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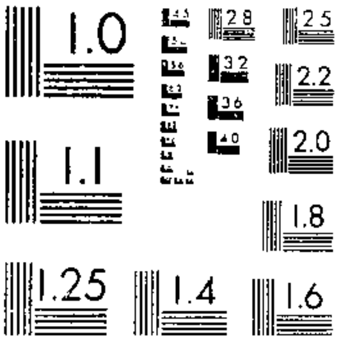

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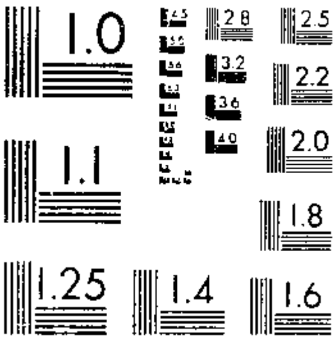

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FEEDING PUNCTURES OF MIRIDS AND OTHER PLANT-SUCKING INSECTS AND THEIR EFFECT ON COTTON

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INTRODUCTION

Observations made in Texas in 1924, after the discovery of an infestation of cotton with the cotton flea hopper (*Psallus seriatus* Reut.), aroused the suspicion that this insect was the carrier of a disease producing a serious disorder of the cotton plant.[2] In severely injured plants the very small squares may be almost completely destroyed, and marked changes in growth characteristics may occur. The studies on which this bulletin is based were begun at Tallulah, La., in 1927, for the purpose of obtaining more exact information as to the character of the damage produced by the cotton flea hopper and other plant-sucking insects.

EARLY OBSERVATIONS AND EXPERIMENTS

Previous field and laboratory work had shown that, in addition to the more general symptoms of injury, small swellings were frequently produced on the stems and leaf petioles of the cotton plants infested with Psallus. These characteristic lesions offered a ready means of identifying cotton flea hopper damage, and a series of experiments had been made to determine whether similar lesions could be caused by artificial inoculations with material from crushed insects or injured plant tissues. Painter,[3] using fine needle points,

[1] The authors are indebted to R. L. McGarr and E. R. Tinkham for assistance in the field work during 1928 and 1929.

[2] HUNTER, W. D. THE SO-CALLED COTTON FLEA. Jour. Econ. Ent. 17: 604. 1924.
——— THE COTTON HOPPER OR SO-CALLED "COTTON FLEA." U. S. Dept. Agr. Circ. 361, 15 p., illus. 1926.

[3] PAINTER, R. H. A STUDY OF THE COTTON FLEA HOPPER, PSALLUS SERIATUS REUT., WITH ESPECIAL REFERENCE TO ITS EFFECT ON COTTON PLANT TISSUES. Jour. Agr. Research 40: 485-516, illus. 1930.

reported successful inoculations from crushed insects, but the writers' experiments with similar methods were not conclusive, the punctures with sterile needles often showing as much damage as those made with the crushed insects. Even needle points ground as finely as possible are still very much larger than the piercing parts of the insect's proboscis and almost always cause a reaction in the plant due to the mechanical injury. Pending the development of a more suitable inoculation technic, feeding tests with live insects were begun in 1927, and the present bulletin is restricted to the results obtained by this method of experimentation.

LESIONS CAUSED BY THE FEEDING OF INDIVIDUAL INSECTS IN TESTS

If the lesions in the cotton plant were due to a pathogenic organism transmitted by insects, it was thought highly improbable that all individuals of the species involved would prove to be infective. The first of the present experiments were therefore designed to determine what proportion of the flea hoppers might cause the swellings. In the first tests each specimen was incaged in a small vial attached to a cotton stem or leaf petiole and watched until it began and finished feeding. When the proboscis was withdrawn the place was marked and examined daily for development of a lesion. If the same specimen could be induced to feed several times, each place was tagged separately. As this method of handling and watching the separate insects proved to be tedious and time-consuming, the vials were later fastened to the plants by means of a narrow strip of adhesive tape (Pl. 1, A) and left attached to one point for a minimum period of about 24 hours. Since in warm weather the majority of these insects must feed one or more times in order to survive as long as 24 hours, this procedure was finally adopted for most of the routine work. Part of the experiments were carried out where general insect infestation was light on unprotected cotton, but, in order to eliminate possible complications of the results from feedings by insects other than those in the tests, the major part of the work during this and the following seasons was done with protected plants grown in large screen cages.

