STRUCTURAL CHANGES IN THE LEAVES OF *SORGHUM BICOLOR* (LINN.) MOENCH INDUCED BY INSECTICIDE PHYTOTOXICITY

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PREFACE

Generally, Phytotoxicity has been evaluated as damage in the form of local injury, or necrosis of plant tissues. So far, only external deformities resulting from insecticide applications have been stressed. To determine the sequence of changes in the structural pattern and chemical constituents of the leaves after insecticide treatment, the following study was initiated.

Each chapter of the thesis is written, with minor modifications, in the style of biological journals to which it will be presented.

I wish to express my deep appreciation to my major adviser, Dr. D. E. Howell, Head, Department of Entomology, for his constant encouragement and invaluable suggestions during the course of my graduate work and this study. I am also very much indebted to Dr. Harvey L. Chada, Entomology Research Division, U.S. Department of Agriculture, for suggesting the problem and making this study possible, and for his helpful ideas throughout the study. I also acknowledge my gratitude to Dr. Margaret H. Brooks, Associate Professor, Botany and Plant Pathology, for providing the laboratory facilities, and for her valuable criticism of the manuscript. Indebtedness is also expressed to Dr. James S. Brooks, Professor of Agronomy, for his assistance and cooperation in my graduate program.

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INTRODUCTION

Apart from its benevolent factor, the increasing utility of insecticides for controlling insects has evolved another aspect on the crop economy of the modern world. The problem of differential phytotoxic susceptibility and resistance of crop varieties to several insecticides has been a fascinating field of study in recent years. The tenets of research on this aspect have particularly covered the areas of crops in relation to their decreasing yield after insecticidal application. Phytotoxic effects have been observed on treated soils or following foliar application on trees, vines and other plants. Emphasis has been put on decreased germination of treated seeds, the loss of flavor of the edible parts of the plants, improper coloring of fruits, etc. Generally, phytotoxicity has been evaluated as damage in the form of local injury, or necrosis of plant tissues.

So far, only the external deformities resulting from insecticidal applications have been stressed. To the present, there is meager published work concerning internal disarray which results in a decrease in yield. In other words, the field of internal relations of plant tissues to the insecticidal applications has not been widely studied.

The morphological deformities resulting from the phytotoxic effects may be as a consequence of the internal structural disorganization as well as the upset of the normal metabolic processes.

The decrease in yield is mainly correlated with the inability of the photosynthetic system of the plant to cope with the increased rate

of respiration resulting from damage due to phytotoxicity. The present investigation has been undertaken to determine the sequence of changes in the structural pattern and chemical constitution of the leaves after treatment with several insecticides. With this perspective the study involving grain sorghum has been divided into three facets: (1) the resulting morphological and anatomical abnormalities; (2) the chemical composition of the resultant complex molecular organizations, and (3) the apparent modification of the cell organelle (the structure of chloroplast).

REVIEW OF LITERATURE

1. Introduction

Insecticide phytotoxicity is a very fascinating problem to economic entomologists because of widespread interest in the use of insecticides. Annual losses to crops and rangelands due to insect pests is estimated to be more than two billion dollars (U.S. Agri. Dept., 1965). The physiologists and economic entomologists hence prompted the application of insecticides.

The insecticides when applied to the soils often produce adverse effects, or following foliar application on trees, vines and other plants, and possible hazards arise in the form of residues with chemical persisting in the product.

Phytotoxic effects of insecticides have been noted as retarding the rate of germination of treated seeds, tainting the flavor of edible parts of the plants such as leaves, stems, fruits, etc., retarded growth, reduction in yield, improper coloring of fruits, etc. Generally, the damage is in the form of local injury or necrosis of plant tissues. The knowledge of the effect of insecticides on plants is very essential in facilitating their economic uses. As both insects and plants are composed of the same fundamental protoplasm, it is scarcely surprising that an agent toxic to one is also likely to damage the other (West and Hardy 1961). The phytotoxic responses of plants varies greatly with different insecticides and the concentrations used. Some insecticides toxic to one variety may not necessarily be harmful to another. Similarly, if a

variety is susceptible to one insecticide it may be resistant to other insecticides.

2. Phytotoxicity of insecticides

Since the Seventeenth Century mineral oils, petroleum and turpentine have been known to injure and even kill treated plants. However, in the 1940's when a number of entirely new synthetic compounds for insect control were developed the interest involving their hazards to plants has been increased. During 1946 reports of DDT injury of cucurbits were received from workers in many states.

Results of an experiment on arsenical spray injury to peach trees by Haenseler and Martin (1925) indicated that spray injury appeared mainly as leaf burning or necrotic areas at the older nodes of the new growth. Weak trees were more susceptible than vigorous ones.

Studies of deOng (1926) on the phytotoxicity of quick-breaking emulsions showed that the acute effect of oils was noticeable in the form of defoliation, twig, and fruit injury. There was an apparent disturbance of physiological processes of the trees and fruits. This was manifested by slight delay in the ripening of the fruits and forced dropping of tree-ripe lemons and oranges.

Dustan (1946) reported the phytotoxicity of DDT on cucurbits. He also observed the adverse effect of DDT on young tomato seedlings, while older and well established plants were saved. While studying the reaction of different varieties of squashes and pumpkins to DDT phytotoxicity, Carruth and Harvey (1947) showed that foliage injury and yield reduction produced by DDT dust was associated with the setting point of the DDT dust. The 3% DDT dust having a setting point of 90 C was more injurious than similar dusts prepared from DDT having setting points of 103 C to 105 C.

Stearns et al. (1947) noticed severe chlorosis followed by necrosis on cucumbers and cantaloups and a slight yellowing of the terminal growth on potatoes following application of toxophene 20% wettable powder. Yellowing and a slight stunting of all varieties of bush lima beans were noticed following the application of DDT dust and spray formulations.

King and Hudson (1949) reported spray injury on apple trees following heavy dosages of parathion. The injury was characterized by wilted, curled, and dead terminals. Injury on fruits was in the form of dark colored spots scattered over the undersides of the fruits. Glass (1950) observed the injury caused by parathion to apple foliage. It occurred mostly on the young leaves before full maturity was reached. Allen and Casida (1951) described a standardized laboratory technique for evaluating insecticidal phytotoxicity; mode of action of insecticides on plant aerial growth. This procedure provided a rapid screening technique that was used to test for both inhibitory and stimulating effects of the insecticides. The evaluation of insecticidal phytotoxicity was based largely on gross detection of damage to plants recorded as the phytotoxic index (percent inhibition of growth due to toxicants).

Hacskaylo (1957) reported that when cotton plants were grown in sand culture containing different levels of thimet, all plants initially wilted, but recovered later. The accumulation of reducing sugars, sucrose, and starch with reduction of soluble and protein nitrogen was observed with increasing levels of thimet.

Studies of Krishnaswamy (1954) on the insecticidal and adverse effect of DDT and BHC on vegetables showed the BHC formulations inhibited plant growth and produced a scorching effect on brinjal. All vegetable crops treated with BHC suffered from off-flavor.

While working on mangolds and beans, Lord (1955) determined that substances which inhibit cholinesterases and plant esterases may occur in plants treated with organophosphorous insecticides.

Mukerjee and Wadhi (1956) noted the phytotoxicity of toxophene and dieldrin on maize and DDT on Momardica charantia, and Lagenaria sicera-ria, the latter was severely burnt.

Hacskaylo and Scales (1959) worked on the effect of different in-secticides on cotton plants and reported that dieldrin-DDT mixtures re-tarded flowers formation, boll set, and plant growth, whereas plants treated with (azenaphosmethyl) Guthion at 0.25 lbs per acre produced more flowers than the untreated plants. All insecticides reduced boll size. Guthion-DDT, and dieldrin-DDT mixtures caused stunting of the plants by killing some of the meristematic regions of the stem.

Kirk and Wilson (1960) reported the adverse effect of phorate on germination of seeds of wheat. Both phorate and SD-3562 (Bidrin®) were very phytotoxic to wheat seeds. Phytotoxic effects on seed viability increased as the interval between treatment and seeding was prolonged.

Dennis and Edward (1961) working with chlorinated hydrocarbons and organophosphorous compounds on various vegetable plants, noticed that tomato and cucumber plants were sensitive to lindane, aldrin, and DDT emulsion. Plants showed mottled chlorosis of upper leaves, marginal scorch, and wilting. Similar slight effects were also found on peas, beans, and cabbage. Brown et al. (1962) showed that the growth and yield of cotton plants were not affected when treated with toxophene, DDT, and methyl parathion, while plants treated with calcium arsenate showed burning of the young leaves, and the margins of the leaves were crinckel-ed.

In many recorded cases, the degree of phytotoxicity ranged from high

to low with the concentration of insecticides. Razvi (1964) observed that the severity of phytotoxic effects of BHC and Sevin when applied to bottle-gourd plants of high levels resulted in an increase in percentage of reducing sugars. She reported severe chlorosis, necrosis, and even death of the plants in case of BHC on bottle-gourd, whereas BHC merely retarded the growth of tomato plants. Hacskaylo and Boling (1966) noted the deleterious effect on the growth characteristics of cotton seedlings when UC 21149 (Temik®) was applied to soil either alone or in combination with herbicides.

3. Insecticidal Phytotoxicity to Sorghum

Sorghum ranks high among the cultivated crops of the world, ranking fourth to wheat, rice and maize in total acreage planted. It is a major cereal constituent of the staple food in the tropics of the Old World. In recent years much agricultural research has been concentrated on tropical crops. The major insect pests causing severe damage to sorghums in the United States are chinch bugs, sorghum midge, sorghum webworms, and corn earworms. The studies on application of insecticides to control the pests have received much attention in recent years, following the recognition of severe phytotoxic responses to some insecticides by some sorghums.

Unpublished previous work of Dahms and Wood, Jr. in Oklahoma in 1954 showed a differential response of sorghum varieties to the insecticides tested. Among the insecticides, Ethyl parathion, diazinon, demeton (systox), and malathion caused little or no phytotoxicity while severe burning was observed by methyl parathion and chlorthion. However, the rapid recovery of all the varieties after 10 days of application was noted.

Everly and Pickett (1960) obtained aphid control on grain sorghum during early growth following treatment of seeds with two and four pounds of phorate per 100 pounds of seed, but yields were reduced, probably due to reduced plant stand. Randolph (1961) in his experiment with sorghum hybrid RS 610 found that methyl parathion and parathion caused discoloration of the leaves, but the yield of grain and forage was not affected.

Burkhardt (1963) did not notice any phytotoxicity to sorghum varieties Frontier 400 C and DeKalb 62 when sprayed with four and eight ounces of Phosdrin (mevinphos) per acre where the heads emerged two weeks before treatment; however, slight injury was observed after application of Phosdrin at the rate of 12 and 16 ounces per acre.

Chada et al. (1964) noted differential insecticide phytotoxicity in different hybrids and parental lines of sorghum. They observed severe leaf injury caused by naled and methyl parathion, with moderate injury by toxophene, while carbaryl and endrin appeared to have little or no effect on all varieties tested. They correlated the degree of phytotoxicity with the parentage of plants, showing that phytotoxicity was genetically controlled. They also found that yield reductions up to 50% occurred on hybrid sorghums susceptible to insecticide injury. Harding (1965) tested Northrup King 210 and Lindsey 788 hybrid grain sorghum with different insecticides to determine the effect of insecticidal phytotoxicity and aphids on grain sorghum yield. The results of his test indicated that plots treated with toxaphene exhibited the highest degree of leaf damage, but no reduction in yield, while the application of Bidrin® and dimetilan resulted in slight leaf burn.

Experiments on the evaluation of insecticides for sorghum midge

control by Harding (1965) indicated yield reduction in Lindsey 788 sorghum hybrid due to phytotoxicity of Bidrin®, SD 9129 (Azodrin), Bomyl , Niran (parathion), toxaphene, and methyl parathion.

4. Physiological Basis of Phytotoxicity

Phytotoxic effects could be due to some transitory disturbances in physiology of the plant, such as accelerating or retarding transpiration, photosynthesis, or respiration. In considering the effect of herbicides and other spray materials, most attention has been dedicated to the effect on stomatal aperture. It is believed that stomatal closure may depend on an increased acidity in the guard cells resulting from an increase either of CO_2 or of organic acid content. Thus, herbicides may effect the rate of transpiration by an effect on stomatal opening resulting from a modification of certain biochemical processes in the guard cells or neighboring epidermis. Player (1950) found that the sprays of indole-3-acetic acid (IAA), 2-naphtyoxy-acetic acid (Noxa), 4-chlorophenoxyacetic acid (4CPA), 2,-4-dichorophenoxy acetic acid (2,4-D), etc., reduced the transpiration rate of castor bean plants. Dewey et al. (1956) and Juniper (1959) reported that herbicides increase cuticular transpiration.

Continued changes in the photosynthetic rate and hence of available photosynthate have far reaching effect on growth, well being, chemical composition, and yield of the plant. Higher concentrations of herbicides have an adverse effect on the rate of photosynthesis (Wedding et al. 1956, Wort 1957, 1959, and 1962). Plants treated with herbicides were depleted rapidly of their carbohydrates, apparently by the increased rate of respiration. Increases of up to 80% have been obtained in CO_2 evolution or oxygen consumption following the application of 2-4-D to ripening pears and young pea stem tissue, according to Smith's

review of respiratory changes in relation to toxicity. It was indicated that 2-4-D exerts influence on the aerobic phase of respiration (Smith, 1951). Linden (1954) suggested that 2-4-D brings about an increase in respiratory rate by affecting phosphate metabolism and by its union with metabolic products and enzymes.

Insecticides may influence plant growth directly by affecting cell division and cell enlargement. While discussing the fungicide phytotoxicity Horsfall (1945) indicated that the cell number is not affected, but rather the process of cell enlargement is curtailed. The chemicals in the spray enter the tissues and harden the middle lamellae. As a consequence of this, the plant remains dwarfed and low in yield.

Apparently, to date meager attention has been paid to the use of insecticides in studying the physiological basis of phytotoxicity. The study by Hall (1951) with the strong and dilute solutions of TEPP (tepp) on the tomato plants is the first of its kind. He observed increase in stem length due to dilute solutions and decrease in the same caused by strong solutions of TEPP. The latter also decreased total carbohydrates with an increase in respiration rate and nutrient content. He considers these effects are analogous to those produced by hormone weed killers.

FIELD TESTS

In recent years several workers have reported the differential response of sorghum varieties to the insecticidal phytotoxicity. Chada et al. (1964) conducted systematic experiments to determine differential phytotoxicity of five insecticides at recommended rates using seven parental lines and five hybrids of sorghum. The results of this experiment were in accordance with field observations of replicated grain yield plots of sorghum at Mangum, Oklahoma. Naled (Dibrom) and methyl parathion produced severe injury on the variety Texas 660. However, hybrid RS 610, combine 7078, and caprock were the least injured, whereas combine 60 Kafir was severely damaged by both the insecticides.

The present study was undertaken as a continuation of the above to determine factors responsible for differential insecticide phytotoxicity among sorghum hybrids and to study possible physiological changes in the plants when phytotoxicity occurred.

