INTERACTION BETWEEN GREENBUG (HOMOPTERA: APHIDIDAE) AND SUSCEPTIBLE AND RESISTANT WHEAT PLANTS: FEEDING BEHAVIOR, QUALITATIVE ULTRASTRUCTURE, QUANTITATIVE ULTRASTRUCTURE

By

ALIA TAHER MORGHAM

Bachelor of Science
University of El-Fateh
Tripoli, Libya
1977

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1983

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
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Thesis Approved:

Paul E. Richardson
Thesis Advisor

Glenn W. Todd

Norman N. Durham
Dean of the Graduate College

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TABLE OF CONTENTS

Chapter Page

PART III

LIST OF FIGURES

Figure Page

PART I

PART II

PART III

INTRODUCTION

This investigation is composed of three manuscripts written for submission to the Annals of the Entomological Society of America. Each manuscript is written as a separate section. The first manuscript (Part I) entitled "Probing Behavior and Correlation of Electronically Recorded Waveforms with Probing Activities of Greenbug (Homoptera: Aphididae) Biotype E on Resistant and Susceptible Wheat Plants," describes the probing behavior of greenbug biotype E on susceptible and resistant wheat and the correlation of the waveform with the stylet positioned in the leaf tissue. The second manuscript (Part II) is entitled "Ultrastructural Responses of A Resistant and A Susceptible Wheat to Infestation by Greenbug Biotype E (Homoptera: Aphididae)". This part is concerned with ultrastructural changes in cells and organelles of the susceptible cultivar, 'Sturdy', and the resistant line, 'Largo', following infestation with GBE. The third manuscript (Part III) is entitled "Stereological Analysis of A Chloroplast Changes Induced by Greenbug Biotype E (Homoptera: Aphididae) Feeding on Susceptible and Resistant Wheat Plants". This work quantifies changes occurring in the chloroplasts of susceptible and resistant wheat postinfestation with GBE.

Approval for presenting the thesis in this manner is based upon the Graduate College's policy of accepting a thesis written in manuscript form and is subjected to the Graduate College's approval of the major professor's request for a waiver of the standard format.

For: Annals, Entomological Society of America

Send correspondence to:

Dr. P. E. Richardson

Dept. of Botany and Microbiology

318 LSE

Oklahoma State University

Stillwater, OK 74078

405/624-5559

Probing Behavior and Correlation of Electronically Recorded Waveforms with Probing Activities of the Greenbug (*Homoptera: Aphididae*) Biotype E on Resistant and Susceptible Wheat Plants

ALIA T. MORGHAM, P. E. RICHARDSON, AND R. L. BURTON[2]

Department of Botany and Microbiology, and Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater, Oklahoma 74078

ABSTRACT. The feeding behavior of greenbug biotype E (GBE), *Schizaphis graminum* (Rondani), on susceptible and resistant wheat leaves was electronically monitored for 1 h. Three wave forms which were correlated with salivation, phloem ingestion and non-phloem ingestion are described. Phloem ingesting and the ingestion duration of GBE was longer on the susceptible than on the resistant plant. GBE, when monitored on the resistant line, exhibited brief separate probes, non-phloem ingestion, and longer salivation. The X-waveform always preceded phloem ingestion and the stylet/stylet sheath could be traced to the phloem tissue.

THE GREENBUG, *Schizaphis graminum* (Rondani), is a major insect pest of wheat, barley, sorghum, and oats in the central and southern Great Plains area of the United States. Five biotypes of the greenbug have been identified: A, B, C, D (Starks and Burton 1977), and E (Porter et al. 1982). Biotype E (GBE) constitutes most of the current field populations. This biotype damages the wheat line 'Amigo' which was formerly resistant to biotype C (GBC).

McLean and Kinsey (1964) introduced a technique of electronically monitoring the feeding behavior of aphids. The principle of the technique is that when an aphid probes into an electrified substrate with its stylet filled with saliva an electric circuit is completed and the voltage can be recorded on an oscilloscope or a strip-chart recorder. Distinctive sequences in waveforms corresponding to voltage changes are associated with salivation and ingestion in specific plant tissue. Four different waveforms can be identified. The s-waveform correlates with active stylet movements within the plant tissue and non-phloem ingestion corresponds to ingestion from tissue other than the phloem. The X-waveform correlates with a penetration of phloem and the phloem ingestion waveform occurs when there is corresponding ingestion from phloem.

McLean and Kinsey (1967) studied the probing behavior of the pea aphid. Three distinctive patterns were recorded during aphid probes and a distinctive curve pattern was always recorded when the stylet contacted sieve elements. These authors also determined and correlated the plant tissue contacted by the aphid stylet with the generation of the different waveforms.

Campbell et al. (1982), in describing the feeding behavior of GBC on susceptible and resistant lines of sorghum, recorded waveforms corresponding to salivation, phloem ingestion, and non-phloem ingestion. The aphid increased the number of separate probes with less time for phloem ingestion on resistant plants as opposed to that on susceptible plants. The authors concluded that toxic material associated with phloem sap in the resistant line retarded aphid feeding. Montllor et al. (1983) monitored the feeding

behavior of GBC and GBE on 'IS 809' sorghum, resistant to GBC and susceptible to GBE. GBE accomplished phloem ingestion in a shorter period of time, spent more time ingesting from the phloem, and made fewer separate probes than GBC. A relatively short sieve-element ingestion period and longer ingestion period from other tissues were characteristic of the aphid *Myzus persicae* (Sulzer) when feeding on a resistant sugarbeet (Haniotakis and Lange 1974). Short total ingestion and sieve-element ingestion were also characteristic of *Therioaphis maculata* (Bukton) when feeding on a resistant alfalfa line (Nielson and Don 1974). Kennedy et al. (1978) found more stylet sheaths ending in mesophyll than in the phloem when *Aphis gossypii* fed on resistant varieties of muskmelon. Ryan et al. (1987) studied the feeding behavior and honeydew production of GBC and GBE on susceptible and resistant wheat. GBC showed 2-3 fold more probes, salivation, phloem penetration, and phloem ingestion on the resistant wheat line 'Amigo' than did GBE, to which this genotype is susceptible. Similar results were obtained when GBC and GBE fed on the resistant line "Largo".

The purpose of this investigation was to electronically record the waveforms of GBE, feeding on the susceptible and resistant wheat plants 'Sturdy' and 'Largo' and to correlate the recorded waveforms with the histological location of the aphid stylets in the leaf tissue. The probing behavior of the aphid was also compared in the resistant and susceptible plants.

MATERIALS AND METHODS

Colonies of *S. graminum* biotype E (GBE) were reared on sorghum plants in insect cages in a greenhouse at approximately 23°C and LD 14:10. Seeds of two wheat plants were used; 'Sturdy' which is susceptible to GBE, and 'Largo' which is resistant. The seeds were planted in small pots and kept in a growth chamber at 25°C and LD 14:10. The leaves used in this study were the second leaves of three-week-old plants.

The probing behavior of mature greenbugs was monitored using a feeding monitoring electronic system (McLean and Kinsey 1967; Brown and Halbrook 1976; Ray et al. 1987). An aphid was allowed to feed at one site on the second leaf for at least 1 h. Aphids were killed in situ on the leaves by administrating a drop of FPA fixative directly to the aphid while it was still feeding. Leaf segments one cm^2 containing the attached aphid were excised from the plant and transferred to FPA fixative (Sass 1958). Segments were then fixed for 48 h, dehydrated in a TBA series, embedded in paraffin, and sections cut on a rotary microtome at 12-15 μm. The sections were stained in Safranin O and fast green FCF. With this combination, the stylet sheaths stained bright red and the stylets appeared a golden brown. Representative selected sections were photographed under the light microscope.

RESULTS

Probing Behavior on Susceptible and Resistant Cultivars. Typical sequences of waveforms of GBE probing on wheat leaves are illustrated in Figs. 1-3. These typical wave pattern sequences were recorded on the strip-chart recorder during aphid feeding on the resistant and susceptible wheats studied. These patterns were: S-NPI— salivation followed by non-phloem ingestion (Fig. 1); S-X-PI— salivation, X-wave, phloem ingestion (Fig. 2); and S-X-PI-S-X-PI-S-X-PI— three X-waves each followed by phloem ingestion. One of the most pronounced feeding behaviors noted in this study, brief separate probes occurred when GBE fed on the resistant plant 'Largo' (Fig. 4). It also occurred when GBE fed on the susceptible 'Sturdy', however, it was not the predominate wave form when feeding occurred on this line. Another very commonly seen wave form, the S-NPI pattern, occurred primarily on the resistant line in three different patterns (Fig. 1, 5, and 6) and was also recorded to some extent on the susceptible (Fig. 1). However, on the susceptible cultivar, the aphid produced primarily the S-X-PI pattern (Fig. 2). GBE feeding on the resistant plant resulted in a longer salivation time than on the

susceptible. GBE, probing either the resistant or susceptible plants, usually produced only one, rarely 2 or 3 X-wave forms before PI. In all the patterns that were recorded, PI was always preceded by an X-wave form. In this short (1-2 h) monitoring period, the susceptible plant showed more X-wave forms, more PI, less NPI, and less salivation than the resistant plant.

Correlation of Waveform Patterns with Stylet and Stylet Sheath Positions in Leaf Tissue. Examination of recordings from 25 separate feeding sessions showed that when a waveform pattern S-X-PI (Fig. 2) was recorded, the stylet tips or stylet sheath were located in phloem tissue (Fig. 7, 8, 9, and 13). The waveform pattern S-NPI (Fig. 1, 5, and 6) was associated with stylet tips located in the mesophyll parenchyma (Fig. 10, 11, 12, and 16), or in the xylem (Fig. 14) but never in the phloem tissue. Only when an X-wave form was recorded could the stylet tip be traced to the phloem tissue. In cross sections the location of the stylet in the phloem tissue could be seen. However, specific phloem tissue cell types contacted were often difficult to determine. The stylet path appeared to be primarily intercellular, but mixed intercellular and intracellular paths were common (Fig. 7, 11, and 15). The aphid usually produced two sheath branches (Figs. 8, 9, 11, and 12) which may result from the difficulty in reaching appropriate tissue.

