PROBING BEHAVIOR AND PLANT DAMAGE OF TWO BIOTYPES OF AN APHID IN A SUSCEPTIBLE AND A RESISTANT BARLEY

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AND A RESISTANT BARLEY

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INTRODUCTION

This investigation is composed of two 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) is entitled "Histology of the penetration site of two Biotypes of an Aphid (Homoptera: Aphididae) in a Susceptible and a Resistant Barley," and describes probing behavior of greenbug biotypes C and E on a susceptible and resistant barley. The second manuscript (Part II) is entitled "Levels of Silicon and Phenolic Compounds Surrounding the Penetration Site of two Biotypes of an Aphid (Homoptera: Aphididae) in a Susceptible and a Resistant Barley," and showed that the levels of the silicon and phenolic compounds changed in damaged leaf tissue after aphid feeding.

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 subject to the Graduate College's approval of the major professor's request for a waiver to the standard format.

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Histology of the penetration site of two Biotypes of an Aphid (Homoptera: Aphididae) in a Susceptible and a Resistant Barley

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ABSTRACT

The probing behavior of two biotypes (C and E) of the greenbug *Schizaphis graminum* (Rondani) on a susceptible ('Rogers') and on a resistant ('Will') cultivar were compared. Biotype C predominantly entered the host plant tissue intracellularly through the epidermis regardless of cultivar while biotype E entered predominantly intercellularly. The path of both biotypes in both cultivars in the mesophyll tissue was predominantly intercellular. Biotype C showed a higher percentage of deep penetrations as well as a higher percentage of tracks oriented toward a vascular bundle. Aphids feeding on Rogers showed more branches per penetration than aphids feeding on Will. The majority of the stylet sheaths in all four treatment groups ended in the mesophyll parenchyma.

INTRODUCTION

The greenbug, Schizaphus graminum (Rondani) is a serious pest of cereal crops in the great plains and is responsible for the loss of millions of dollars each year (Starks and Burton 1977). This aphid causes damage by inserting its stylets into and feeding on plant tissue. The aphid may also inject a toxin into the plant, (Starks and Burton 1977). Heteroptera are known to produce two types of salivary secretions: the stylet sheath material and a watery saliva (Miles 1958). The stylet sheath material gels rapidly to form a 'track' lining the path of the stylets during the penetration of plant tissue. Sheath material of the milkweed bug, Oncopeltus fasciatus (Dall), is composed of a lipoprotein, rich in tyrosine, with traces of tryptophan (Miles 1960). The watery saliva which is exuded independently of the sheath material "originates as an alkaline secretion including phenolases produced in the accessory salivary glands. This saliva also contains hydrolytic enzymes from the posterior main gland and free amino acids from both glands" (Miles 1969). Pectinases and cellulases may aid in stylet penetration, however, content of the saliva may vary greatly from one species to another (McAllen and Adams 1961).

McAllan and Adams (1961) studied 19 aphid species and proposed four classes of penetration based on the presence or absence of pectin-hydrolysing enzymes in the saliva as follows:

(1) If pectinases are absent, mechanical penetration will produce an intracellular route.

(2) If pectinases are present, they may not necessarily be employed so that the stylet path is still intracellular.

(3) When the insect partially employed the enzyme, the stylet path will show intercellular and intracellular penetrations in the same probe.

(4) When or if stylet penetration relies almost entirely on pectinases hydrolysing the middle lamella and not on mechanical pressure so that the route is intercellular.

The feeding behavior of the greenbug was first studied by Chatters and Schlehuber (1951) on 11 cultivars of wheat, seven barleys, an oat. They compared the relationship of four physical characteristics to resistance. The four characteristics were leaf thickness, amount of mechanical tissue, stomata number per leaf unit area, and mode of stylet penetration. According to their conclusions the amount of mechanical tissue in the leaf and stem showed no correlation to greenbug resistance but resistant cultivars had thicker leaves than susceptible. There was no relationship between numbers of stomata per unit area and resistance. Injection of saliva resulted in damage regardless of whether feeding occurred and damage severity depended partly upon the genus of cereal affected. The path of the stylet through the tissue was intercellular. Large and small globules and oyster-shell

like bodies were formed in the protoplasm of affected parenchyma cells and modification of the cell wall and/or formation of cavities usually resulted.

Chatters and Schlehuber (1951) also found that greenbugs caused considerable parenchyma tissue breakdown in barley. Saliva diffused transversely and produced cell content plasmolysis followed by cell wall breakdown with cavern-like breaks between upper and lower epidermis. Occasionally cavities produced in parenchyma were lined with a substance staining bright red with Safranin O. This substance was brittle and often chipped out of the cavity, and during preparation it was displaced in the mounting medium. Saliva passed through phloem elements and diffused laterally causing plasmolysis and eventual destruction of mesophyll parenchyma and phloem. Sieve tubes were plugged with a blue-green substance when stained with safranin and fast green.

Feeding in oats appeared to be intercellular but tissue effects are slightly different than those found in barley. Walls of epidermal and mesophyll cells remained intact but seemed to undergo marked changes in chemical make-up. These walls stained with safranin O suggesting that they were possibly composed of, or impregnated with lignins, tannins, or suberin-like substances. This resulted in rusty-looking areas on affected leaf surfaces. Cells were not plasmolyzed until some time after cell wall alterations.

Effects in wheat appeared to be a combination of those found in barley and oats (Chatters and Schlehuber 1951). Cell walls were

altered as in oats but cell contents were plasmolyzed soon after penetration as in barley. Lysis occurred as in barley, followed by lining of the cavity with redish material similar to that of oats and barley. In some cases entire cavities were filled by pigmented masses. In all cases termination of the stylet appeared to be in the phloem.

The appearance of a new aphid biotype in the 1960's resulted in the initiation of a histological and histochemical study comparing biotype A and B on a resistant ('Will') and susceptible ('Rogers'), cultivar of barley (Saran, unpublished). The two cultivars of barley were stated to have no anatomical differences except for a slightly higher deposition of lignin in the epidermal cells of Will as compared to Rogers. Biotype A predominantly feeds intercellularly, although, there were deep intracellular penetrations lasting over two hours. Biotype B was reported to exhibit intracellular and sometimes intercellular penetrations-deep and shallow-which lasted 5-15 minutes followed by penetrations lasting more than two hours. Biotype B produced stylet tracks which appeared to end in the mesophyll, bundle sheath, xylem and phloem. Copious amounts of saliva were produced which seemed to be correlated with greater damage produced. Biotype A caused less damage, produced less saliva, and feeding tracks ended predomnantly within the phloem. Biotype A damage appeared more limited to the sieve elements ruptured by the stylet while biotype B

caused necrosis and collapse of adjacent cells. Biotype B feeding sites contained massive callose deposits and slime plugs in the phloem which were not apparent in biotype A feeding sites.

Further studies by Saxena and Chada on Rogers barley (1971) indicated that biotype A produced plant damage mainly in the phloem. Phloem cells appeared completely collapsed with no distinction between phloem parenchyma and sieve tube elements. Biotype B fed inter- and intracellularly producing straight stylet sheaths. Chlorosis resulted when saliva was injected into epidermal cells. When saliva contacted cell contents, there was marked plasmolysis, and protoplasm usually became concentrated in the region of contact. Cell contents became disorganized, the nucleus swelled considerably, and eventually became a darkened irregularly shaped mass. Cell walls finally ruptured. Adjacent cells were affected resulting in large vacuolar spaces in the mesophyll parenchyma.

A third biotype, C, was found in sorghum fields in 1968 (Wood et al. 1969). Feeding of this aphid has been monitored on sorghum using an electrical device in which different waveforms are produced corresponding to salivation, phloem ingestion and nonphloem ingestion (Campbell et al. 1982). It was demonstrated that aphids feeding on resistant plants registered a reduced ingestion from the phloem while showing an increase in separate probes and duration of

nonprobes (i.e., stylet withdrawn from the plant). Stylet penetration by biotype C is considered intercellular through the epidermis (Campbell et al. 1982).