Materials and Methods

An experiment was set up in 1966 to measure the degree and effects of phytotoxicity of three recommended insecticides on two sorghum hybrids. A randomized block design consisting of three replicates of each hybrid was used, involving hybrids RS 610 and OK 612. Each block was divided into three plots each 12 rows by 18 feet long. Insecticides used were emulsifiable concentrates and rates of application were: methyl parathion, 0.5 lbs.; Bidrin, 0.25 lbs.; and diazinon, 0.5 lbs. per acre. The

untreated check was sprayed with water. The insecticides were applied with a compressed air sprayer at the rate of 7.25 gallons per acre and a pressureof 40 psi. The temperature at the time of application was 75 F, and wind velocity 0-5 mph, with bright sunshine.

Two rows in each plot were sprayed, leaving two border rows unsprayed, thereby avoiding drift of one insecticidal application to another. In general insect attack on sorghum appears after the sorghum heads emerge from the boot. Hence, to observe the adverse effect of insecticides at that stage, the insecticides were sprayed at medium-to-hard dough stage. Insects were not a problem at the time of application.

The evaluation of phytotoxicity was based on visible leaf area damaged six days after the application of the insecticide according to the rating method by Chada et al.(1964).

Percentage of leaf area damaged	Rating
0-5	1
6-36	2
37-67	3
68-99	4
100	5

Observations

Bidrin has been shown to cause more rapid and severe phytotoxic effects than the other insecticides tested. Within six hours after spraying, light green streaks appear between the main veins of the leaves. These streaks became more distinct and wider between 12 hours and 24 hours after spraying, and developed into dark red lines on the leaf surface with heavy marginal burning after 24 hours. Gradually this reddening increased until it covered the entire leaf surface and ultimately

(within 60 hours after insecticide spray) the leaf turned dry and rigid. The effect was so severe that four days after the application of insecticide, 85-90% of the leaves were dried and the entire row appeared dead. The response of OK 612 to Bidrin was more severe than that of RS 610, where little phytotoxicity was noticed.

Phytotoxic effects of methyl parathion appeared 36 hours after application but were not very conspicuous. However, the principal symptom on the leaves sprayed with methyl parathion was the appearance of water soaked lesions on the laminae. Occasionally the injury appeared initially on both the margins of the lamanae and as isolated spots distant from the margin. This type of injury spread across the entire leaf blade, and 48 hours after the insecticide treatment dark red areas with discolored white patches appeared on the surface of leaves with marginal burning. These affected parts of the leaves became dry three to four days after spraying.

The hybrids did not show any phytotoxic symptoms when sprayed with diazinon. Data on the reaction of the sorghum hybrids to the spray applications are presented in Table I.

Discussion

The evaluation of phytotoxic effects by the rating method indicated a significant differential response of the two hybrids to the insecticides applied. It is apparent from Table I that Ok 612 is more susceptible to Bidrin and methyl parathion than is RS 610. Methyl parathion affected the plants less severely than did Bidrin.

In Table I it is shown that there is a difference in the phytotoxic reaction of the two hybrids. OK 612, with a phytotoxicity rating of 1.8

and 3.8 for methyl parathion and Bidrin sprays, respectively, was damaged by these sprays, but diazinon did not cause any injury. RS 610 was uninjured by any of the treatments. The injury rating of 1.3 for the Bidrin spray was of little significance, since a rating of 1.0 indicates an uninjured plant, and slight discolorations on unsprayed sorghum plants are normal.

Table 1

Phytotoxicity of Insecticides to Sorghum Hybrids

(Stillwater, Oklahoma 1966)

Insecticides	Leaf Injury Rating	
	RS 610	OK 612
Methyl Parathion	1	1.8
Bidrin	1.3	3.8
Diazinon	1	1
Check	1	1

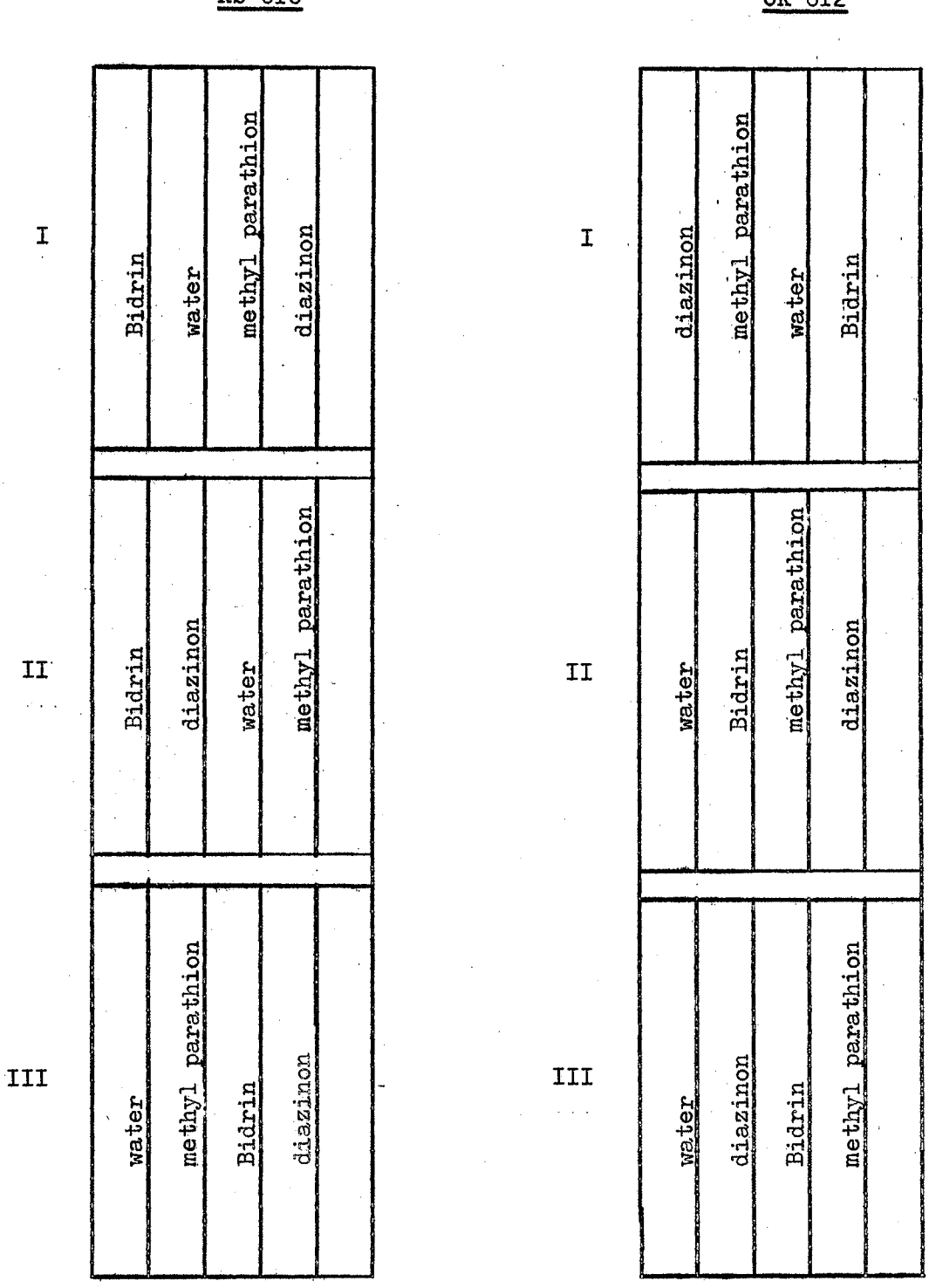

Fig. 1 Arrangement of experimental plots to determine phytotoxicity of insecticides.

ANATOMY OF INJURED LEAVES

The genus Sorghum has not been studied anatomically in great detail. Artschwager (1948) discussed the anatomy and morphology of Sorghum vulgare and reported two types of vascular bundles in sorghum leaves: (1) small round bundles in groups of 7 to 15 alternating with (2) large oval vascular bundles. The latter represents the principal parallel veins of the leaf occupying the entire leaf cross section. Smaller bundles, on the other hand, are embedded in Parenchyma close to the lower epidermis. Metcalfe (1961) described three types of vascular bundles in Sorghum halepense: (1) small, (2) crowded and angular, and (3) large. The small vascular bundles are not associated with sclerenchyma, whereas the large vascular bundles have both adaxial and abaxial girders of sclerenchymatous strands. He also described bulliform cells, mesophyll with distinctly radiate chlorenchyma and bundle sheaths. Sheath cells around the large vascular bundles are not of the same size as those around smaller vascular bundles.

The phytotoxicity of insecticides on crop plants has been studied by various workers only with reference to either the disturbance of the growth pattern or morphological disarray. The information available indicates that the structural details of the injured organs of the plants have not been worked out. Some work on the structural alteration of plant tissues following treatment with herbicides has been reported. Ashton et al. (1963) studied the histological changes in Phaseolus vulgaris induced by the phytotoxic effects of atrazine, a herbicide.

They reported the disintegration of chlorplasts in leaves, a cessation of cambial activity, and a decreased thickness of the cell walls of vascular elements in the stem.

The object of the present study was to investigate the disruption of the cellular organization in the leaves as a consequence of the toxic effects of the insecticides.

Materials and Methods

Two grain sorghum hybrids, RS 610 and OK 612, were sprayed with three insecticides: Bidrin, methyl parathion, and diazinon; the details of treatments have been stated in the previous chapter.

Samples of the leaves were collected over a period of 60 hours at intervals of 12 hours. Pieces, 3x4 mm, were cut from the damaged spots on the leaves and stored at a temperature of -2 C to -5 C for a period of two to four days.

Sections were cut on a freezing microtome using the technique suggested by Pickering (1966) with slight modifications. Various concentrations of gelatin were tried as embedding media. Embedding the leaves in a 2% solution of gelatin gave satisfactory results. The time for quick freezing varied greatly and depended upon the texture of the leaves as affected by the phytotoxicity of insecticides.

The leaf pieces were embedded in a large drop of 2% gelatin on the tissue holders and frozen for 30-60 seconds. The temperature of the microtome was kept constant at -10 C and was found suitable for this material. Using the regular microtome knife the sections were obtained at 16μ thickness. They were individually transfered to clean slides kept at room temperature. The slides were moved to hot plates for 2-3

seconds and then were allowed to dry for 15-20 minutes at room temperature.

Before passing the sections to an alcohol-aqueous series, a 0.5% collodion solution was thinly spread on the slide with the help of a dropper. After draining off the excess collodion the slides were passed on to 95% alcohol. This worked as an adhesive for the sections. The dry slides processed as above were dehydrated in an alcohol series and stained according to the procedure described by Himes and Moriber (1956) for staining DNA, protein, and polysaccharides.

Reagents:

1. The bleaching solution was prepared by mixing 5 ml of 10% sodium metabisulfite, 5 ml 1N HCl and 90 ml of distilled water.
2. 0.5 grams of Azure A was dissolved in 100 ml of freshly prepared bleach.
3. 0.5 grams of basic fuchsin and 0.5 grams of sodium metabisulfite were dissolved in 100 ml of 0.15 N HCl to prepare schiff reagent. The mixture was stirred at every three hours and was kept at room temperature for 24 hours. After the addition of 300 mgms of decolorized charcoal the mixture was filtered through whatman filter paper No. 44. The process of filteration was repeated several times until the filtrate obtained was colorless.
4. One gram of naphthol yellow S was dissolved in 100 ml of 1% acetic acid. The solution was diluted with 1% acetic acid to prepare 0.2% naphthol yellow S.
5. 0.8 grams of periodic acid was dissolved in 90 ml of water and 10 ml of 0.2 M sodium acetate solution.

Chart I

Staining Procedure:

Graded series of alcohol to water.

↓

Hydrolysis in 1N HCl for 12 min. at 60 C.

↓

Rinse in water.

↓

Azure A-schiff solution for 5 minutes.

↓

Rinse in water.

↓

Bleach 4 minutes.

↓

Periodic acid solution for 2 minutes.

↓

Rinse in water.

↓

Basic fuchsin-Schiff for 2 minutes.

↓

Rinse in water.

↓

Bleach for 2 minutes (2 changes).

↓

Rinse in water.

↓

0.2% naphthol yellow S for 2 minutes.

↓

Tertiary butyl alcohol (2 changes of 2 minutes each).

↓

Xylene for two minutes.

↓

Mounted in kleermount.

Observations

A. Details of leaf anatomy:

Sorghum is the genus within Andropogoneae of the class Monocotyledonea. Hence, the leaves of Sorghum are parallelly veined with cross connecting veinlets. The epidermis of the leaf is covered with a thin layer of cuticle. Bulliform cells mostly occur either as solitary cells or in pairs. They do not occur as frequently on the lower epidermis as on upper. The epidermal cells on the lower side of the leaf are larger than that on the upper side.

The vascular bundles are surrounded by a jacket of large, oval chlorenchymatous cells containing numerous large plastids, forming the bundle sheath. The mesophyll of the leaf consists of relatively compact chlorenchyma with plastids that are smaller and numerous than the chlorophyllaceous sheath surrounding the vascular bundles. There is no well developed palisade layer. Apparently, the vascular bundles fall into three different types depending on the size and the structural differences: small, medium, and large.

The small bundles are round in shape and are surrounded by a continuous layer of thin walled, elongated mesophyll cells containing conspicuous plastids. These cells are arranged in a radiate pattern around the bundle sheath. The xylem is not well differentiated into protoxylem and metaxylem in these bundles. The absence of hypodermal sclerenchyma differentiates the small bundles from the medium and large ones (Figure 3).

The medium sized bundles have I shaped girders of a single layer of 2-7 cells of sclerenchyma adjacent to the upper and lower epidermis. The circular continuity of the radiate mesophyll cells is disrupted by

the presence of 2-3 Parenchymatous cells located between the bundle sheath and the Sclerenchyma. The vascular part of the bundle consists of a few pitted xylem cells and a group of phloem cells (Figure 4).

The cells of the bundle sheath in large bundles are relatively smaller than those of small and medium ones. Moreover, the bundle sheath often does not form a continuous ring. On both the upper and lower epidermis. The large bundles are supported by 2-3 layers of sclerenchyma. Each bundle is jacketed by a layer of narrow lignified cells which are in continuity with hypodermal sclerenchyma. These cells are thin walled and paranchymatous close to the xylem vessels. There is a single discontinuous layer of the radiate chlorenchyma around the bundle sheath towards the marginal sides of the leaf (Fig. 2).

The metaxylem of the vascular bundles appears to be endarch, protoxylem being situated in close promixity to the phloem. Two large vessels of metaxylem are found on the marginal side of the bundle. They are often surrounded by thin walled small parenchymatous cells towards the peripheral sides and thick walled lignified cells towards the central side of the bundle.

There are five to seven small bundles situated between two medium bundles and two to three medium bundles present between two large bundles. There is a row of one to two large parenchymatous cells running across from the lower to the upper epidermis, often terminating in the stomatal apparatus on both sides.

The hybrids of Sorghum bicolor, OK 612 and RS 610, are similar to each other anatomically. The frequency of occurrance of bulliform cells in the upper epidermis of the leaves of OK 612 is less than that in RS 610. In OK 612 the bulliform cells are absent in the lower epidermis.

