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Mechanics of Feeding Of the Greenbug (*Toxoptera graminum* Rond.) On *Hordeum*, *Avena*, and *Triticum*

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OKLAHOMA AGRICULTURAL EXPERIMENT STATION

in cooperation with

UNITED STATES DEPARTMENT OF AGRICULTURE

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Mechanics of Feeding of the Greenbug (Toxoptera graminum Rond.) On Hordeum, Avena, and Triticum

By R. M. CHATTERS AND A. M. SCHLEHUBER[1]

There has been much interest in the feeding habits of the greenbug (*Toxoptera graminum* Rond.) as these habits relate to resistance and susceptibility on the part of the host plants. This bulletin deals with an attempt to apply to a common problem the methods of cytology and agronomy along with the extensive greenhouse and field experience on the greenbug of R. G. Dahms.[2]

On the basis of cytological, entomological, and agronomic observations, the implications are that resistance and susceptibility are expressions of physiological differences.

MATERIALS AND METHODS

In this study, 11 varieties of wheat, seven of barley, and one of oats were selected for observation of the several characteristics described herein.

The wheat varieties were Comanche (C.I.[3] 11673), Early Blackhull (C.I. 8856), Pawnee (C.I. 11669), Red Chief (C.I. 12109), Wichita (C.I. 11952), Tenmarq (C.I. 6936), Triumph (C.I. 12132), Kharkof (C.I. 1442), Clarkan (C.I. 8858), Quanah (C.I. 12145), and a local selection of *Triticum* sp. X *Agropyron elongatum* Stillwater Selection 504364 grown during the 1950 season at Stillwater. Barley strains were Ward (C.I. 6007), Omugo (C.I. 5144), Tenkow (C.I. 646), Harbine (C.I. 7524), Dobaku (C.I. 5238), C.I. 4202-2, and C.I. 7530. The oat used was Wintok (C.I. 3424).

For the study on stem characters, sections of stem were taken from field-grown plants. In each case, sections were removed from the same position on the stem, this point being 3 cm. above the first node below the spike.

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[1] Respectively: Associate Cytologist, Oklahoma Agricultural Experiment Station; and Senior Agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, USDA, and Agronomist, Oklahoma Agricultural Experiment Station. Grateful acknowledgement is expressed to Paul Holloway, Laboratory Technician, and Oran Steffey, Research Associate, of the Botany and Plant Pathology Department for their part in the preparation of slides and photographs.

[2] Entomologist, Oklahoma Agricultural Experiment Station and Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.

[3] C.I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

Seedlings were grown in the laboratory and in the greenhouse for use in preparation of all leaf sections whether with or without greenbugs feeding.

Stem sections were killed in F.A.A. solution wherever slides were prepared for a study of mechanical tissue. Leaves were killed and fixed in F.A.A., Nawaschin's and Carnoy's solutions. It was found to be particularly advantageous to use Carnoy's solution for killing and fixing leaves bearing feeding greenbugs as this agent killed the insects so quickly that they did not have an opportunity to withdraw their stylets from the tissues. This permitted observation of the leaf and the feeding insect on the same microscope slide preparation. All tissues were processed by the butyl-ethyl alcohol method and imbedded in a mixture of paraffin to which was added varying amounts of beeswax, depending upon the amounts of mechanical tissue present in the specimen. Several staining methods were employed including the ferric chloride-tannic acid and the safranin O-fast green F.C.F. methods. The latter proved to be most satisfactory and was finally used to the exclusion of all others.

Photomicrographs were prepared, using a Leitz Micam camera with Kodalith Orthochromatic Type 2 film and a Leitz 35 mm. camera with Microfile and Kodachrome film.

RELATIONSHIP OF MECHANICAL TISSUE TO RESISTANCE

Detailed studies on the microstructure of the leaves and young stems of 11 varieties of wheat and seven varieties of barley indicated no direct correlation between the amounts of mechanical tissue and the susceptibility or resistance to the greenbug. On the contrary, the stem of one variety of barley, C.I. 4202-2, showed in cross section a considerable amount of very delicate parenchymatous tissue underlying the epidermis (Figure 1). This strain of barley was considered to be highly resistant as compared with Ward (Figure 2) which had more mechanical tissue and was classed as susceptible. Presence or absence of mechanical tissue then appears not to account for the differences in susceptibility to the greenbug in the genera studied.

THICKNESS OF LEAVES IN RELATION TO SUSCEPTIBILITY

A preliminary study on seven varieties of barley has shown that the resistant varieties are characterized by having thicker leaves than the susceptible varieties. At the mid-vein position, the leaves of the three susceptible varieties studied ranged in average thickness from 182 in Harbine to 195 microns in Ward and Tenkow, and of the four resistant varieties from 260 for Dobaku and C.I. 7530 to 312 microns for Omugi. It was also observed that the thickness of the leaves between the mid-vein and the first lateral vein paralleling it measured 104 microns in each of three susceptible varieties and from 146 to 156 microns in the four resistant varieties.