In the first series of 24-hour feedings, and also in those which were watched continuously, only a part of the feeding points showed typical swellings within two or three days while others remained entirely negative externally. This evidence, therefore, tended to support the idea of an infective agent. Some of this plant material, however, was prepared for microtomic sectioning, and a careful study of the stained serial sections at the end of the season showed that nearly all the punctures which were negative externally had produced a condition of the internal tissues less extensive but otherwise similar to that found in the definite swellings. In the discussion of the experiments those feeding points will be described as negative where no injury, either external or internal, developed.

The results obtained may be indicated by the following records selected from this series: One nymph applied to seven different places on a cotton plant for about 24 hours each (developing to an adult in the meantime and dying at the last place) caused no external swellings. Six of these places, upon being sectioned, were found to have internal lesions, and one was doubtful. Another nymph applied the same number of times caused one doubtful injury and six external swellings, all of which were positive upon being sectioned. No external swellings were observed in the case of one female which was observed to feed at three different places and which was afterwards left in a vial attached to the plant for 24 hours. Upon sectioning, internal lesions were found in the tissues in all three places where the specimen had been observed to feed and a questionable lesion in the place where it was confined for 24 hours. This specimen was left attached for a fifth feeding but died before the end of the 24-hour period. A male with the same history caused five external swellings, having fed before dying at the last place. From the tests in the series for 1927, which were ultimately checked up by microscopical examination of sectioned plant material, there was only one individual that produced no identifiable reaction. This one (a nymph) had been observed to puncture the plant in three places.

METHODS EMPLOYED FOR IDENTIFICATION AND STUDY OF LESIONS

The results of this series showed, for one thing, that external swellings could not be relied upon for identifying the damage. During 1928 it was found after a number of trials that comparatively thick free-hand sections stained with safranin provided a rapid and fairly satisfactory method of determining the presence or absence of internal lesions, and this method was employed in much of the routine examination thereafter. When a more careful study was desired, the material was imbedded in paraffin by the usual cytological methods and sectioned with a rotary microtome.

Though various standard killing and fixing reagents were tried, formalin-acetic acid-alcohol was employed as the fixative for most of the material, largely because of its convenience, since it could be used as a preservative as well as a fixative. Free-hand sections from 100 to 200 microns in thickness were cut with the aid of a hand microtome, transferred to the formalin-acetic acid-alcohol fixative, and stained in safranin. The paraffin-imbedded material was cut 10 to 12 microns thick, the sections mounted serially, and though various stains were tested, the material was stained for the most part with Flemming's triple stain, iron haematoxylin, or safranin and light green.

SPECIES INCLUDED IN THE EXPERIMENTS OF 1928

Four other species of Miridae in addition to *Psallus seriatus* were included for comparative observations in the experiments of 1928. Two of these, *Lygus pratensis* (L.) and *Adelphocoris rapidus* (Say), have been definitely connected with field damage to cotton, whereas the other two, *L. apicalis* Fieb. and *Poeciloscytus basalis* Reut., are very seldom found on this host plant.[4] Individuals of each of these species were tested on cotton stems and leaf petioles by the methods previously described and were found to cause lesions which were

[4] EWING, K. P. EFFECTS ON THE COTTON PLANT OF THE FEEDING OF CERTAIN HEMIPTERA OF THE FAMILY MIRIDAE. Jour. Econ. Ent. 22: 761-765, illus. 1929.

indistinguishable from those of *Psallus seriatus*, although the average severity of the damage varied somewhat among the different species.

CHARACTER OF MIRID LESIONS

Small series of experiments in 1927, as well as many others in 1928 and 1929, proved that individuals of the same species vary greatly as to the effect of their feeding punctures, though consecutive feeding punctures of the same individual produced lesions of fairly uniform size. In stained sections the typical mild lesion is usually found to be confined to the cortex or to the cortex and collenchyma (pl. 1, B), and the affected cells are circularly arranged about the center. The terms "lesion" and "hopper damage" have been applied to all such affected areas although in the smaller forms there may be no evidence of any destruction of tissue. The cells are increased in number, often enlarged, and usually stain more densely than those in the normal surrounding tissue. The nuclei are large and prominent, and the general appearance is one of cell activity with more or less distortion due to pressure. From the smallest lesion, in which only a half dozen or so cells are affected, all stages may be encountered up to the most severe forms in which a half or more of the stem or petiole has been affected and a good part of the tissue has been destroyed. (Pl. 2, A and B.)