The first ultrastructural study of host plant tissue affected by the greenbug was carried out by Al-Mousawi et al. (1983). These studies compared biotype C leaf tissue damage in a resistant as opposed to a susceptible wheat cultivar. The susceptible cultivar showed early phloem-parenchyma degeneration with affected parenchyma cells losing all detail. Cells with ruptured plasmalemma were found close to greenbug saliva sheaths. Chloroplasts of mesophyll cells became round with intact membranes at 2 days post-infestation, and small vesicles occurred between plasmalemma and cell wall. By 3-4 days, chloroplast lamellae appeared separated from one another, and osmiophilic granules had increased in size. By 10 days post-infestation chloroplasts in damaged areas were devoid of contents except for enlarged osmiophilic granules. The nucleus was the last organelle to degenerate. This occurred after it became densely osmiophilic at 10 days.

The resistant cultivar plants showed less damage than the susceptible ones. Plasmolysis of phloem cells occurred. However, there seemed to be less saliva sheath material associated with it. At 2 days post-infestation, damaged mesophyll cells were surrounded by healthy cells, and by 10 days, vascular and mesophyll tissue had returned to normal except for a few necrotic mesophyll cells. The saliva

sheath in both the resistant and susceptible cultivars was seen as a densely stained fibrillar material, usually found in intercellular spaces.

The purpose of the current study was to examine host plant tissue damage and the probing behavior of greenbug biotype C and biotype E. Biotype E is a fifth biotype discovered in 1980 (Porter et al. 1982). These studies utilized a susceptible (Rogers) and a resistant (Will) cultivar of barley. Will is resistant to biotype C, but it has lost its tolerance and maintained most of its antibiosis against biotype E (Starks et al. 1983). Rogers is susceptible to both biotypes C and E.

METHODS AND MATERIALS

Seeds of 'Will' and 'Rogers' barley were germinated in flasks of aerated, distilled water. The germinated seeds were then planted in pots and grown under greenhouse conditions. Aphid biotypes C and E were placed on 10 day old barley seedlings with a small paint brush. The aphids were allowed to probe for a maximum of one hour or the aphid terminated probing naturally in less than one hour. A dot of india ink was placed on the opposite side of the leaf from the feeding site to help in locating the saliva sheath. Probing times varied from 5 minutes to 1 hour and samples were taken at 0.5, 1, 2, 3, 4, and 10 days.

Three experiments were done and a total of 106 samples were obtained. In the first experiment ten plants of Will were used. A single aphid was placed on each of the ten plants. Five of the aphids used were biotype C and five were biotype E. The second experiment was done in the same manner using Rogers instead of Will barley. In the third experiment ten plants of each cultivar were used and four aphids were placed on each plant. Five of the Rogers plants received biotypes E aphid and the other five received biotype C aphid. Five of the Will plants received biotype E aphid and the other five received biotype C aphid.

In the first two experiments the aphids were placed in petri dishes on moist filter paper and starved for one hour before

feeding. In the third experiment the aphids were placed directly on the plant without starvation.

Leaf tissue was fixed for 24-48 hours in FPA (formalin-proprionic acid-alcohol), dehydrated in a graded series of tertiary butyl alcohol, infiltrated, and embedded in paraffin. Ten micrometer serial sections were cut on a rotary microtome and stained with safranin and fast green.

Statistical analysis was done using a computer SAS program. Analysis of variance (ANOVA) using a general linear model (GLM) was used to analyze the number of stylet sheath penetrations and the number of stylet sheath endings. The type of entry of the stylet sheath, depth and direction of the stylet sheath, and tissue reached by the stylet sheath was analyzed using a X^2 test.

RESULTS

The number of stylet sheaths found in 'Will' and 'Rogers' barley are shown in Table 1. Column one indicates the number of paraffin blocks in which stylet sheaths were found. Each block corresponds to a single aphid. Column two indicates the total number of penetrations seen for each group. For example, in the first group, Rogers barley on which biotype C fed, aphid stylet sheaths were found in 15 blocks. Some blocks contained two to four penetrations each, resulting in a total of 29 for that group. The last column indicates the total number of stylet sheath endings seen for each group. Often the stylet sheath branches (Fig. 1) anywhere from two to four times resulting in multiple stylet sheath endings.

Statistical analysis was done to compare total penetrations and total branches (stylet sheath endings). There was no statistically significant difference in total penetrations between biotypes (F=0.4, df=51) or between cultivars (F=0.11, df=51). The interaction between biotypes and cultivars also showed no significant difference in total penetrations (F=1.97, df=51). There was no difference in the total number of stylet sheath endings between biotypes (F=0.49, df=51) or between cultivars (F=0.89, df=51). The interaction between biotypes and cultivars also showed no difference in the total number of stylet sheath endings (F-0.97, df=51).

When the number of branches per penetration was analyzed there was no difference between biotypes (F=0.58, df=51), however, a difference (P < 0.10) was found between varieties (F=2.72, df=51). Rogers had an average of 1.84 branches per penetration while Will had an average of 1.52 branches per penetration. When least square means were compared between the four treatment groups the only significant difference (P < 0.10) in the number of branches per penetration was between biotype C on Will barley and biotype E on Rogers barley. Biotype C on Will barley had an average of 1.4 branches per penetration and biotype E on Rogers had an average of 1.9 branches per penetration.

Entry of the stylet sheath into the plant tissue is shown in Table 2. The first column indicates the number of entries seen. Entry of the stylet sheath was not located for every penetration observed. The remaining columns indicated four types of stylet entry: intercellular (Fig. 2), in which case the stylet enters between two epidermal cells; intercellular with partial penetration (Fig. 3), in which case the stylet enters between two epidermal cells but partially penetrates into one or both of the adjacent epidermal cells; intracellular (Fig. 4), in which case the stylet penetrates directly through an epidermal cell; and stomatal (Fig. 5), in which case the stylet enters between two guard cells.

In biotype C feeding on Rogers and on Will the majority of entries were intracellular. Rogers had 56% intracellular entries

and Will 60%. The second largest category for both of these groups was intercellular with partial penetration of Rogers 20% and Will 40%. Biotype C feeding on Rogers also had 16% intercellular penetration and 8% stomatal penetration.

In biotype E feeding on Rogers and Will half of the entries are intercellular. Rogers had 50% and Will had 54% intercellular entry. The second major category for both of these groups is intercellular with partial penetration. Rogers had 22% intercellular with partial penetration and Will had 31%. Biotype E on Rogers also had 17% intracellular penetrations and 11% stomatal penetrations. Biotype E on Will had 15% intracellular entry.

Statistical analysis between biotypes shows there is a statistically significant difference (X^2=18.4, $P<0.001$) in the preference of the entry site between biotypes, but no significant difference (X^2=4.1) in preference between cultivars. Biotype C most frequently enters intracellularly while biotype E most frequently enters intercellularly.

Two intracellular entries by biotype C feeding on Rogers and one by biotype E feeding on Rogers end in the epidermal cell.

Table 3 shows adaxial vs. abaxial penetration. Aphids were placed on the adaxial surface of the leaf which is where over 80% of the penetrations were made by biotype C feeding on Will, and biotype E feeding on Will and Rogers. Biotype C on Rogers had 59% adaxial penetration and 41% abaxial penetration.

The depth of penetration of the stylet sheath and direction of penetration is shown in Table 4. Shallow penetration is considered to be one or two cells into the tissue, not including the epidermal cells. Direction of penetration indicates whether the penetration is directed toward mesophyll parenchyma or toward a vascular bundle.

Biotype C feeding on Rogers and biotype E feeding on Rogers and Will had over 60% deep penetrations. Biotype C on Will had 88% deep penetration. Over half of the penetrations of biotype C feeding on Rogers (57%) and Will (65%) and of biotype E on Will (57%) are directed toward a vascular bundle.

Statistical analysis between biotypes shows biotype C to have a higher frequency of deep probes than biotype E (X^2=2.7, $P<0.10$) while there is no difference (X^2=0.13) in frequency between cultivars. Biotype C also shows a higher percent (X^2=5.1, $P<0.05$) of probes directed toward a vascular bundle with no difference (X^2=.007) between cultivars.