DISCUSSION

A definite correlation was found between the waveforms obtained during monitoring and the position of the aphid stylet in plant tissue. During the short feeding time (1-2 h) of GBE on the resistant line, the aphid was unable to locate the phloem and attempted to ingest food from other leaf tissue (e.g. parenchyma cells and xylem) whose contents may be less nutritional than the phloem. This apparent inability to locate the phloem may be due to the absence of factors responsible for the orientation of stylet to phloem tissue (Chatters and Schlehuber 1951). Even when the aphid was able to locate the phloem in

the resistant line the phloem ingestion period was shorter than for the susceptible. This suggests that there may be repellent substances associated with the phloem sap or that the sap is of less nutritive value which may prevent the aphid from carrying out effective phloem ingestion. Long periods of non-probing, short separate probes, increased amounts of salivation, increased ingestion from tissue other than phloem, and short durations of phloem ingestion characterized the behaviors of the aphid when feeding on a resistant plant. Our finding that GBE made more separate probes on the resistant than on the susceptible cultivar is in agreement with the results obtained when GBC probed resistant lines of sorghum and a non-host plant (Campbell et al. 1982) and when GBC and GBE fed on 'Largo' and 'Sturdy' (Ryan et al. 1987). Most aphids make several probes before settling down for prolonged feeding which may provide the aphid with information to determine whether it is on an acceptable host.

The frequency of behaviors related to phloem ingestion and phloem penetration obtained in this study when GBE fed on either line is not in complete agreement with the results obtained by Ryan et al. (1987). This may be explained by the fact that in their study, they monitored aphid feeding for a 24 h period whereas in our study we monitored feeding for only 1-2 h. The relatively longer time required by the aphid to reach and ingest from the phloem of the resistant plant may account for this apparent contradiction in the results.

Stylet tips or stylet sheaths were located in the phloem tissue when an S-X-PI waveform pattern was recorded by the feeding monitor. This was consistent with the results obtained by McLean and Kinsey (1967). They also showed that the stylet tips were in contact with the phloem sieve elements when X-waveforms had been recorded. They suggested that the X-waveform may be due to a buildup of callose around the wound of the penetrated sieve elements, an accumulation of p-protein around the stylet tip. Or, possibly the X-waveform occurs when p-protein is broken down by spurts of saliva. The X-waveform signal was noted before penetration into the sieve element of cabbage plant

by *Brevicoryne brassicae* (Kimmins 1986). Evert et al. (1973) traced stylet paths to sieve elements in barley leaves using electron microscopy. They detected no callose around the point of penetration of the stylet into the sieve elements. Since the sieve elements of barley leaves lack p-protein, they concluded that prior to penetration of a sieve element the aphid flushes its stylets in order to clear them for food ingestion which also could cause X-waveform production on the recording. The X-waveform signal appears as a result of drops in voltage potential which may indicate penetration of the plasmalemma. Since living plant cells exhibit membrane potential differences between the cytoplasm and external media, any disruption of the membrane could cause an alternation in electrical potential (Findly and Hope 1976).

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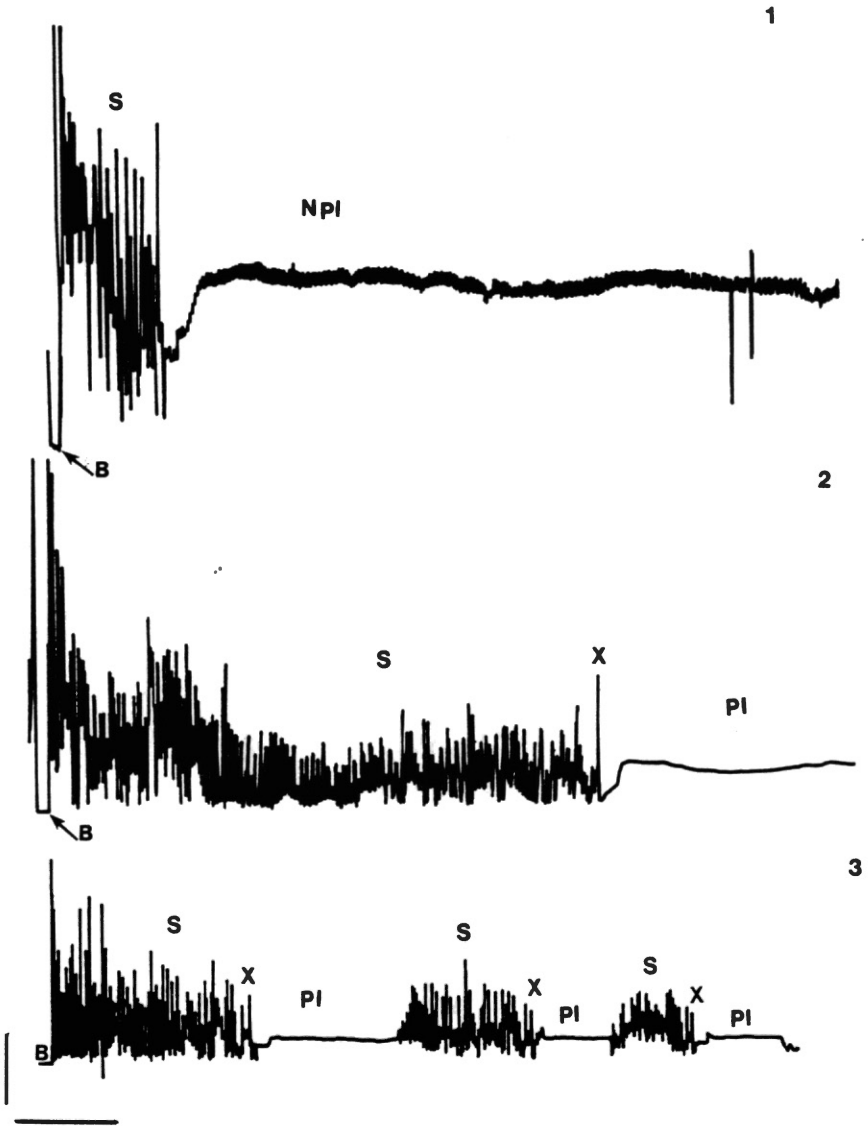
Footnotes:

[2]ARS-USDA, Plant Science and Water Conservation Lab., P.O. Box 1029, Stillwater, OK 74076.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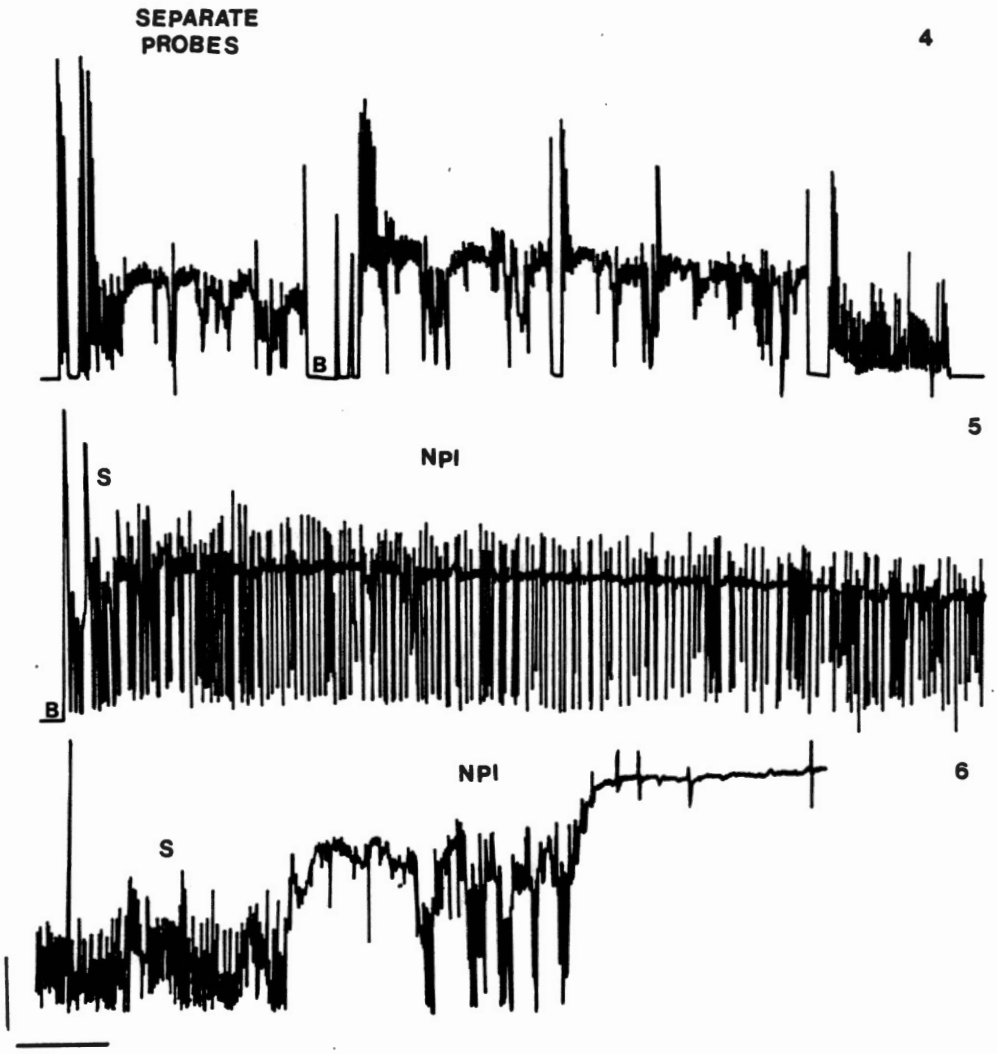
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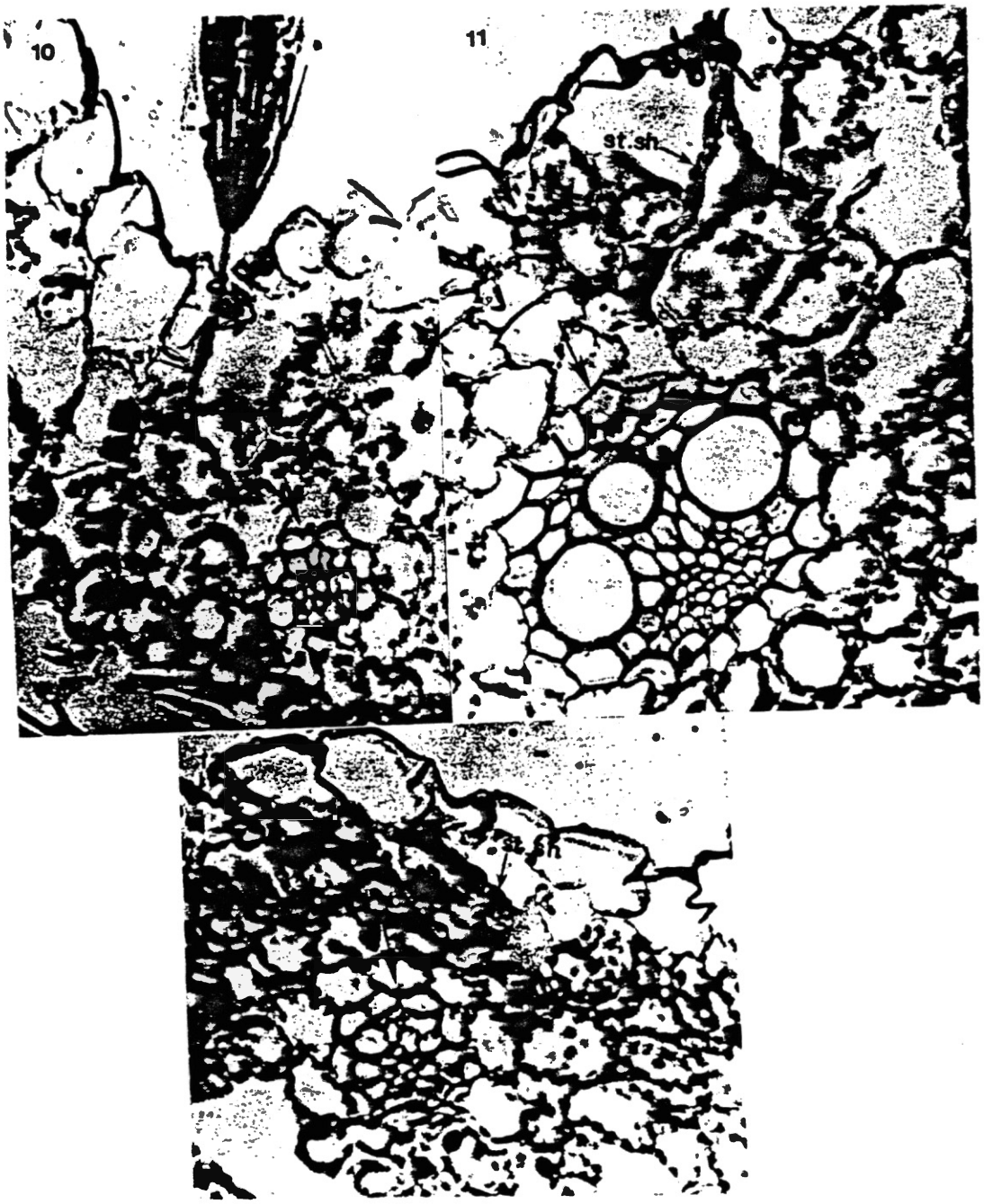
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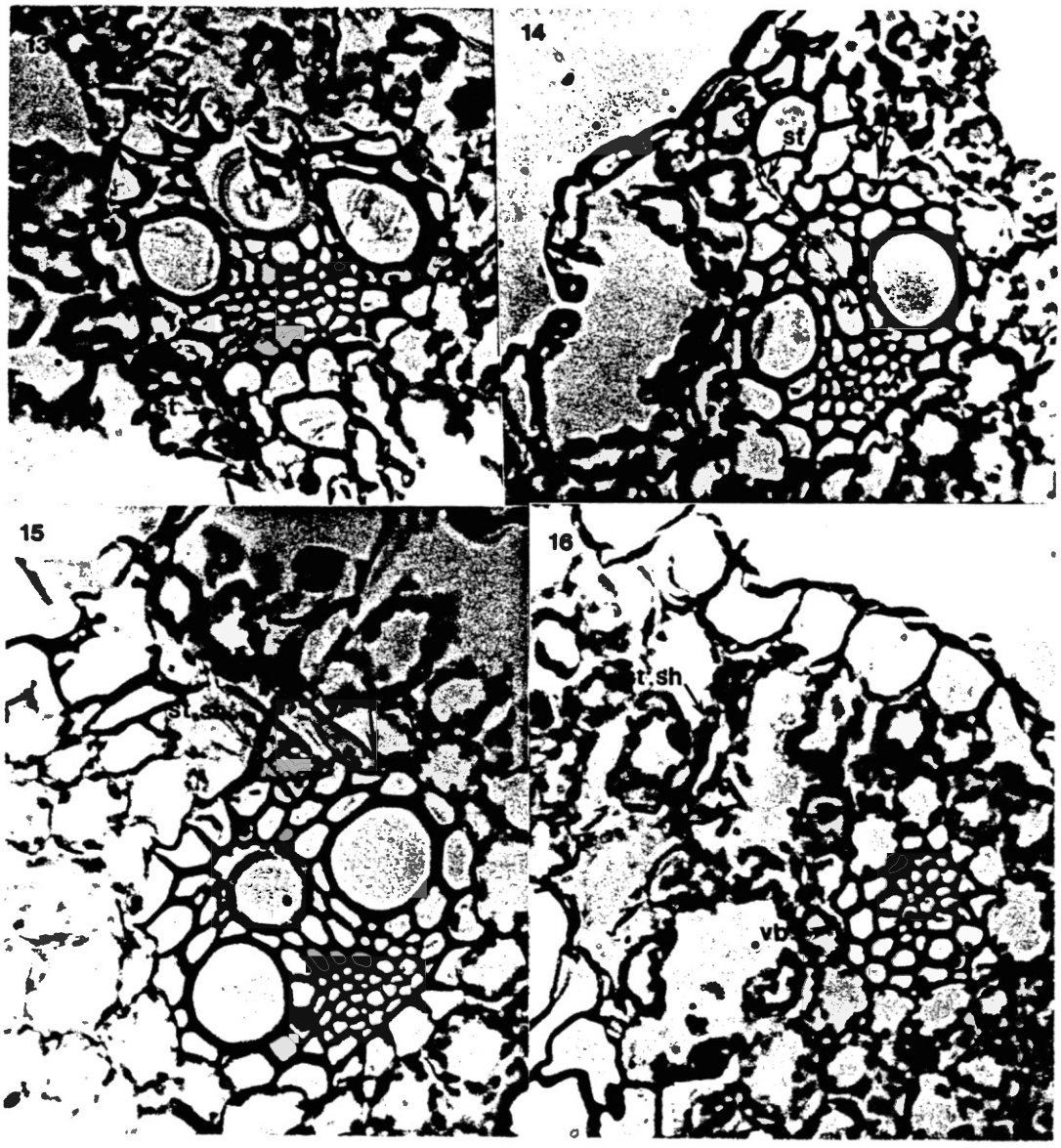
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Figs. 1-3. Representative waveforms recorded during electronic monitoring of probing by greenbug biotype E on wheat leaves. (1) Base line (B), S-NPI pattern, salivation (S) followed by non-phloem ingestion (NPI), ingestion from leaf tissue other than the phloem. (2) Base line (B), S-X-PI pattern, salivation (S), X-waveform (X), and phloem ingestion (PI). (3) S-X-PI-S-X-PI-S-X-PI pattern. Three X-waves, eachpreceded by salivation and followed by phloem ingestion. Horz. bar 5 min, vert. bar 48 mV, and base line 0 mV.

Figs. 4-6. The wheat line, 'Largo', resistant to GBE. (4) Brief separate probes Base line (B). (5) Base line (B), salivation (S) followed by non-phloem ingestion (NPI) (6) Another S-NPI pattern. Horz. bar 5 min, vert. bar 48 mV, and base line 0 mV.

Figs. 7-9. The wheat cultivar, 'Sturdy', susceptible to GBE. (7) Cross section of leaf showing penetration of stylet (st) to vascular bundle (vb) and ending in phloem (ph) (X320) and the stylet (st) surrounded by stylet sheath (st. sh) taken while monitoring the stylet probe that produced an S-X-PI pattern. (8) Cross section of leaf. Stylet sheath (st. sh) ending in phloem tissue (X320). (9) Longitudinal section of leaf (X350). Branched stylet sheath (st. sh) ending in phloem tissue.

Figs. 10-12. The wheat cultivar, 'Sturdy'. (10) Cross section of leaf (X350) showing stylet (st) terminating in the mesophyll parenchyma that produced an S-NPI pattern. (11) Cross section of leaf (X350) showing branched stylet sheath (st. sh). In this case, the aphid probe monitor produced an S-NPI pattern. (12) Cross section of leaf (X350) showing stylet sheath (st. sh) in the mesophyll parenchyma.

Figs. 13-14. The wheat line, 'Largo'. (13) Cross section of leaf (X350) showing aphid stylet (st) ending in the phloem which produced an S-X-PI pattern. (14) Cross section of

leaf (X350) showing stylet (st) ending in xylem. This produced the S-NPI pattern shown in Fig. 6.

Figs. 15-16. The wheat line, 'Largo' (15). Cross section of leaf (X350) showing the stylet sheath (st. sh) ending near the xylem; the aphid produced the S-NPI pattern shown in Fig. 5. (16) Cross section of leaf (X350) containing stylet sheath (st. sh) penetrating the mesophyll parenchyma.