Studies on this phase of the investigation have not been extended to wheat and oats. In order to evaluate the findings on the barley properly

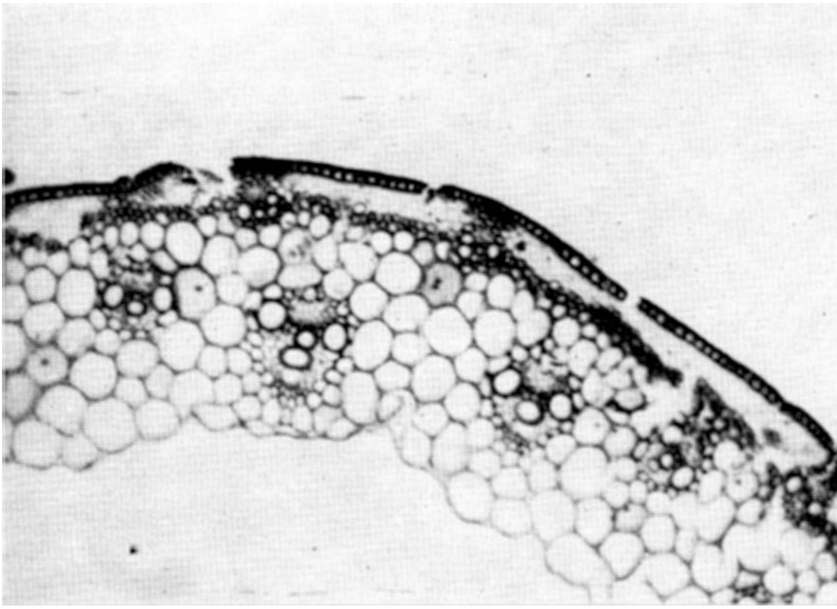

Figure 1.—Transverse section of stem of a greenbug-resistant Hordeum **(C.I. 4202-2) showing areas of thin-walled parenchyma cells underlying the epidermis.**

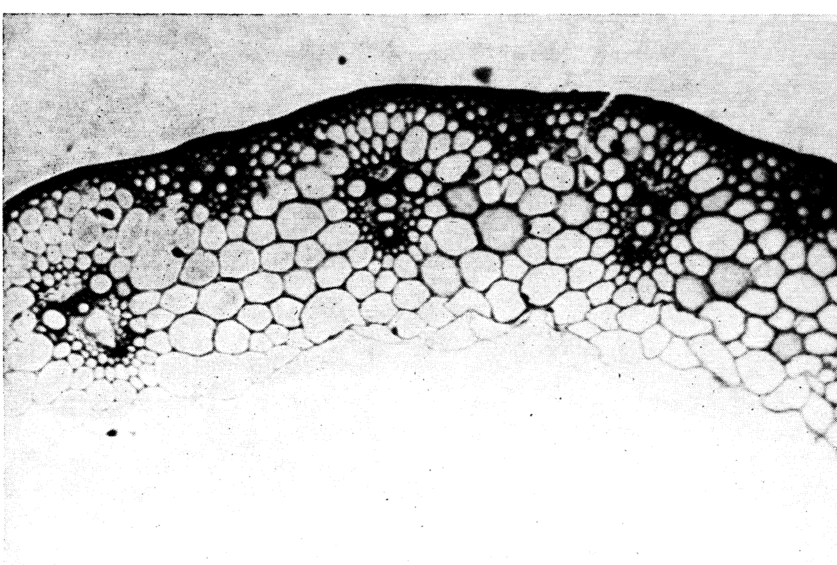

Figure 2.—Transverse section of stem of a susceptible Hordeum **(Ward C.I. 6007) showing thick layer of mechanical tissue underlying the epidermis.**

as they pertain to susceptibility or resistance, it would be necessary to acquire some information on the stylet length of the greenbug. Thickness of leaves and length of stylets may possibly bear some relationship to resistance and susceptibility. At present, however, we have no basis for such a claim.

STOMATAL NUMBERS AND RESISTANCE

Another point of interest regarding feeding habits of the greenbug pertains to susceptibility and the number of stomata present on the surface of the leaves of the various cereals. The leaves of these seven varieties of barley were studied in detail to determine what relationship existed between stomatal numbers and invasion by the greenbug. No correlation was found since C.I. 7530, the most resistant of the varieties examined showed an unusually high number of stomata. The average numbers ranged from 64 to 100 stomata per unit of surface area studied in the resistant varieties, and from 75 to 83 in some of the susceptible varieties. Later studies on the piercing habits of the greenbug confirmed these findings and further illustrated the point that entrance of the stylets through stomata in barley was very rare and perhaps purely fortuitous.

MODE OF PENETRATION OF STYLETS

To obtain information as to the mode of penetration of greenbug stylets, a series of slides was prepared showing the entrance into the leaf tissues of these mouth parts on both resistant and susceptible varieties. Invariably, the stylets were introduced intercellularly and continued their passage into the tissues in the same manner. Figures 3 to 6 illustrate the nature of this stylet insertion.