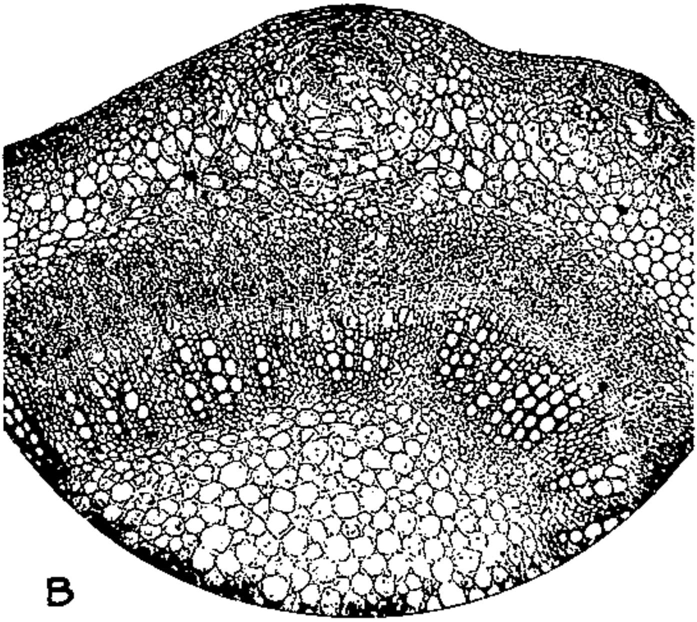
As the area involved becomes larger, collenchyma and epidermis are forced outward to form the swellings which appear externally.[5] When the pressure becomes too great the swellings split open lengthwise of the stem (pl. 2, B), and the exposed tissues on dying form grayish or brownish scars. When the punctures are comparatively deep, extending close to the vascular tissues or into the pith, the swellings may merely compress the tissues internally, and fairly large areas have been noted which did not show at all externally.

In medium-sized lesions the pressure from the increase and enlargement of the cells causes much cell distortion, progressing to the point where the central ones are apparently ruptured. In larger lesions a considerable central area of broken-down cells may be thus involved. (Pl. 2, A.) The remains of cell walls and contents form a conglomerate mass staining brown or red with the stains employed.

That cell multiplication occurs and is involved in the formation of the swellings is shown by the finding of nuclei in various stages of mitosis in the younger lesions. The smaller lesions and those in early stages of development are recognized in the stained sections principally by the large, prominent nuclei in a small group of cells, some of the cells themselves also being enlarged and irregular in outline. (Pl. 2, B.) Iron haematoxylin stains are especially good in bringing out the nuclei. The cell contents are not well preserved in free-hand sections and in these the identification of the smaller lesions depends on the distortion of the cells and deeper staining of the affected area.

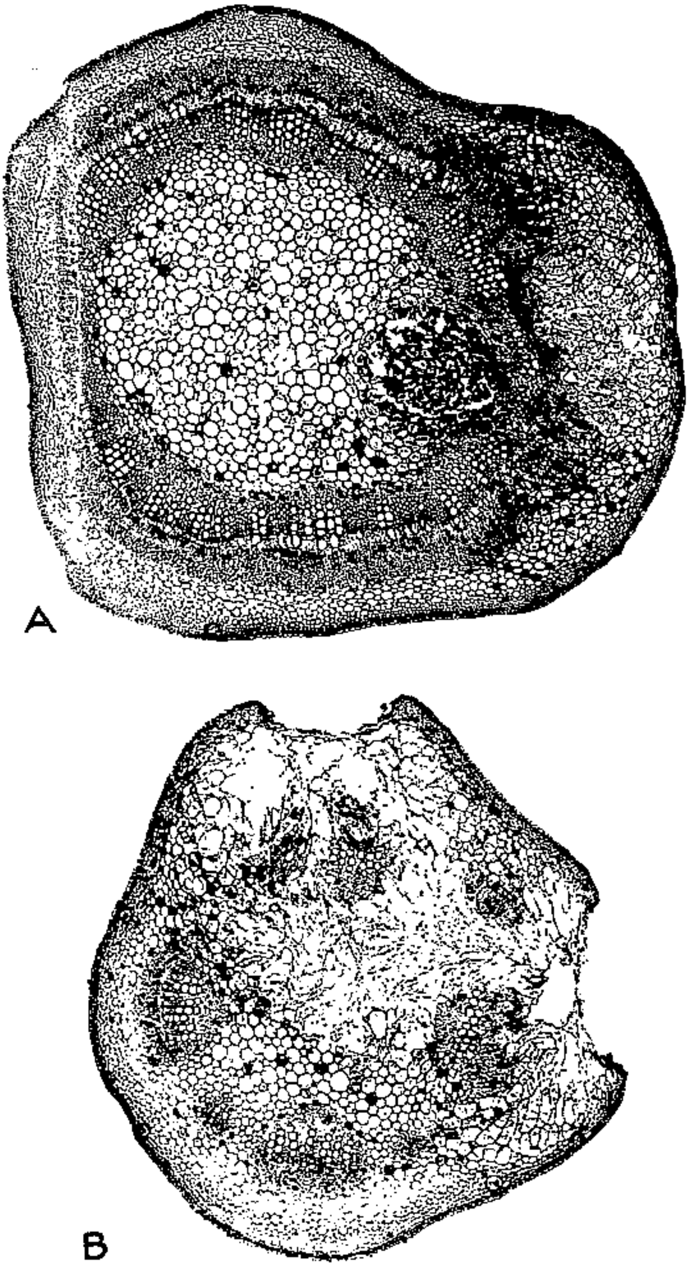
The external swellings usually appear on the second or third day, occasionally on the fourth or fifth. There is one record of a *Lygus pratensis* causing split lesions which appeared in two instances on