Tissue reached by the stylet sheath is shown in Table 5. In all four groups the tissue most often reached by the stylet sheath was the parenchyma. There was no difference in the tissue reached between biotypes (X^2=0.5) or between cultivars (X^2=0.8). Many times the stylet sheath was directed toward the vascular bundle but stopped just short of it in the mesophyll or ended in the bundle sheath.

In biotype C feeding on Rogers, stylet sheath endings were found in the xylem 11% of the time and in Will 24% of the time. In biotype E feeding on Rogers barley stylet sheath endings were found in the xylem 15% of the time and in Will 7% of the time. The sheath often branched several times in the xylem resulting in endings in one or more of the vessel elements as well as in xylem parenchyma before either ending there or continuing on to the phloem. Often when the sheath ended in a vessel element it produced a substance at the tip of the sheath which stained blue with safranin and fast green (Fig. 6). It was not unusual for the stylet sheath to travel through the xylem to reach the phloem, but the sheath never traveled through the phloem to reach the xylem.

In all four groups few stylet sheaths ended in the phloem. In biotype C feeding on Rogers, 11% ended in the phloem and in Will 9% ended there. In biotype E feeding on Rogers 8% ended in the phloem and in Will 14% ended there. In several cases the stylet sheath appeared to end near a sieve tube element but it was not possible to determine exactly in which cell it was located (Fig. 7).

The path of the stylet sheath through the mesophyll tissue was usually intercellular in all four groups (Fig. 8). Occasionally a mesophyll cell would be punctured along the path of the stylet and often termination of the sheath would be in a mesophyll cell. Even the path of biotype C which usually entered the tissue intracellularly turned intercellular once past the epidermal cells.

Samples were taken from barley leaves 0.5, 1, 2, 3, 4, and 10 after feeding. However, since stylet sheaths were found in only about half of the samples taken, for many of the time periods there is only one (or sometimes no) sample. This makes a detailed account of tissue damage impossible, however, some observations may still be made.

Damage to the mesophyll cells can be seen as early as 12 hours but is usually most pronounced in tissue taken four and ten days after feeding. Sometimes damage is confined to the mesophyll cells in direct contact with the stylet sheath but damage may also be seen three or four cells away from the sheath. On occasion, disorganization in the mesophyll cells can be seen in a wide area around the sheath extending from the upper to lower epidermis. In all four groups formation of cavities in the mesophyll was seen.

Damaged mesophyll cells became enlarged and rounded and the protoplast disorganized. The cell walls of the mesophyll cells became thickened and stained red with safranin. By ten days the cell walls are very thick and stained a brownish red and areas in between the mesophyll cells stain bright red. In two cases the stylet sheath could no longer be distinguished at this point however, the portion of the sheath inside the vascular bundle remained intact (Fig. 9).

In all four groups there were instances in which a mesophyll cell or bundle sheath cell would contain a solid mass of round

granular looking bodies which either stained red, reddish brown or dark brown (Fig. 10). This occurred only in a single cell in each instance and the cell seemed to have been punctured by the stylet sheath.

DISCUSSION

There are few differences in the probing behavior of biotypes C and E on Will and Rogers barley. Biotype C predominantly enters the host plant intracellularly regardless of cultivar while biotype E enters predominantly intercellularly. The path of both biotypes in both cultivars in the mesophyll tissue was predominantly intercellular.

The aphid entered the leaf most frequently on the adaxial surface of the leaf in all groups except biotype C on Rogers barley in which 59% penetrated adaxially and 41% penetrated abaxially. This is not a true indication of the preference of the aphid because all aphids were placed on the adaxial surface, however, this may help explain the path taken by the aphid. If the aphid enters on the adaxial surface of the plant the sheath must travel through or around the xylem to reach the phloem tissue. If penetration is on the abaxial surface there is a more direct path to the phloem and in fact, sheaths were often seen traveling through the xylem to reach the phloem but sheaths never traveled through the phloem to reach the xylem.

Biotype C shows a higher percentage of deep penetrations than E as well as a higher percentage of tracks oriented toward a vascular bundle during an hour feeding time.

There was no difference between biotypes or varieties in the number of penetrations or the number of stylet sheath endings.

There was a difference in number of branches per penetration between cultivars with Rogers containing stylet sheaths with more branches per penetration than Will.

It was shown by Campbell et al. (1982) that biotype C made more probes on resistant sorghum cultivars than on susceptible, indicating that the aphid made a more direct path to the phloem tissue in susceptible cultivars. In this study, although there is no difference in the number of probes, the increase in the number of branches per penetration in Rogers indicates more random probing in the susceptible than the resistant cultivar. This could indicate that susceptibility may be due in part to an increase in damage to mesophyll parenchyma as well as phloem ingestion. Cavity formation, however, was seen in the mesophyll parenchyma in all four treatment groups, with no single group showing more mesophyll damage than another.

The majority of the stylet sheaths in all four treatment groups ended in the mesophyll parenchyma, following an hour or less probing time. Feeding time should probably be longer than one hour to determine the destination of the stylet. Saxena and Chada (1971) allowed the aphid to feed continuously for over 48 hours to determine the destination of the stylets in biotypes A and B. The aphids in this study did not have enough time to reach their destination and as a result this study is an indication of aphid probing rather than aphid feeding.

Termination of the stylet sheath in the xylem was usually in a large vessel element. The aphid usually produced something at the tip of the sheath which stained light green with safranin and fast green. Pollard (1971) felt the aphid used such a substance to seal off the sheath after the aphid entered the less preferred xylem tissue. McLean and Kinsey (1967) found that mouth parts of pea aphids starved 24 hours before feeding would pass through phloem before ingestion of liquid from the xylem. In two of the three experiments conducted for this paper the aphids were starved for one hour before feeding which may have resulted in an increase in the number of stylet sheaths ending in the xylem.

When the stylet sheath ended in the phloem tissue it was difficult to discern in which cell it terminated. Since the aphids were removed after feeding, the stylets did not remain in the tissue. It has been shown that only the maxillary stylets open into the sieve tube with the mandibulars reaching only to the cell wall, thus, when the stylet is withdrawn the stylet sheath may appear to extend only to the cell wall and not into the cell (McLean and Kinsey 1967; Pollard 1971). In several instances the stylet sheath appeared to end next to a sieve tube element.

Evaluation of damage to the plant tissue was difficult due to the lack of samples for some groups. Also, feeding times were not accurate making it difficult to compare samples. For example, when comparing two one hour feeding samples taken four days after

feeding, one sample showed extensive damage, while other samples showed little damage. Some aphids probably did not actually probe for an hour. In the future evaluation of feeding and plant damage should involve histology in conjunction with electrical monitoring of aphid feeding so that accurate feeding times may be recorded.

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TABLE 1. NUMBER OF STYLET SHEATHS FOUND

VARIETY AND BIOTYPE	NUMBER OF APHIDS	NUMBER OF PENETRATIONS	NUMBER OF STYLET SHEATH ENDINGS
Biotype C on Rogers Barley	15	29	47
Biotype C on Will Barley	15	24	34
Biotype E on Rogers Barley	13	20	39
Biotype E on Will Barley	9	17	28

There are no significant differences between test groups.

TABLE 2. TYPE OF ENTRY OF THE STYLET SHEATH

VARIETY AND BIOTYPE*	NUMBER OF ENTRIES SEEN	TYPE OF ENTRY			
		INTERCELLULAR	INTER. W/PARTIAL PENETRATION	INTRACELLULAR	STOMATAL
Biotype C on Rogers Barley[a]	25	(4) 16%	(5) 20%	(14) 56%	(2) 8%
Biotype C on Will Barley[a]	15	(0) 0%	(6) 40%	(9) 60%	(0) 0%
Biotype E on Rogers Barley[b]	18	(9) 50%	(4) 22%	(3) 17%	(2) 11%
Biotype E on Will Barley[b]	13	(7) 54%	(4) 31%	(2) 15%	(0) 0%

*Test groups followed by the same letter are not significantly different, test groups followed by different letters are significantely different ($P<.001$, X^2 test).