Upon insertion of the stylets, the greenbugs, like other aphids (1, 3, 4)[4], appear to inject considerable amounts of saliva into the host tissue with striking resultant effects. The alterations within the plant tissues vary considerably in degree and kind, depending upon the genus of the cereal affected. In the three cereals investigated, stylet sheaths are formed which aid in tracing the path which is taken by the stylets in the tissues. Davidson (1) has suggested that entrance is made into the tissue by the intercellular route because the insect's stylet requires something to keep it rigid after leaving the labium where it is held quite firmly in place by the muscles in this organ. Other possible explanations are that the pectin substances of the middle lamella may break down more easily than the cellulosic or ligno-cellulosic cell walls, or that by passing intercellularly and secreting saliva, which is highly enzymatic, the insect is able to feed upon two or more cells at once. Whatever the reasons, movement of the stylets through the tissues is invariably intercellular.

[4] Figures in parentheses refer to Literature Cited, page 18.

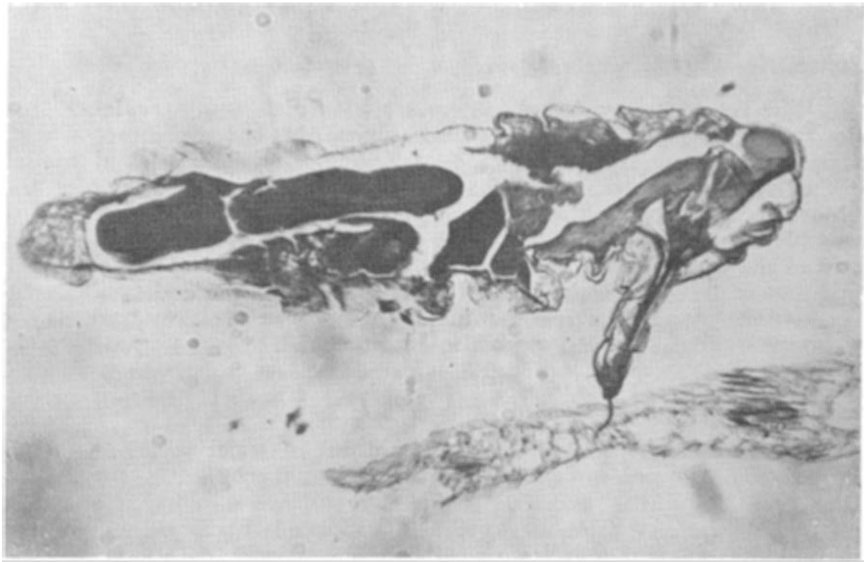

Figure 3.—Mid-saggital section of greenbug showing entrance of stylet into leaf of Hordeum.

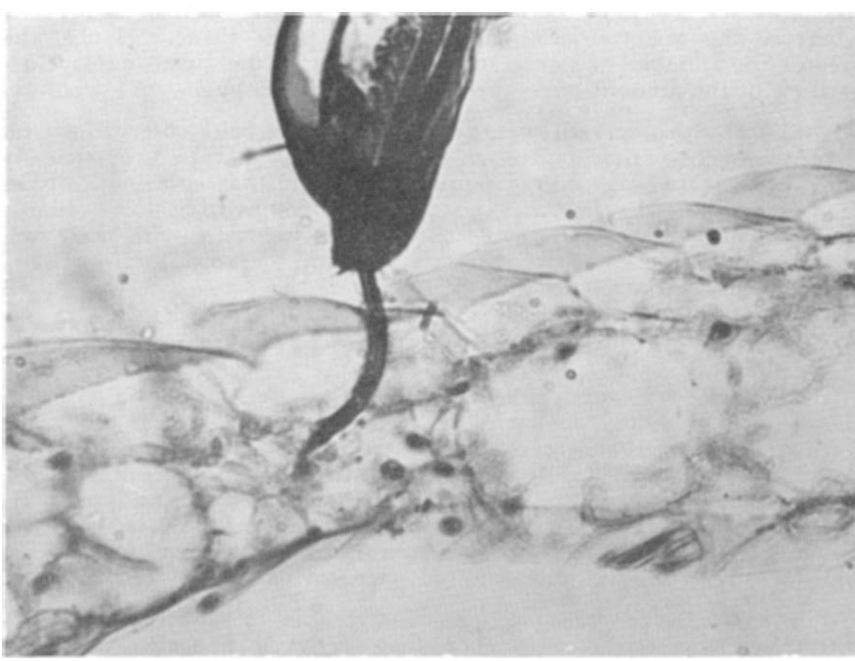

Figure 4.—Portion of Figure 3 magnified to show detail of intercellular penetration of stylet in Hordeum **leaf.**

Stylets Are Highly Selective Organs

The results of our many observations furnish ample evidence that the stylets do not enter the tissues haphazardly, but are directed with marked precision (Figures 4 to 8). Note particularly how in Figure 8 the stylet turns back after having, seemingly, been thrust past the phloem. This investigation has not progressed far enough to determine whether the sensitivity of the stylet is due to pH or carbohydrate content of the desired cells, whether it is due to nerve control by the greenbug or whether the sensitivity is due to other undetermined factors. Investigations along this line should prove to be of great interest to the plant physiologist as well as to the entomologist.

