A COMPARATIVE STUDY OF THE MORPHOLOGY OF THE FEEDING OF THE TWO BIOTYPES OF THE CORN LEAF APHID, RHOPALOSIPHUM MAIDIS (FITCH), ON RESISTANT AND SUSCEPTIBLE SORGHUMS

by

PHILIP BONE MORGAN

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INTRODUCTION

This study was made to examine the relationship of the feeding punctures of biotype KS-1 and biotype KS-2 of the corn leaf aphid, Rhopalosiphum maidis (Fitch), on resistant and susceptible sorghums. White Martin was used as the susceptible host and rooted cuttings of the plant Piper Sudan 428-1 were used as the resistant host.

A statistical analysis was made of the data obtained from the comparative count of the stylet sheaths; character of the stylet sheaths, whether they were non-branched, two-branched or multiple-branched; objective of the stylets; and site of the feeding puncture.

Additional information was obtained on the type of penetration by the stylets and cell damage to internal tissues.

LITERATURE REVIEW

The plant Piper Sudan 428-1, from which rooted cuttings were obtained, was discovered by Howitt and Painter (1956). They concluded that the plant Piper Sudan 428-1 demonstrated resistance approaching immunity to the corn leaf aphid during intensive antibiotic tests. Also it would appear then that

the plant Piper Sudan 428-1 carries a high level of resistance in the form of antibiosis.

The two biological races of corn leaf aphid, biotype KS-1 and biotype KS-2, were discovered by Cartier and Painter (1956). They found that biotype KS-2 had a greater survival and reproductive capacity on resistant varieties and under comparable conditions produced heavier adults than biotype KS-1.

Further studies concerning the feeding of the corn leaf aphid were conducted by Pathak and Painter (1957). They discovered two more biological races and found that all biotypes gained significantly more weight on certain susceptible plants than on resistant ones. The weight loss was significantly less than the loss when the aphids were kept without food. Biotype KS-2 took in more food from the resistant plants than did the other biotypes. The behavior of the other biotypes in taking some food when kept hungry overnight, from the resistant plants, but losing weight later, suggests the presence of a repellent material that must be tasted in the resistant plants.

Studies have been made of the feeding of Hemiptera-Homoptera type insects. Evidence presented by Davidson (1923), Horsfall (1923), Smith (1926), Painter (1928), Smith and Poos (1931), Tate (1937), Chatters and Schlehuber (1951) and Diehl and Chatters (1956) indicated that the phloem was usually the source of food supply, and hence the probable objective of the stylets. There have been studies conducted concerning the method used by the Hemiptera-Homoptera type insects to locate the internal

plant tissue. Horsfall (1923) concluded that the route followed by the proboscis is more or less sinuous and the various branches of the path indicate the trial and error method of reaching the vascular bundle. Evidence was presented by Fife and Frampton (1936) that a pH gradient was involved because the phloem was more alkaline than the surrounding tissue. Painter (1951) concluded that among insects that suck the juices of plants there may exist an ability to distinguish tissues inside of plants. Kennedy and Mittler (1953) found that severing the part of the aphid's rostrum which remains outside the plant during feeding results in sap exuding from the severed stylet. Also, Mittler (1953) found that the sap that exudes from the severed stylets is similar in chemical composition to the honey dew excreted by the aphids.

Adams and McAllan (1956) concluded that pectinase is present in the salivary glands. Their findings may possibly indicate why the stylets penetrate the plant tissue intercellularly.

The histological methods that were used in this problem can be found in Johansen (1940), and in addition to those mentioned above, publications by Artschwager (1940) and Eams and MacDaniels (1947) were used as guides in studying the stylet sheaths and plant tissue.

METHODS AND MATERIALS

Histological Procedure

A leaf on a host plant was selected and examined for the absence of exuviae. If the leaf was found to be satisfactory a specially designed plastic cage, which presented a feeding area one millimeter wide and ten millimeters long, was then placed on the leaf.

Five or ten late instar aphids were placed in each feeding cage and the following information was recorded: host plant, biotype of Rhopalosiphum maidis (Fitch), size of feeding area, tissue number, length of leaf, distance of feeding area from leaf whorl, time allowed for feeding and number of aphids placed in cage (Tables 1,2,3, and 4).

At the end of the 48-hour feeding period, the feeding area was marked with India ink and the plant tissue was placed in a formalin-aceto-alcohol fixative, which was prepared according to the following formula:

Ethyl alcohol 70% ..... 90 cc.
Glacial acetic acid 99% ..... 5 cc.
Formalin 40% ..... 5 cc.

This formula was found to be the most desirable since the tissue, after a 24-hour fixation period, could be left in the fixative indefinitely or transferred directly to the 70 per cent ethyl tertiary-butyl alcohol mixture of the tertiary-butyl alcohol dehydrating procedure.

The tertiary-butyl alcohol method of dehydration was found to be the most useful for plant tissue and was prepared by the following formulae:

	70%	85%	95%	100%
Distilled water	30 cc.	15 cc.		
Ethyl alcohol 95%	50 cc.	50 cc.	45 cc.	
Tertiary-butyl alcohol	20 cc.	35 cc.	55 cc.	75 cc.
Ethyl alcohol 100%				25 cc.