TABLE 3. ADAXIAL VS. ABAXIAL PENETRATION

VARIETY AND BIOTYPE	NUMBER OF ENTRIES	ADAXIAL	ABAXIAL
Biotype C on Rogers Barley	29	(17) 59%	(12) 41%
Biotype C on Will Barley	24	(21) 87%	(3) 13%
Biotype E on Rogers Barley	20	(17) 85%	(3) 15%
Biotype E on Will Barley	17	(14) 82%	(3) 18%

TABLE 4. STYLET SHEATH DEPTH AND DIRECTION OF PENETRATION

VARIETY AND BIOTYPE*	NUMBER OF SHEATH ENDINGS	DEPTH OF PENETRATION		DIRECTION OF PENETRATION	
		SHALLOW	DEEP	PARENCHYMA	VASCULAR BUNDLE
Biotype C on Rogers Barley[a]	47	(15) 32%	(32) 68%	(20) 43%	(27) 57%
Biotype C on Will Barley[a]	34	(4) 12%	(30) 88%	(12) 35%	(22) 65%
Biotype E on Rogers Barley[b]	39	(15) 38%	(24) 62%	(26) 67%	(13) 33%
Biotype E on Will Barley[b]	28	(9) 32%	(19) 68%	(12) 43%	(16) 57%

*Test groups followed by the same letter are not significantly different, test groups followed by different letters are significantly different (depth of penetration, $P < 0.1$, direction of penetration, $P < 0.05$, X^2 test).

TABLE 5. TISSUE REACHED BY THE STYLET SHEATH

VARIETY AND BIOTYPE	NUMBER OF SHEATH ENDINGS	TISSUE REACHED		
		PARENCHYMA	XYLEM	PHLOEM
Biotype C on Rogers Barley	47	(37) 79%	(5) 11%	(5) 11%
Biotype C on Will Barley	34	(23) 68%	(8) 24%	(3) 9%
Biotype E on Rogers Barley	39	(30) 77%	(6) 15%	(3) 8%
Biotype E on Will Barley	28	(22) 79%	(2) 7%	(4) 14%

There are no significant differences between test groups.

FIGURE CAPTIONS

Fig. 1.--Light micrograph of the stylet sheat (SS) of biotype E on Rogers barley showing multiple stylet sheath endings. There are three sheaths all of which end in the xylem (X). Vascular bundle showing the bundle sheath (BS), xylem (X), and area of the phloem (P) (x 2,700).

Fig. 2.--Light micrograph of the stylet sheath of biotype C on Rogers barley showing intercellular penetration between two collapsed epidermal cells (CE). The sheath ends in a stomatal chamber (SC) (x 2,700).

Fig. 3.--Light micrograph of the stylet sheath (SS) of biotype C on Rogers barley showing intercellular entry with partial penetration into the adjacent epidermal cell (E) (x 2,700).

Fig. 4.--Light micrograph of the stylet sheath (SS) of biotype C on Will barley showing intracellular penetration through an epidermal cell (E) (x 2,700).

Fig. 5.--Light micrograph of the stylet sheath (SS) of biotype E on Roger barley showing penetration through the guard cells (GC) of a stomate (x, 2,700).

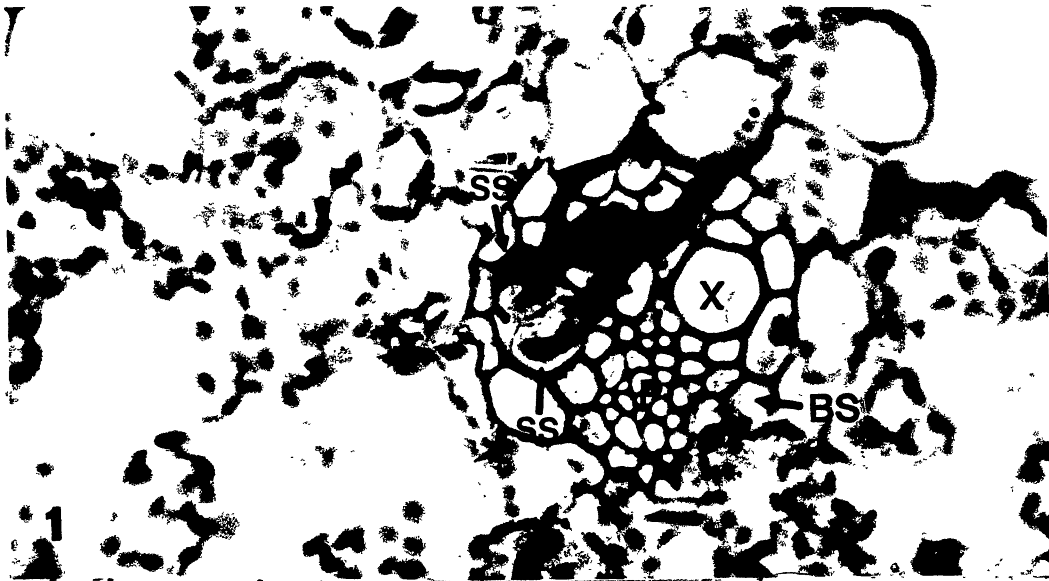
Fig. 6.--Light micrograph of the stylet sheath (SS) of biotype C on Will barley with two branches ending in a vessel element (V) and a protoxylem lacuna (PL) in the xylem tissue (x 2,700).

Fig. 7.--Light micrograph of the stylet sheat (SS) of biotype E on Will barley ending in the phloem tissue (P) of the vascular bundle (x 2,250).

Fig. 8.--Light micrograph of the stylet sheath (SS) of biotype E on Rogers barley showing an intercellular path through the mesophyll tissue (M) (x 2,700).

Fig. 9.--Light micrograph of the penetration site of biotype C on Rogers barley 10 days post-infestation. Arrow indicates thickened mesophyll (M) cell walls (CW) which stain red with safrainin (x 675).

Fig.10.--Light micrograph of the feeding site of biotype C on Rogers barley showing an affected mesophyll cell filled with round granular bodies (GB). Also shown are mesophyll cells (M) and the bundle sheath (BS) of a nearby vascular bundle (x 2,700).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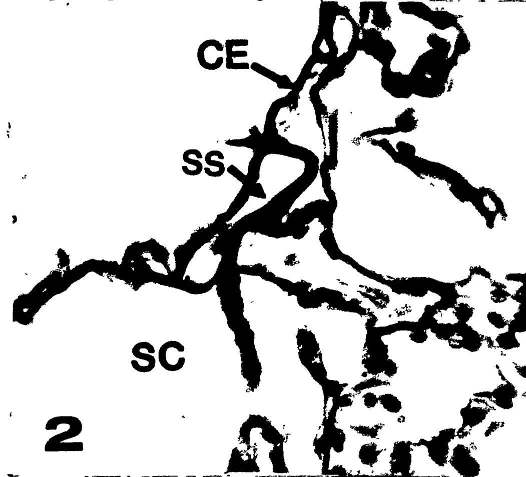
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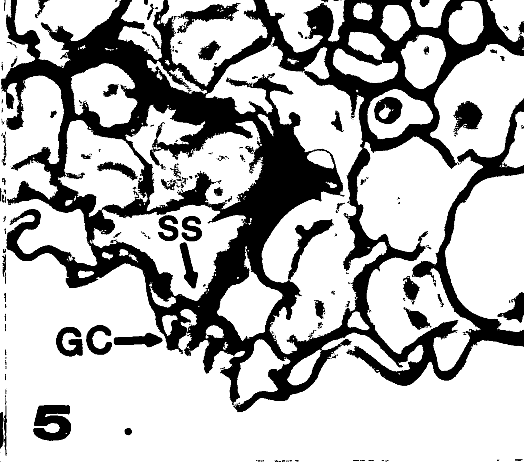
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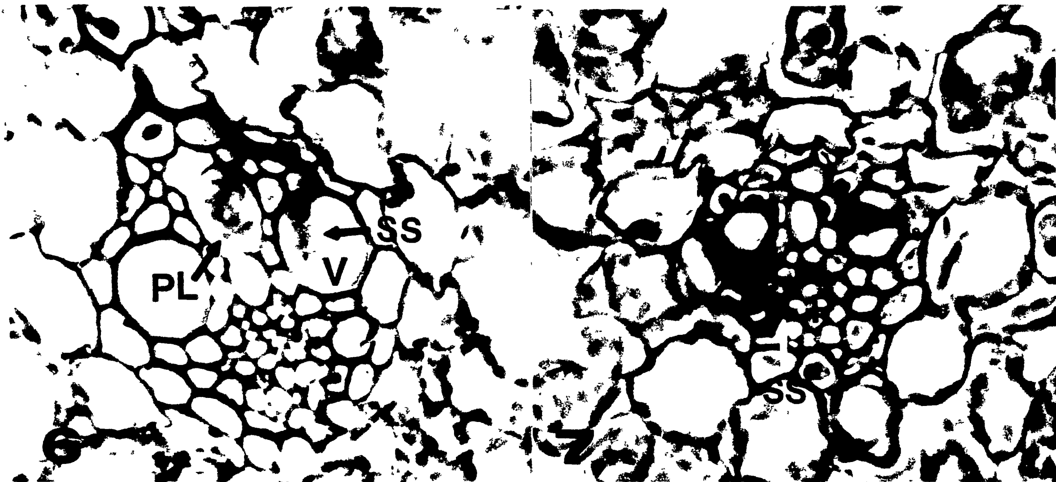