B. Insecticidal injury to the treated leaves:

The morphological appearance of the leaves in case of both the hybrids showed a somewhat similar pattern of injury, whereas their response to insecticides differed in the earliness and intensity of toxic symptoms. Treatment with methyl parathion: the first indication of phytotoxicity observed 36 hours after insecticidal application was damaged epidermis, and injured mesophyll tissue. The intensity of the injury increased after 48 hours and covered the various tissue systems, including the disintegration of bundle sheathchloroplast (Figure 18), blocking of xylem vessels and shrinkage of parenchymatous cells (Figure 17). After 60 hours, the cells in both the upper and lower epidermis were filled with dark granulated protoplast; the choroplast of the bundle sheath was almost absent; xylem vessels in all the three types of vascular bundles were observed to be blocked (Figures 19 and 20), and the radiate chlorenchyma as well as the column or parenchymatous cells were found shrunken and often collapsed. After this the leaves became dry and discolored. The response of sorghum leaves to methyl parathion at various time intervals after application are presented in Table 2.

Treatment with Bidrin: the symptoms of phytotoxicity in the leaves treated with Bidrin appear earlier and the consequences are more pronounced compared with those observed in leaves treated with methyl parathion.

Within the first 12 hours after insecticidal spraying light green streaks become clearly appearant on the upper surface of the leaves. Sections of leaves cut after 24 hours indicated deformed cell walls of the parenchymatous cells and shrunken chloroplasts of the bundle sheath

parenchyma (Figure 5). However, after 36 hours the bundle sheath chloroplasts were dilated (Figure 6). In many cases this dilation indicated the first sign of disintegration. Moreover, the mesophyll cells sandwiched in between the parenchymatous column and the bundle sheath were slightly shrunken (Figure 6). The intensity of the effects reached its peak after 48 hours of insecticidal application where besides the damages indicated above, the blockage of xylem vessels and disappearance of chloroplasts in many cells was observed (Figures 7, 8, 9, 10, 11, 12).

Certain difficulties were faced in sectioning the leaves 60 hours after the insecticide treatment, because the leaves were nearly dry and rigid. The change in the concentration of the embedding medium solved this problem. Almost all the tissue systems within the leaves were in a highly disorganized state packed inbetween the upper and lower epidermal cells (Figures 13, 14, 15). The cell walls of all the living tissues were distorted excepting those of the upper and lower epidermal cells (Figure 16). The response of sorghum leaves to Bidrin at various time intervals after application are presented in Table 3.

Discussion

Both of the insecticides had similar adverse effects on the internal organization of the leaves of sorghum hybrids. These included (a) Injured epidermal cells; (b) shrinkage and collapsing of parenchymatous cells; (c) disintegration followed by ultimate destruction of chloroplasts; (d) blockage of the tracheary elements. The primary acute symptoms consisting of light green streaks appear on the leaves treated with Bidrin. Whereas in the case of the leaves treated with methyl parathion they appear as discolored patches, later turning into small

greyish areas that become water soaked in appearance, and finally necrotic in both the treatments.

Internally, this was accompanied with the coagulation of protoplast observed in the epidermal cells in the vicinity of stomata on the upper surface of leaves, indicating the stomatal penetration through the walls of substomatal chambers. As proved by Dybing and Currier (1959), the stomatal penetration of herbicides may be extremely effective if the stomata are open at the time of application. The immediacy of the phytotoxic effects produced by Bidrin and methyl parathion in the present investigation can be explained on this basis.

Since the plants were sprayed during the daytime the penetration of spray molecules is greatly enhanced because of high humidity prevailing inside the air spaces of the transpiring leaves. Presumably, high humidity and the attendant high water content of the leaves results in the water continuum of the leaf cells providing a continuous path for the diffusion of molecules from the surface into the aquous phase of the inner apoplast and thence to the symplast.

The widely accepted symplast continuum concept is advanced by plant physiologists in which the apoplast makes up the continuous non living cell wall phase that surrounds the symplast, which is the living, functionally integrated unit composed of interconnected protoplast.

Following the injury to the stomatal chambers and the adjacent cells the effects are observed on the apoplast and symplast of the parenchymatous column. The effects are concentrated more towards the symplast of these cells, rather than apoplast. The further translocation takes a trend towards injuring the integrated system of the symplast of the chlorenchymatous cells adjacent to the parenchymatous column, which

results in the progressive disintegration of the chloroplast. The nature of the process of disintegration of chloroplasts in mesophyll and bundle sheath show easily recognizable patterns: in the former the plastids aggregate and shrink continuously till completely disintegrated, whereas chloroplast of bundle sheath cells first shrink to a small extent, dilate to fill up the whole cell lumen, and finally shrink rapidly until destroyed. This pattern, however, may indicate two different structural organizations of the constituents within the chloroplast, as shown by electron micrographs in the leaves of maize, sugercane, and other grasses. These structurally distinct entities have been shown by Laetsch et al., (1966) to possess different functions in the metabolism of the plant.

The pathway of the insecticidal translocation from the bundle sheath parenchyma and undifferentiated sclerenchymatous endodermal system, can be traced to the tracheary elements of the vascular system of leaves, joining thereafter the ingredients of the transpiration stream in leaves. The accumulation of toxic compounds and their persistent binding in the xylem vessels lead to the plugging of the tracheary lumen. The injury to the mesophyll and xylem vessels causes toxic effects; such injury by stopping photosynthesis and plugging the tracheary elements retard the translocation of necessary materials to various regions (Crafts 1948; Blackman 1950). Apparently, the disorganization of the tissue system of the leaves results in the deprivation of available water and food. There is no doubt that the insecticides migrate by both apoplastic and symplastic movements within the leaves. Their action seems to be closer to that produced by substituted urea, in that the insecticides move in the apoplast with water of transpiration but which do not enter the phloem and move with food (Woodford et al, 1958).

In the final stage of injury all cells of the mesophyll become filled with substances which stain darkly, and the cells are collapsed. This collapse of cells is thought to be related to membrane exchanges. This probably results in leakage of celluler content and ultimate break down of the osmotic system. As a consequence of this injury the mechanical stress on thin walled cells and phloem elements disrupts the apoplastic integrity, which accompanied the complete loss of the continuum of the symplast, and results in the death of the treated leaves.

In general, the resultant phytotoxic injury is primarily due to the formation of toxic compounds with the constituents present in the protoplasm. These toxic molecular aggregates block the passage of metabolites through xylem vessels and disrupts the flaccidity of the transpiration stream. It is possible that these substances are physically free radicals effected photochemically in the presence of oxygen damaging the biological system (Smith et al. 1961).

Table 2. Effect of methyl parathion on the anatomy of sorghum leaves at different time intervals.

Effect of methyl parathion on:	Time after treatment			
	24 hours	36 hours	48 hours	60 hours
1. Epidermis	No effect	Slight effect on upper epidermis	Upper & lower epidermis injured	Upper epidermis more damaged than lower
2. Parenchyma	No effect	No effect	Shrinkage of cell walls	Cell walls collapsed
3. Chlorenchyma	No effect	Chloroplast slightly injured	Shrinkage of cell walls and chloroplast	Walls collapsed and chloroplast contracted
4. Sheath chloroplast of small bundle	No effect	No effect	Disintegrating	Disintegrating & in most cells disappearing
medium bundle			"	"
large bundle			"	"
5. Xylem of small bundle	No effect	No effect	Blocked	Blocked
medium bundle			"	"
large bundle			"	"
6. Phloem of small bundle	No effect	No effect	No effect	Walls affected
medium bundle				"
large bundle				"

Table 3. Effect of Bidrin on the anatomy of sorghum leaves at different time intervals.

Effect of Bidrin on:	Time after treatment			
	24 hours	36 hours	48 hours	60 hours
1. Epidermis	Little effect on upper epidermis.	Upper epdiermis affected.	Coagulation of protoplast in both upper & lower epidermal cells.	Both upper & lower epidermis damaged, stomatal apperture closed, air chamber shrunken, complementary & guard cells lost their identity.
2. Parenchyma	Apoplast deformed	Apoplast deformed	Shrinkage of walls.	Severely injured.
3. Chlorenchyma	No effect	Slight shrinkage of walls	Chloroplast disintegrate, cell wall shrunken.	Cell walls collapsed chloroplast highly contracted.
4. Bundle sheath chloroplast of, small bundle	Slightly shrunken	Dilated	Dilated & disintegrated	Mostly disintegrated sometimes dilated
medium bundle	"	"	"	Disappeared
large bundle	"	"	"	"
5. Xylem of small bundle	No effect	No effect	Blocked	Blocked
medium bundle			"	"
large bundle			"	"
6. Phloem of small bundle	No effect	No effect	Cell wall affected	Walls crushed
medium bundle			"	"
large bundle			"	"

HISTOCHEMICAL STUDIES

The detailed structural changes suggested the transpiration stream as the pathway of the insecticidal translocation in the leaves by which the phytotoxicity results. Major injury was obviously concentrated in the chloroplasts and the vascular system, xylem vessels in particular. Microscopic observations invariably suggested the blocking of the xylem with the deposition of an insoluble chemical complex in the lumen. The disintegration and ultimate disappearance of the chloroplast is believed to be coincident with the qualitative changes in the nature of the chemical composition of the cells containing chloroplasts (Ashton et al., 1963).

The study reported here was undertaken with a view to chemically identifying the fundamental nature of the chemical compounds involved in the clogging of xylem vessels. The attempt to reveal the general chemical changes in the composition of the symplast resulting from the toxicity of the insecticides is projected.

Materials and Methods

Two hybrid grain sorghums, OK 612 and RS 610, were sprayed with three insecticides. The damaged leaves of OK 612 were collected and sectioned on the freezing microtome (described in detail in the previous chapter). The sections were cut 16 μ thick, spread on a slide, dehydrated and stained as follows.

Periodic acid-Schiff (PAS) Reaction.

Total carbohydrates of insoluble polysaccharides can be detected cytochemically by the Periodic Acid-Schiff's reaction as suggested by Hotchkiss (1948) and McManus (1948). Their method as slightly modified by Jensen (1962) is used in the present studies.

The freshly cut sections were passed through different grades of alcohol and water of the following percentages:

Absolute ethyl alcohol ⟶ 95% alcohol ⟶ 70% alcohol ⟶ 50% alcohol ⟶ 30% alcohol ⟶ water. The time allowed for the slides in each grade was two minutes.

Reagents:

0.5 grams of basic fuchsin and 0.5 grams of sodium metabisulfite were dissolved in 100 ml of 0.15 N HCl to prepare Schiff reagent. The mixture was stirred every three hours and was kept in the dark under room conditions for 24 hours. After the addition of 300 miligrams of decolorizing charcoal the mixture was filtered through Whatman No. 44 filterpaper. The process of filteration was repeated several times until the filtrate obtained was colorless.

One gram of naphthal yellow S was dissolved in 100 ml of 1% acetic acid. The solution was further diluted with 1% acetic acid to prepare 0.2% naphthol yellow S to be used as a counter stain.

Procedure:

Chart II

Graded series of alcohol to distilled water.

↓

0.5% periodic acid for 20 minutes

↓

Water for 10 minutes (2 changes)

↓

Schiff's reagent for 12 minutes

Water for 2 minutes
↓
2% sodium metabisulfite for 2 minutes
↓
Water for 15 minutes
↓
Naphthal yellow S for 2 minutes
↓
Graded series of alcohol to absolute alcohol
↓
Xylene for 5 minutes
↓
Mount in kleermount

Triple stain technique:

Himes and Moriber (1956) have suggested a triple stain technique for the microchemical detection of DNA, protein, and polysaccharides.

The reagents and procedure are desoribed in detail in the previous chapter.

Sudan black B technique:

The total lipids were localized by using the Sudan black B reagent prepared according to Baker (1947). The staining procedure was followed as suggested by Jensen (1962).

Reagents:

An excess of sudan black B dye was dissolved in 50 ml of 70% alcohol. The solution was stirred thoroughly and kept overnight at 37C. Some more sudan black was added to the solution in the morning and stirred well several times during the course of the day, during which it was kept at 37C. This was filtered before use.

Procedure:

Chart III

50% alcohol for 5 minutes
↓
Saturated solution of sudan black for 10 minutes
↓
50% alcohol for 1 minute (3 changes)
↓
Glycerine and mount.

Results and Discussion

A closer look at the chloroplast, both in bundle sheath cells and mesophyll cells, suggested the gradual changes resulting in the discoloration of chloroplasts. The deep green initial color of the chloroplast converted into a yellowish green at the time of disintegration and ultimately a yellow resulting at last in the accumulation of vesicular structure in the corner of the cell leaving most of the lumen blank.

The histochemical tests in the leaves of untreated plants showed that both the chloroplasts of mesophyll and bundle sheath cells stained yellow with naphthol yellow S, indicating the presence of the basic group of proteins (Fig. 21). Near the limit of the phytotoxic injury the presence of proteins and lipids was indicated in the disintegrating chloroplast. The absence of polysaccharides in the chloroplasts of the treated leaves was indicated by the negative PAS test; in contrast to this, the presence of PAS positive granules in the chloroplast of untreated leaves suggested that the changes connected with polysaccharide metabolism were involved with Phytotoxicity. Similar findings have also been reported in Phaseolus vulgaris susceptible to phytotoxic effects resulting from the herbicidal action of atrazine (Ashton et al. 1963).

While working on the action of AAMT [3-(α-amioethyl) - 5 methyl tetronic acid] on Zea mays, Signol (1961) reported that the dilation of chloroplasts which is structurally the swelling of compartments in the expansion of grana results in the breakdown of the plastid membranes. The presence of free lipids and fatty acids, stained greenish black with Sudan dye, at the disintegration stage of chloroplasts indicates the liberation of the broken membranous structure which is reportedly composed of lipids and proteins in close conjugation (Korn 1966). This apparently

elaborates the vacuolar nature of the vesicles formed from the disappearing chloroplast, upsetting the metabolic processes related to photosynthesis. Moreover, the function of the bundle sheath chloroplast has been suggested to be the storage of starch (Laetsch et al. 1966). Hence, the absence of starch in the chloroplast of bundle sheath cells of treated leaves suggests the increase in the degradatory processes accompanied with the retardation in synthetic activities.

At the height of injury the accumulation of the complex compound in the epidermal cells (Figs. 22&24), and in collapsed mesophyll chlorenchyma (Figs. 25, 26 & 30), showed a very high affinity for the dyes. That the major part of this complex was polysaccharide in nature was inferred from the PAS positive test. With the triple stain technique however, the compound stained blue (Figs. 23, 25 & 26). In the leaves of the untreated plants the polysaccharides stained reddish purple with the triple stain technique. The details of the method suggest that the chemical reactivity of Azure A is directed towards the sugars of nucleic acids; in which the aldehyde group has been made available through hydrolysis (Orstein 1951). This reaction is considered to be specific for riboses of nuclear material. According to Himes and Moriber (1956) the dark blue staining of polysaccharides in rare cases, might be due to the reversible reaction resulting from two consequent applications of Schiff's reagents which in turn readily reacts with highly available 1-2 glycol groups of polysaccharides.

Considering these two explanations the observations lead to the conclusion that the change in the nature of polysaccharides was due to the incorporation of the phytotoxic substances with the protoplasmic contents. Under controlled conditions the polysaccharides in no case stain blue using the triple stain technique, whereas, after the maximum phytotoxic

injury has occurred the nature of the component chemical groups of polysaccharides is so changed that in the initial step of the method the compound immediately gets the stain indicating higher affinity for the dye. The typical absence of blue color in the less severely affected region of the leaves further supports this conclusion. Interestingly enough, the polysaccharides in such tissue systems are stained reddish purple, as observed in the untreated plants. It is apparent that the incorporation of the toxic substance with the polysaccharides modifies the product of hydrolysis which readily becomes available to the first dye used in the triple stain technique.