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Send correspondence to:
Dr. P. E. Richardson
Dept. of Botany and Microbiology
Oklahoma State University
Stillwater, OK 74078
405/624-5559

Ultrastructural Responses of A Resistant and A Susceptible Wheat to Infestation by Greenbug Biotype E (*Homoptera: Aphididae*)

ALIA T. MORGHAM, P. E. RICHARDSON, AND R. L. BURTON[2]
Department of Botany and Microbiology, and
Oklahoma Agricultural Experiment Station,
Oklahoma State University, Stillwater,
Oklahoma 74078

ABSTRACT. Individuals of biotype E greenbug, *Schizaphis graminum* (Rondani), were allowed to feed for at least 1 h on leaves of a resistant and a susceptible wheat plants. Tissue responses were studied by means of transmission electron microscopy. The greenbug caused severe degenerative changes in vascular cells of susceptible plants as early as 1 h postinfestation. In the mesophyll tissue, cells near the stylet path were most affected and less damage was incurred as the distance from the stylet path increased. The ultrastructural features of affected cells included disruption of chloroplast and cellular membranes and enlargement of the plastoglobuli within chloroplasts. Wall appositions were observed in susceptible tissue 1 day postinfestation and became readily noticeable by 4 days. By 7 days it was nearly impossible to distinguish the remnants of original structural features. In resistant plants, few mesophyll cells appeared to be pierced by the aphid stylets but cellular debris was found in intercellular spaces. Saliva sheaths were not noted after 4 days postinfestation. Bacterial cells were observed with the cellular debris in intercellular spaces at feeding sites in resistant plants. Resistance in wheat to specific greenbug biotypes appeared to depend on the ability of the host plant to resist chemical alterations induced by virulent greenbugs in susceptible cultivars.

THE GREENBUG, *Schizaphis graminum* (Rondani), is one of the more destructive cereal insects in some of the major regions of wheat production. The aphid causes significant losses to wheat in the Southern Great Plains of the United States (Rogers et al. 1972). Several varieties of wheat, barley, oats, and sorghum resistant to various greenbug biotypes have been developed (Joppa et al. 1980; Webster and Starks 1984; Boozaya-Angoon et al. 1981; and Starks et al. 1983). However, new greenbug biotypes that overcome existing resistance have appeared. The existence of biotype E greenbug was first noted in 1979 in Bushland, Texas when biotype C resistant Amigo wheat was damaged (Porter et al. 1982). Biotype E is now the predominant biotype in the field (Kindler et al. 1983).

Feeding mechanisms of aphids as related to host plant resistance and susceptibility has attracted the attention of many workers (McLean and Kinsey 1968; and Pollard 1973). Roberts (1940) studied the feeding of *Myzus persicae* (Sulzer) and *Macrosiphum gei* (Koch) on tobacco and found that the penetration path is intercellular and intracellular ending in the phloem tissue. Chatters and Schlehuber (1951) studied the feeding mechanism of *S. graminum* on wheat, barley, and oats. They stated that host plant tissue damage caused by aphid feeding is a result of stylet penetration and saliva injection and not organic compound removal. It is also their interpretation that there is no correlation between the amount of host plant mechanical tissue and susceptibility or resistance to the greenbug. Gibson (1971) related aphid resistance of potato cultivars to the presence of glandular leaf hairs. Resistance was also considered to be due to physiological and biochemical factors. That is, physiological factors such as those concerned with sap pH, which in turn affect phloem-guidance (McMurtry and Stanford 1960). In barley, one form of resistance to *S. graminum* is determined by the presence of benzyl alcohol (Juneja et al. 1972). The presence of phenolic compounds is considered to be generally correlated with plant resistance as well (Miles 1969a). Constitutive alkaloids can render tobacco plants resistant to aphids, although some other insects can metabolize them (Guthrie et al. 1962).

As reported by Saxena and Chada (1971), the mouthparts of biotype A greenbug enter plant tissues intercellularly and finally reach the phloem by a somewhat circuitous route causing damage mainly in the phloem and phloem parenchyma. The sieve tubes appeared completely collapsed. They also reported that biotype B enters plant tissues mostly intracellularly and most damage occurs in the mesophyll cells of the leaves. Wood et al. (1969) compared the reactions of small grains to biotypes A, B, and C, and reported that feeding mechanisms of the three biotypes were different. They indicated that biotypes A and C fed in the phloem sieve-tubes and B fed in mesophyll parenchyma and exhibited no phloem feeding.

Campbell et al. (1982) reported that greenbug biotype C feeding on resistant varieties of sorghum shows less imbibition of phloem sap and shows longer duration of non-probing feeding behavior than is the case on susceptible varieties. The authors suggested that resistance in sorghum may be due to toxic materials associated with the phloem sap of the resistant plant. Esau et al. (1961) studied progressive damage in susceptible sugar beet tissue following *Myzus persicae* infestation. They noted that the first organelle to be affected are the chloroplasts followed by cell necrosis. Al-Mousawi et al. (1983) reported two different types of damage in susceptible wheat caused by biotype C. The first type of damage was observed in the vascular tissue. The second kind of damage occurred in the mesophyll cells along the aphid feeding tracks. The aphid caused more damage in the vascular bundles than in the mesophyll cells along stylet tracks. More damage occurred in the susceptible than in the resistant variety and feeding damage could no longer be found in resistant leaves at 10 days postinfestation. Since few ultrastructural studies have been reported concerning the infestation of wheat by greenbugs, the current study was undertaken to provide additional information about mechanisms of aphid damage to the host plant and to aid in better understanding the biology of the host plant/aphid interaction. This information should be of value to future efforts such as breeding plants for resistance to the greenbug.

MATERIALS AND METHODS

Colonies of greenbug biotype E were reared on sorghum in insect cages in the greenhouse at approximately 23°C and LD 14:10. Resistant and susceptible wheat plants were used in this study; 'Largo', resistant to biotype E and 'Sturdy' which is susceptible to it. Seeds of the two were germinated in petri dishes for 2 days and then planted one per pot and kept in growth chambers at 25°C and LD 14:10. Three-week-old plants were used in all experiments.

Mature greenbugs were allowed to feed and were electronically monitored at single sites on second leaves of wheat seedling for an average of 1-2 h. At the end of the feeding period the feeding sites were marked with India ink and the aphids were removed. Control samples were taken from uninfested areas of the same leaves. Leaf segments (1 mm^2) from the feeding sites were collected at 1 h, and 1, 2, 4, and 7 days postinfestation and fixed for 2 h in 4% glutaraldehyde on 0.1 M sodium and potassium phosphate buffer, pH 7.3. The leaf segments were washed in the same 0.1 M phosphate buffer, post-fixed in 2% osmium tetroxide for 4 h, and dehydrated in a graded ethanol series. Tissue was infiltrated with Spurr's epoxy resin, firm mixture (Spurr 1969).

Thin sections were cut with a diamond knife on a Sorvall MT-2 ultramicrotome and collected on 200-mesh copper grids stained with 5% uranyl acetate and 0.4% lead citrate and examined and photomicrographed with a JEOL-100C XII transmission electron microscope.

RESULTS

Feeding Damage. Macroscopic necrotic feeding lesions, each surrounded by a chlorotic halo, occurred 3 to 4 days postinfestation on leaves of the susceptible cultivar, 'Sturdy', and persisted throughout the period of the study. The resistant line, 'Largo', showed no chlorosis; however, white chlorotic flecks appeared at the feeding site by the

third day postinfestation and disappeared a day later. Two types of microscopic damage were observed in the susceptible cultivar. The first was to the mesophyll cells along the aphid stylet paths and the second type damage was found in the vascular tissue, especially in the phloem conductive tissue, which is considered to be the ultimate destination of the aphid stylet.

Ultrastructural Responses. In the susceptible wheat cultivar, Sturdy, densely stained materials could be detected in intercellular spaces among mesophyll cells within a few hours after feeding (Fig. 1). Vascular tissue showed severe degeneration and cell plasmolysis one hour postinfestation. Phloem parenchyma cells were densely stained and the internal organelles completely destroyed (c.f. Fig. 2 and 3).

By 1 to 2 days postinfestation all the internal cell structures of the affected vascular bundles were severely damaged and most of the cell organelles were indistinguishable. Densely stained nuclei were noted as well as large numbers of small membrane bound vesicles (Figs. 4 and 5). Densely stained fibrillar materials were deposited in cells of the tracheary tissue. We believe these are similar to or part of the saliva sheath which enters xylem cells as a result of the aphid feeding in the vascular bundles (Fig. 6). Ultrastructural changes occurred in the mesophyll cells by 1 to 2 days postinfestation. At one day host cell organelles such as mitochondria and nuclei and their membrane systems were comparable to those located in uninfested control cells (c.f. Fig. 7 and 8). Chloroplasts were relatively unaffected except for the increase of the size and number of plastoglobuli. The rough endoplasmic reticulum (ER) and plasmodesmata were very pronounced and this suggests that the host cells are engaged in active metabolism (Fig. 8). At this stage, plasma membranes began to separate from the cell wall and fibrillar material (wall apposition) was deposited between the cell wall and the plasma membrane. By 2 days postinfestation, the chloroplasts were rounded with large numbers of plastoglobuli and chloroplast bounding membranes and their granal and stromal lamellae were poorly

defined (Fig. 9). Vesiculation and wall apposition were observed near broken plasma membranes and cells had electron dense cytoplasm with granulation. Some chloroplasts in damaged cells showed pleomorphism (Fig. 10 arrows). Some cells appeared much more severely damaged than others (Fig. 11). In these cases, cell wall collapse was severe, the chloroplast fretwork was disrupted totally and grana and stroma were disintegrated in cells which contained fibrillar materials in a milieu in which there were almost no discernable organelles (Fig. 11).

The ultrastructural effects on infested susceptible plants at 4 days included plasma membrane separation from cell walls and prominent fibrillar materials or wall appositions deposited on the plasma membrane side of the cell walls. Mitochondria were severely disrupted and chloroplasts had swollen grana thylakoids (Fig. 12). The saliva sheaths in the intercellular spaces between mesophyll cells were very densely stained and the cell walls adjacent to them were even more deformed compared with the controls, which showed no such effects. Frequently the walls appeared partially dissolved. Mitochondria had swollen cristae and contained densely stained materials. The nuclei often had several invaginations and, at this time, were irregularly spaced with a globular body in each. The outer envelope of the chloroplasts were breaking down and the cytoplasm had a granular appearance (Fig. 13). Damaged cells were often devoid of cytoplasm and intact organelles. A large number of vesicles appeared in the intercellular spaces as a result of cell breakdown (Fig. 14). The cytoplasm became electron dense and finally contained condensed ribosomes and the chloroplasts were in the final stages of degeneration. Severely damaged vascular cells contained branched stylet sheaths (Fig. 15, a-e).