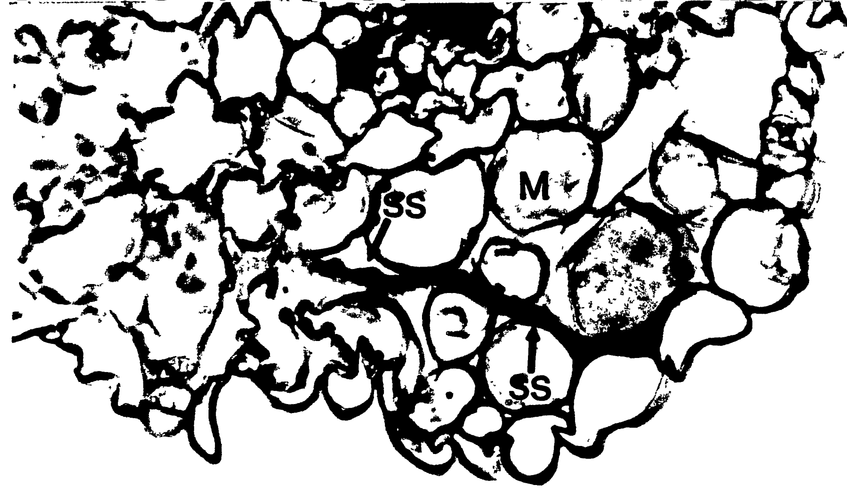

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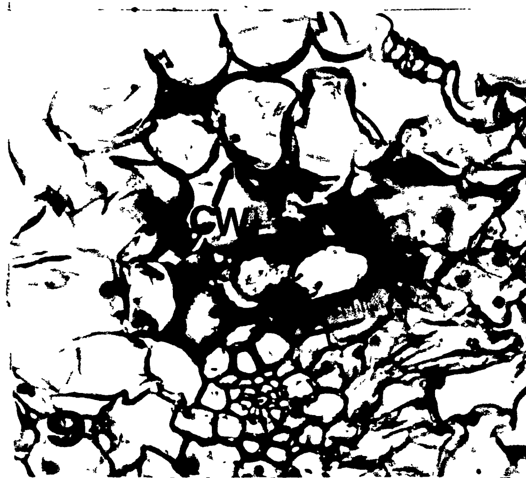

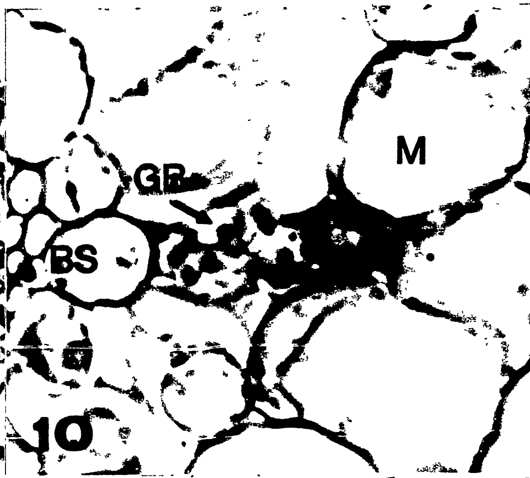

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Levels of Silicon and Phenolic Compounds Surrounding the Penetration Site of two Biotypes of an Aphid (Homoptera: Aphididae) in a Susceptible and a Resistant Barley

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ABSTRACT

The level of silicon and phenolic compounds around the penetration site of two biotypes (C and E) of the greenbug *Schizaphis graminum* (Rondani) were evaluated using scanning electron microscopy and x-ray microanalysis. All four treatment groups showed an increase in silicon in the damaged area. The adjacent vascular bundle areas of biotype C and E in Rogers barley (susceptible) showed an increase in silicon while in Will barley (resistant) the silicon level remained similar to that of the control. The Will barley control showed a higher level of silicon in the vascular bundle area than the Rogers barley control, while levels were similar between the two controls in the epidermal area.

Experiment one demonstrated no differences in the level of phenolic compounds between any of the treatment groups. However, experiment two showed the resistant Will barley contained more phenolic compounds than did the susceptible Rogers barley. Phenolic compound levels remained similar to control levels in damaged and adjacent vascular bundles of Rogers barley. Phenolic levels are similar to the control in biotype C on Will barley. Phenolic levels are drastically decreased after biotype E damage.

INTRODUCTION

Accumulation of silicon around the penetration site of powdery mildew fungi on barley may be related to the primary resistance reaction (Kunoh and Ishizaki 1975, 1976; Sargent and Gay 1977). Accumulations are found following powdery mildew infection in wheat (Kunoh and Ishizaki 1976) and in the non-host plant, French bean, after cowpea rust infection (Heath 1979).

In halo regions, the outer epidermal wall of barley becomes incrusted with opaline silica in association with cuticular lipid deposition (Sargent and Gay 1977). Silicon deposition appears as a local wall thickening. This wall thickening is also seen in mesophyll cells of corn following rust infection (Leath and Rowell 1966, 1969), in French bean, after cowpea rust infection (Heath 1979) and in sunflower leaves infected by *Verticillum dahliae* (Robb et al. 1977). These wall thickenings are seen as electron-opaque deposits in the transmission electron microscope and identified as containing the element silicon with the aid of energy dispersive x-ray analysis (Heath 1979; Sargent and Gay 1977).

Silica deposition in vessel elements of woody perennials may be due to an increase in the concentration of monosilicic acid in the transpiration stream in response to evapotranspiration or stagnation of apoplastic flow (Scurfield et al. 1974). An increase in evapotranspiration following contact of the appressorium with the epidermis of the host plant has also been cited as the cause

cause for silica accumulation around the penetration site of barley powdery mildew. However, this does not account for accumulation on mesophyll cells which lie in the humid environment of the leaf (Heath 1979). Water in the cell walls of mesophyll cells moves as a liquid to the cell wall on the cuticle side of epidermal cells where it either evaporates or moves across the cuticle as a liquid (Nobel 1974). There is evidence that an intact epidermal cytoplasm is necessary for halo formation in barley following mechanical damage (Sargent and Gay 1977), while formation of wall deposits of infected mesophyll cells have been inhibited by actinomycin D, cycloheximide, and blasticidin S (Heath 1979).

The association between polyphenols and silica is described in ray parenchyma cells of several species of broad-leaved trees (Scurfield et al. 1974). Infrared absorption studies indicate that silicon in the sap of horsetail is not present as monosilicic acid but as an organic complex containing phenyl rings with silicon attached to a phenyl group (Lovering and Engel 1967). However, study of the xylem sap of oats, bean, horsetail, and clover by a molecular sieve technique completely refutes this idea showing silicon in the exudates to be present in the form of monosilicic acid (Hartley and Jones 1972).