A similar complex has been found to fill the lumen of the xylem vessels often clogging them entirely (Figs. 27, 28 & 29). The PAS positive test and blue stain by triple stain technique confirmed that the substance is a complex of phytotoxic product incorporated with polysaccharides.

The translocation of phytotoxic polysaccharide complex follows the pathway of the transpiration stream. The absence of polysaccharides in the bundle sheath cells of the severely affected leaves shows that while passing through these cells the insecticides might have formed the phytotoxic product--polysaccharide complex; this complex following the transpiration stream might have gone to xylem vessels through the intercellular connections established within bundle sheath cells and undifferenciated endodermal cells and from the latter to xylem vessels. The stomatal closure at the extreme of the phytotoxic effects might have resulted in the increased pressure within the system. As a consequence, the complex substance was pushed to mesophyll chlorenchyma and oozed out by cuticular transpiration developing the apparent morphological deformities (necrosis) of the affected leaves.

ELECTRON MICROSCOPIC STUDIES

Electron microscopic studies of Hodge et al. (1955) with the fine structure of maize chloroplasts showed that in the bundle sheath cells chloroplasts, the lamellae normally extend the full width of the disc shaped plastids and grana are absent. The mesophyll chloroplasts, however, contain numerous grana of fairly regular cylindrical form. These consist of highly ordered stacks of dense lamellae, the interlamellar spacing being 125A. Similar chloroplast have been reported by Brown and Johnson (1964) in some grasses, where the mesophyll chloroplasts have definite discs of grana while bundle sheath cells consist of only stroma lamellae. Laetsch et al. (1966) described the two kinds of chloroplasts in sugar cane leaves. The chloroplast in the bundle sheath cells do not have grana but contain numerous starch grains. The mesophyll cells adjacent to bundle sheath cells have relatively small chloroplasts than bundle sheath chloroplasts and are characterized by the presence of grana but are scarce in starch grains. They concluded that the chloroplast in the bundle sheath cells have an important role in starch metabolism.

As far as information available, there are no previously published electron microscopic studies on the effect of insecticides on plant tissues. Some work on the structural alteration of chloroplasts following treatments with other chemicals and mineral deficiencies has been reported.

The present study of the fine structure of chloroplast was undertaken to evaluate the alterations of fully developed chloroplasts affected by insecticide applications.

Materials and Methods

Reagents:

Phosphate buffer--A 0.15 M solution of dibasic sodium phosphate was mixed with 0.15M solution of monobasic potassium phosphate at the ratio of 161 ml to 39 ml, respectively, and adjusted to pH 7.4.

Buffered glutaraldehyde fixative --25% glutaraldehyde in water was mixed with 0.15 M phosphate buffer to give a final concentration of 4% glutaraldehyde in 0.15M buffer at pH 7.4.

Osmium tetroxide fixative--0.5 grams of osmium tetroxide was dissolved in 50 ml of the phosphate buffer to give a final concentration of 1% osmium tetroxide. The solution was kept under refrigeration and in the dark.

Epoxy resin mixture (Luft 1961)

Mixture A was prepared by mixing 62 ml of Epon 812 and 100 ml of Dodecenyl succinic anhydride (DDSA). Mixture B was prepared by mixing 100 ml of Epon 812 and 89 ml of methylnadic anhydride (MNA). Mixtures A and B were mixed in the proportion of 7:3 respectively, and 1.5% of 2,4,6-tri(dimethyl aminomethyl) phenol (DMP-30) was added as accelerator and mixed thoroughly.

Procedure:

Leaves of sorghum hybrid OK 612, unsprayed and at various stages of insecticide injury i.e. 36, 48 and 60 hours after application (described in detail in previous chapters) were collected and were cut into pieces about 0.1 mm^2. The sections were transferred to bottles containing 4% buffered glutaraldehyde and were stored at 3-5 C, 10-12 days.

The tissues were washed with 0.15 M phosphate buffer for three hours with three changes. Then they were fixed with 1% buffered osmium

tetroxide for one hour.

The tissues were then dehydrated with ethyl alcohol in successive changes of 30, 50 70, 90, and 100%. They remained in 30, 50, 70% alcohol 30 minutes each, and were left overnight in 90% alcohol at 3-5 C. Tissues were further dehydrated with three changes of 100% alcohol over a period of 36 hours.

After dehydration, the tissues were soaked in propylene oxide (1, 2-epoxy propane) for 45 minutes (three changes) at room temperature. The embedding schedule was followed as described by Luft (1961). In the last change of propylene oxide an equal quantity of the complete mixed resin was added and mixed by swirling for two hours. Then the tissues were transferred to other vials containing 75% resin and 25% propylene oxide and kept overnight with continuous swirling. Tissues were further infiltrated in 100% resin mixture for 24 hours at room temperature and were stirred continuously. Then they were transferred to aluminium boats containing resin mixture and subjected to the following polymerization schedule:

(a) Incubation overnight at 35 C.

(b) Incubation for 24 hours at 45 C.

(c) Incubation for 2 days at 60 C.

After polymerization, the resin was hard and the blocks were removed from the aluminium boats. Tissue pieces were sawed out of the resin blocks, trimmed to about 1 mm square and glued with epoxy glue to the base formed from the same material molded in a gelatin capsule. The tissue blocks were trimmed into a tiny pyramid with the help of a razor blade and a dissecting scope. Sections were cut with a diamond knife on a Porter-Blum microtome. The sections were stained with uranyl acetate and examined with RCA EMU 3-G microscope.

Observations

The chloroplast containing cells in sorghum are restricted to the bundle sheath and to a single layer surrounding the bundle sheath. Two different kinds of chloroplasts have been noted in sorghum leaves. The chloroplasts in the bundle sheath cells are characterized by the presence of stroma lamellae and the absence of grana. The chloroplasts of parenchymatous cells adjacent to the bundle sheath are smaller and they possess well developed grana. Similar chloroplasts have been reported in Zea mays (Hodge et al. 1955), in some grasses (Brown and Johnson 1964), and in sugar cane (Laetsch et al., 1966).

Chloroplasts of insecticide treated leaves show a drastic alteration of structure. The typical differences between chloroplasts of the bundle sheath and adjacent mesophyll cells were not apparent in the affected portion of the leaves. The initial changes can be seen as early as 36 hours after the insecticide application and the final stage of injury was found within 60 hours after treatment with insecticides where the entire chloroplasts were destroyed.

The initial stage of injury as detectable with the electron microscope was the enlargement of compartments of grana in the mesophyll chloroplast. The expansion of grana caused the whole lamellar system to become disorganized and due to the disruption of the fret system, the connections between grana were destroyed. With the increasing stages of injury the enlargement and expansion of grana was extensive, and finally, the grana were reduced to dilated tubules of the vesicles and was found near the periphery of the cell. According to Thomason and Weier (1962) some of these vesicles represent swollen fret.

The bundle sheath chloroplast without grana was found to reduce to

long irregular lamellae with the enlargements at different spots of the lamellar system. Further, the stroma created long chains of vesicles which ultimately were reduced to dilated vesicles.

In the final stage of injury, it is difficult to distinguish the two types of plastids.

Discussion

The general effects of insecticides on the internal structure of sorghum leaves were disintegration of chloroplasts of bundle sheath as well as mesophyll cells.

There are several concepts of chloroplast structure and the most common widely accepted view envisions the grana as arrays of aligned discs with the flattened faces parallel, thus forming a column of discs. Each disc is separated by stroma. The discs are held in place by a larger parallel system of stroma lamellae. Thus, a disc is a thickened region in a more extensive lamellar system.

At the time of insecticide application, the plants were in the medium-to-hard-dough stage, thus the observations described above were related to the destruction and alteration of matured chloroplasts caused by the insectidides. Under the light microscope, the injury to the chloroplasts of the treated leaves appeared in the form of: (1) shrinkage of chloroplasts; (2) chloroplasts became dilated and filled the lumen of the bundle sheath cells; (3) then finally disintegrated and disappeared from many cells. In addition, under the electron microscope, it has been found that the chloroplasts undergo the following structural changes: (1) the disruption of the fret system (Fig. 31); (2) the enlargement of compartments of the grana and stroma lamellae; (3) disorganization of the lamellar system (Fig. 32); (4) and ultimately the chloroplasts were

reduced to vesicles (Fig. 33). These changes in the fine structure of chloroplasts could be due to the modifications of membranes induced by insecticide toxicity. The changes in the fine structure may cause the changes in the form of the chloroplasts, like the dilation of bundle sheath chloroplasts which filled the whole lumen of the cells. This could be due to the swelling of the lamellar system. These membranes did not disrupt in the early stages but broke down in the final stage of injury when the whole chloroplasts were destroyed.

It was mentioned in the previous chapters that insecticide phytotoxicity was primarily due to the formation of the toxic substances with the contituents of the protoplasm. These toxic substances might be the cause of the modification of membranes and the destruction of chloroplasts. Due to the adverse effects of insecticides, the disorganization of the lamellar system would result in the abnormal photosynthetic activity.

The structural changes of chloroplasts in Bidrin treated plants resemble those of dihydrostreptomycin treated Zea mays plants, as reported by Signol (1961), in which the swelling of compartments occurred when plastids were destroyed and ultimately reduced to chain of vesicles. Ashton et al. (1963) reported a similar disorganization of the chloroplast membrane in atrazine treated plants in the presence of light. Thus, some of these alterations in the fine structure of chloroplasts are not only caused by the insecticides but also caused by the action of these substances which have similar effects on the swelling of compartments of the grana.

SUMMARY AND CONCLUSION

Two sorghum hybrids were sprayed with Bidrin, methyl parathion and diazinon. Phytotoxicity was measured with respect to visible leaf injury. Data indicated that the hybrid OK 612 is more susceptible to insecticide injury than RS 610. Because of the severity of injury caused by Bidrin and methyl parathion to OK 612, these insecticides are not satisfactory for sorghum insect control. Diazinon was safe on both the hybrids.

Sections of injured leaves examined under a light microscope showed that both the insecticides, Bidrin and methyl parathion, had in common adverse effects on the internal organization of the leaves of sorghum. These included: (1) injury to the epidermal cells; (2) shrinkage and collapsing of parenchymatous cells; (3) disintegration and ultimate destruction of chloroplasts in the bundle sheaths as well as in mesophyll cells; (4) blockage of xylem vessels.

The toxic effects were first observed on the stomatal and adjacent cells and then to the apoplast and symplast of the parenchymatous column. The effects were more concentrated towards the symplast. Further effects were noted towards the symplast of the chlorenchymatous cells adjacent to the parenchymatous column, resulting in the progressive disintegration of chloroplasts. The further translocation of insecticides towards the vascular system of the leaves leads to the plugging of the tracheary lumen, which retards the translocation of necessary materials to various regions, apparently resulting in the deprivation of available water and food.

In the final stage of injury all cells were collapsed and were filled with a substance which has great affinity for histochemical dyes.

The histochemical tests showed the absence of polysaccharides in the chloroplasts of the treated leaves; while polysaccharide granules were present in the chloroplasts of untreated leaves. This suggested that the changes connected with polysaccharide metabolism were involved with phytotoxicity. The accumulation of a complex compound in the epidermal cells, and in collapsed mesophyll chlorenchyma showed a great affinity for dyes. PAS and triple stain tests showed that the major portion of the complex is polysaccharide. The observations of the triple stain technique lead to the conclusion that the incorporation of the phytotoxic substances with the protoplasmic contents change the nature of the polysaccharides in the treated leaves. A similar complex has been found to fill the lumen of the xylem vessels, often clogging them entirely.

The increased pressure within the system due to phytotoxicity might have resulted in the stomatal closure at the extreme of the phytotoxic effect. As a consequence, the complex substance was pushed from xylem vessels to mesophyll chlorenchyma, and oozed out by cuticular transpiration, developing apparent morphological changes such as necrosis of the affected leaves.

Under the electron microscope, the fine structure of chloroplasts of sorghum has shown two distinct patterns. The well differentiated grana was present in the mesophyll chloroplasts while bundle sheath chloroplasts were characterized by the absence of grana and the presence of starch grains.

The fine structure of chloroplasts was altered by the treatment with Bidrin and methyl parathion. The fret system was destroyed, resulting in the disorganization of the arrangement of grana in the mesophyll

chloroplasts, the compartments of the grana ultimately disintegrated and were found in the groups, and the hollow vesicles were dispersed in the stroma. Finally, grana was reduced to dilated tubules of the vesicles. The bundle sheath chloroplasts were reduced to long irregular lamellae which ultimately were reduced to dilated vesicles. In the final stage of injury, the typical differences between chloroplasts of the bundle sheath and adjacent mesophyll were not apparent.

On the basis of above observations, it is concluded that insecticides migrate by both apoplastic and symplastic movements within the leaves. Their actions seem to be closer to that produced by substituted urea, where insecticides moved in the apoplast with water of transpiration but did not enter the phloem.

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A P P E N D I X

PLATE I

Fig. 2. Transection of mature sorghum leaf of untreated plant enlarged, showing large vascular bundle. x 530

B.	Bundle sheath
M.	Mesophyll
P.	Parenchyma
Ph.	Phloem
S.	Stomata
Sc.	Sclerenchyma
UE.	Upper Epidermis
XV.	Xylem vessel

Fig. 3. Transection of mature sorghum leaf of untreated plant, showing small vascular bundles. x 370