[5] In the great majority of cases the swellings from single punctures are not more than 2 or 3 mm. in diameter. An idea of the usual range in size may be gained from the two sections shown in Plates 1, B, and 2, A.



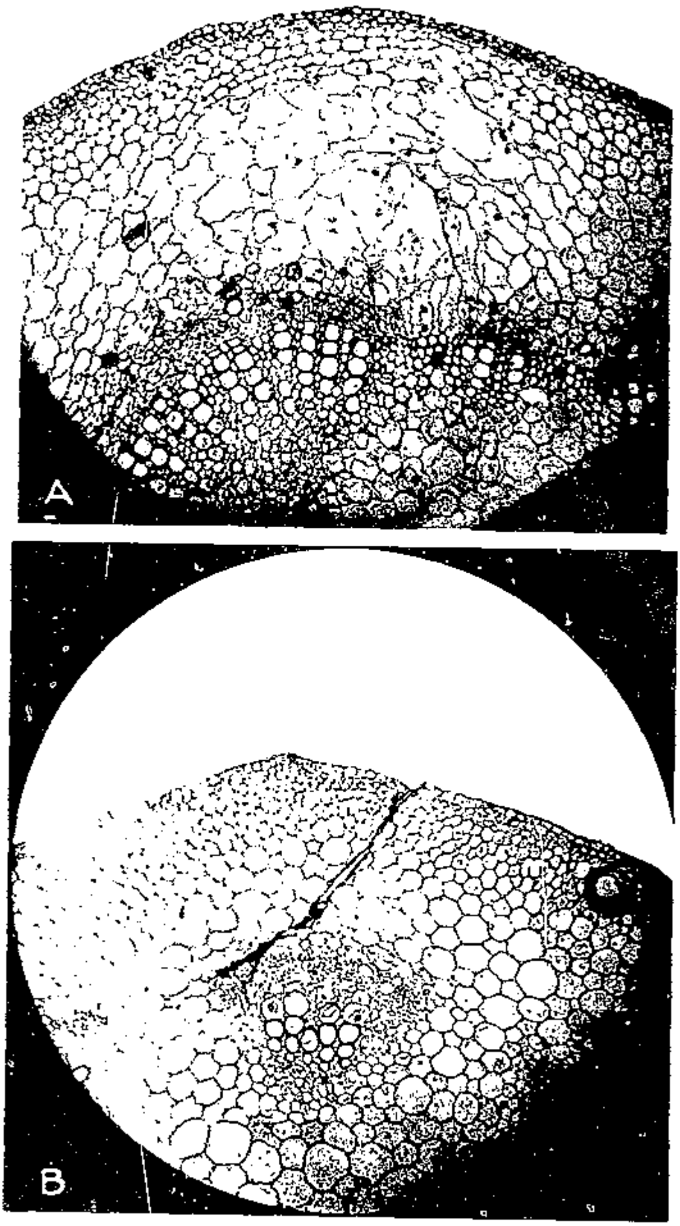
FEEDING PUNCTURES OF INSECTS

A, Glass vial attached to a fruiting branch on a cotton plant. In each vial an insect was confined so it could feed upon a small portion of the stem. A piece of cotton lint or absorbent cotton on each side where the rim does not come in contact with the stem prevented the escape of the insect. B, A comparatively mild lesion confined to the cortex and collenchyma of a cotton stem. This was caused by an observed feeding of an adult of *Psallus seriatus* and produced a slight swelling which was visible externally. The material was preserved for sectioning five days after the feeding. Flemming's triple stain was used. × 50.