Leaves which are damaged mechanically (Parry and Smithson 1963) or by chilling (Beddows and Jones 1958) show a higher amount of silica deposition in epidermal cells as well as accumulation of

phenolic compounds (Johnson and Schaal 1957; Liebermann et al. 1958). It has been reported that o-diphenols enhance the solubility of monosilicic acid at pH 7.5 and 30°C, indicating that phenolics might facilitate the accumulation of silica in cells which produce them (Bauman 1960, 1963; see Scurfield et al. 1974). However, mesophyll cells which contain silicon wall deposits show small amounts of phenolic compounds when compared to walls of similar cells lacking deposits in the immediate vicinity of the fungus which show higher levels of phenolic compounds (Heath 1979).

The purpose of this study was to determine levels of silicon and phenolic compounds around the penetration site of two biotypes (C and E) of the greenbug *Schizaphis graminum* (Rondani) in a susceptible ('Rogers') and a resistant ('Will') cultivar of barley. Five biotypes of *S. graminum* have been reported: A, B, C, D, (Starks and Burton 1977) and E (Porter et al. 1982). Biotypes C and E are present in fields today.

Rogers is susceptible to both biotypes C and E. Will is resistant to biotype C while it has lost its tolerance but maintained its antibiosis against biotype E (Starks et al. 1983.)

MATERIALS AND METHODS

Seeds of 'Will' and 'Rogers' barley were germinated in flasks of aerated, distilled water, planted in pots, and grown under greenhouse conditions. Biotypes C and E of the greenbug were placed on barley seedlings at 10 days. Greenbugs were allowed to feed for two days and then removed. Leaf samples were taken ten days after feeding. Six experimental groups were designated as follows: Will on which biotype C fed, Will on which biotype E fed, Will on which no aphids fed, Rogers on which biotype C fed, Rogers on which biotype E fed and Rogers on which no aphids fed.

X-ray analysis was done of two areas around the damaged site: (1) the epidermis and (2) the vascular bundle. Samples were analyzed using a JEOL JSM-35 scanning electron microscope with a Tracor Northern 2000 x-ray microanalyzer. Accelerating voltage was 15 KV.

Full width at half maximum was determined for each of the spectral regions of interest (in keV): Si (1.64-1.84), Fe (6.30-6.50). The background was subtracted from each spectrum and x-ray peaks were integrated to obtain total counts for each region of interest. Counts for the ten samples of each treatment group were totaled and the averages were compared. For the averages to be statistically different they must differ by a magnitude of 2. (Tracor Northern, personal communications).

X-ray analysis of the epidermal area for silicon. Ten leaf samples 1 cm in diameter were taken of the damaged area and placed on carbon stubs with carbon paint. One sample from each of the six experimental groups was placed on a carbon stub. There was a total of ten stubs for the experiment with 6 samples on each stub. Fresh, untreated plant material was used. Five counts, 60 seconds each were taken from each sample, culminating in a 300 second count for each spectrum. The actual area analyzed was a raster that measured approximately 7.5 x 7.5 um (a 3 cm x 3 cm raster on the viewing screen). A magnification of 100x was used.

X-ray analysis of the vascular bundle area for silicon and phenolic compounds. Tissue taken for vascular bundle analysis was labeled for phenolic compounds with ferric chloride (Brisson, et al. 1977). Phenolic compounds were identified on the x-ray spectrum as an iron peak. Hand cut cross sections of the damaged area 1 mm in thickness, were fixed in 5% gluteraldehyde in 3% ferric chloride. The tissue was fixed for 6 hours, washed in distilled water, dehydrated in a graded ethanol series and critical-point dried. Samples were placed on carbon stubs with carbon paint. One 60 second count was made of each sample using a selected area of 3 cm x 3 cm. A magnification of 1000x was used.

Three vascular bundle areas were compared: (1) the vascular bundle area around the damaged site, (2) the vascular bundle area adjacent to the damaged site and (3) a vascular bundle from a control plant on which no aphids fed.

RESULTS

X-ray analysis of silicon around the penetration site of the epidermal area. X-ray analysis of the epidermal area showed an increase in the amount of silicon in all four treatment groups which were infested with aphids (Fig. 1). Biotype C on Rogers contained 8 times more silicon than the control while biotype E on Rogers contained 6 times more silicon than the control. Biotype C on Will contained 5 times more silicon than the control and biotype E on Will contained 3 times more silicon than the control. There was no difference in the amount of silicon between the Will and Rogers controls. It was not possible to demonstrate a clearly significant difference in the amount of silicon present between the susceptible and resistant cultivars of barley or between the aphid biotypes.

X-ray analysis of silicon of the vascular bundle area. The damaged vascular bundle area of all four treatment groups showed an increase in silicon after aphid feeding (Fig. 2). Biotype C on Rogers contained 7 times more silicon than the control while biotype E on Rogers contained 10 times more silicon than the control. Biotype C on Will barley contained 3 times more silicon than the control while biotype E on Will contained 4 times more silicon than the control. There was 2 times more silicon in the resistant Will control than in the susceptible Rogers control.

The adjacent vascular bundle areas of biotypes C and E on Rogers showed an increase in silicon. Rogers infested with biotype C and biotype E contained 3 times more silicon than the controls. The adjacent vascular bundle areas of biotype C and E on Will were similar to the controls.

X-ray analysis of iron of the vascular bundle area. There was no difference in the level of iron marker for phenolic compounds in the four treatment groups (Fig. 3). However, an earlier preliminary experiment revealed much different results (Fig. 4). The Will control showed 3 times as much iron marker as did that of Rogers, the susceptible barley. Damaged and adjacent vascular bundle areas of biotype C feeding on Will showed high iron marker levels similar to those of the Will control. Damaged and adjacent vascular bundle areas of biotype E on Will showed a significant decrease in the amount of iron marker when compared to the Will control.

The iron marker levels of the Rogers controls showed no significant differences when compared with damaged and adjacent vascular bundle areas of biotype C on Rogers and biotype E on Rogers.

DISCUSSION

X-ray analysis of biotype C and E feeding sites on Will and Rogers barley indicates that high levels of silicon are present in the damaged area. Chatters and Schlehuber (1951) often found a brittle substance lining cavities produced in the mesophyll tissue after feeding of biotype A of the greenbug on barley, as well as chemical alterations in epidermal and cell walls of oats and wheat. This brittle substance may have been silicon. Silicon is now regarded to have a possible role in fungal pathogen resistance (Heath 1979; Kunoh and Ishizaki 1975, 1976; Sargent and Gay 1977), and its presence may be responsible for the lack of formation of haustoria in French bean after cowpea rust infection (Heath 1979).

All four treatment groups showed an increase in silicon in the damaged area. While the level of silicon in the damaged treatment groups showed quite a bit of variability there was no difference great enough to be statistically different between cultivars or between biotypes. This variability is due in part to the method used to analyze the silicon level, semiquantitative elemental analysis. Variability is due to tissue thickness, variability in depth of penetration of the electron beam into the sample, and smoothness of the sample surface. Because of these variabilities there must be a twofold change in the element in question to be statistically significant.

There was also some variability between samples within a treatment group. Although the area on the viewing screen remained the same there was variability in vascular bundle sizes.

The adjacent vascular bundle areas of biotype C and E on Rogers barley showed an increase in silicon while in Will barley the silicon level remained similar to that of the control. This indicates that damage to the susceptible Rogers may be more widespread while damage to the resistant Will is more localized.

The Will control showed a higher level of silicon than the Rogers in the vascular bundle area, while levels were similar between the two controls in the epidermal area.

Experiment 2 demonstrated no differences in the level of phenolic compounds between any of the treatment groups. This may have been due to lack of binding of the phenolic marker ferric chloride, because experiment 1 showed striking differences between treatment groups. In experiment 1 the resistant Will showed 3 times as much phenolic compounds as did the susceptible Rogers. Phenolic compound levels remained similar to control levels in damaged and adjacent vascular bundles of Rogers. While phenolic levels are similar to the control in biotype C on Will, phenolic levels are drastically decreased after biotype E damage in Will.