The plant tissue was left in the dehydrating solutions according to the following schedule:

Ethyl tertiary-butyl alcohol	70%	Overnight
Ethyl tertiary-butyl alcohol	85%	1 to 2 hours
Ethyl tertiary-butyl alcohol	95%	1 to 2 hours
Ethyl tertiary-butyl alcohol	100%	1 to 2 hours
Tertiary-butyl alcohol	100%	1 to 2 hours
Tertiary-butyl alcohol	100%	1 to 2 hours
Tertiary-butyl alcohol	100%	Overnight

The tissue was removed from the 100 per cent tertiary-butyl alcohol and placed for one hour in a vial containing a mixture of one-half paraffin oil and one-half tertiary-butyl alcohol. The plant tissue and a small amount of the alcohol-paraffin oil mixture were transferred to a vial one-half full of partially solidified Parowax. The vial was placed in a 56° C. embedding oven for a period of two to four hours. The tissue was then transferred to Parowax and during the next six hours transferred into two more changes of Parowax, then into

Table 1. Data obtained from the feeding combination of *Rhopalosiphum maidis* (Fitch), biotype KS-1 on White Martin.

	Replications				
Tissue number	29-A	29-C	29-D	37-C	39-D
Size of feeding area in square millimeters	6	5	6	10	5
Length of leaf in centimeters	22	14	14	21	14
Distance of feeding area from leaf whorl in centimeters	13	7	7	11	5
Feeding time in hours	48	48	48	48	48
Total number of aphids	10	10	10	5	5
Number of aphids per square millimeter	1.66	2.0	1.66	0.2	1.0
Total number of sections examined	134	119	132	240	180
Total number of stylet sheaths	25	21	20	22	6
Number of stylet sheaths per square millimeter	4.16	4.2	3.33	2.2	1.2
Number of stylet sheaths per aphid	2.5	2.1	2.0	4.4	1.2

Table 2. Data obtained from the feeding combination of *Rhopalosiphum maidis* (Fitch), biotype KS-1 on Piper Sudan 428-1.

	Replications				
Tissue number	35-B	35-D	40-B	40-C	40-D
Size of feeding area in square millimeters	10	10	7	7	5
Length of leaf in centimeters	26	26	17	16	10
Distance of feeding area from leaf whorl in centimeters	11	14	8	5	4
Feeding time in hours	48	48	48	48	48
Total number of aphids	10	10	5	5	5
Number of aphids per square millimeter	1.0	1.0	0.71	0.71	1.0
Total number of sections examined	176	159	240	224	138
Total number of stylet sheaths	33	22	10	39	23
Number of stylet sheaths per square millimeter	3.3	2.2	1.44	5.5	4.6
Number of stylet sheaths per aphid	3.3	2.2	2.0	7.8	4.6

Table 3. Data obtained from the feeding combination of Rhopalosiphum maidis (Fitch), biotype KS-2 on White Martin.

	Replications				
Tissue number	32-C	32-D	41-D	41-B	41-A
Size of feeding area in square millimeters	5	8	7	6	8
Length of leaf in centimeters	12	14	10	17	16
Distance of feeding area from leaf whorl in centimeters	5	6	4	8	6
Feeding time in hours	48	48	48	48	48
Total number of aphids	10	10	5	5	5
Number of aphids per square millimeter	2	1.25	0.71	0.83	0.62
Total number of sections examined	130	177	208	215	240
Total number of stylet sheaths	6	6	14	1	3
Number of stylet sheaths per square millimeter	1.2	0.75	2.0	0.16	0.37
Number of stylet sheaths per aphid	0.60	0.75	2.8	0.20	0.60

Table 4. Data obtained from the feeding combination of Rhopalosiphum maidis (Fitch), biotype KS-2 on Piper Sudan 428-1.

	Replications				
Tissue number	34-A	36-A	36-B	36-C	36-D
Size of feeding area in square millimeters	7	5	7	7	5
Length of leaf in centimeters	14	9	13	13	9
Distance of feeding area from leaf whorl in centimeters	6	5	6	6.5	5
Feeding time in hours	48	48	48	48	48
Total number of aphids	10	5	5	5	5
Number of aphids per square millimeter	1.42	1.0	0.71	0.71	1.0
Total number of sections examined	145	138	161	162	191
Total number of stylet sheaths	36	22	27	21	6
Number of stylet sheaths per square millimeter	5.14	4.4	3.85	3.0	1.2
Number of stylet sheaths per aphid	3.6	4.4	5.4	4.2	1.2

Tissuemat for one hour. The tissue was then embedded in Tissuemat and sectioned at a thickness of 12 microns.

Haupt's fixative was found to be the most acceptable for fastening the sections to the slides. It was prepared as follows:

Knox gelatin	1 gram
Phenol crystals	2 grams
Glycerin	15 cc.
Distilled water	100 cc.