At 7 days postinfestation, mesophyll cells in the damaged susceptible plants showed cell plasmolysis and cell organelles at different late stages of degeneration (Fig. 16). Cell wall collapse was obvious at this stage and cells were severely disrupted. Chloroplasts lacked intact membrane systems and thylakoid lamellae were separated. Wall appositions were still prominent in some cells (Fig. 17). The nucleus was the last cell organelle to be

disrupted. Nuclear material was lightly stained and the nuclear envelope was still intact although the double nature of the membrane could not be seen. The cytoplasmic membrane was severely affected and peculiar vacuoles were present (Fig. 18).

At 1 h postinfestation, vascular cells of the resistant plant appeared similar to the control except for some plasmolysis (c.f. Fig. 19 and 20). No darkly stained cells were observed. As early as 1 h postinfestation broken cell organelles such as chloroplast lamellae, mitochondria, and membrane fragments appeared in the intercellular spaces of mesophyll cells (Fig. 21). No such cell debris was observed in control tissue of either the resistant or the susceptible plants. Nuclei, chloroplasts and mitochondria of such controls were normal with clearly defined outer membranes.

At 1 to 2 days, salivary sheaths in the resistant line were noted in the mesophyll intercellular spaces. Chloroplast granal and stromal lamellae were not significantly different from the control. There was an accumulation of starch in the chloroplasts and very few chloroplasts showed pleomorphism. Small wall appositions were noted in a few micrographs (Fig. 22). Damaged cells had small vesicles in the cytoplasm. By 4 days, bacterial cells were found together with cell debris in intercellular spaces. Bacterial cells were not seen in the susceptible plant. The tonoplast was not well defined (Fig. 23). Cell debris was still observed by 4 days postinfestation and the adjacent cell wall was not well defined. Most of the cell components appeared comparable to those located in healthy tissue (Fig. 24). The saliva sheaths were not recognizable after 4 or 7 days postinfestation. At 7 days mesophyll and vascular cells at feeding sites appeared normal (Fig. 25).

DISCUSSION

The resistant and the susceptible wheat plants used in this study responded differently to greenbug biotype E infestation. Largo, which contains a gene for greenbug resistance from an amphiploid of *Triticum turgidum/Triticum tauschii* (Joppa et al. 1982),

showed no macroscopic damage throughout the study. 'Sturdy', which contains no gene for greenbug resistance, showed macroscopic necrotic lesions (1 mm in diameter) surrounded by a chlorotic halo (2-3 mm in diameter) 3 days postinfestation. On the susceptible wheat plants ('Sturdy'), the greenbug appeared to induce modifications of the plant's metabolism and senescence-like symptoms followed. It has been previously reported that the chlorophyll content in susceptible wheat is reduced by greenbug infestation (Ryan et al. 1987). This chlorophyll loss from mesophyll cells is usually accompanied by release and breakdown of protein, especially ribulose bisphosphate carboxylase oxygenase (rubisco), which makes up more than 50% of the protein in wheat leaves (Wittenbach 1979). The released amino acids may enrich the phloem sap and stimulate phloem ingestion by the greenbug. The modification of plant metabolism and the induction of senescence-like symptoms may improve the quality of the susceptible plant as a food source (Dorschner et al. 1987). This is consistent with the finding of MacKinnon (1961) that aphids preferred feeding on excised leaves rather than the intact plant. On resistant plants, the greenbug have a lower potential for growth and reproduction (Sumner et al. 1986). This may be explained by the inability of greenbugs to modify the plant metabolism and to release higher concentrations of amino acids needed for increased growth and fecundity. This idea is supported by the findings of Van Emden and Bashford (1976) who indicate that excised or senescent leaves from resistant plants are as acceptable to aphids as leaves from susceptible plants.

In our work, ultrastructural studies of susceptible plants revealed two types of damage, one is to the vascular cells and the other involves the mesophyll cells along the stylet paths. The earlier damage effects were observed in the vascular cells of the susceptible plants where the phloem parenchyma cells showed severe organelle degeneration which indicated that phloem tissue was the ultimate goal for the greenbug stylets. Xylem tissues are sometimes pierced by the stylets. This is indicated by the presence of a salivary sheath in xylem tracheary tissue. In the resistant plants, intact

vascular cells appeared comparable to controls except for occasional cell plasmolysis. Similar results were obtained by Al-Mousawi et al. (1983) in susceptible wheat cultivar TAM W-101 infested with greenbug biotype C.

In susceptible plants damage to mesophyll cells were observed 2 days postinfestation. The first organelle to be affected is the chloroplast and the most notable change is the increase in size and probably the number of plastoglobuli. These increases in the number of plastoglobuli in damaged chloroplasts has been noted in several host/pathogen interactions. Examples are, zinnia leaves treated with tagetitoxin (Jutte and Durbin 1979), *Beta vulgaris* leaves infected with *Cercospora beticola* (Steinkamp et al. 1979), and in sunflower leaves infected with the vascular pathogen *Verticillium dahliae* (Robb et al. 1977). The increases appeared related to damage and eventual disappearance of internal membranes.

After the appearance and expansion of plastoglobuli, the chloroplasts became rounded and their lamellae were degraded, which could lead to the release of nutrients. This should be of benefit to the aphid. Other cell organelles such as mitochondria were severely affected by 4 days postinfestation. There is little evidence of nuclear damage until fairly late stages of cellular damage. In addition we noticed an increase in the level of rough ER 1-4 days postinfestation. This may indicate that damaged cells produce new metabolites needed for cellular autolysis at later stages of cells distruction. This hypothesis agrees with the findings of EiLamy et al. (1971) and Brady and Tung (1975) that senescenced leaves showed an increase in the level of cytoplasmic polyribosomes, and in the rate of protein synthesis.

Wall appositions are a common response of plants to infection by fungi and in toxin treated plants (Heath 1980; Hanchy 1981). Histochemical investigation of the chemical composition of wall appositions in barley coleoptiles infected by *Erysiphe graminis* showed that wall appositions contained protein, carbohydrate, callose, and phenolic compounds but contained no cutin or suberin (Smart et al. 1986). Resistant hosts

responded to penetration of the fungus by synthesis and deposition of carbohydrates particularly callose and cellulose between cell walls and plasma membranes of invaded cells, while in susceptible hosts, wall appositions are missing or poorly defined (Sherwood and Vance 1980). Wall appositions may prevent transfer of nutrients between host and pathogen or prevent the transfer of toxic materials into host cells. In susceptible plants, several toxins induced plasma membrane invagination and wall appositions (Hanchey 1981). In toxin-treated plants, wall appositions formations were noted after detection of electrolyte leakage which indicate changes in membrane permeability. In diseased or toxin-treated plants, wall appositions may play a protective role over damaged plasma membrane areas (Wheeler,1974).

In plant-bacterial interactions wall appositions were observed only in tissue where the pathogen was not inhibited and their role in resistance is doubtful (Morgham et al. 1987). In the present case prominent wall appositions were observed mostly in susceptible plants and as early as 1-day postinfestation and they were more pronounced by 4 days. Their appearance may have resulted from damage at the plasma membrane caused by toxic material(s) of the saliva or saliva sheath.

In resistant plants, fewer changes were observed in vascular cells. In mesophyll cells the damage was apparently restricted to breakdown of a few cells which were pierced by greenbug stylets, as indicated by the presence of cellular debris in the intercellular spaces. Since the greenbug made more probes and salivated longer on resistant plants (unpublished data) and this ultrastructural study shows destruction of few mesophyll cells, it seems unlikely that the mesophyll cells are the primary target of the greenbug. In the resistant some damage did occur to mesophyll cells near the salivary sheath. These cells showed granular cytoplasm, membrane damage and vesicle formation. There were massive accumulations of starch in their chloroplasts. Similar accumulations of starch have been noted in susceptible plant chloroplasts infiltrated with bacteria (Lallyett 1977) or with virus (Appiano et al. 1977). This starch accumulation may have resulted from

decreased translocation of triose phosphates out of the chloroplasts and/or other factors. Bacteria were noted, probably from the aphid, but since they were not in the susceptible plants, they may have no role in virulence.

Stylet paths are mostly intercellular and may become intracellular as stylets reach vascular cells. Branched salivary sheaths (a-e) occurred in vascular cells (Fig. 15). The totally collapsed cell walls may imply that aphids use salivary pectinases to dissolve the middle lamellae and protrude their stylets between cells.

This ultrastructural study revealed no morphological structural differences between the resistant and the susceptible wheat plants. We speculate that greenbug salivary pectinases may be unable to degrade pectins from resistant plants as has been suggested by Campbell and Dreyer (1985). Another possibility that may account for the tolerance to greenbug infestation is that the chemical structure/quantity of pectin substances in greenbug resistant and susceptible wheat were different (Ryan et al. 1986).

In summary, our interpretation of our data indicate that cells with severe degenerative changes were located in the susceptible plants near the salivary sheaths. On the other hand, the resistant plants showed minor damage. These results agree with the results obtained by Al-Mousawi et al. (1983). In addition, we observed other responses such as wall appositions and an increase in the level of rough ER in the susceptible plants, and large amounts of starch deposition in the chloroplasts of the resistant plants.