SERIOUS INJURIES CAUSED BY THE COTTON FLEA HOPPER

A, A severe lesion extending from the collenchyma to the pith of a cotton stem with some of the vascular tissue affected and with an area of completely broken-down cells in the pith. Caused by an adult kept in the feeding vial 24 hours. The material was preserved for sectioning six days after the attachment of the vial. Flemming's triple stain was used. × 28. B, Two large split lesions in a leaf petiole with over half of the petiole damaged. Caused by a male confined on the plant for 24 hours. Safranin and light green stain used. × 28.



A, A lesion in the cortex of a petiole showing the enlarged cells and nuclei. With a higher magnification one dividing nucleus was seen in this section. The lesion was caused by an adult *Lygus apicalis* confined to the petiole for 24 hours. The material was preserved for sectioning five days afterwards, but the appearance is more typical of the earlier stages of development. Stained with iron haematoxylin. × 92. B, Path of the feeding puncture of an adult *Stictocephala festina* in a leaf petiole. The "sheath" shows a short branch at the tip into the phloem of a vascular bundle. The insect was observed to feed for 30 minutes, and the material was preserved for sectioning six hours later. No cell reaction had as yet developed in the adjacent tissue. Stained with Flemming's triple stain. × 92.

the day following the feeding. These were from watched feedings on protected cotton. The swellings usually reach their maximum size before the fifth day.

A study of the early development of the lesions has been made from plant material killed and fixed for imbedding at various intervals following observed feedings. Preparations from material, made immediately after the punctures were inflicted and 6 and 12 hours thereafter, have not shown definite indications of cell reaction. In several specimens a few enlarged nuclei and in one specimen a dividing nucleus were noted 24 hours after the punctures were made. One preparation, made 24 hours after a puncture from *Lygus apicalis*, showed a comparatively well-developed area in the pith involving about 30 cells (pl. 3, A), whereas nothing definite was found in certain 36-hour and even 48-hour specimens. This series was small and it is probable that some of the latter would not have developed any positive damage.

The lesions may start in almost any part of the tissues. As previously mentioned, the milder forms usually occur in the cortex, but the puncture may go entirely through the vascular tissue and centralize in the pith or, in leaf petioles, in which there is not a complete vascular cylinder, the center may be found between the vascular bundles. Other punctures seem to be very shallow, and the center of the lesion appears in the collenchyma with the affected cells spreading out fanwise into the cortex. In larger swellings the vascular cylinder or a vascular bundle may be involved in the swelling with a portion of it distorted or completely destroyed, and a few instances have been noted in which the center of the lesion seemed to be in the vascular tissue itself.

Injury from the pressure of the rim of the vial has been observed in some instances. This appears as slight depressions or as blistered spots but on sectioning does not extend much below the epidermis.

SPECIES INCLUDED IN THE EXPERIMENTS OF 1929

During 1929 in an effort to find a plant-sucking species which would not cause such lesions in cotton, two more species of Hemiptera and three species of Homoptera that are commonly collected in sweepings from weeds or cotton were added to the experiments. One of the five, a lygaeid, *Geocoris punctipes* (Say), is said to be predacious on other insects and normally not a plant feeder. Another was *Nysius californicus* Stål, a species of the same family. Two of the species, *Homalodisca triquetra* (Fab.) and *Graphocephala versuta* (Say), are cicadellids, and the fifth species, *Stictocephala festina* (Say), is a membracid.

EXPERIMENTAL RESULTS WITH DIFFERENT SPECIES

A summary of the comparative results obtained with the 10 species is given in Table 1. The feedings were all made in the same way by attaching vials containing single insects to leaf petioles or stems (usually branch stems) near the top of immature plants.[6]

[6] No apparent difference was noted between stems and leaf petioles as regards the damage produced. As a rule, young leaf petioles provided better material for sectioning since they contain less woody tissue than do the stems. The places selected, whether petioles or stems, were usually the younger parts of the plant, but those where active growth had been completed or nearly so.

In a few cases the vials were left attached for 48 hours or longer. Otherwise, if the insect was still alive at the end of 24 hours, it was moved to another point and attached as before. The limits of the area exposed to the insect were marked with India ink, and each place was tagged and numbered. The feeding place was then examined daily until about the fifth day after attachment of the vial, when the stem or leaf was cut off and taken to the laboratory. With only a few exceptions, all those which did not show external swellings were sectioned and stained, and the sections of the entire series from the exposed area were examined microscopically. Insects that died or escaped before the end of 24 hours were omitted from the comparative tabulations.