The glass slides were cleaned with scouring powder, rinsed with distilled water and then dried with a lint free cloth. One small drop of Haupt's fixative was placed on each slide and smeared with the finger until the film was very thin. The sections were arranged on the slide and the slide was flooded with a two per cent solution of formalin. The slide was warmed gently on a slide dryer at a temperature of 46° C. until the paraffin sections were free of wrinkles and the formalin had evaporated. The slides were removed from the slide dryer, placed in slide boxes and allowed to dry at room temperature for several days.

Reversing the staining procedure of safranin O and Harris hematoxylin to hematoxylin and safranin O proved to be the most satisfactory and resulted in the following procedure:

Xylol I	10 min.
Xylol II	10 min.
Xylol + 95% ethyl alcohol (1:1) ...	10 min.

Ethyl alcohol 95%	3 min.
Ethyl alcohol 85%	3 min.
Ethyl alcohol 70%	3 min.
Ethyl alcohol 50%	3 min.
Ethyl alcohol 35%	3 min.
Harris hematoxylin	15 min.
Acid distilled water	30 sec.
Tap water (running)	20 min.
Ethyl alcohol 35%	3 min.
Safranin O solution	10 min.
Ethyl alcohol 50%	30 min.
Ethyl alcohol 70%	3 min.
Ethyl alcohol 85%	3 min.
Ethyl alcohol 95%	3 min.
Xylol + 95% ethyl alcohol (1:1) ...	5 min.
Xylol I	5 min.
Xylol II	5 min.

The Harris hematoxylin, safranin O and acid distilled water were made by the following formulae:

Harris Hematoxylin

Hematoxylin crystals	5 grams
Aluminum ammonium sulfate	3 grams
Ethyl alcohol 50% .....1000 cc.	

Dissolve with the aid of heat, then add six grams of mercuric oxide and boil for 30 minutes. Filter, bring to volume

with 50 per cent ethyl alcohol and acidify by adding one drop of concentrated hydrochloric acid to every 100 cubic centimeters of the solution.

Safranin O

Safranin O	2 grams
Ethyl alcohol 95%	100 cc.
Distilled water	100 cc.

The final concentration of this solution will be one per cent.

Acid Distilled Water

Distilled water	200.00 cc.
Concentrated hydrochloric acid ...	0.01 cc.

Comparative Number of Stylet Sheaths

In order to obtain an accurate count of the stylet sheaths, it was necessary to have a method of recording the location of each individual stylet sheath. This was accomplished with the aid of record sheets (Appendix 1). Each record sheet represented one slide and contained rows of rectangles which were equivalent to the tissue sections.

Five pieces of tissue from each of the four feeding combinations were examined for stylet sheaths. As each stylet sheath was located, its position in the section was recorded; and since the sections were cut at a thickness of 12 microns, often parts of one stylet sheath would be found in several

adjoining sections. These stylet sheath parts were connected by lines to show their relationship to each other. When all of the stylet sheaths were located and counted for each piece of tissue, the count was recorded (Tables 1,2,3 and 4).

Random Sampling of Stylet Sheaths

Due to the large number of stylet sheaths, it would have required a great deal of time to have examined each one separately. To shorten the procedure and still get an adequate sample, one stylet sheath from each of the twenty pieces of tissue was selected by random sampling, and examined in detail. This procedure was repeated three times; the information obtained about each stylet sheath being recorded on an especially prepared form (Appendix 2). This form listed all of the kinds of tissue that would be examined in the sections. The following information about each stylet sheath was recorded: if the stylet sheaths were non-branched, two-branched, or multiple-branched; intercellular or intracellular penetration by the stylets; if cell destruction was present or absent and where the stylet sheaths ended. One piece of tissue was found to have only one stylet sheath. To make up the deficit, stylet sheaths were picked at random from two other pieces of tissue in the same feeding combination.

Character of Stylet Sheaths: Non-branched, Two-branched or Multiple-branched Stylet Sheaths

A comparison was made to determine if the branching was related to either the biotypes or host plants. To record information as to whether the stylet sheaths were non-branched, two-branched or multiple-branched, it was necessary to establish a standard to which the stylet sheaths could be compared. A sheath that was non-branched would consist of the main stem only. If the main stem was divided into two branches, the sheath was considered to be two-branched; if there were more than two branches, the stylet sheath was considered to be multiple-branched. When the character of the individual stylet sheath was established the information was recorded (Tables 5,6, 7 and 8).

Objective of the Stylets

A count was made of the stylet sheaths that ended in the mesophyll in comparison with those that reached the vascular bundle. It was also necessary to establish a procedure to which the endings of the stylet sheaths could be compared. The branching of the stylet sheaths was not considered in this study. If a non-branched or a branched stylet sheath ended in the mesophyll the stylet sheath was considered to have ended in the mesophyll. However, if a non-branched stylet sheath reached the vascular bundle it was considered to have ended in the vascular bundle. In addition, if the stylet sheath was branched

Table 5. Data obtained from a random sample of 15 individual stylet sheaths made by Rhopalosiphum maidis (Fitch), biotype KS-1 on White Martin.