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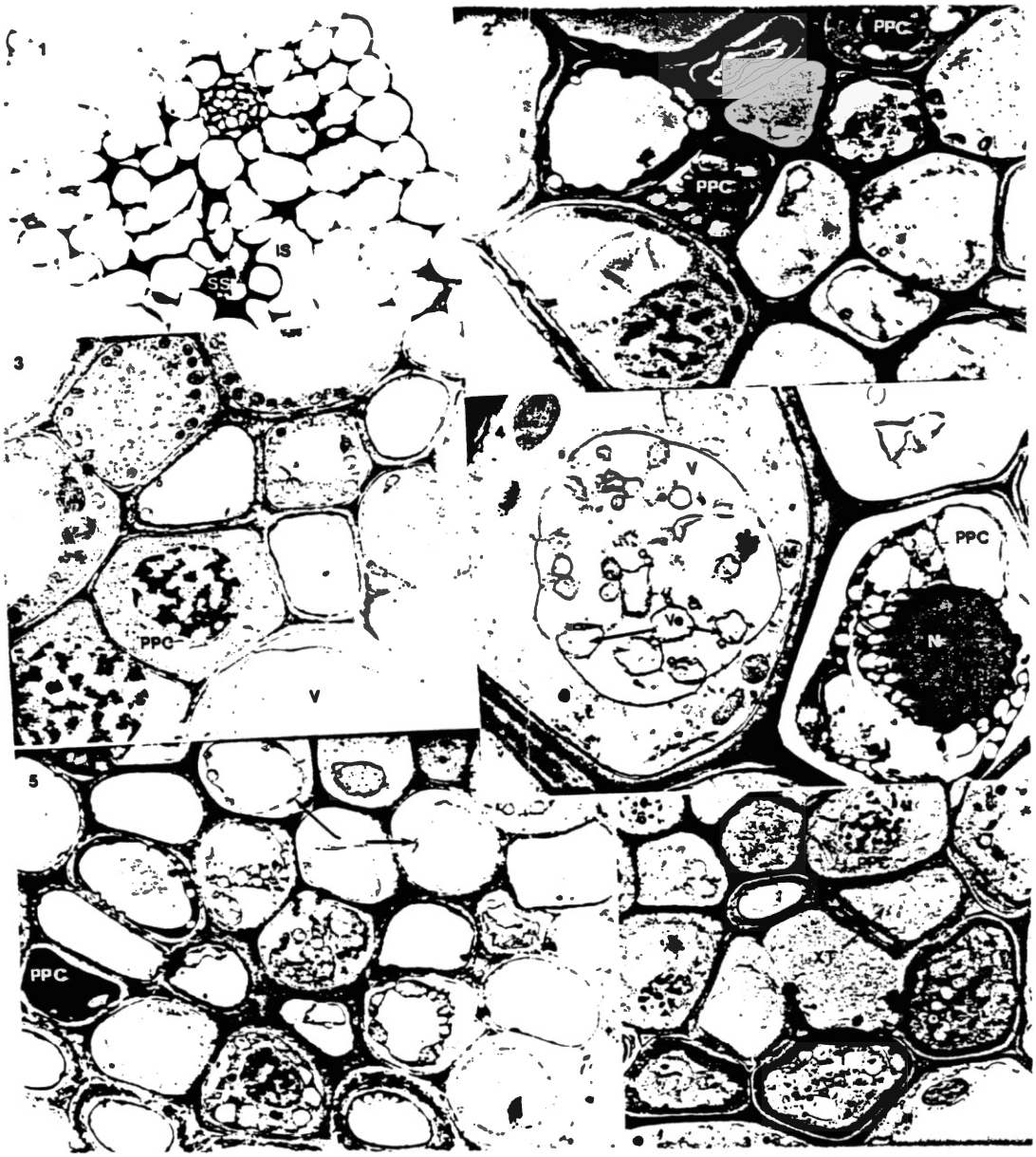
Footnotes:

[2]ARS-USDA, Plant Science and Water Conservation Lab., P.O. Box 1029, Stillwater, OK 74076.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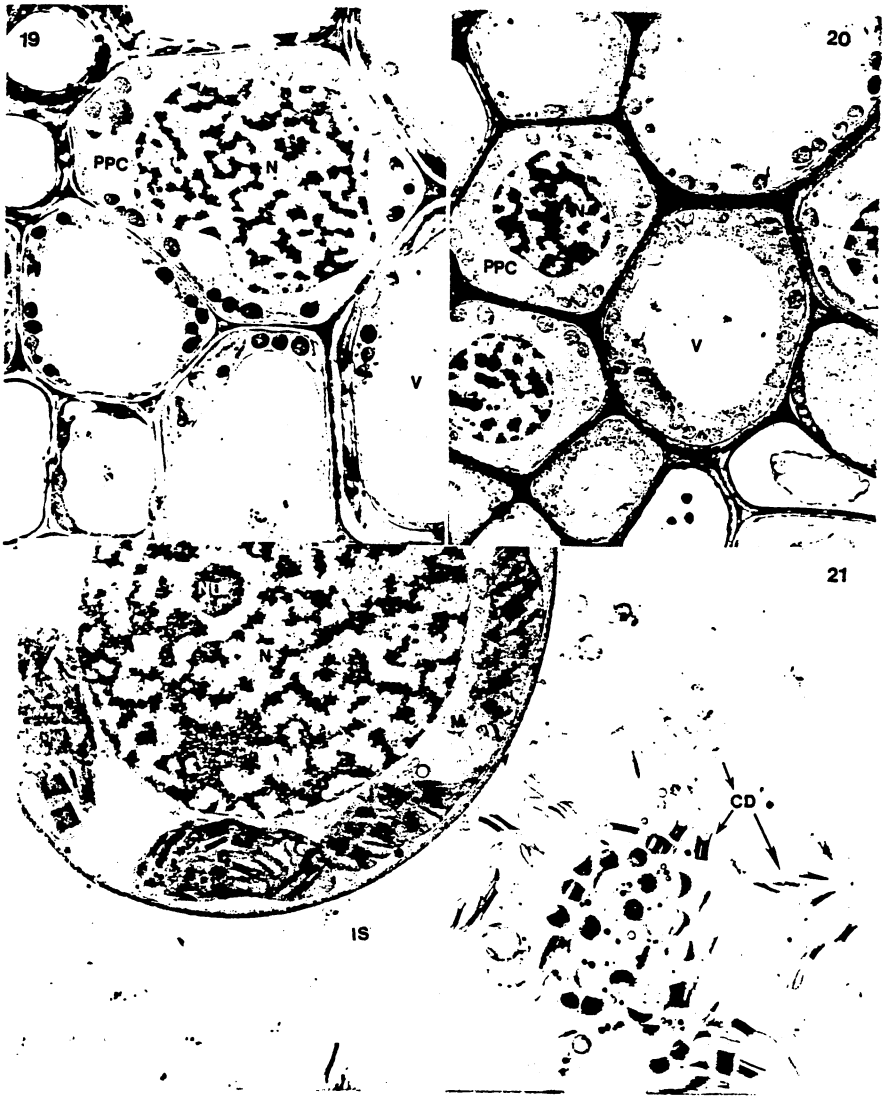
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Figs. 1-6. The wheat cultivar, 'Sturdy', susceptible to biotype E greenbug. (1) Light photographs 1 h postinfestation showing a darkly stained saliva sheath (SS) in the intercellular spaces (IS) of the mesophyll cells (X 1,200). (2) Electron micrograph of vascular cells 1 h postinfestation. Phloem parenchyma cells (PPC) and vacuoles (V) appeared densely stained (X 6,000). (3) Electron micrograph of control vascular cells. All cells appeared normal with clear vacuoles (V) (X 5,800). (4) Electron micrograph of phloem parenchyma cell (PCC) 1 day postinfestation showing a darkly stained nucleus (N) and vacuole (V) with an intact membrane containing small vesicles (Ve) and digested organelles (X 12,200). (5) Electron micrograph of vascular cells 2 days postinfestation, showing cell plasmolysis (arrows) and a densely stained phloem parenchyma cell (PPC) (X 4,500). (6) Electron micrograph of vascular tissue 2 day postinfestation, showing fibrillar material filling xylem tracheids (XT) (X 4,625).

Fig. 7-11. The wheat cultivar, 'Sturdy', susceptible to greenbug biotype E. (7) Electron micrograph of control mesophyll cells showing normal appearing cells, showing cell wall (CW), intercellular spaces (IS), plastoglobuli (P), chloroplast (C), mitochondria (M), nucleus (N), and nucleolus (NU) (X 10,800). (8) Electron micrographs of the saliva sheath (SS) 1 day postinfestation in intercellular spaces (IS) and chloroplasts (C) with darkly stained plastoglobuli (p) (X 7,200). (9) Electron micrograph of mesophyll cell 2 days postinfestation, showing round chloroplasts (C) with a large number of plastoglobuli (p) and a saliva sheath (SS) with fibrillar appearance (X 10,800). (10) Electron micrograph of chloroplasts (C) 2 days postinfestation exhibiting pleomorphism (arrows) (X 7,200). (11) Electron micrograph of completely damaged cell (DC) 2 days postinfestation showing disrupted cell organelles and a cell filled with fibrillar materials (X 7,200).

Fig. 12-18. The wheat cultivar, 'Sturdy', susceptible wheat cultivar. (12) Electron micrograph of a mesophyll cell 4 days postinfestation that show wall apposition (Ap) between wavy cell wall and stretched plasma membrane. Mitochondria (M) appear disrupted and lack an intact boundary membrane (X 17,500). (13) Electron micrograph of mesophyll cell 4 days postinfestation. Mitochondria (M) contain densely stained vesicles, nucleus (N) irregular in shape and cell wall appears dissolved in certain areas (X 8,700). (14) Electron micrograph of intercellular space (IS) that contains small vesicles (Ve) released from broken cells (X 4,200). (15) Vascular cells contained branched salivary sheaths (a-e) (X 4,500). (16) Electron micrograph of plasmolyzed cells 7 days postinfestation that shows cell organelles at a final stage of degeneration and a saliva sheath (SS) darkly stained with a smooth appearance (X 8,500). (17) Electron micrograph of collapsed cell 7 days postinfestation, showing wall apposition (Ap) and a degenerated cell with scattered cellular contents (X 8,200). (18) Electron micrograph, showing a degenerated nucleus (N) 7 days postinfestation and a broken cell wall (arrow) (X5,800).

Fig. 19-21. The wheat line, 'Largo', resistant to biotype E greenbug. (19) Electron micrograph of vascular cells from the control plant (X 6,000). (20) Electron micrograph of vascular cells 2 days postinfestation, showing normal phloem parenchyma cells (PPC). Some cells show plasmolysis (X 6,000). (21) Electron micrograph of a mesophyll ceil 1 h postinfestation. The intercellular space (IS) is filled with broken cell organelles. The adjacent cell appeared comparable to that from the control (X 7,200).

Fig. 22-25. The wheat line, 'Largo', resistant to biotype E greenbug. (22) Electron micrograph of mesophyll cells 2 days postinfestation, showing chloroplasts (C) with large number of starch grains (S) and the intercellular space (IS) containing a saliva sheath (SS) (X 7,250). (23) Electron micrograph of mesophyll cells 4 days postinfestation showing

bacterial cells (B) and cell debris (CD) in the intercellular space (IS). Cell organelles do not appear significantly different from those of control cells (X 6,000). (24) Electron micrograph of cell debris (CD) filling the intercellular space (IS) 4 days postinfestation. The nucleus (N), chloroplasts (C) and mitochondria (M) appear normal (X 7,250). (25) Electron micrograph of mesophyll cells 7 days postinfestation showing clear intercellular spaces (IS) and normal appearing cells (X 9,000).