TABLE 1.—*Summary of the results in 1929 of individual feedings by plant-sucking insects on cotton stems and petioles*

[Adults and nymphs exposed in vials for 24 hours or longer]

Family and species	Stage	Individuals	24-hour feedings	Positive reactions		Positive reactions with external swellings	Split lesions
		Number	*Number*	*Number*	*Per cent*	*Per cent*	*Per cent*
Miridae:							
Psallus seriatus	Adult	71	116	115	99.1	89.6	11.3
	Nymph	38	64	64	100.0	98.4	6.3
	Both	109	180	179	99.4	92.7	9.5
Lygus pratensis	Adult	69	165	163	98.8	90.2	33.7
	Nymph	13	18	18	100.0	88.9	22.2
	Both	82	183	181	98.9	90.1	32.6
Lygus apicalis	Adult	25	60	57	95.0	63.2	7.0
	Nymph	6	7	7	100.0	71.4	0
	Both	31	67	64	95.5	64.1	6.3
Adelphocoris rapidus	Adult	29	60	49	81.7	77.6	18.4
	Nymph	16	31	29	93.5	55.2	6.9
	Both	45	91	78	85.7	69.2	14.1
Poeciloscytus basalis	Adult	40	67	67	100.0	100.0	58.2
Lygaeidae:							
Geocoris punctipes	do	4	16	11	68.8	0	0
Nysius californicus	do	6	21	16	76.2	6.3	0
Cicadellidae:							
Homalodisca triquetra	Nymph	5	5	4	80.0	50.0	25.0
Graphocephala versuta	Adult	7	15	15	100.0	33.3	0
Membracidae:							
Stictocephala festina	do	9	28	28	100.0	71.4	10.7

The column headed "Individuals" shows the number of insects that completed at least one 24-hour exposure. The number of exposures ranged from 1 to 8 and averaged about 2 per insect.[7]

The proportion of positive results among the species of Miridae is very high and shows only slight variations between adults and nymphs of the same species or between different species, except the percentage for adults of *Adelphocoris rapidus*, which was somewhat below the average. Some of the largest split lesions have been caused by this species; and as the adults are large and difficult to

[7] The maximum numbers of feedings by single insects were obtained in 1928 when one adult *Lygus apicalis* was kept attached to the plant at different points for 10 days, and an individual of *Lygus pratensis* was observed to feed 16 times in two days.

handle in vials, it is suspected that most of the negative results were due to the failure of the insects to feed. This is borne out by the fact that of 13 negative results with this species 8 were from insects which gave positive reactions at other points.

The lowest proportions of positive reactions were obtained with the two species of Lygaeidae.

The last two columns in Table 1 give comparisons of the relative toxicity of the different species. The percentages are based upon the number of feedings producing positive reactions, and it may be noted that *Poeciloscytus basalis* takes first place in the proportion of external as well as split swellings[8] produced. When confined on cotton plants in cages this species also causes the blasting of squares. Fortunately it does not occur commonly on field cotton.

Lygus pratensis also produces a high proportion of both external and split swellings, but *Psallus seriatus* produces relatively few split swellings. Among the species of which a fair number of nymphs were tested, the adults produce more damage as measured by split lesions, although the nymphs in all cases produce a slightly higher percentage of positive reactions. The proportion of external swellings is variable, but the nymphs of *P. seriatus* produce a higher percentage than the adults.

Among the Homoptera, *Stictocephala festina* produces a fairly high proportion of observable and split swellings, whereas the figures for *Graphocephala versuta* indicate milder effects In four of the five tests with nymphs of *Homalodisca triquetra* lesions were produced, two of which were visible externally, one being a split lesion.

PROPORTION OF INDIVIDUALS GIVING POSITIVE REACTIONS

The proportion of individuals giving at least one positive reaction, which is not shown in Table 1, is even more significant than the percentage of positive reactions based on separate feedings. A summary of this for the five species of Miridae may be given as follows: Of 46 feedings in 1928 the results of which were negative or doubtful (including both watched feedings and 24-hour feedings), 42 were from 25 individuals which gave positive results at other punctures, 2 individuals fed only once, and 1 gave negative results in each of two tests. In 1929 of 19 feedings (including 13 of Adelphocoris) that were listed as negative or doubtful after examination of the sectioned and stained plant material, 14 were from insects that caused lesions at other points, 3 of the individuals completed only one 24-hour exposure, and only 1 completed two feedings, neither of which was definitely positive in result.