	Random Sample Number			
	1	2	3	Total
Stylet Sheaths:				
Non-branched	2	4	2	8
Two-branched	2	1	2	5
Multiple-branched	1	0	1	2
Stylet Sheath ended in:				
Mesophyll	2	1	2	5
Vascular bundle	3	4	3	10
Site of the feeding puncture:				
Between the vascular bundles	4	2	4	10
Over the vascular bundles	1	3	1	5

Table 6. Data obtained from a random sample of 15 individual stylet sheaths made by Rhopalosiphum maidis (Fitch), biotype KS-1 on Piper Sudan 428-1.

	Random Sample Number			
	1	2	3	Total
Stylet Sheaths:				
Non-branched	2	1	1	4
Two-branched	2	2	3	7
Multiple-branched	1	2	1	4
Stylet Sheath ended in:				
Mesophyll	4	0	2	6
Vascular bundle	1	5	3	9
Site of the feeding puncture:				
Between the vascular bundles	4	2	4	10
Over the vascular bundles	1	3	1	5

Table 7. Data obtained from a random sample of 15 individual stylet sheaths made by Rhopalosiphum maidis (Fitch), biotype KS-2 on White Martin.

	Random Sample Number			
	1	2	3	Total
Stylet Sheaths:				
Non-branched	2	3	5	10
Two-branched	3	2	0	5
Multiple-branched	0	0	0	0
Stylet Sheath ended in:				
Mesophyll	1	1	3	5
Vascular bundle	4	4	2	10
Site of the feeding puncture:				
Between the vascular bundles	5	3	1	9
Over the vascular bundles	0	2	4	6

Table 8. Data obtained from a random sample of 15 individual stylet sheaths made by Rhopalosiphum maidis (Fitch), biotype KS-2 on Piper Sudan 428-1.

	Random Sample Number			
	1	2	3	Total
Stylet Sheaths:				
Non-branched	1	1	1	3
Two-branched	1	2	3	6
Multiple-branched	3	2	1	6
Stylet Sheath ended in:				
Mesophyll	3	0	0	3
Vascular bundle	2	5	5	12
Site of the feeding puncture:				
Between the vascular bundles	3	2	2	7
Over the vascular bundles	2	3	3	8

and one branch ended in the mesophyll and one branch reached the vascular bundle, the stylet sheath was considered to have ended in the vascular bundle.

Site of the Feeding Puncture

A count of the stylet sheaths that entered the leaf between the vascular bundles or over the area of the vascular bundle was made to ascertain whether the feeding punctures were made at random or if there were evidences that the aphids might be able to determine the type of tissue beneath the leaf surface. The distance between the small vascular bundles, where most of the feeding occurred, was approximately equal to the diameter of the vascular bundles.

Penetration of the Stylets and Cell Damage to Internal Tissue

The stylet sheaths of the four feeding combinations that were picked at random were examined for intercellular or intracellular penetration and for cell damage.

Since the stylet sheath appeared to be situated between the cells it was assumed that intercellular penetration had occurred. If there appeared to be some cell damage, it was assumed that intracellular penetration had occurred, or a possible reaction had taken place following diffusion of materials from the insect through the cell wall as the stylet penetrated between the cells.

RESULTS

Comparative Number of Stylet Sheaths

The number of stylet sheaths formed per aphid was determined and recorded (Tables 1, 2, 3 and 4). For each of the four feeding combinations, the average number of stylet sheaths was determined. It was found that biotype KS-1 on White Martin had an average of 2.44 stylet sheaths per aphid and on Piper Sudan 428-1 an average of 3.98 stylet sheaths per aphid. Biotype KS-2 on White Martin had an average of 0.99 stylet sheaths per aphid and on Piper Sudan 428-1 3.76 stylet sheaths per aphid (Table 9). It is possible that all of the aphids of biotype KS-2 on White Martin did not feed, resulting in the low average.

Character of Stylet Sheaths: Non-branched, Two-branched or Multiple-branched Stylet Sheaths

Out of a total of 60 stylet sheaths examined from the four feeding combinations, it was found that biotype KS-2 on White Martin had ten non-branched, five two-branched and no multiple-branched; biotype KS-1 on White Martin had eight non-branched, five two-branched and two multiple-branched; biotype KS-2 on Piper Sudan 428-1 had three non-branched, six two-branched and six multiple-branched; and biotype KS-1 on Piper Sudan 428-1 had four non-branched, seven two-branched and four multiple-branched (Tables 5, 6, 7 and 8). There was a total of 25 non-branched, 23 two-branched and 12 multiple-branched stylet sheaths.

Table 9. Mean number of stylet sheaths per aphid found after the feeding of each of two biotypes of corn leaf aphid on each of two hosts for 48 hours.

