aphid Feeding And Salivation. Nature 4939:1358-1359.

McLean, D. L. and M. G. Kinsey. 1965. Identification of Electrically Recvorded Curve Patterns Associated With Aphid Salivation And Ingestion. Nature 4976:1130-1131.

Murashige, T and F. Skoog. 1962. A Revised Medium For Growth And Bioassays With Tobacco Tissue Cultures. Physiol. Plant. 14:473-497.

Nabors, M. W., A. Daniels., L. Nadolny, and C. Brown. 1974. Sodium Chloride Tolerant Lines of Tobacco Cells. Plant Sci. Lett. 4:155-159.

Olonju-Dixon, A. G., P. J. Bramel-Cox, and J. C. Reese. 1990. Feeding Behavior of Biotype E Greenbug (Homoptera: Aphididae) and Its Relaitonship to Resistance in Sorghum. J. Econ. Entomol. 83:241-246

Orton, T. J. 1979. Comparison of Salt Tolerance Between Hordeum vulgare and H. jubatum in Whole Plants And Callus Cultures. Z. PFlanzenphysiol. Bd. 98:105-118.

Painter, R. H. 1951. Insect Resistance in Crop Plants. University of Kansas Press, Lawrence, KS, 521 pp.

Peiretti, R. A., I. Amini, D. E. Weibel, K. J. Starks, and R. W. McNew. 1980. Relationship of "bloomless": (bmbm) Sorghum to Greenbug Resistance. Crop Sci. 20:173-176.

Pickett-Heaps, J. D. 1967. The Effects of Colchicine on the Ultrastructure of Dividing Wheat Cells, Xylem Wall Differentiation And Distribution of Cytoplasmic Microtubules. Developm. Biol. 13:206-236.

Roberts, K., J. Burgress, I. Roberts, and P. Lindstead. 1985. Microtubule Rearrangement During Plant Cell Growth And Development: An Immunofluorescebce Study. In. Botanical Microscopy. Ed. A. M. Robards. PP 105-127. New York / Oxford Univ. Press.

Ryan, J. D., R. C. Johnson, R. D. Eikenbary, and K. W. Dorschner. 1987. Drought/Greenbug Interactions: Photosynthesis of Greenbug Resistant And Susceptible Wheat. Crop Sci. 27:283-288.

SAS Institute. 1988. SAS User's Guide: Statistics, Version 6. Gary, NC.

Schuster, D. J. and K. J. Starks. 1973. Greenbug: Componets of Host-Plant Resistance in Sorghum. J. Econ. Entomol. 66:1131-1134.

Smith, R. H., S. S. Bhaskaran, and K. Schertz. 1982. Variation in Drought Tolerance Characteristics From Tissue Culture Derived Sorghum. In. A. Fujiwara Ed. Plant Tissue Culture. Tokyo pp 489-490.

Spurr, A. R. 1969. A Low-Viscosity Resin Embedding Medium For Electron Microscopy. J. Ultrastr. Res. 26, 31.

Teetes, G. L., J. W. Johnson, and D. T. Rosenow. 1975. Response of Improved Resistant Sorghum Hybrids to Natural And Artificial Greenbug Populations. J. Econ. Entomol. 68:546-548.

Todd, G. W., F. W. Ingram, and C. A. Stutte. 1962. Relative Turgidity as an Indicator of Drought Stress in Cereal Plants. Proc. of the Okla. Acad. of Science. 42:55-60.

Van Der Valk, P., P. J. Rennie, J. A. Connolly, and L. C. Fourke. 1980. Distribution of Cortical Microtubules in Tobacco Protoplasts. An Immunofluorescence Microscope and Ultrastructural Study. Protoplasma. 105:27-43.

Venable, J. H. and R. Coggeshall. 1965. A Simplified Lead Citrate Stain for Use in Electron Microscopy. J. Cell Biol. 25, 407.

Volfova, A., C. Chvojka, and J. Hankovska. 1977. The Orientation of Cell Wall Microtubules in Wheat Coleoptile Segments Subject to Phytochrome Treatment. Biol. Plant. 19 (6):421-425.

Weibel, D. E., K. J. Starks, E. A. Wood Jr, and R. D. Morrison. 1972. Sorghum Cultivars And Progenies Rated for Resistance to Greenbugs. Crop Sci. 12:334-336.

Wick, S. M. 1985. Immunofluorescence Microscopy of Tubulin And Microtubule Arrays in Plant Cells. III. Transition Between Mitotic/Cytokinetic Interphase Microtubule Arrays. Cell Biol. Int. Reports. 9:367-371.

Wick, S. M., R. W. Seagull, M. Osborn, K. Weber, and B. E. S. Cunning. 1981. Immunofluorescence Microscopy of Organized Microtubule Arrays in Structurally Stabilized Meristematic Plant Cells. J. Cell Biol. 89:685-690.

VITA 2

Mijitaba Hamissou

Candidate for the Degree of

Doctor of Philosophy

Thesis: ULTRASTRUCTURAL AND BEHAVIORAL STUDY OF GREENBUG FEEDING ON SORGHUM PLANTS UNDER DROUGHT STRESS; MICROTUBULE DISTRIBUTION IN SORGHUM CALLUS

Major Field: Botany

Biographical:

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

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, 1973-75; received the B.S. (1985) and M.S. (1987) in Agronomy from Oklahoma State University; completed the requirements for the Doctor of Philosophy Degree at Oklahoma State University in May 1992.

Professional Experiences: Trainee in Sorghum and Millet Breeding at the International Crops Research Institute for the semi-Arid Tropics, India, 1975-76. Director of the Agricultural Experiment Station, INRAN, Kolo, 1976-77. Technical Training Officer, Centre Nationale 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. Teaching Assistant, Department of Botany, Oklahoma State University, Stillwater, Oklahoma, 1987-1991. Chairman of the All-African Student Conference, 1989-1991, member of Oklahoma Acad. of Science and Oklahoma Soc. for Electron Microscopy.