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Send correspondence to:

Dr. P. E. Richardson

Dept. of Botany and Microbiology

Oklahoma State University

Stillwater, OK 74078

405/624-5559

Stereological Analysis of A Chloroplast Changes Induced by Greenbug Biotype E (*Homoptera: Aphididae*) Feeding on Susceptible and Resistant Wheat

ALIA T. MORGHAM, P. E. RICHARDSON, AND R. L. BURTON[2]

Department of Botany and Microbiology, and

Oklahoma Agricultural Experiment Station,

Oklahoma State University, Stillwater,

Oklahoma 74078

ABSTRACT. Sections from leaves of susceptible and resistant wheat infested with biotype E greenbug (GBE), *Schizaphis graminum* (Rondani), were analyzed stereologically to detect quantitative changes in the chloroplasts and therefore extend our qualitative observations of morphological responses to GBE infestation. In the susceptible plants, a significant decrease in the volume fraction of chloroplasts (Vvchl) was observed 2 days postinfestation. By 4 days Vvchl was highly reduced. The reduction of Vvchl was parallel to the increase in the chloroplast volume fraction and numerical density of plastoglobuli, Vvp and Nvp, respectively. Significant increase in Vvp and Nvp were observed as early as 1 day postinfestation. In the resistant plants the Vvchl was not significantly different from the controls until 4 days postinfestation when it was significantly increased. The volume fraction of starch grains was markedly increased postinfestation, while Vvp and Nvp were not significantly changed. We conclude that these data are consistent with our prior interpretation of the ultrastructural changes which occurred in the chloroplasts and that the primary site of the greenbug feeding damage is the chloroplast.

MORPHOLOGICAL EVALUATION of diseased tissues and cells has been based on qualitative descriptions based on interpretations of light micrographs from sections prepared for electron microscopic study. Descriptive morphology is very important in plant pathology; however, in order to establish structure-function relationships, the qualitative studies should be complemented by quantitative analysis. This can be achieved by using stereological methods. Stereology is the study of the relationship between actual three-dimensional objects and the sections produced by microtoming the objects and producing biological sections for study. The sections appear as a series of thin profile views of cells and cell parts in two-dimensional planes interpretable by the methods of morphology.

The principles of stereology were developed and applied by the French geologist Delesse. He demonstrated that the volume fraction of a component i (Vvi), is equal to the area fraction (A_{Ai}) occupied by the component profiles in plane section through the object of study. Delesse's principle was extended in 1933 by Glagoleff (Weibel 1969). He demonstrated that Vvi could be very closely approximated by superimposing a test point grid over a micrograph of the sample section. The number of test points lying over the component in question (Pi) in relation to the total number of test points lying over the entire structure (P_T) is proportional to the volume occupied by that component in the solid. Therefore,

$$Vvi = \frac{P_i}{P_T}$$

where Vvi (volume fraction) = volume of the component i within the unit volume of a given reference of space.

P_i = points over the profiles of a given component i.

and P_T = total number of test points per test area.

In recent years stereological methods have been developed which allow efficient and reliable quantitative evaluation of cells, cell parts and organelle numbers, surface volumes,

etc. in sectioned tissue examined with the electron microscope. The analysis of morphological changes in the ultrastructure of damaged tissue can be extended quantitatively using stereological techniques (Weibel 1979; Steer 1981).

Gerloff and Ortman (1971) studied the physiological changes in susceptible barley leaves (*Hordeum vulgare* L.) caused by greenbug biotype A (GBA), *Schizaphis graminum* (Rondani) feeding stress. According to that study, chlorophyll content and the rate of photosynthesis declined 6 days postinfestation as compared to noninfested controls. No resistant plants were analyzed.

Infestation of a susceptible wheat, 'Sturdy', by greenbug biotype E (GBE) produced necrotic lesions surrounded by chlorotic halosand these symptoms were accompanied by a reduction in the rate of carbon assimilation and total chlorophyll content (Ryan et al. 1987). The ultrastructural study of 'Sturdy' wheat infested with GBE showed a severe disruption to the photosynthetic apparatus (unpublished data). Similar results are obtained when greenbug biotype C (GBC) fed on the susceptible wheat cultivar 'TAM-101' and the resistant cultivar 'TAM-101 x Amigo' (Al-Mousawi et al. 1983). Our main objective in this study was to quantify the ultrastructural changes of chloroplast components in mesophyll cells of GBE infested susceptible and resistant wheat plants since few stereological analyses of ultrastructural changes induced by insects or plant pathogens have been reported. To accomplish this, we measured the volume fraction of chloroplasts in cells, the volume fraction of starch grains and plastoglobuli in chloroplasts, and also estimated the numerical density of plastoglobuli per chloroplast volume.

MATERIALS AND METHODS

GBE was used to infest susceptible and resistant plants; the hard red winter wheat cultivar,'Sturdy' (susceptible to GBE), and the breeding line, 'Largo' (resistant to GBE). Wheat seeds were pregerminated, planted in small pots, and kept in a growth chamber at 30°C and LD 14:10. Three week-old plants were used in all experiments. Colonies of

GBE were reared on sorghum plants in insect cages in a greenhouse at approximately 23°C and LD 14:10.

The experiment was designed as a randomized complete block, with wheat genotype and sampling time as experimental factors. There were fifteen replications. Adult aphids were allowed to feed at a single feeding site for 1 h then removed and the feeding site marked. Tissue samples were taken at 1, 2, and 4 days postinfestation. The host plant tissue was fixed in 4% buffered gluteraldehyde for 2 h. Tissue samples were subsequently washed and postfixed with 2% osmium tetroxide for 4 h, dehydrated in a graded series of water-ethanol solutions and imbedded in the firm formulation epoxy resin of Spurr (Spurr 1969).

Three thin sections of thickness, 90-60 Å from each resin-embedded block (6-blocks for each sampling time), were cut with a diamond knife using an MT-2 ultramicrotome. Sections were collected on 200-mesh grids and stained with 5% uranyl acetate and 0.4% lead citrate and examined with a JOEL-100 CXII transmission electron microscope. A series of approximately 650 micrographs of damaged mesophyll cells and 150 micrographs of control mesophyll cells was recorded and printed with a final magnification of 42,000. Square double lattice grids of 456 major points and 1,824 minor points were used in the stereological analysis. The grid point spacing was determined by the formula: $a > d^2$ where a = area of the component in question, d = distance between points of grid spacing (Weible, 1979).

The volume fraction of chloroplasts (Vvchl) in a cell, the volume fraction of starch grains (Vvst) in a chloroplast and the volume fraction of plastoglobuli (Vvp) in a chloroplast were estimated using the point-counting procedure (Weibel, 1979). The numerical density of plastoglobuli (Nvp) per chloroplast volume was estimated according to Weibel and Gomez (1962). The stereological data were subjected to an analysis of variance with multiple comparisons and separation of means by least significant difference test for determination of differences at the 5%, and 1% level of significance. The analysis

of variance was conducted with the SAS general linear models procedure (SAS Institute 1982, 139-199).

RESULTS

Vvchl of uninfested tissue (controls) from either the susceptible or the resistant plants had no volume changes throughout the study when examined at 1 and 4 days (Fig. 1). By 1 day postinfestation only susceptible plants had a slight but not statistically significant, decreases in the volume fraction of the chloroplasts in the cell (Vvchl) when compared to the control (Fig. 1). At 2 days postinfestation, a significant decrease in Vvchl of the susceptible plants was observed, while the chloroplasts of the resistant plants showed no volume change. when they were compared to the controls from the 1 day postinfestation. Further significant reduction in Vvchl of infested susceptible plants was observed 4 days postinfestation. On the other hand Vvchl of infested resistant plants showed a slight but significant volume increase at 4 days (Fig. 1).

There was a significant increase in the volume fraction of starch grains in chloroplasts (Vvst) of infested resistant plants at one day postinfestation (Fig. 2). By 2 and 4 days postinfestation the Vvst greatly increased. GBE feeding damage in the susceptible plants had no statistically significant effect on the Vvst.

GBE infestation had statistically significant effects on the volume fraction of plastoglobuli in chloroplast (Vvp) and the numerical density (Nvp) of the plastoglobuli per chloroplast volume in the susceptible plants. By 1 and 2 days postinfestation the Vvp was significantly increased when compared to the controls (Fig. 3). At 4 days postinfestation, a large portion of the chloroplasts was occupied by the plastoglobuli. Compared with resistant plants, no significant change in the Vvp was observed in the infested resistant plants. In infested susceptible plants not only the Vvp but also the Nvp increased (1 day postinfestation) (Fig. 4). The Nvp was slightly but significantly greater at 1 day postinfestation. Large numbers of plastoglobuli were observed 2 and 4 days later. There

was no statistically significant difference between the Vvp or the Nvp in the infested and uninfested resistant plants (Figs. 3 and 4).

DISCUSSION

Although the result of this study did not clearly indicate the mechanism of GBE feeding damage in susceptible plants, it is clear that changes in Vvchl, Vvst, Vvp, and the Nvp were different between the resistant and susceptible plants. The results indicate that the Vvchl of the infested susceptible plants was similar to the controls until 2 days postinfestation (Fig. 1). This volume then significantly decreased. This point coincides with the ultrastructural finding that chloroplast membrane degradation occurs 2 days postinfestation (unpublished data). The reduction of Vvchl could probably result from the break-down of granal and stromal lamellae. This is in support of the findings that the rate of photosynthesis and chlorophyll content are decreased after greenbug feeding on susceptible plants (Gerloff and Ortman 1971; Ryan et al. 1987). The fact that Vvchl of the resistant plants was not significantly different from the controls until 4 days postinfestation is in agreement with our fine structural observations that the chloroplasts were relatively unaffected. The slight increase in the Vvchl of the resistant plants at 4 days postinfestation may be a result of the accumulation of starch grains (Fig. 2).