B.	Bundle sheath
LE.	Lower Epidermis
M.	Mesophyll
PC.	Parenchymatous Column
Ph.	Phloem
S.	Stomata
X.	Xylem

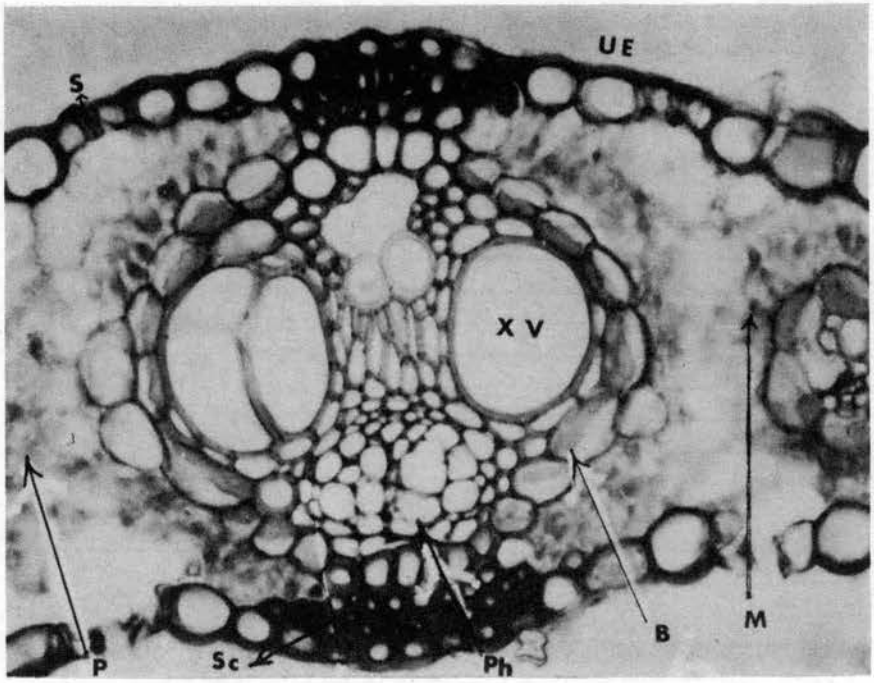

Fig. 2

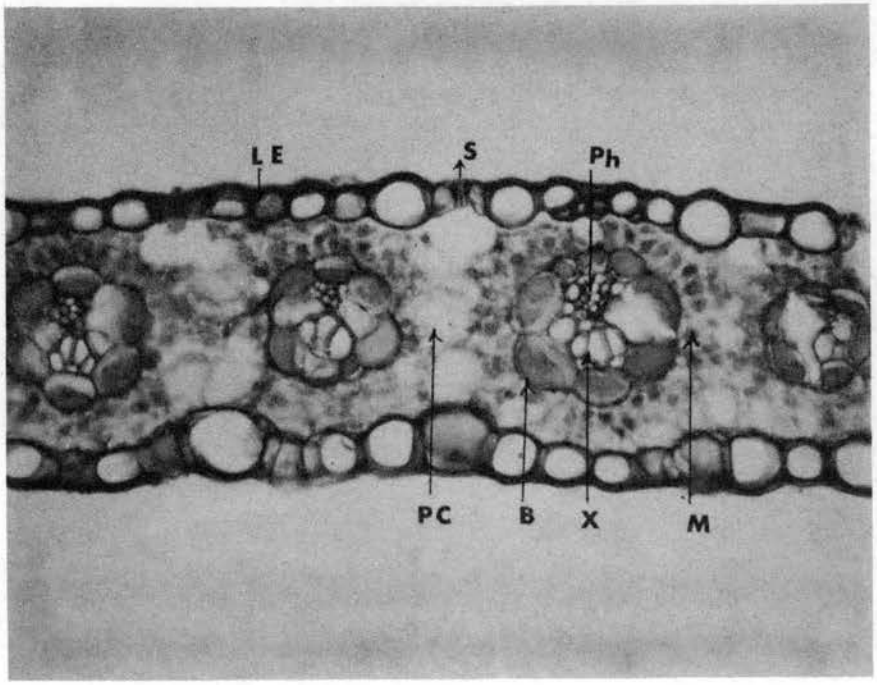

Fig. 3

PLATE II

Fig. 4. Transection of mature sorghum leaf of untreated plant, showing medium vascular bundle. x 420

B.	Bundle sheath
LE.	Lower Epidermis
PC.	Parenchymatous Column
Ph.	Phloem
S.	Stomata
Sc.	Sclerenchyma
X.	Xylem

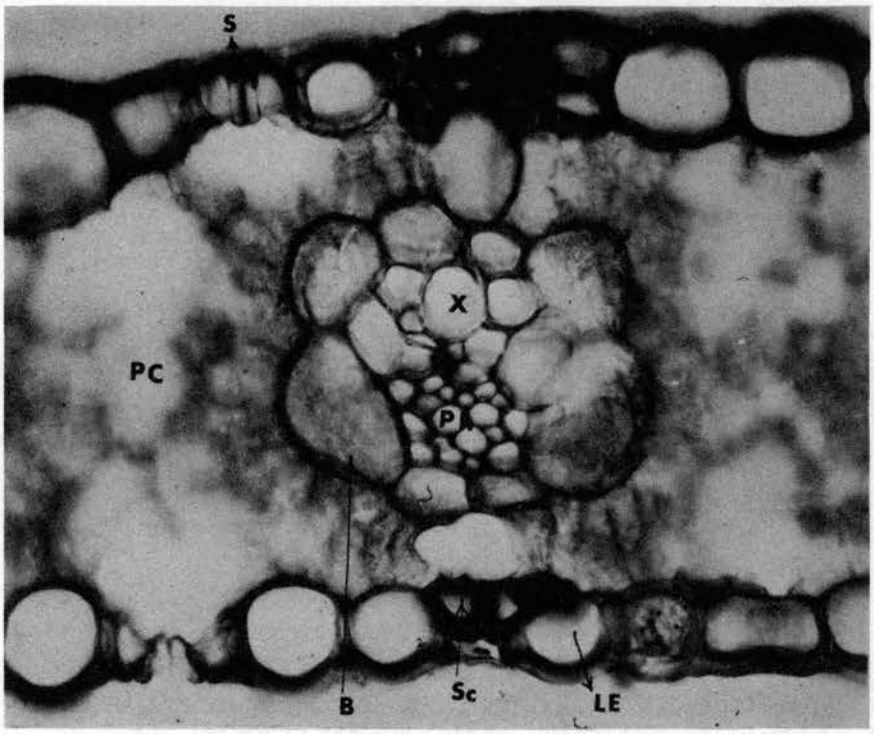

Fig. 4

PLATE III

Fig. 5. Bidrin Injury to Sorghum leaves, 24 hours after application. x 420

b ——— Slight shrinkage of bundle sheath chloroplast.

Fig. 6. Bidrin Injury to Sorghum leaves, 36 hours after application. x 420

C ——— Slight shrinkage of chlorenchymatous cell walls.

D ——— Dilated chloroplast of bundle sheath cells.

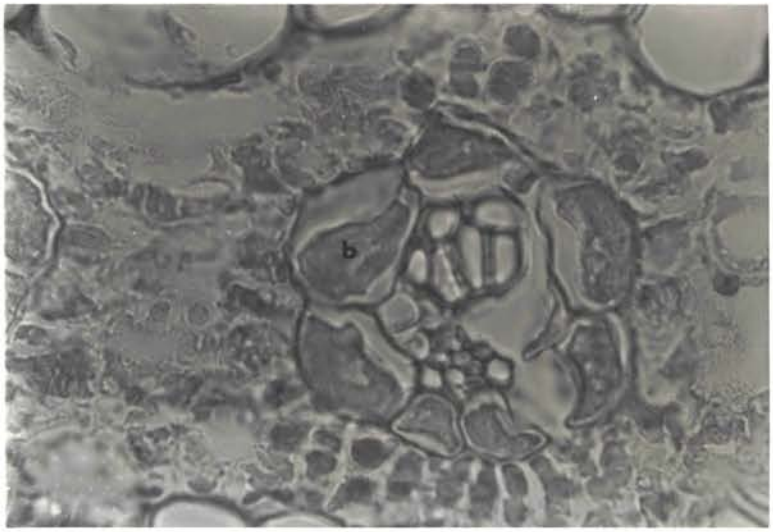

Fig. 5

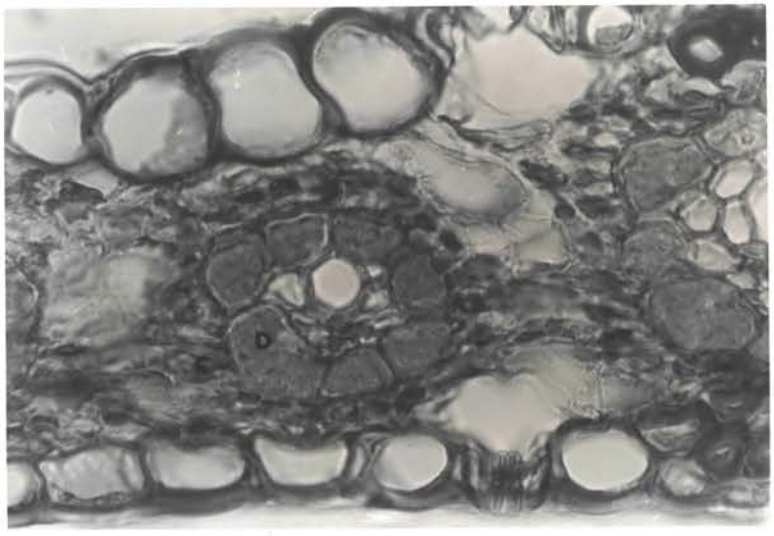

Fig. 6

PLATE IV

Fig. 7 Bidrin Injury to Sorghum leaves, 48 hours after application. x 420

C ——— Shrinkage of chlorenchymatous cell wall.
D ——— Dilated chloroplast of bundle sheath cells.
P ——— Shrinkage of cell walls of parenchymatous column.

Fig. 8 Bidrin injury to Sorghum leaves, 48 hours after application. x 420

A ——— Shrinkage of air chambers.
C ——— Shrinkage of cell walls of chlorenchyma cells.
D ——— Dilated chloroplast of bundle sheath cells.
P ——— Shrinkage of cell walls of parenchymatous column.
SM ——— Shrinkage of chloroplast of mesophyll cells.

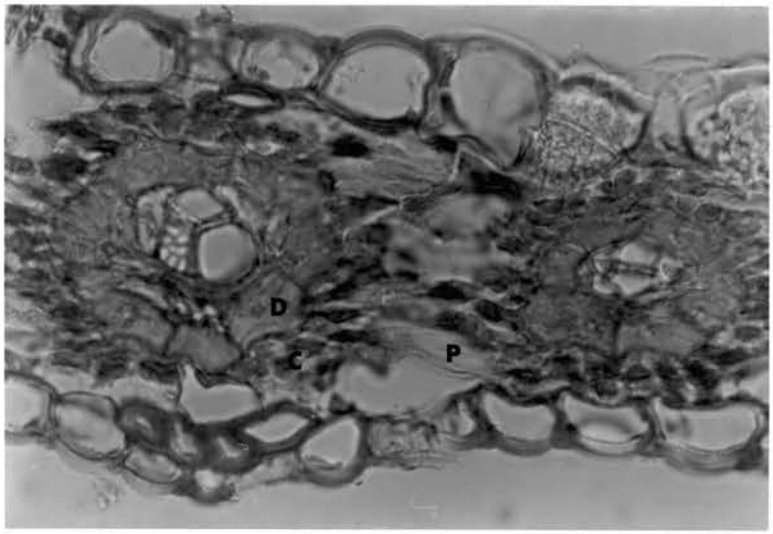

Fig. 7

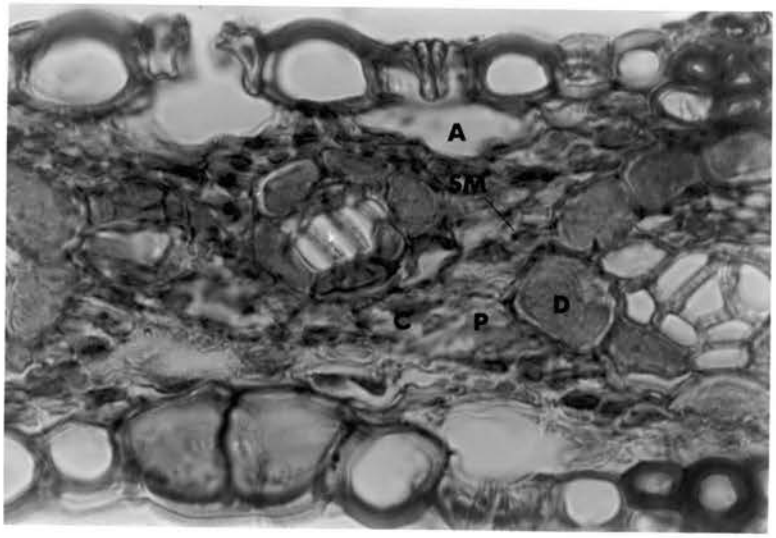

Fig. 8

PLATE V

Figs. 9, 10 Bidrin Injury to Sorghum leaves. 48 hours after application, showing initiation of breakdown of chloroplast at and around medium bundles. x 420

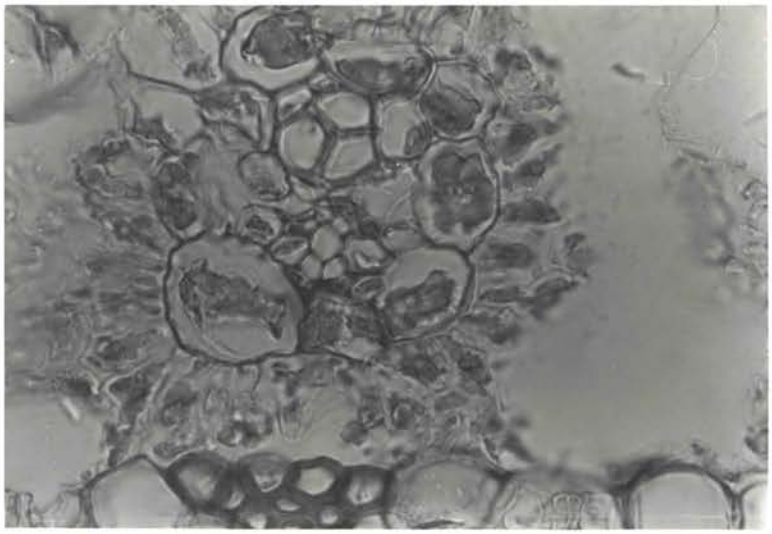

Fig. 9

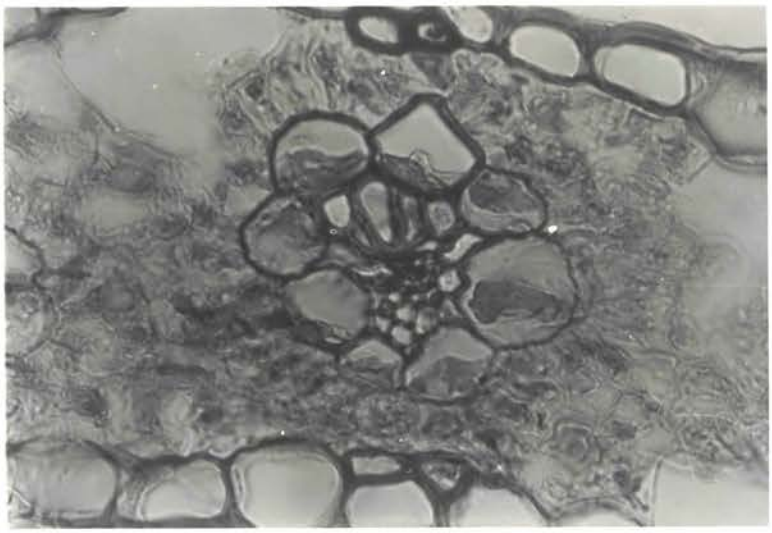

Fig. 10

PLATE VI

Fig. 11 Bidrin Injury to Sorghum leaves, 48 hours after application, showing injury to the large vascular bundle. x 400

Fig. 12 Bidrin Injury to Sorghum leaves, 48 hours after application, showing injury to small vascular bundle and adjacent cells. x 420