Stylet Sheaths as Indicators of Damage

The presence, number, and distribution of stylet sheaths serve as indicators of the amount of feeding which has taken place in the tissues. Davidson (1), in his report on *Aphis rumicus,* has stated that one does not see all the stylet sheaths which are produced in the tissues, or that many of the sheaths may stain so lightly as to be practically indiscernible. However, when observable, they do give some clue as to the degree of damage done to the tissues. It is worthy of note that mere insertion of stylets, production of sheaths, and breakdown of tissues does not presuppose that the greenbug has withdrawn food substances from the tissues involved. Of practical significance is the fact that damage results wherever the insect injects saliva into the plant tissue. Hence, the greater the number of penetrations, the greater the tissue damage regardless of the amount of food uptake by the greenbug.

The above observation further substantiates field observations on the several cereals that damage to tissues tends to increase geometrically with an increase in greenbug population rather than arithmetically as might be expected. In other words, as the competition for available food and space increases, the number of stylet insertions with their concomitant secretion of saliva increases very considerably. Serious damage or death to the plants follows.

PHLOEM AS THE PRINCIPAL FEEDING SITE

The phloem tissue appears to be the ultimate goal of the stylets in these cereals, although in barley and wheat it would appear that some feeding occurs in the parenchymatous tissues as well (Figures 5 to 8). According to Davidson (1), preference for the phloem by *Aphis rumicus* lies in its high carbohydrate content; whereas Fife and Frampton (2), reporting on the feeding behavior of the beet leaf hopper (*Eutettix tenellus*) on sugar beets, claim that the phloem is preferred because the phloem is basic while the less-desired parenchyma is acidic.

In carefully prepared sections, one is able to observe that saliva introduced into the phloem moves along in the phloem elements, altering the cells and their contents as it does so. Further, it has been observed that there is also a lateral diffusion of the saliva from the phloem, resulting in an alteration of the cell contents through plasmolysis or lysis (Figure 7). In the phloem, the cell walls and cell contents undergo such changes that polychrome effects are observed after staining with safranin O and fast green F.C.F. Such changes in staining reaction indicate a chemical change in cell constitution.

EFFECT OF INSECT SALIVA ON PARENCHYMA

When saliva from the greenbug is introduced into the parenchyma tissues, the first visible effect is one of protoplasmic modification and destruction. Large and small globules (Figure 9) supplant the homogeneous protoplasm. Also, oyster-shell-like bodies (Figure 9) form in the