The chloroplasts of the resistant plants responded differently to GBE infestation. Significant starch accumulation was observed at 1, 2, and 4 days postinfestation. Starch accumulation is observed in other systems, such as susceptible cucumber cotyledon plastids germinated in tentoxin (Halloin et al. 1970) and in host/pathogen interactions (Lallyett 1977; Appiano et al. 1977). However, in those studies starch accumulation was only observed in the susceptible plants, whereas, in this study starch accumulation occurred only in the infested resistant plants and this may indicate that utilization or transportation of photosynthate, rather than production of photosynthate is the process most sensitive to greenbug feeding damage. Two mechanisms may be responsible for

starch accumulation. First, the reduction of triose phosphate transportation out of the chloroplasts as a result of inorganic phosphate deficiency may occur. Second, sucrose transportation out of the photosynthetic cells may be reduced. The mechanism by which the greenbug affects starch accumulation is unknown.

Vvp and Nvp were significantly increased only in the infested plants. The increased increment was parallel to the reduction increment of the Vvchl in the infested susceptible (Fig. 1). The amount of lipoquinone was parallel to the size of the plastoglobuli in spinach leaves and the size and the frequency of the plastoglobuli were higher in old leaves than in young leaves (Lichtenthaler 1969). Our results indicated that the Vvp and Nvp may be increased as a result of accumulation of released lipoquinones as a result of disruption of chloroplast thylakoids before comprehensive chloroplasts degradation take place.

In summary, the reduction of Vvchl of infested susceptible plants is parallel with the increases of Vvp and Nvp. In the resistant plants only the Vvs is greatly increased. We conclude that the results obtained from the stereological analysis of chloroplasts of infested susceptible and resistant plants were consistent with our ultrastructural observations (unpublished data).

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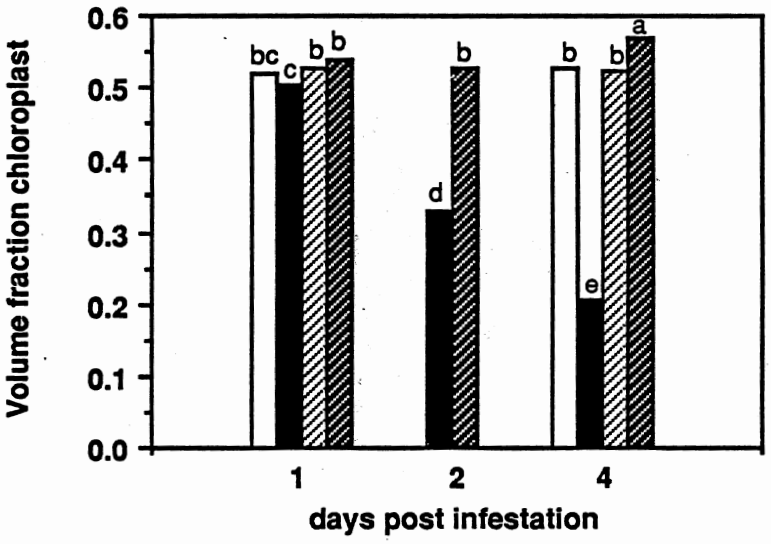
Footnotes:

[2]ARS-USDA, Plant Science and Water Conservation Lab., P.O. Box 1029, Stillwater, OK 74076.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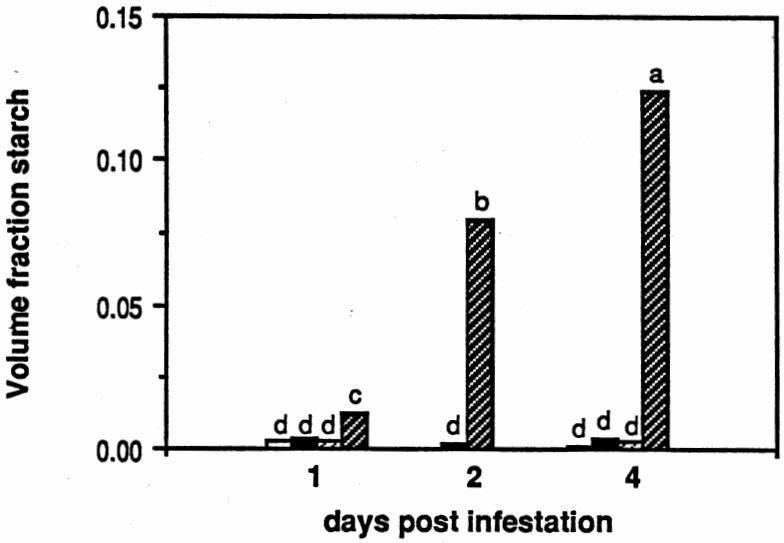
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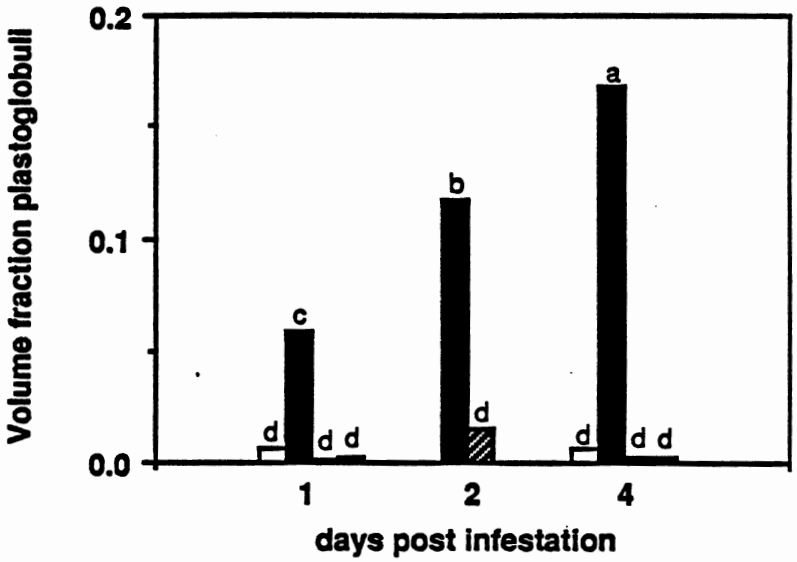
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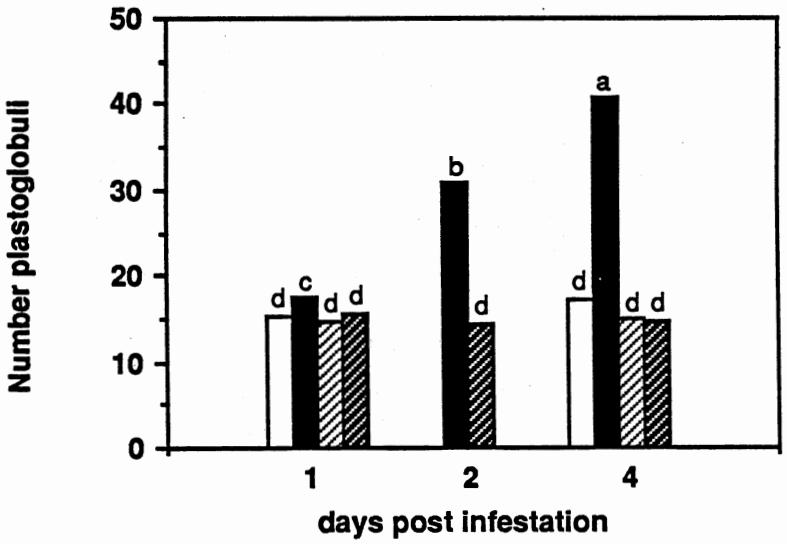
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Figs. 1. The volume fraction of chloroplasts in the cell (Vvchl) of susceptible and resistant wheat infested plants with GBE. All data were subjected to analysis of variance and separation of means by Least Significant Difference (LSD) test at the 5% level of significance. Mean within bar followed by the same letter is not significantly different. Susceptible uninfested (□), susceptible infested (■), resistant uninfested (▨), and resistant infested (▩).

Fig. 2. The volume fraction of starch grains in chloroplast (Vvst) of susceptible and resistant wheat infested with GRE. All data were subjected to analysis of variance and separation of means by LSD test at the 1% level of significance. Mean within bars followed by the same letter are not significantly different. Susceptible uninfested (□), susceptible infested (■), resistant uninfested (▨), and resistant infested (▩).

Fig. 3. The volume fraction of plastoglobuli in chloroplast (Vvp) of susceptible and resistant wheat infested with GBE. All data were subjected to analysis of variance and separation of means by LSD test at the 1% level of significance. Mean within bar followed by the same letter is not significantly different. Susceptible uninfested (□), susceptible infested (■), resistant uninfested (▨), and resistant infested (▩).

Fig. 4. The numerical density of plastoglobuli per chloroplast volume (Nvp) of susceptible and resistant wheat infested with GBE. All data were subjected to analysis of variance and separation of means by LSD test at the 5% level of significance. Means within bars followed by the same letter is not significantly different. Each value given is the mean ± SE of the mean. Susceptible uninfested (□), susceptible infested (■), resistant uninfested (▨), and resistant infested (▩).

VITA

Alia Taher Morgham

Candidate for the Degree of

Doctor of Philosophy

Thesis: INTERACTION BETWEEN GREENBUG (HOMOPTERA: APHIDIDAE) AND SUSCEPTIBLE AND RESISTANT WHEAT PLANTS: FEEDING BEHAVIOR, QUALITATIVE ULTRASTRUCTURE, QUANTITATIVE ULTRASTRUCTURE

Major Field: Botany

Biographical:

Personal Data: Born in Tripoli, Libya, January 21, 1956, the daughter of T. E. Morgham and K. M. Sasi.

Education: Graduated from Tripoli High School, Tripoli, Libya June 1973; attended University of El-Fateh, Tripoli, Libya; received the Bachelor of Science Degree from University of El-Fateh in 1977; attended Oklahoma State University, Stillwater, Oklahoma; received the Master of Science degree from Oklahoma State University in 1983; completed requirements for the Doctor of Philosophy degree at Oklahoma State University in December, 1987.

Professional Experience: Teaching Assistant, Department of Botany, College of Science, University of El-Fateh, 1978-1979; teaching assistant, Department of Botany, Oklahoma State University, 1985-1987.

Professional Organizations: Member of the Honor Society of Phi Kappa Phi.