The largest number of negative results in 1928 came from a series of watched feedings with *Lygus pratensis*, which was rather surprising in view of other results with this method as well as with this species. One of these insects gave negative results in 4 feedings and positive results in 12; and another gave negative results in 3 cases, questionable results in 2, and a positive reaction in 1. A possible explanation of this may be found in the fact that in order to induce them to feed immediately, the insects for these tests were starved for

[8] A description of the split swellings is given under the heading Character of Mirid Lesions.

longer or shorter periods before being placed on the plants, and some of them probably became so weak that they were unable to complete the feeding in all cases, even after the proboscis was inserted. Something similar has been observed with weakened mosquitoes.

The records are further summarized in Table 2.

TABLE 2.—*Summary of negative results obtained in 1928 and 1929 from feedings by plant-sucking bugs on cotton stems and petioles*

	1928	1929	Total
	Number	*Number*	*Number*
Feedings recorded as negative or doubtful	46	19	65
Individual insects involved	28	18	46
Individual insects which caused positive reactions at other points	25	14	39
Insects which fed only once	2	3	5
Insects which gave two negative results	1	1	2

Among the small series of Psallus feeding in 1927, one nymph, as mentioned previously (p. 3), produced negative results in two cases and doubtful results in one. This makes a total of only three individuals out of the entire number tested that produced more than one negative and no positive reactions. Five others were tested only once and possibly did not feed. The general conclusion to be drawn is that nearly all individuals of these species may be expected to cause a reaction in the plant similar to that which is recognized as hopper damage. A very few may be actually nontoxic.

In view of these results it was not altogether surprising to find that the homopterous species caused similar lesions and a high percentage of positive reactions from feedings. It may be well, however, to warn against the conclusion that all these species are potential pests of cotton. Several of them, although taken occasionally or frequently on cotton, have not been connected in any way with field damage. The two so-called sharpshooters, *Homalodisca triquetra* and *Oncometopia undata* (Fab.), which are often abundant on cotton, apparently confine their attention to the stems, and as the lesions produced here are usually mild, little or no measurable damage seems to result.

THE PATH OF THE FEEDING PUNCTURES

Of special interest in the case of the three species of Homoptera is the fact that the feeding punctures produce a sharply defined sheath which stains a bright red with safranin. (Pl. 3, B.) This makes it possible to follow the path of the proboscis through the tissues and shows quite definitely that these insects search out the vascular tissues in which to feed. If the path does not go straight toward the cylinder or a bundle it is usually branched toward the tip, because of the probing of the insect in different directions, and one of the branches may be found to end in the phloem or occasionally the xylem. Well-formed sheaths have been found in tissues killed for sectioning immediately after the puncture of *Stictocephala festina*.

In the case of the Miridae, material preserved soon after punctures were observed shows nothing to indicate the path taken by the proboscis and usually little or no evidence of injury. At the most, two or three collapsed cells that seemed to have been punctured have been found. In older material preserved after the lesions are well developed, occasional specimens show an irregular brownish streak, which may have been the line of the original path, running for a short distance through the center of the lesion. Deadened cell walls take a reddish or brownish stain and may also appear through the lesion as a short streak, but these are as apt to be at right angles to the direction of the proboscis as otherwise and are usually rather easily distinguished from the broad sheath of material around the path of the Homoptera puncture. It should be mentioned, however, that in a few microtome-sectioned specimens of 5-day-old lesions caused by Stictocephala there are certain indications that the sheath material is gradually absorbed or diffused by the plant, as in these sections the paths are broken and not well defined.

Of further interest in connection with these observations is the fact that the sheaths are similar to, and perhaps identical with, those described by Horsfall[9] and others around the feeding punctures of several species of aphids in various plants. This writer discusses the nature of the sheath material and is inclined to the opinion that it is a secretion of the cells as a reaction to the wound stimulus. In view of the fact that well-defined sheaths have been found immediately after the puncture, it seems likely that the red staining substance results from the action of the saliva of these species on the middle lamella (the intercellular layer) which the paths seem mostly to follow.

In addition to the sheaths, cell multiplication occurs in the area about the punctures made by the species of Homoptera, developing to the point of tissue destruction, and the effect is quite similar in general to that in mirid lesions.

DISCUSSION OF RESULTS

From the experiments and a histological study of affected plants no indications have developed that a living virus is involved. The insects tested have been obtained from various host plants, and the fact that practically all individuals of 10 species (some of them widely separated systematically) produce a condition in cotton stems and leaf petioles similar to that recognized as true hopper damage almost certainly shows that it is normally associated with the feeding punctures and is probably due to the saliva injected. The toxicity of different individuals is decidedly variable, and this would seem to be consistent with the idea of a chemical irritant.