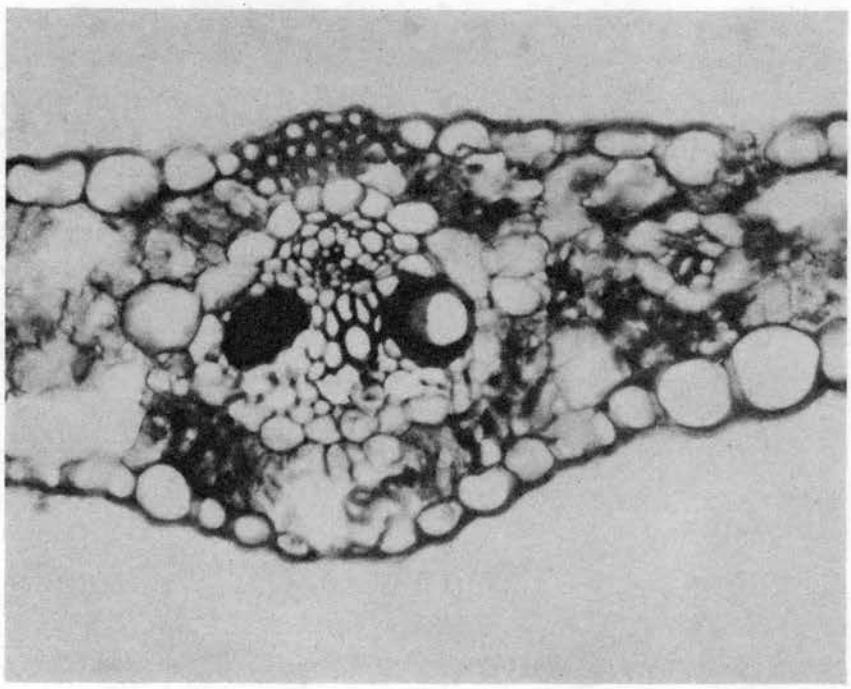

Fig. 11

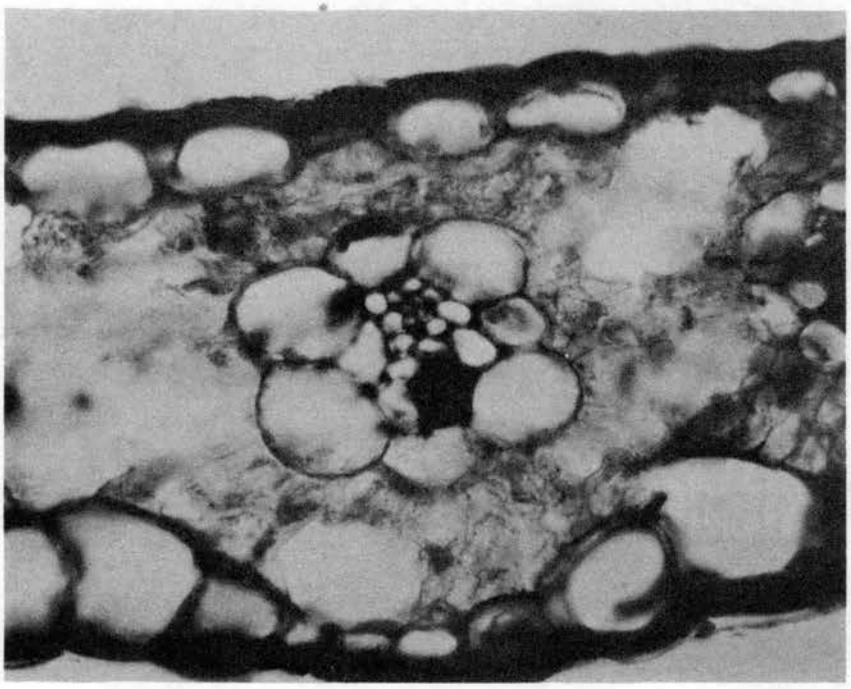

Fig. 12

PLATE VII

Fig. 13 Bidrin Injury to Sorghum leaves, 60 hours after application, showing complete breakdown of chloroplast at and around small vascular bundles. x 370

Fig. 14 Bidrin Injury to Sorghum leaves, 60 hours after application. x 420

A ——————————— Shrinkage of air chamber.
SI ——————————— Injury to stomata, guard cells and complementary cells.

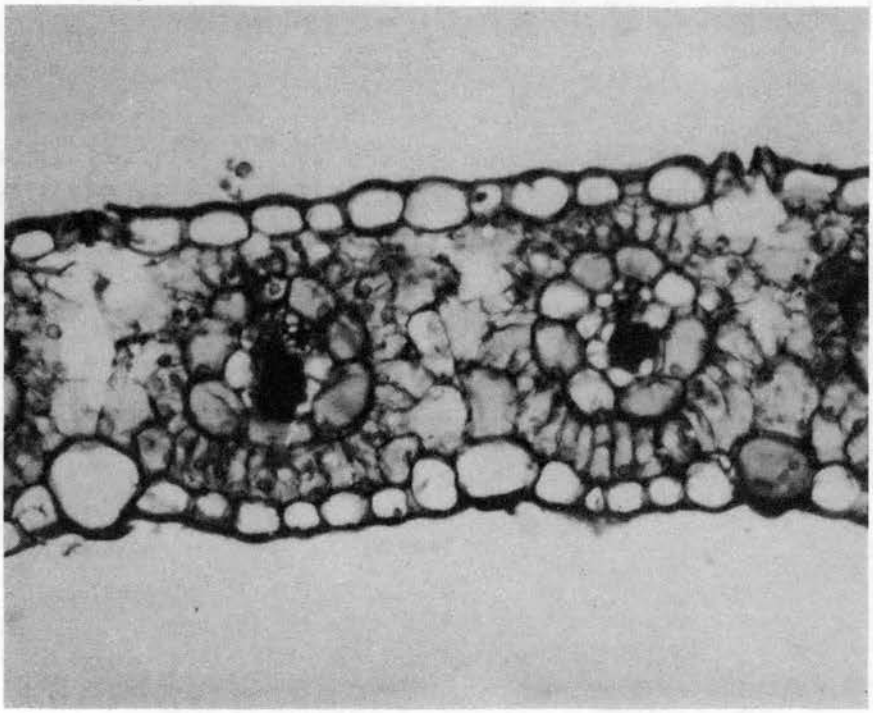

Fig. 13

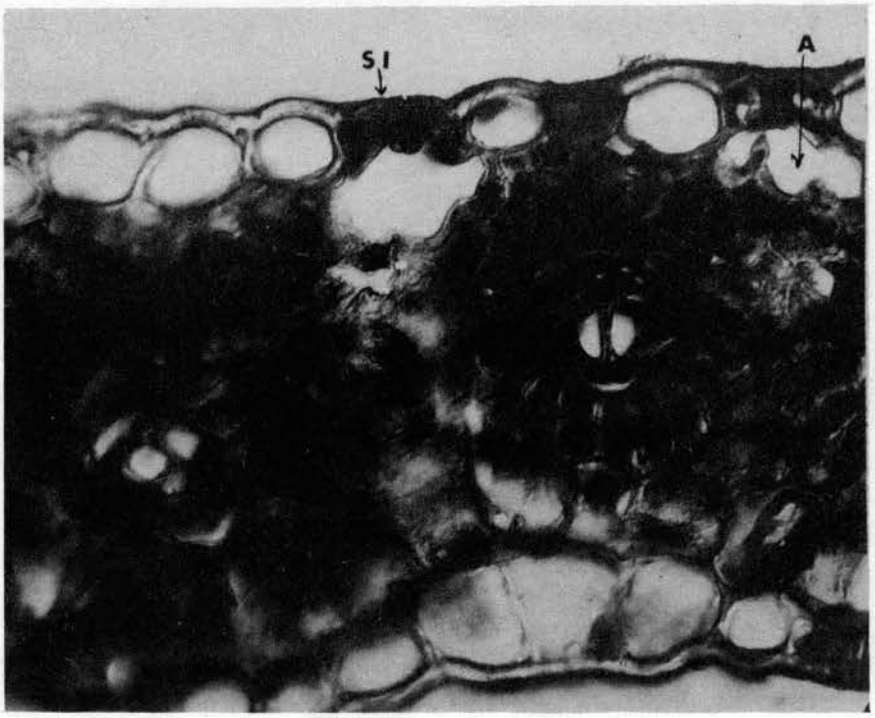

Fig. 14

PLATE VIII

Fig. 15 Bidrin Injury to Sorghum leaves, 60 hours after application, showing complete blockage of cross vein. x 310

Fig. 16 Bidrin Injury to Sorghum leaves, 60 hours after application, showing zone of complete collapse of tissue system. x 380

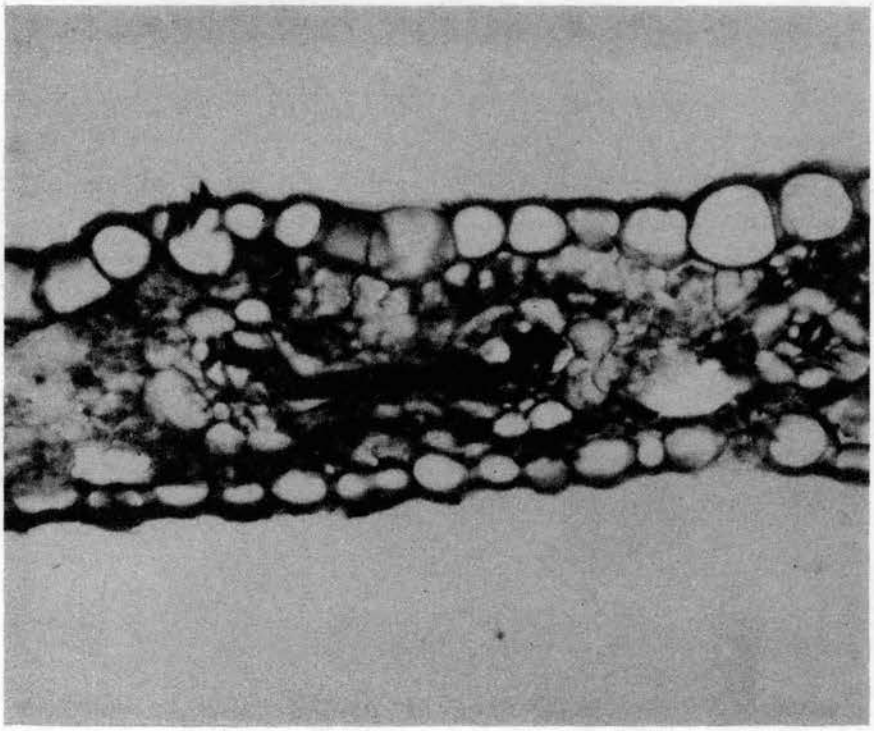

Fig. 15

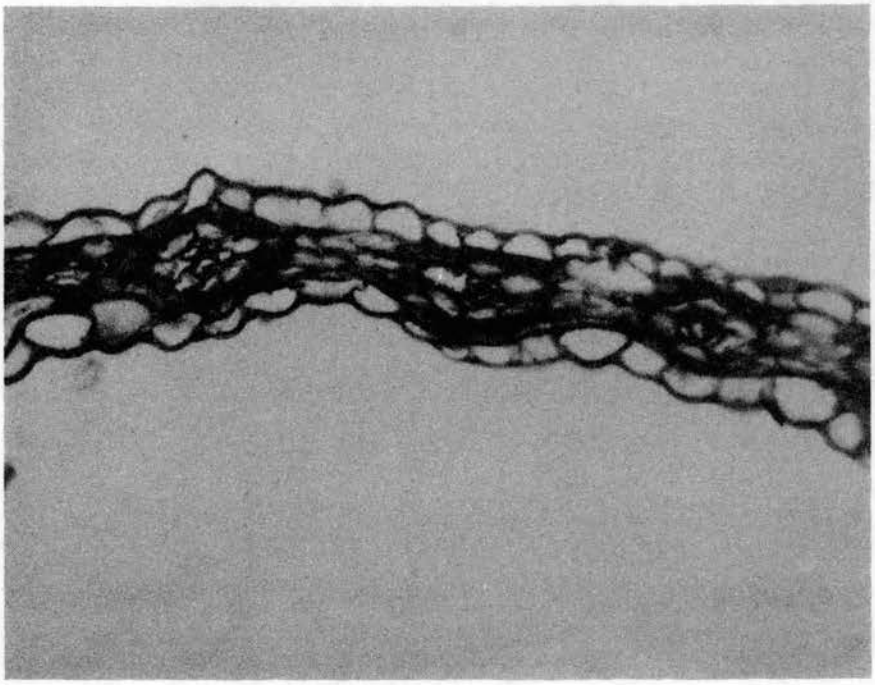

Fig. 16

PLATE IX

Fig. 17 Methyl parathion injury to Sorghum leaves, 48 hours after application. x 370

D ——— Dilated chloroplast of bundle sheath cells.

P ——— Shrinkage of cell walls of parenchymatous column.

Fig. 18 Methyl parathion Injury to Sorghum leaves, 48 hours after application. x 370

SB ——— Shrinkage and disintegration of chloroplast of bundle sheath cells.

SM ——— Shrinkage and disintegration of chloroplast of mesophyll cells.

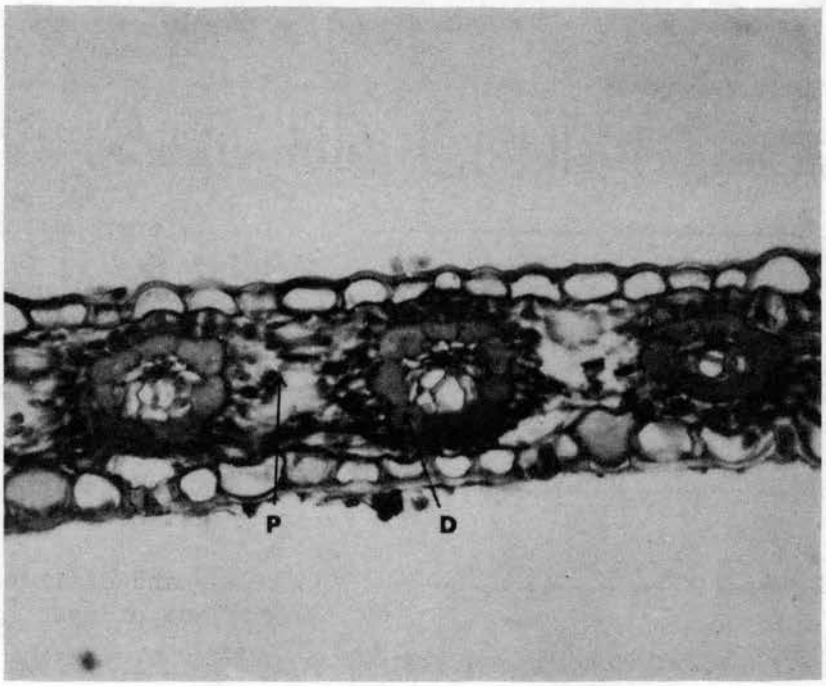

Fig. 17

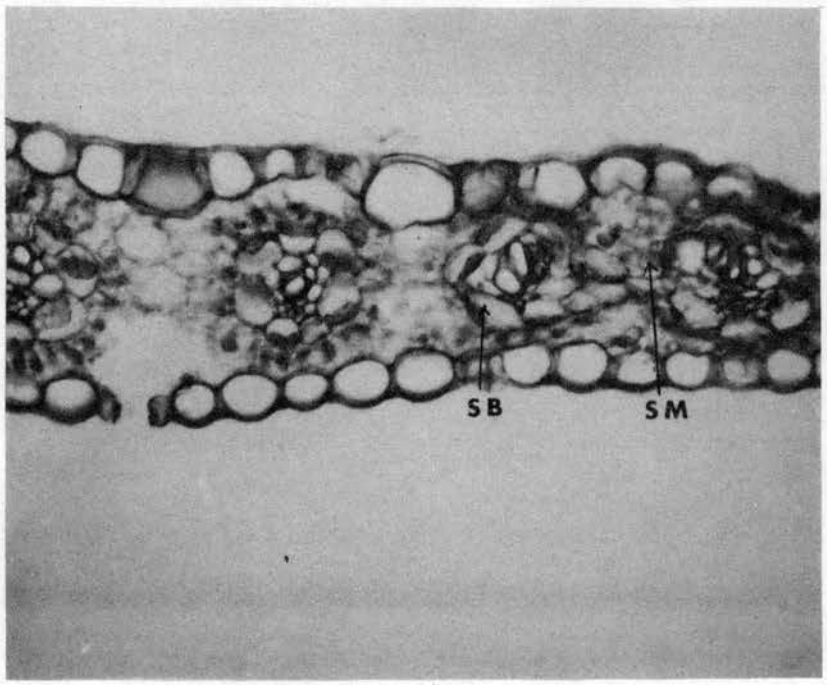

Fig. 18

PLATE X

Fig. 19 Methyl parathion injury to Sorghum leaves, 60 hours after application, showing injury to the large vascular bundle. x 530