Biotypes	:	Host Plants: White Martin		: Piper Sudan 428-1
KS-1		2.44	n.s.	3.98
		n.s.		n.s.
KS-2		0.99	*	3.76

* Significant beyond 0.5% level. L.S.D. = 2.18
n.s. Not significant

Objective of the Stylets

A study of biotype KS-2 on Piper Sudan 428-1 showed that three stylet sheaths ended in the mesophyll and 12 stylet sheaths reached the vascular bundle; with biotype KS-2 on White Martin, five stylet sheaths ended in the mesophyll and ten stylet sheaths reached the vascular bundle. In the case of biotype KS-1 on Piper Sudan 428-1, six stylet sheaths ended in the mesophyll while nine reached the vascular bundle; and with biotype KS-1 on White Martin, five stylet sheaths ended in the mesophyll and ten reached the vascular bundle (Tables 5, 6, 7 and 8). A total of 19 stylet sheaths ended in the mesophyll and 41 stylet sheaths reached the vascular bundles.

Site of the Feeding Puncture

A total of 60 stylet sheaths were examined from the four feeding combinations and the counts were as follows: biotype KS-2 on Piper Sudan 428-1 had seven stylet sheaths which

penetrated the leaf between the vascular bundles and eight stylet sheaths which penetrated the leaf over the vascular bundle; biotype KS-2 on White Martin had nine stylet sheaths which penetrated the leaf between the vascular bundles and six stylet sheaths which penetrated the leaf over the vascular bundle; biotype KS-1 on Piper Sudan 428-1 had ten stylet sheaths which penetrated the leaf between the vascular bundles and five stylet sheaths which penetrated the leaf over the vascular bundle; and biotype KS-1 on White Martin gave ten stylet sheaths which penetrated the leaf between the vascular bundles and five stylet sheaths which penetrated the leaf over the vascular bundle (Tables 5,6,7,8). Totaling the counts, it was found that 36 stylet sheaths entered the leaf between the vascular bundles and 24 stylet sheaths entered the leaf over the vascular bundle.

Penetration of Stylets and Cell Damage to Internal Tissue

The penetration of the tissue by the stylets of the four feeding combinations appeared to be somewhat similar.

The stylets appeared to have penetrated the epidermis intercellularly, while the mesophyll and bundle sheath seemed to be penetrated both intercellularly and intracellularly. There apparently was intracellular penetration of the xylem. It was difficult at times to determine the type of cell penetration of the phloem due to the small size of the sieve cells and companion cells; but it was assumed that intracellular penetration had occurred.

There seemed to be some variation in cell damage in the four feeding combinations, although there was no apparent cell damage

in the epidermis.

Studying biotype KS-1 on White Martin and Piper Sudan 428-1, cell damage was apparent in the mesophyll, bundle sheath and phloem. The xylem did not have any visible cell damage; however, some of the smaller xylem vessels appeared blocked in Piper Sudan 428-1.

Biotype KS-2 on White Martin apparently caused cell damage in the mesophyll and phloem, while the bundle sheath and xylem did not appear to have any cell damage. On Piper Sudan 428-1 cell damage was apparent in the mesophyll, bundle sheath, xylem and phloem. There appeared to be some blockage of the xylem and phloem cells.

DISCUSSION

Comparative Number of Stylet Sheaths

A statistical comparison of the averages of the stylet sheath counts was made. No significant difference was found between the means of the following: biotype KS-1 on Piper Sudan 428-1 and White Martin, biotype KS-1 and biotype KS-2 on Piper Sudan 428-1, or biotype KS-1 and biotype KS-2 on White Martin. Significant differences were found between the means of biotype KS-2 on Piper Sudan 428-1 and White Martin (Table 9).

This indicates that the mean number of stylet sheaths produced by biotype KS-1 does not differ significantly when these insects are fed on Piper Sudan 428-1 compared to White Martin, but the mean number of stylet sheaths made by KS-2 does differ significantly when Piper Sudan 428-1 as a host is compared with

White Martin. The observed difference in the mean number of sheaths when the two biotypes are compared is not significant for either Piper Sudan 428-1 or White Martin but approaches significance with White Martin.

Character of Stylet Sheaths: Non-branched, Two-branched or Multiple-branched Stylet Sheaths

When grouped there were totals of 25 non-branched, 23 two-branched and 12 multiple-branched stylet sheaths. Chi square gave a value of 12.39, which with six degrees of freedom would be a probability of 0.05 plus (Table 10).

Table 10. The numbers of stylet sheaths classified as to character of branching made by the two biotypes of corn leaf aphid on two host plants.

	Biotype KS-1		Biotype KS-2		
Character of stylet sheath	White Martin	Piper Sudan 428-1	White Martin	Piper Sudan 428-1	Totals
Non-branched	8	4	10	3	25
Two-branched	5	7	5	6	23
Multiple-branched	2	4	0	6	12

Chi square = 12.39

Degrees of freedom = 6

Probability = 0.05 plus

The counts of sheaths of the biotype KS-1 in Piper Sudan 428-1 and White Martin were combined, and the same was done with KS-2 on Piper Sudan 428-1 and White Martin. Chi square gave a value of 0.09 plus, which with two degrees of freedom would be a probability of 0.95 (Table 11). That is, the character of the branching of the stylet sheaths made by the two biotypes did not differ more than might be expected due to chance.