Another point is that the lesions are quite definitely limited and show no tendency to extend throughout the plant. It is possible that a virus might enter the vascular system and be carried to other parts. In no instances, however, either in the present work or in that of Painter, have young squares been caused to drop by the feeding of the insects on other parts of the plant, except within a very short distance of the squares themselves. A certain similarity between the initial reaction of the plant cells to the feeding punctures

[9] HORSFALL, J. L. THE EFFECTS OF FEEDING PUNCTURES OF APHIDS ON CERTAIN PLANT TISSUES. Penn. Agr. Expt. Sta. Bul. 182, 22 p., illus. 1923.

and to wound stimuli (as represented by needle punctures) is also indicative of the local character of the lesions.

In connection with the subject of infection, reference should be made to a peculiar, deeply staining, granular material which sometimes occurs in the cells of the plant. This was noted by Painter as resembling the slime mold found in clubroot of cabbage and was suspected by him of being related to cotton flea hopper damage. Cells packed with this substance may be found scattered through the pith, cortex, and other tissues, and at times they are extremely numerous. In some of the preparations more or less of an accumulation of the material occurs in the lesions, but with these exceptions it has been found as abundantly in apparently normal, protected plants as in those on which insects have fed. It is not regularly or usually associated with lesions in early stages of development, and an accumulation comparable to that in the older lesions has been observed in tissues reacting to sterile needle punctures. While the nature or origin of the substance is not known, its connection, if any, with the disorder must be purely secondary or accidental.

SUMMARY

Experiments were begun in 1927 and carried through three years, at Tallulah, La., for the purpose of studying the lesions produced in stems and petioles by the cotton flea hopper and other plant-sucking insects. The object was to determine, if possible, whether the injury to cotton following the feeding of these insects was the result of a transmissible virus or due to mechanical or chemical injury.

External indications of injury seemed to prove that only a part of the many insects tested had affected the plants in the way considered characteristic of hopper injury, but later examination by microtome sections through the feeding points showed that practically all punctures resulted in the same type of internal injury. The swelling and breaking open of the lesions in some cases seemed to be the result of more extensive injury rather than injury of another kind.

The size of the lesions produced by one individual in consecutive feedings was often fairly uniform, but the toxicity of different individuals was decidedly variable.

The early reaction of the internal tissues of the plant is recognizable as an enlargement of nuclei and cells at some point along the feeding puncture. This has been observed in material preserved for sectioning 24 hours after the feeding. Dividing nuclei occasionally found in the preparations show that cell division is stimulated, and in this respect the lesions are comparable to the reaction of the plant to wound stimuli. The milder lesions may show only evidence of cell activity and more or less cell distortion, whereas in more severe forms areas of broken-down tissue develop. The full development of the lesion is rapid; and the external swellings, when produced at all, are usually visible by the second or third day.

Individuals of 10 species (7 Hemiptera and 3 Homoptera) were included in the tests. The average severity of the damage by different species was variable. Of the two more important field pests, the percentage of split lesions was 32.6 for *Lygus pratensis* and 9.5 for *Psallus seriatus*. About 7 per cent of the lesions caused by *P. seri-*

atus and 10 per cent of those caused by *L. pratensis* did not show externally. *Poeciloscytus basalis*, a species which is seldom taken on cotton, caused external swellings at every feeding point and produced the highest percentage of split lesions (58.2) of any of the species tested. Of all the individuals that fed more than once only two failed to produce the injury at some feeding point.

The feeding punctures of the three species of Homoptera used in the tests can be traced through the tissues by a well-defined "sheath" which stains a bright red with safranin. This may be found in sections made immediately after the feeding of the insects and is similar in appearance to the sheaths which have been described in aphid punctures. As in the case of the aphids, the paths usually end in the vascular tissue.

No sheath material was found about the punctures made by mirids, and the path taken by the proboscis of the species on which observations were made could not be definitely traced. At the most, in plant material preserved for sectioning soon after the feedings, a few ruptured cells have been found.

The experiments with the 10 species of plant-sucking insects, some of which do not feed naturally on cotton, have shown that nearly all individuals cause a reaction in the tissues of cotton stems and leaf petioles similar to that produced by the cotton flea hopper, *Psallus seriatus*. This is taken to indicate that hopper damage is due to injected substances normally present in the insects and toxic to the plant, rather than to a transmissible disease.

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