Fig. 20 Methyl parathion injury to Sorghum leaves, 60 hours after application, showing injury to the large vascular bundle and cross vein. x 530

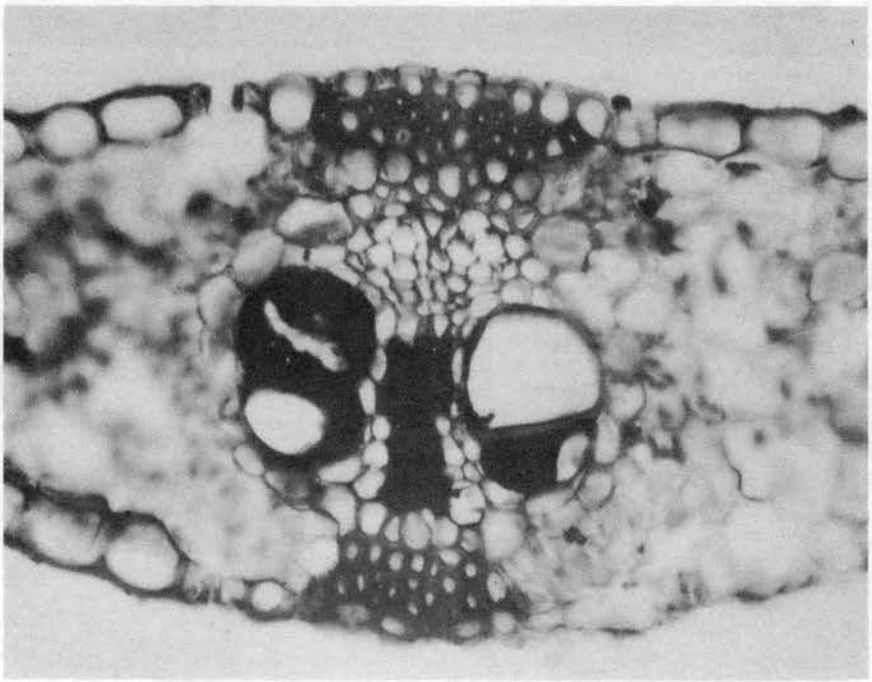

Fig. 19

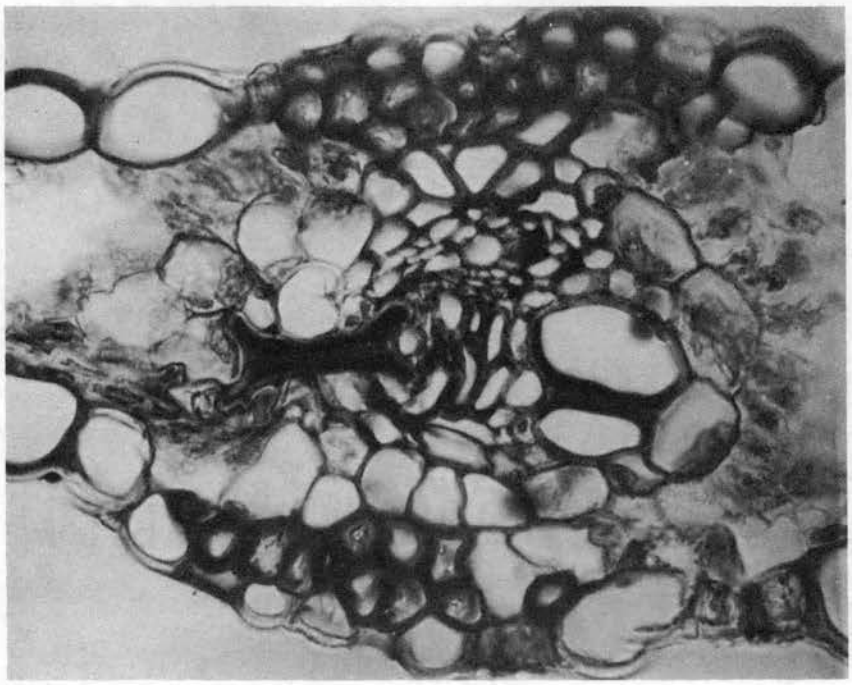

Fig. 20

PLATE XI

Fig. 21 Cross section of mature Sorghum leaf from untreated plant. Triple stain. x 370

Fig. 22 Cross section of mature Sorghum leaf from Bidrin-treated plant, 48 hours after insecticide spraying, showing disappearance of chloroplast from bundle sheath and mesophyll cells, and coagulation of protoplast in epidermal cells. Triple stain. x 370

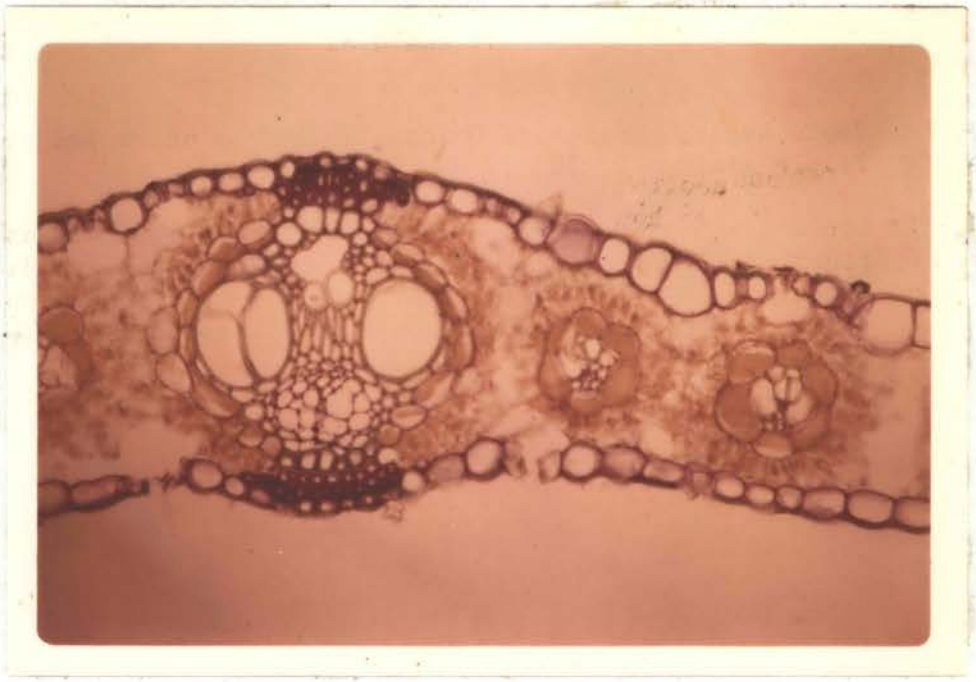

Fig. 21

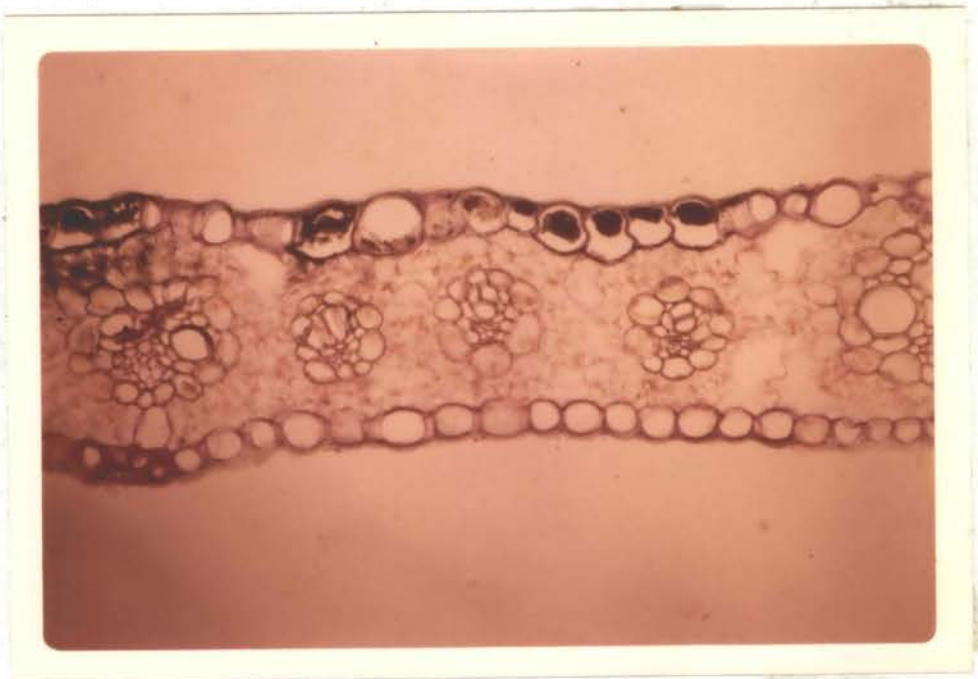

Fig. 22

PLATE XII

Fig. 23 Internal injury to leaves from methyl parathion-treated plant, 48 hours after insecticide application, showing disintegration of chloroplast, clogging of xylem, injury to epidermal cells and collapse of chlorenchyma. Triple stain. x 370

Fig. 24 Internal injury to leaves from methyl parathion treated plant, 48 hours after insecticide application, showing coagulation of protoplast in epidermal cells, and disintegration of chloroplast. Triple stain. x 370

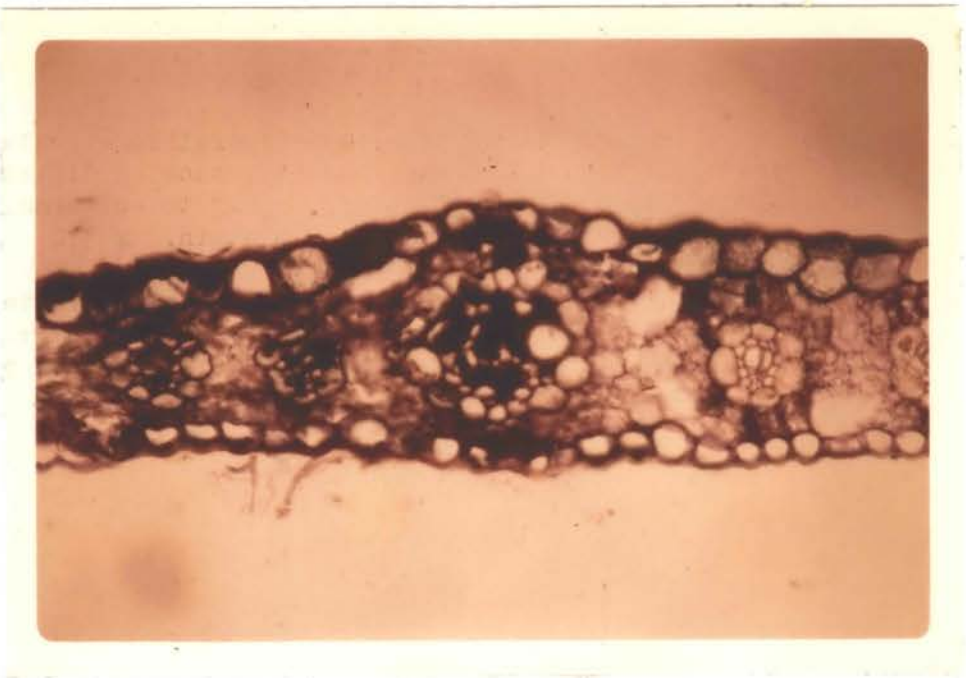

Fig. 23

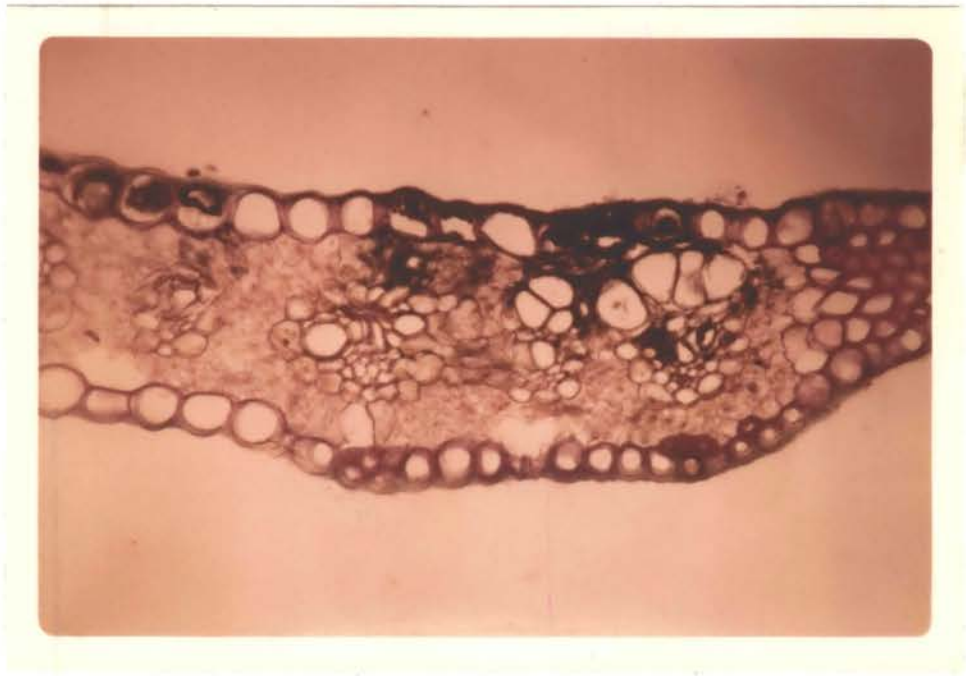

Fig. 24

PLATE XIII

Fig. 25 Cross section of Sorghum leaf from Bidrin-treated plant, 48 hours after spraying, showing clogging of xylem vessels and cross vein, injured chlorenchyma and parenchyma. Triple stain. x 370

Fig. 26 Internal injury to Sorghum leaf from Bidrin treated plant, 60 hours after insecticide spray, showing extensive collapse of tissue system. Triple stain. x 370