It is not surprising that the resistant Will contained more phenolic compounds than the susceptibel Rogers as it has been shown that phenolic compounds act as feeding deterrents when fed to the

greenbug in artificial diets (Dryer and Jones 1981, Todd et al. 1971). The date from experiment 1 suggests that the new biotype E may be injecting phenolases in its saliva into the plant tissue. These phenolases then breakdown phenolic compounds rendering Will no longer completely resistant to biotype E.

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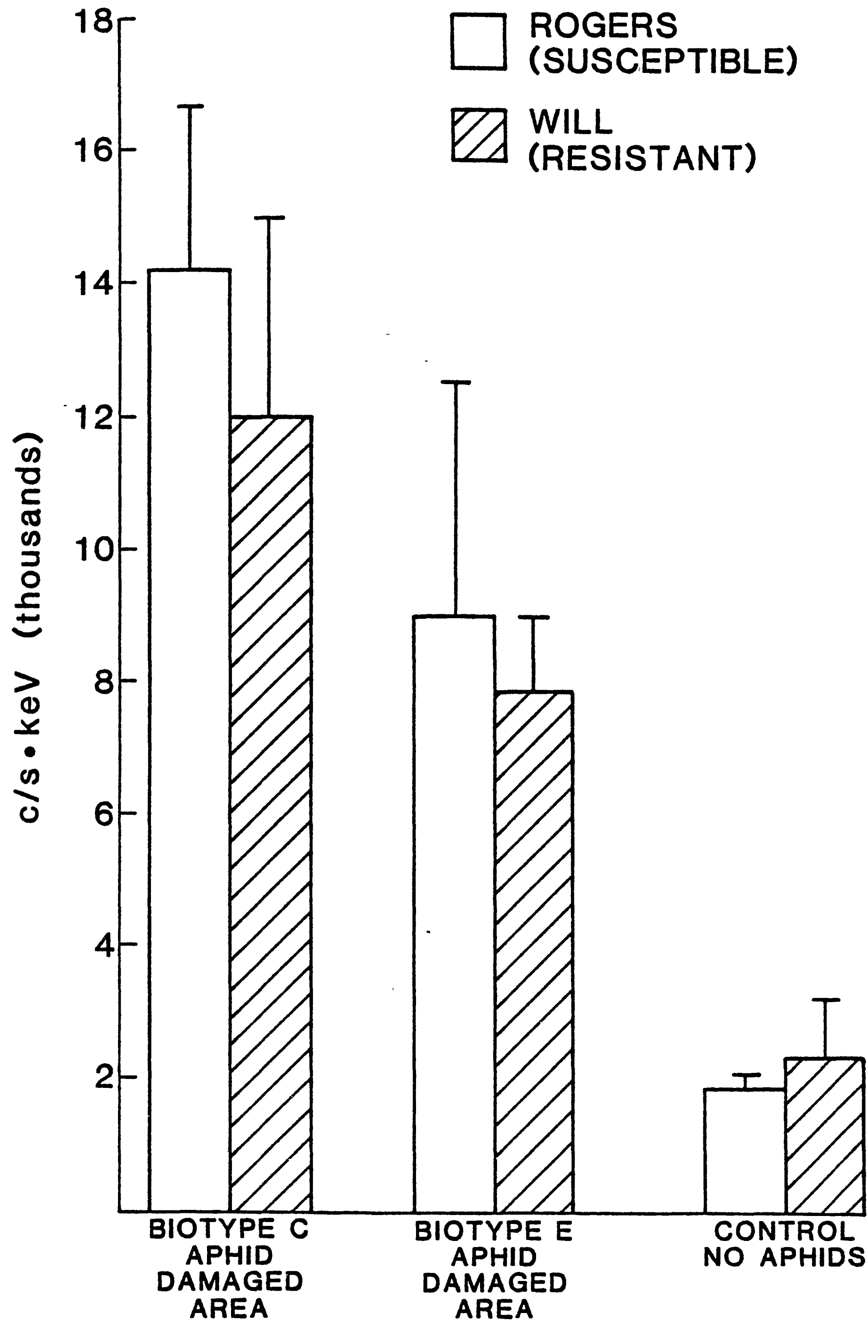

Fig. 1. Comparison of the average area of the silicon peak of the epidermal area. C/S x keV = counts per second times energy of the element.

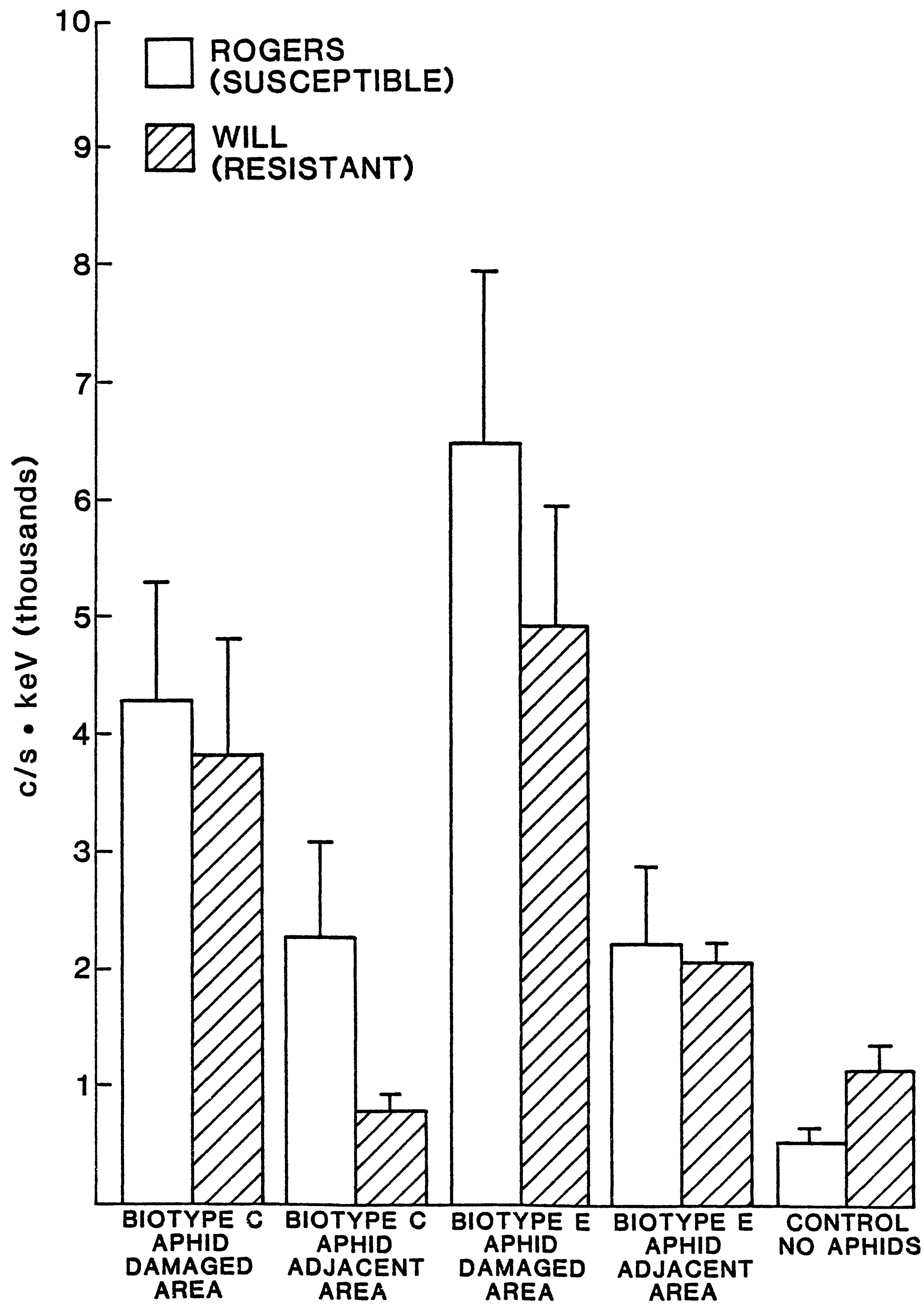

Fig. 2. Comparison of the average areas of the silicon peak of the vascular bundle area. C/S x keV = counts per second times energy of the element.