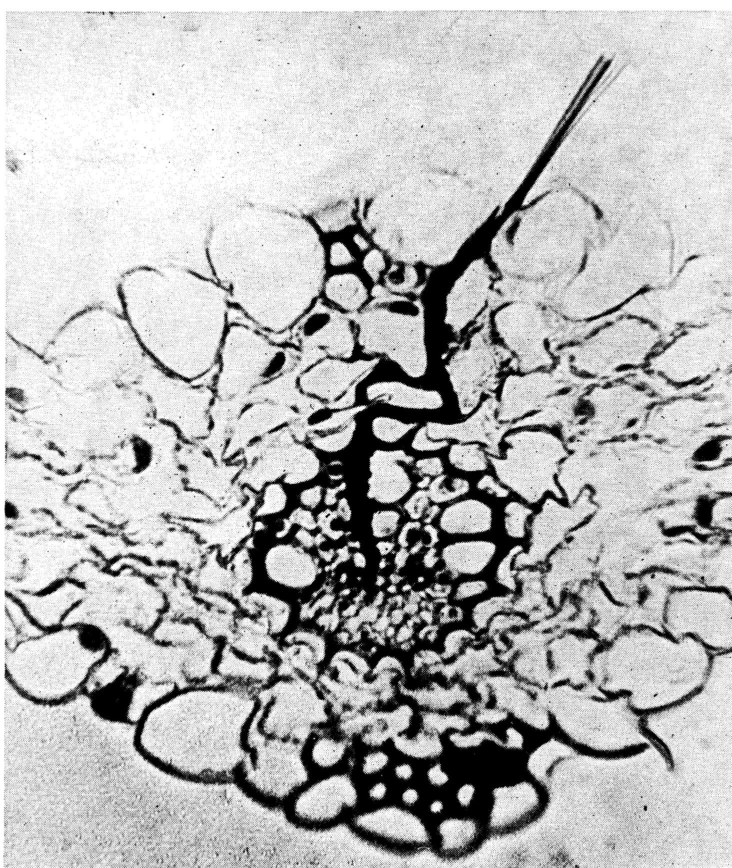

Figure 5.—Mode of penetration of stylet in Triticum **leaf.**

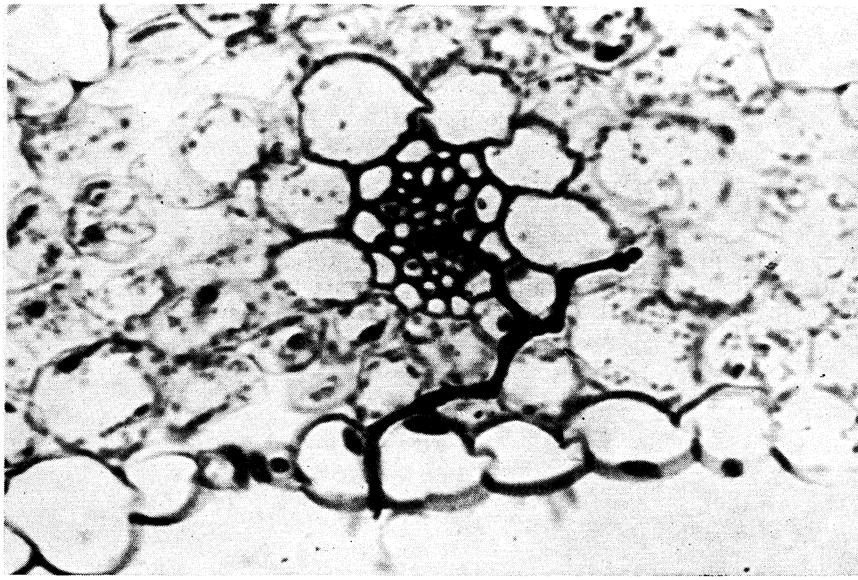

Figure 6.—Mode of penetration and path of stylet in leaf section of Hordeum.

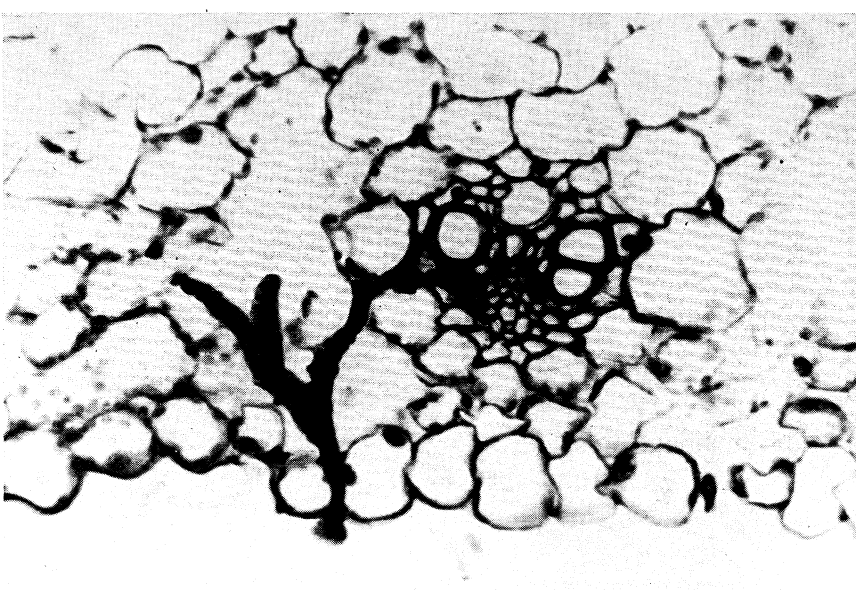

Figure 7.—Mode of penetration and path of stylet in Hordeum **leaf. Note cavity in parenchyma of mesophyll layer.**

cells as they undergo their several changes preceding necrosis. Modification of cell walls and/or formation of cavities usually results (Figures 7, 10, 11).

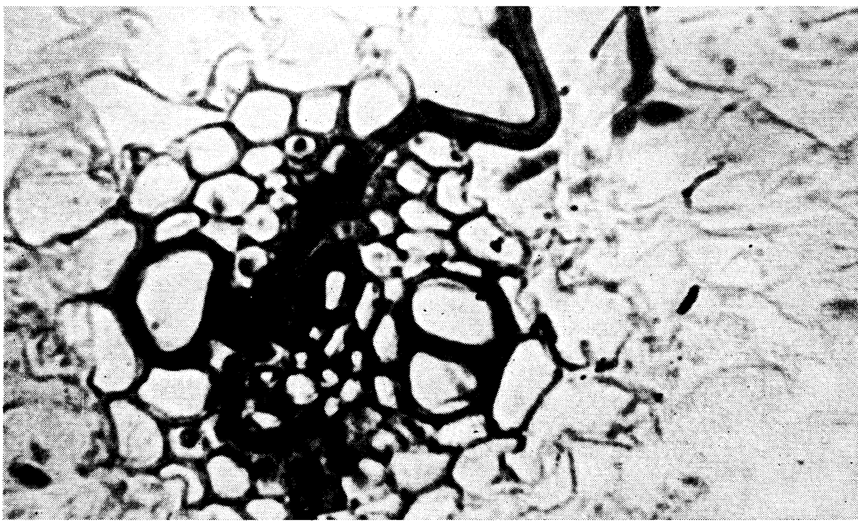

Figure 8.—Stylet penetrating tissue of Hordeum **leaf. Stylet has turned back into phloem tissue at an acute angle.**