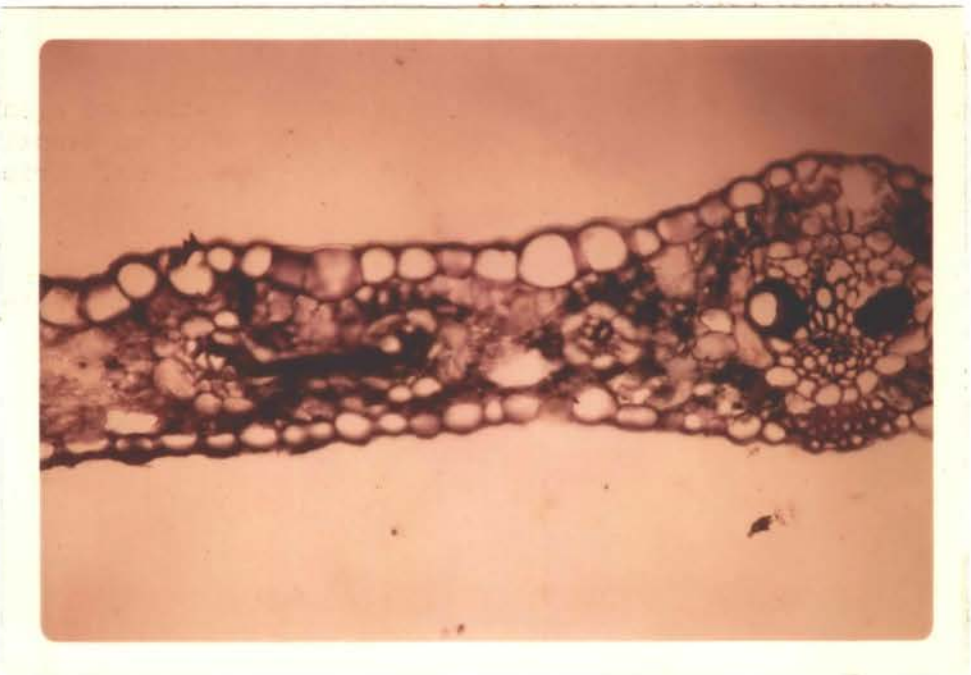

Fig. 25

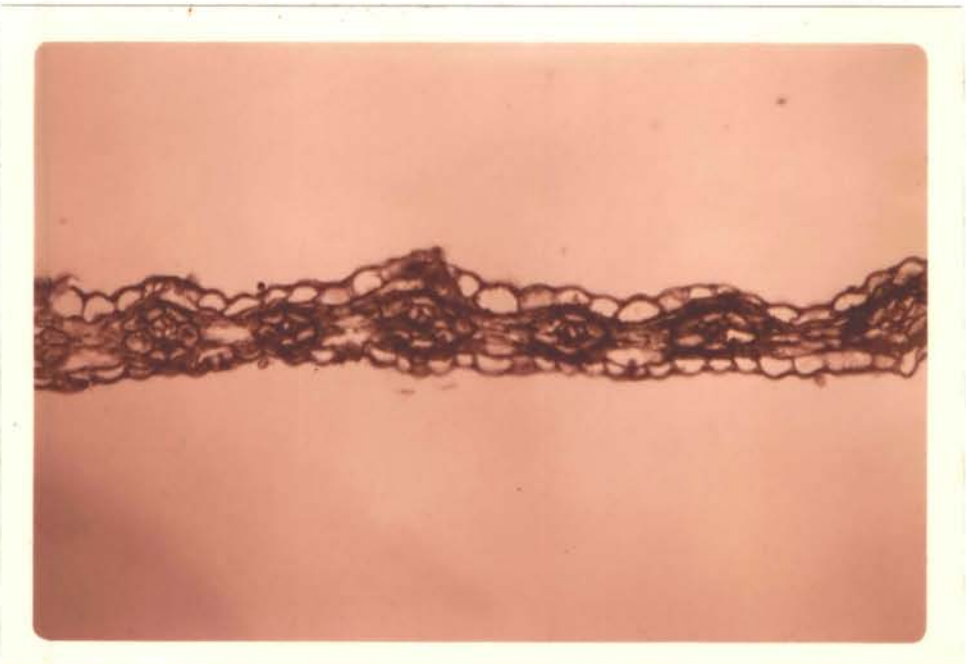

Fig. 26

PLATE XIV

Fig. 27 Bidrin injury to the mature leaf of sorghum, 48 hours after insecticide spraying, showing progressive disintegration of chloroplast, slight shrinkage of chlorenchymatous cell wall, and injury to xylem. PAS Test. x 370

Fig. 28 Bidrin injury to the mature leaf of Sorghum, showing internal symptoms 48 hours after insecticide spraying. PAS Test. x 370

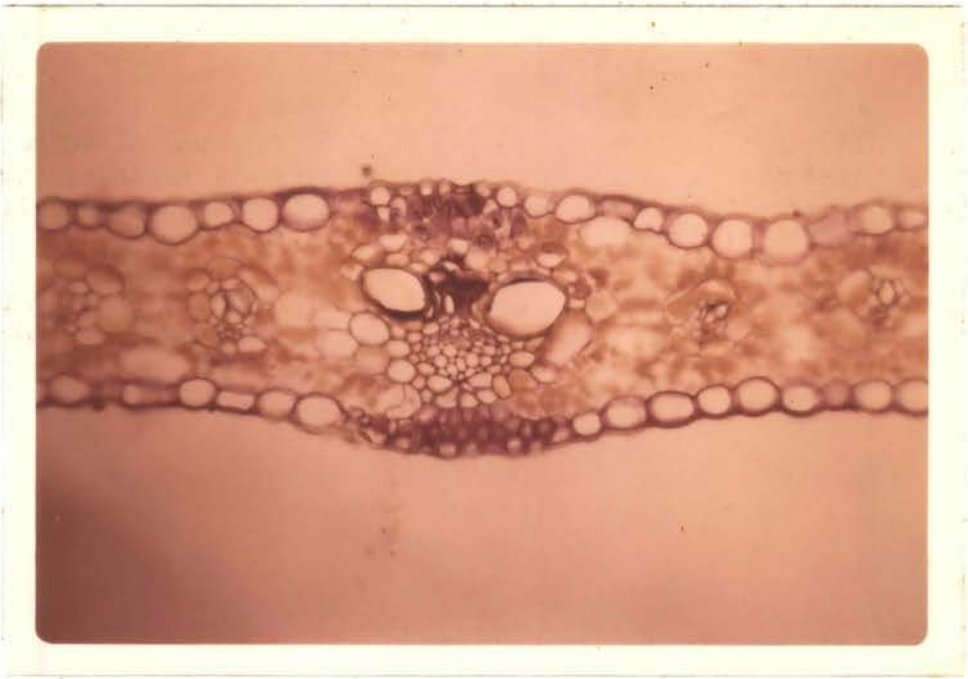

Fig. 27

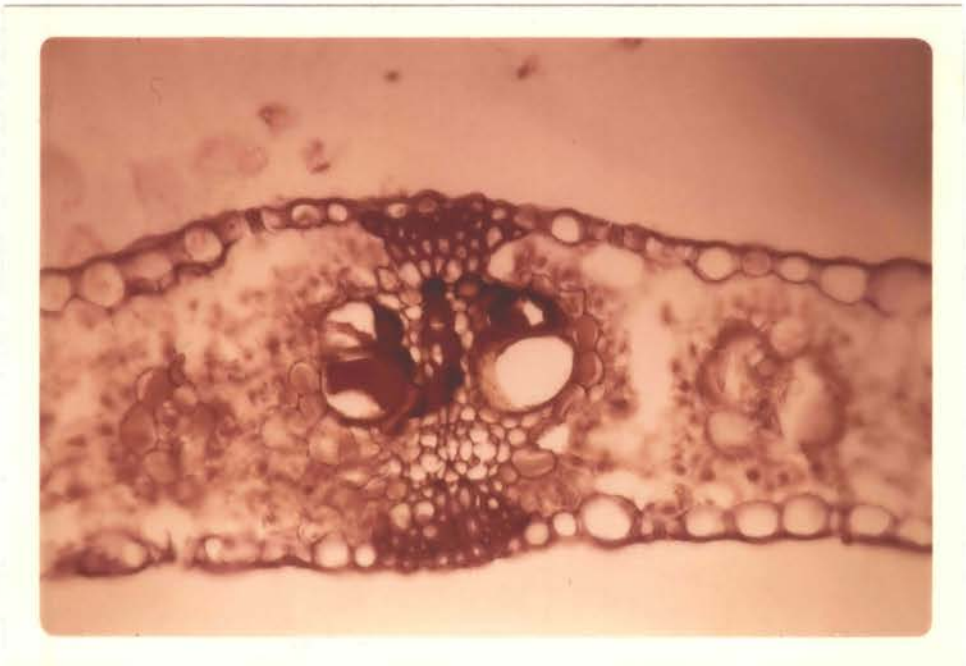

Fig. 28

PLATE XV

Fig. 29 Internal injury to Bidrin-treated sorghum leaf, 48 hours after insecticide spraying, enlarged, showing injury to small vascular bundle and surrounding chlorenchyma cells. PAS Test. x 370

Fig. 30 Internal injury to Bidrin-treated sorghum leaf, 60 hours after insecticide spraying, showing complete collapse of tissues. PAS Test. x 370

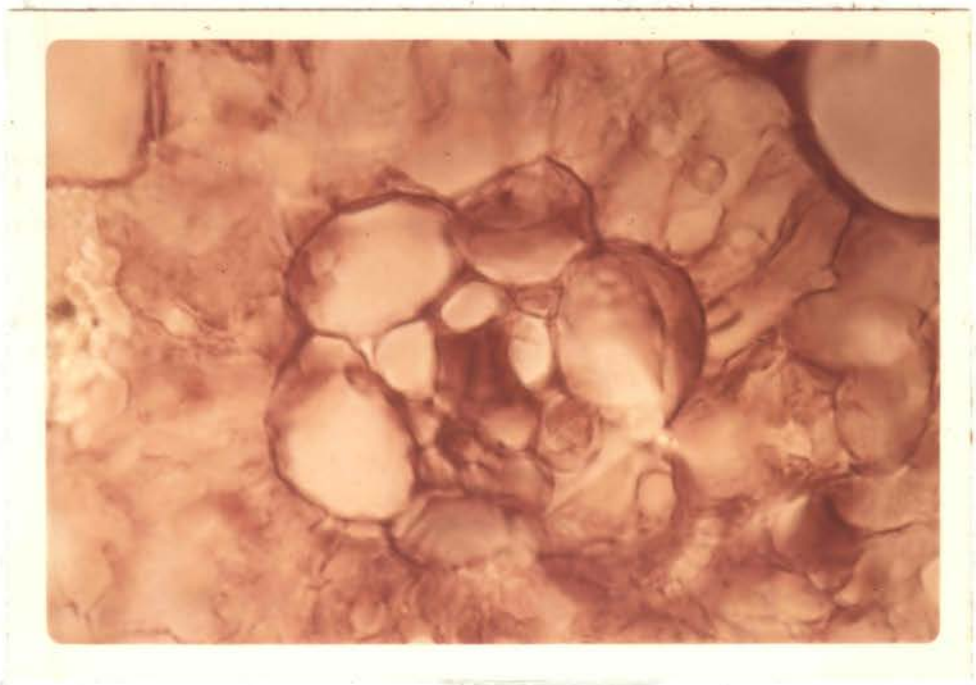

Fig. 29

Fig. 30

PLATE XVI

Fig. 31 Chloroplast alteration in an OK 612 sorghum leaf, treated with Bidrin, showing the breakdown of frets. x 43,500

Fig. 32 Chloroplast alteration in an OK 612 sorghum leaf, treated with Bidrin, showing disorganization of lamellae. x 43,500

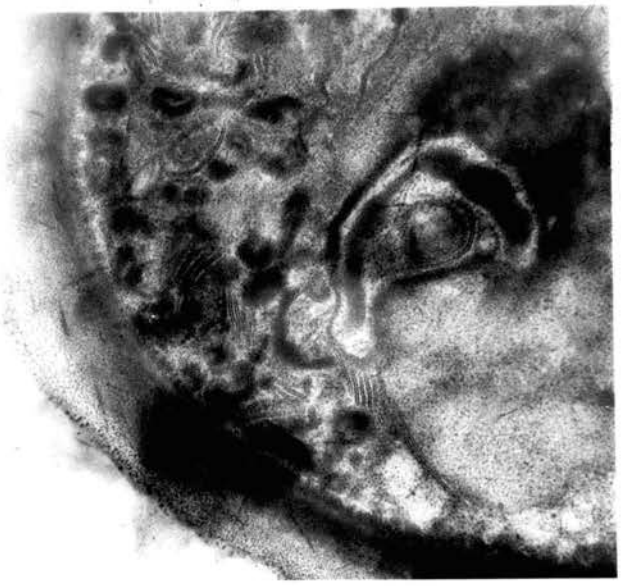

Fig. 31

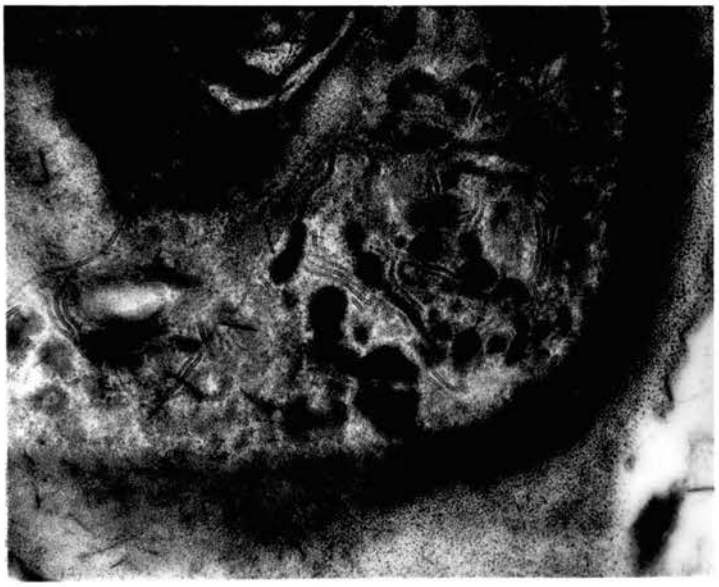

Fig. 32

PLATE XVII

Fig. 33 Final stage of alteration of chloroplast in an OK 612 sorghum leaf, treated with Bidrin, showing the vesicles. x 30,000

M ——————————— Mitochandria
V ——————————— Vesicles

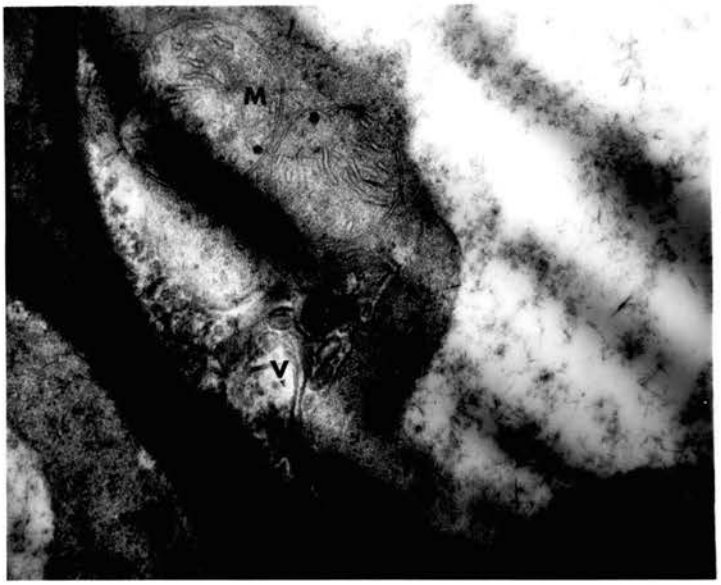

Fig. 33

VITA

Talat Ara Razvi

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Doctor of Philosophy

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