The counts of the stylet sheaths made by the two biotypes on each host plant were combined as to host plant. Chi square gave a value of 10.56, which with two degrees of freedom would be a probability of 0.005 (Table 12). This indicated that the

Table 11. The numbers of stylet sheaths on White Martin and Piper Sudan 428-1 classified as to character of those made by each biotype.

Character of stylet sheaths	Biotype KS-1	Biotype KS-2	Totals
Non-branched	12	13	25
Two-branched	12	11	23
Multiple-branched	6	6	12

Chi square = 0.09

Degrees of freedom = 2

Probability = greater than 0.95

Table 12. The numbers of stylet sheaths of the corn leaf aphid found on the two hosts when classified as to kind of branching.

Character of stylet sheaths	Host Plants: White Martin	Piper Sudan 428-1	Totals
Non-branched	18	7	25
Two-branched	10	13	23
Multiple-branched	2	10	12

Chi square = 10.56

Degrees of freedom = 2

Probability = 0.005

character of branching of the sheaths was not the same on the two hosts.

The numbers of stylet sheaths produced by the four feeding combinations were studied separately, but with the two-branched and multiple-branched records combined, making a total of 25 non-branched compared with 35 branched stylet sheaths. Chi square gave a value of 8.98, which with three degrees of freedom would be a probability of less than 0.025 (Table 13). That is, there are differences between the two-branched and multiple-branched stylet sheaths.

The larger number of non-branched stylet sheaths made by both biotypes in White Martin may possibly indicate less

Table 13. The numbers of stylet sheaths of the two biotypes of corn leaf aphid on two host plants with the numbers of branched contrasted with the non-branched records.

Character of stylet sheath	Biotype KS-1		Biotype KS-2		Totals
	White Martin	Piper Sudan 428-1	White Martin	Piper Sudan 428-1	
Non-branched	8	4	10	3	25
Branched	7	11	5	12	35

Chi square = 8.98

Degrees of freedom = 3

Probability = less than 0.025

repellent material, and therefore, the number of branches caused by false starts and endings as compared to both biotypes on Piper Sudan 428-1 is reduced. The pH gradient (Fife and Frampton 1936) or other gradient may be more definite in White Martin, enabling the aphids to locate the phloem more easily.

A comparison between the number of feeding punctures and the branching of the stylet sheaths revealed a similarity between the low number of feeding punctures and the number of unbranched stylet sheaths of biotype KS-2 on White Martin. Returning to the theory of the pH gradient (Fife and Frampton 1936) or other gradient, it is possible that the biotype KS-2 is more sensitive than KS-1 to such a gradient. This would

possibly explain the lower number of feeding punctures and higher number of unbranched stylet sheaths of biotype KS-2 on White Martin.

Objective of the Stylets

A statistical analysis of the counts of the four feeding combinations was made. Chi square had a value of 1.46, which with three degrees of freedom gave a probability of somewhat greater than 0.50 (Table 14). This would indicate no significance between the four classifications in the objective of the stylets.

Considering that these counts obtained from the four feeding combinations were simply Rhopalosiphum maidis (Fitch) on Sorghum vulgare Pers. and using the hypothesis that the aphids are equally likely to feed in the mesophyll and the vascular bundle, chi square was found to have a value of 8.07, which with one degree of freedom gave a probability of less than 0.01. This would cause the hypothesis to be rejected, indicating the vascular bundle was the objective of the stylets.

The counts of the stylet sheaths reaching the vascular bundle showed that biotype KS-2 reached the vascular bundle slightly more frequently than biotype KS-1 on Piper Sudan 428-1. This may indicate that biotype KS-2 is more able to adapt itself to any repellent material which may be in the vascular bundle and not in the mesophyll of Piper Sudan 428-1.

Table 14. The numbers of the stylet sheaths of the two biotypes of the corn leaf aphid that ended in the mesophyll or reached the vascular bundle on two host plants.

Objective of stylet sheath	White Martin : Biotype KS-1	White Martin : Biotype KS-2	Piper Sudan 428-1 : Biotype KS-1	Piper Sudan 428-1 : Biotype KS-2	Totals
Mesophyll	5	5	6	3	19
Vascular bundle	10	10	9	12	41

Chi square = 1.46

Degrees of freedom = 3

Probability = greater than 0.50

Site of the Feeding Puncture

An analysis was made of the counts of stylets which penetrated between the vascular bundles compared with those which started over the vascular bundle. Chi square was found to have a value of 1.11, which with one degree of freedom would show a probability of somewhat greater than 0.25 (Table 15). This would suggest that the aphids are unable to determine the type of tissue beneath the surface of the leaf, and that the feeding punctures were made at random; also that the biotypes do not differ in this regard although biotype KS-2, having fewer feeding punctures between the vascular bundles, may have been slightly better able to distinguish between tissues than biotype KS-1.

Table 15. The number of stylet sheaths penetrating between the vascular bundles or over the area of the vascular bundle, combined as to biotype.