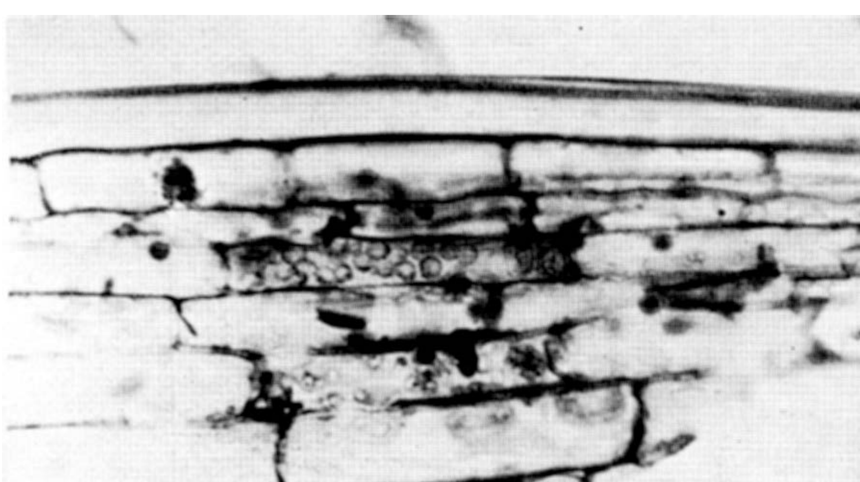

Figure 9.—Plasmolysis of cells in Hordeum **caused by the greenbug.**

RESPONSE OF DIFFERENT CEREALS TO GREENBUGS

Sections of leaves were studied in detail in order to determine whether the effect of the greenbug was the same on barley, oats, and wheat. It was seen that each of these cereals responds differently trom the others following a feeding invasion by the greenbug.

Effect upon *Hordeum*

Greenbugs feeding on barley cause a considerable breakdown in the parenchyma tissue lying between the upper and lower epidermis. It would appear that the greenbug injects the saliva into the mesophyll layer. From the point of stylet insertion, the saliva diffuses transversely. The saliva produces plasmolysis of the cell contents followed by breakdown of the cell walls in such a manner as to produce a cavern-like break between the upper and lower epidermis (Figures 9 and 10). Apparently, this cavitation is a result of chemical lysis brought about by potent enzymes in the insect's saliva. Whether the insect feeds upon these breakdown products or whether in passing the stylet into the phloem these cells become damaged by chance is a moot question.

It has also been observed in *Hordeum* that occasionally the cavities produced in the parenchyma tissue are lined with a substance which stains bright red with safranin O. This substance is of a brittle nature,

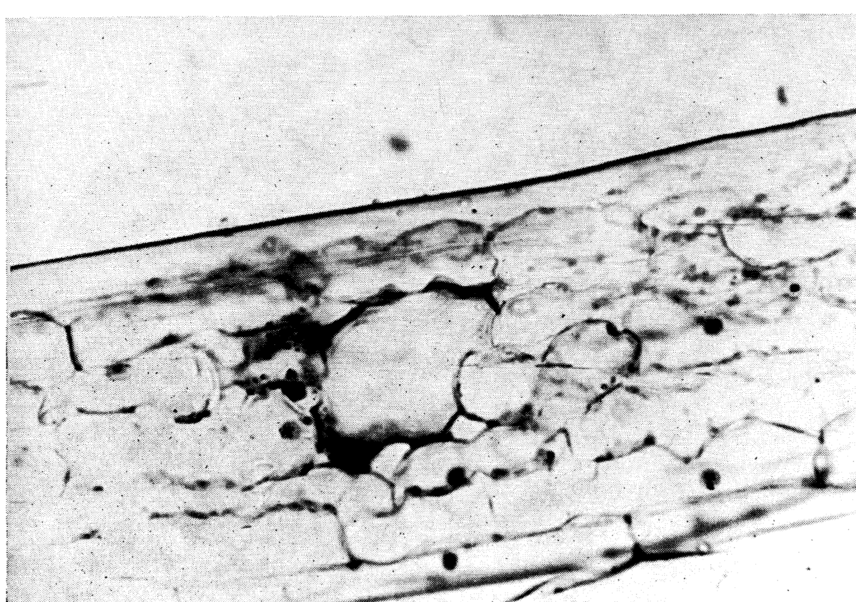

Figure 10.—Plasmolysis and cavitation of tissue in Hordeum **leaf caused by the greenbug.**