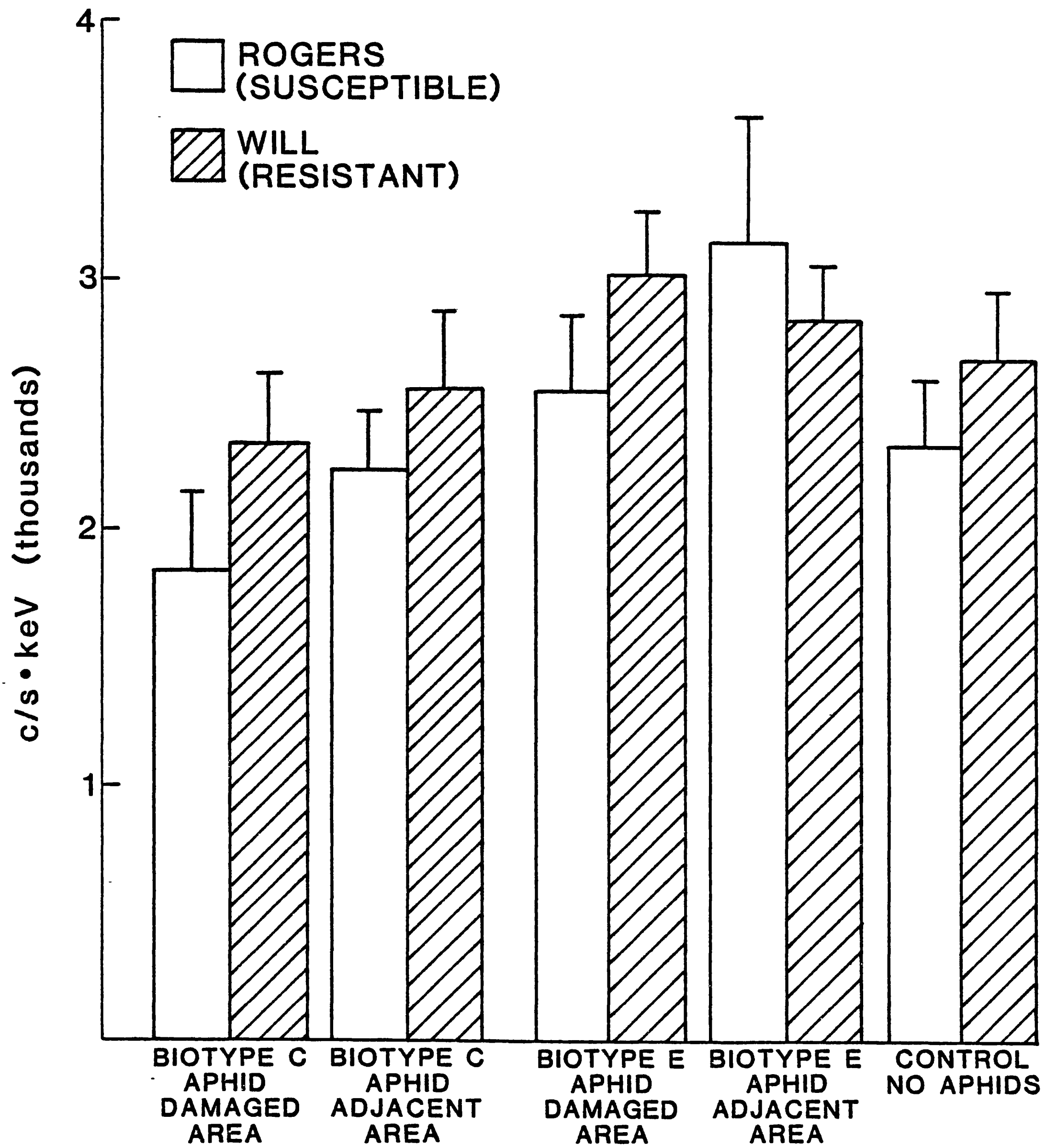

Fig. 3. Comparison of the average area of the iron peak of the vascular bundle area. Experiment 2. C/S x keV = counts per second times energy of the element.

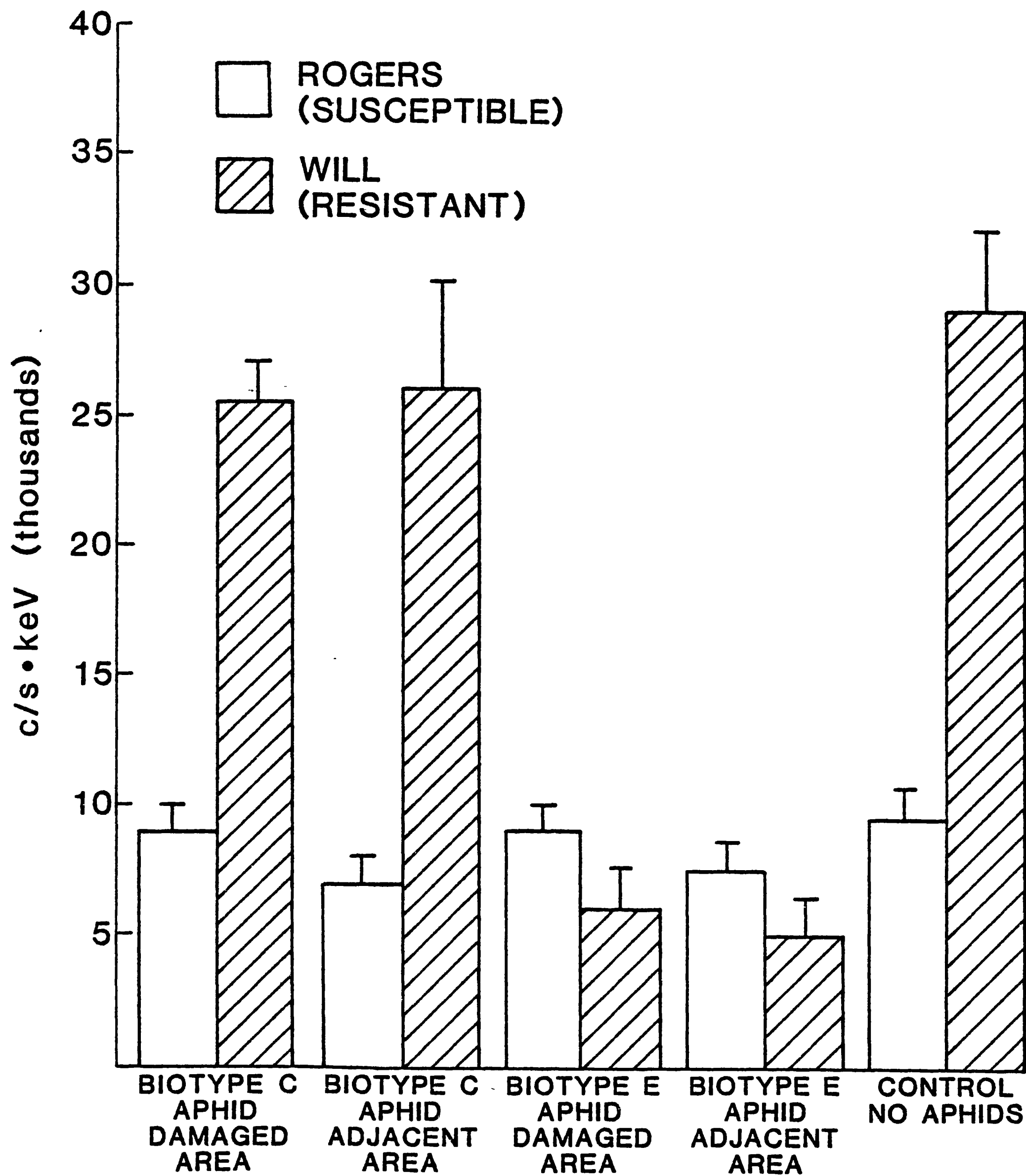

Fig. 4. Comparison of the average area of the iron peak of the vascular bundle area. Experiment 1, background not subtracted. C/S x keV = counts per second times energy of the element.

VITA

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Canidate for the Degree of

Master of Science

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