Site of feeding puncture	Biotype KS-1	Biotype KS-2	Total
Between the vascular bundles	20	16	36
Over the area of vascular bundle	10	14	24

Chi square = 1.11

Degrees of freedom = 1

Probability = greater than 0.25

Penetration of Stylets and Cell Damage to the Internal Tissue

The method of tissue penetration being highly similar for the four feeding combinations would possibly indicate that the structures of the host plant were not the cause of the feeding variation.

The cell damage that was observed indicates that some secretions from the insect enter the plant. Biotype KS-1 caused cell damage in the mesophyll, bundle sheath, and phloem on both host plants. Biotype KS-2 caused cell damage in the mesophyll and phloem on White Martin and in the mesophyll, bundle sheath, xylem, and phloem on Piper Sudan 428-1. This

may be related in some way to the fact that biotype KS-2 has a better survival and reproductive capacity on the resistant host than biotype KS-1.

This study gives some explanation of information that has been obtained from previous studies. The fact that the stylet sheaths formed by KS-2 on Piper Sudan 428-1 reached the vascular bundle more often than KS-1 on Piper Sudan 428-1 may be related to Cartier and Painter's (1956) findings that biotype KS-2 had a greater survival and reproductive capacity on the resistant sorghums than biotype KS-1. It is also in agreement with the findings of Pathak and Painter (1957) who concluded that biotype KS-2 took in more food from the resistant plants than did the other biotypes.

The studies cited (Kennedy and Mittler 1953) suggested that aphids may receive liquids from plants without using their pharyngeal pump. If so, it comes probably from the phloem due to pressure in the vascular system. Such pressure should be much less from the mesophyll tissue.

SUMMARY

Twenty pieces of tissue were sectioned, stained, and examined for stylet sheaths.

The histological procedure consisted of fixation with formalin-aceto-alcohol, the paraffin method of embedding and the use of tertiary-butyl alcohol as a dehydrating medium. The tissue was stained with Harris hematoxylin and safranin O.

A total of 373 stylet sheaths were found in the 3,129 sections that were examined. Sixty of these stylet sheaths were picked by random sampling and studied in detail.

Some of the points studied consisted of comparative records of the number of stylet sheaths; whether they were non-branched, two-branched, or multiple-branched; objective of the stylets; and site of the feeding puncture for each combination of biotype and host.

Additional information was obtained on the type of penetration by the stylets and possible damage to internal tissues. The intercellular and intracellular penetration of the leaf tissue by the stylets was somewhat similar for both biotypes.

The number of stylet sheaths formed per aphid was not related to the resistance or susceptibility of the host plant with biotype KS-1, but was with biotype KS-2. Therefore, there was a difference in the biotypes in this respect.

The branching of the stylet sheaths was not influenced by which biotypes made them nor by the host plant when resistant, but was different when made by different biotypes on the susceptible host.

The conclusion was reached that the vascular bundle was definitely the objective of the stylets.

The feeding punctures were made at random, therefore, the aphids appeared to be unable to determine the type of tissue beneath the surface of the leaf.

Biotype KS-1 caused damage in the mesophyll, bundle sheath and phloem on both White Martin and Piper Sudan 428-1, while biotype KS-2 not only caused possible damage to the mesophyll and phloem in White Martin but also to the mesophyll, bundle sheath, xylem and phloem in Piper Sudan 428-1.

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APPENDIX

EXPLANATION OF APPENDIX 1

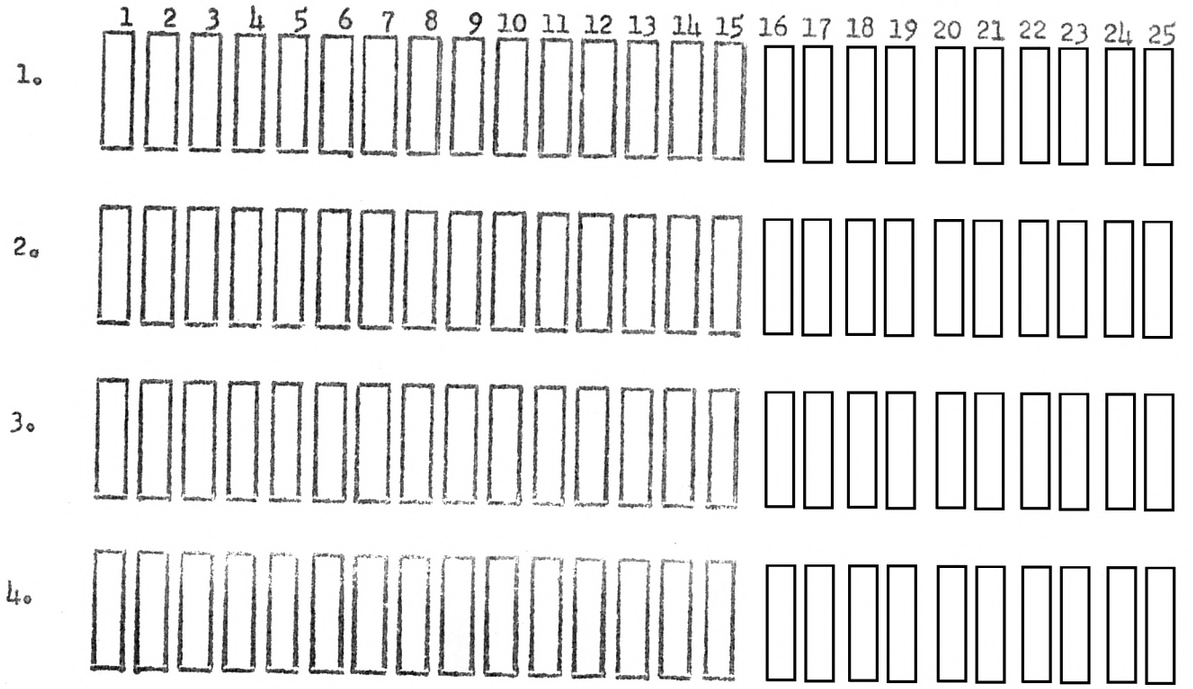
Form used to represent an individual slide upon which were recorded the position of each individual stylet sheath found in each section represented by the rectangles.