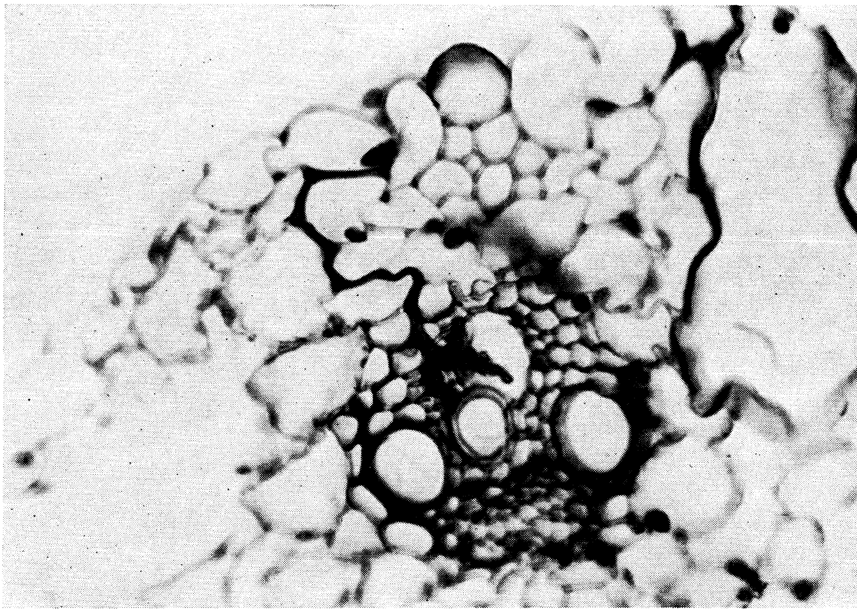

Figure 11.—Stylet path and extensive lysis in Hordeum **leaf. One branch of stylet sheath has entered protoxylem lacuna.**

either naturally or due to the action of the reagents employed in processing the tissues. During the sectioning and staining process the above described lining often is chipped out of the cavity and will be found lying in the mounting medium.

When the saliva enters the phloem tissue, it passes along in the phloem elements and diffuses laterally (Figure 8), resulting in plasmolysis and ultimate destruction of either mesophyllic parenchyma or phloem. The phloem appears to be more resistant to the salivary enzyme than is the parenchyma of the adjacent areas.

Sieve tubes appear to be plugged with substance which stains bluish-green when the safranin-fast green technique is employed.

Feeding by the greenbugs upon the mid-vein was not observed on barley.

Effect upon *Avena*

The observations on barley are in sharp contrast to those on oats. Here with the very same insect, inserting its stylet in the same manner and injecting the same kind of saliva, strikingly different effects upon the tissues are produced.

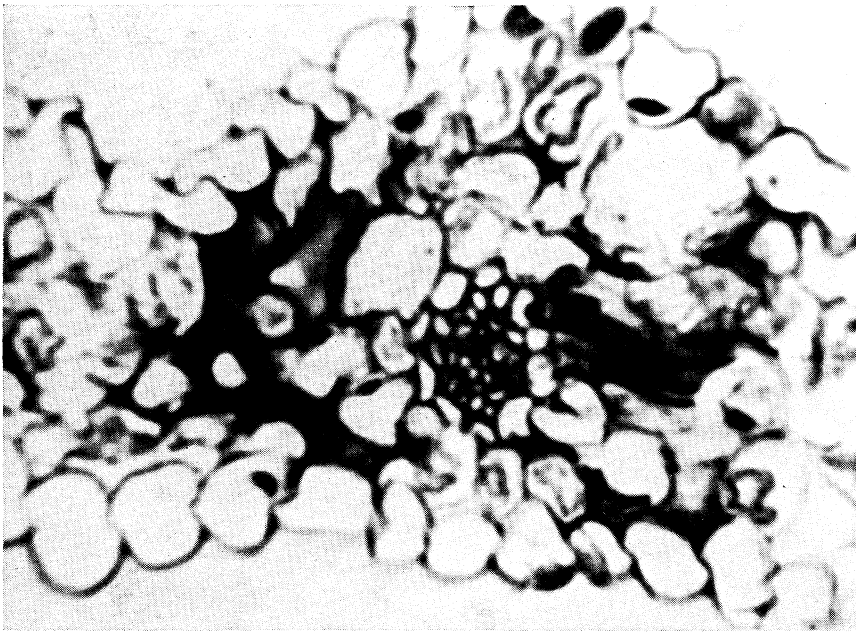

Figure 12.—Cell wall change produced by the greenbug in leaf of *Avena.*

The usual diffusion of saliva is observed as seen in barley, but the end result is different. Instead of a general breakdown of the cell walls and a collapse of the epidermal layers, the walls of the epidermal and mesophyll cells appear to remain intact but to undergo marked changes in chemical make-up (Figure 12). These walls, which previously had taken up fast green F.C.F. in the manner characteristic for this stain, now become highly stained with safranin O when treated with this dye. The response to this brilliant red dye would suggest that the walls were now possibly composed of, or impregnated with, lignins, tannins, or suberin-like substances resulting in the appearance of rusty-looking areas on the surfaces of the affected leaves.