Date_____
Slide No._____
Host Plant_____
Biotype_____
_____
_____

Stylet Sheaths:

1. Red------Non-branching
2. Blue-----Branching
3. Green---Multiple Branching

Row No. Section Number



Comments:

EXPLANATION OF APPENDIX 2

Form used to record data obtained from studying individual stylet sheaths of *Rhopalosiphum maidis* (Fitch) on *Sorghum vulgare* Pers.

Tissue No.	Slide	
Slice No.		
Row No.		
Section No.		
Stylet No.		
Non-branched		Stylet Sheaths
Branched		
Mult.-branched		
Yes	Stained	
No		
Intercellular	Stylets Penetrated	Epidermal Cells
Intracellular		
Yes	Cells Destroyed	
No		
Yes	Stylet Ends	
No		
	Stomate	
Intercellular	Stylets Penetrated	Motor Cells
Intracellular		
Yes	Cells Destroyed	
No		
Yes	Stylet Ends	
No		
Intercellular	Stylets Penetrated	Sclerenchyma Cells
Intracellular		
Yes	Cells Destroyed	
No		
Yes	Stylet Ends	
No		
Intercellular	Stylets Penetrated	Collenchyma Cells
Intracellular		
Yes	Cells Destroyed	
No		
Yes	Stylet Ends	
No		
Intercellular	Stylets Penetrated	Mesophyll Cells
Intracellular		
Yes	Cells Destroyed	
No		
Yes	Stylet Ends	
No		
Intercellular	Stylets Penetrated	Bundle Sheath
Intracellular		
Yes	Cells Destroyed	
No		
Yes	Stylet Ends	
No		
Intercellular	Stylets Penetrated	Xylem
Intracellular		
Yes	Cells Destroyed	
No		
Yes	Stylet Ends	
No		

EXPLANATION OF APPENDIX 2 (concl.)

Form used to record data obtained from studying individual stylet sheaths of Rhopalosiphum maidis (Fitch) on Sorghum vulgare Pers.

Tissue No.	Slide	
Slide No.		
Row No.		
Section No.		
Stylet No.		
Intercellular	Stylets Penetrated	Phloem
Intracellular		
Yes	Cells Destroyed	
No		
Yes	Stylet Ends	
No		
Intercellular	Stylets Penetrated	Mid-rib
Intracellular		
Yes	Cells Destroyed	Mesophyll
No		
Yes	Stylet Ends	
No		

A COMPARATIVE STUDY OF THE MORPHOLOGY OF THE FEEDING OF THE TWO BIOTYPES OF THE CORN LEAF APHID, RHOPALOSIPHUM MAIDIS (FITCH), ON RESISTANT AND SUSCEPTIBLE SORGHUMS

by

PHILIP BONE MORGAN

B. S., Fort Hays Kansas State College, 1950

AN ABSTRACT OF A THESIS

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MASTER OF SCIENCE

Department of Entomology

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OF AGRICULTURE AND APPLIED SCIENCE

1957

The purpose of this study was to examine the differences in the feeding punctures of biotype KS-1 and biotype KS-2 of the corn leaf aphid, Rhopalosiphum maidis (Fitch), on resistant and susceptible sorghums. This study was a continuation of the work that has been done with the two biological races and Piper Sudan 428-1.

The aphids were allowed to feed in an area one millimeter wide and ten millimeters long. Twenty pieces of tissue were sectioned, stained, and examined for stylet sheaths.

The histological procedure consisted of fixation with formalin-aceto-alcohol, the paraffin method of embedding and the use of tertiary-butyl alcohol as a dehydrating medium. The tissue was stained with Harris hematoxylin and safranin O.

A total of 373 stylet sheaths were found in the 3,129 sections that were examined. Sixty of these stylet sheaths were picked by random sampling and studied in detail.

Some of the points studied consisted of comparative records of the number of stylet sheaths; whether they were non-branched, two-branched, or multiple-branched; objective of the stylets; and site of the feeding puncture for each combination of biotype and host.

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