In contrast with the cellular effects on *Hordeum,* it seems that the cell contents are not plasmolyzed for some time after the alterations in the cell wall make-up described above have begun to appear. As a result, the saliva may diffuse across a considerable number of cells before the cells surrounding the stylets or stylet sheaths die.

Invasion of the mid-vein was rather frequent in *Avena,* whereas it was not observed in any *Hordeum* specimens.

Entrance of the stylets close to or through the stomatal apparatus was observed more frequently in *Avena* than in *Hordeum.*

Effect upon *Triticum*

Triticum proved to be particularly interesting in that the damage done by the greenbug was the result of a combination of the changes observed in *Hordeum* and *Avena* (Figure 13).

The cell walls were altered by the saliva as seen in *Avena* but the cell contents were plasmolyzed soon after penetration as in *Hordeum.* Lysis was also seen to take place just about as it did in barley. This change was followed by the lining of the cavity by a reddish substance similar to that described above for *Hordeum* and *Avena.* In some cases, entire cavities were filled by a pigmented mass.

The ultimate terminal point for the stylets was the phloem, where the entrance of saliva tended to plug up the sieve tubes.

Stomata penetration and mid-vein invasion were observed but were not common occurrences.

SUMMARY

1. Observations on barley, wheat, and oats indicate that there is no relationship between amounts of mechanical tissue in leaves and stems and resistance to the greenbug.

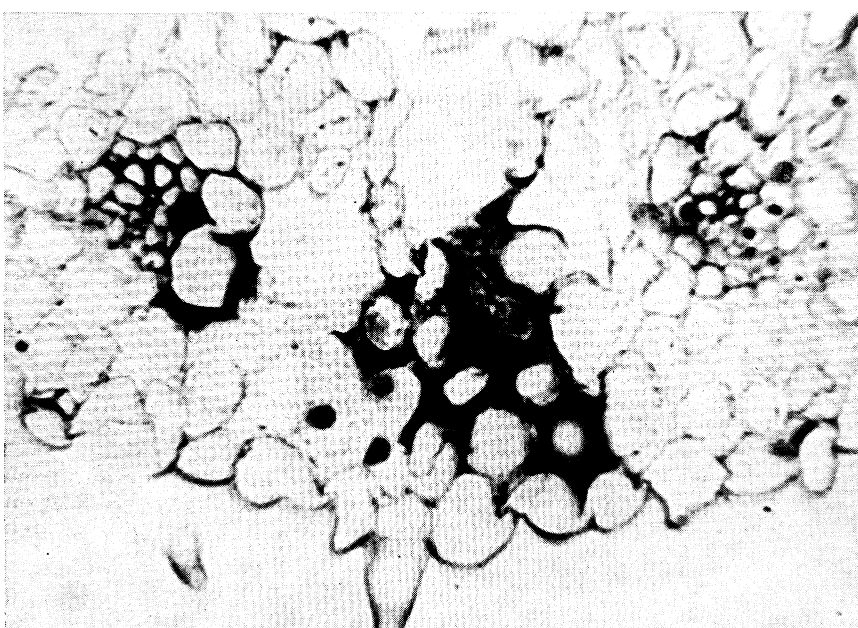

Figure 13.—Triticum **leaf cross section showing nature of cell and cell wall damage produced by the greenbug.**

2. Stomatal numbers are not related to resistance or susceptibility in barley. This character was not studied in oats and wheat.

3. A preliminary study on seven varieties of barley showed greater thickness of leaves in resistant than in susceptible varieties.

4. Stylets tend to enter tissues intercellularly and less frequently through the stomatal apparatus.

5. Stylets are highly sensitive organs which do not enter tissue haphazardly but appear to be directed toward a specific tissue.

6. Numbers of stylet sheaths serve as indicators of amount of damage done to tissues.

7. Increase in number of greenbugs on a plant or leaf results in damage which tends to increase geometrically rather than arithmetically, due to competition for available vascular tissue.

8. Injection of saliva and not the uptake of food appears to be the primary cause of tissue damage.

9. The phloem appears to be the ultimate feeding site for the greenbug.

10. Greenbug damage varies in effect in *Hordeum, Avena* and *Triticum* from lysis to cell wall modification to a combination of lysis and cell wall modification in the three genera respectively.

11. If structural differences account for any resistance it would be related to the leaf thickness and length of the extended stylet. There is, however, insufficient evidence to substantiate such an hypothesis from the work reported in this paper.

12. Implications from these studies are that resistance to the greenbug is physiological.

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