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JOURNAL OF AGRICULTURAL RESEARCH

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TEMPORARY IMMUNITY IN ALFALFA ORDINARILY SUSCEPTIBLE TO ATTACK BY THE PEA APHID[1]

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INTRODUCTION

Outbreaks of the pea aphid (*Macrosiphum pisi* (Kalt.)) on alfalfa in the Middle West are sporadic in occurrence. It has been observed that individual plants known to be highly susceptible in the nursery in some seasons will not support an aphid population in other seasons. Aphids placed on such plants after they have been brought into the greenhouse in November failed to live on the stems developed in the field; however, when these plants were cut back to permit new growth they became so heavily infested that they were quickly killed. The intermittent resistance of alfalfa to the pea aphid is referred to in the present paper as temporary immunity. The intent of this paper is to point out the causes of this form of immunity and to indicate the danger of confusing temporary immunity with continuous immunity.

EFFECT OF LIGHT, TEMPERATURE, AND MOISTURE ON RESISTANCE OF ALFALFA TO APHIDS

Davidson (*1*),[3] in discussing the feeding of the bean aphid (*Aphis rumicis* L.) on the English broadbean, or Windsor bean (*Vicia faba* L.), observed that the aphids did not feed so readily during some weeks as during others, and that they fed very little on the older tissue at any time. He also noted that plants under reduced light, although succulent, had small populations of aphids. His suggestions to explain this situation were: (1) The cell sap may be unfavorable for apid development during the period of low reproduction; (2) there is a reduced carbohydrate production by the plant; and (3) the food in the older tissues is unsatisfactory for assimilation. Tottingham stated that during the different seasons in the Temperate Zones the intensity and duration of light radiation received by plants varies greatly, and that at an altitude of 30° the sun delivers only about one-third as much light as at 65°.[4] Miller (*7*) stated that changes in the acidity of plant tissues are associated with diurnal and seasonal fluctuations in light intensity and temperature. He explained that with the absence of light the production of carbohydrates is reduced and sugar is oxidized to form an acid molecular structure.

[1] Received for publication September 15, 1944.

[2] The author is indebted to R. T. Cotton, of the Bureau of Entomology and Plant Quarantine, for suggestions and guidance in the preparation of this paper; and to Margaret A. Newcomb, E. C. Miller, J. C. Frazier, Geo. A. Dean, R. C. Smith, and R. H. Painter, of Kansas State College, for criticisms of the work. The photomicrographs were made by F. J. Hanna, also of Kansas State College.

[3] Italic numbers in parentheses refer to Literature Cited, p. 43.

[4] TOTTINGHAM, W. E. PLANT BIOCHEMISTRY. Rev. ed. 2, 249 pp., illus. Minneapolis, Minn. 1937. [Processed.] (See p. 206.)

For about 4 weeks in December and January, when the noonday sun shines more obliquely from the south than in other months, the greenhouse at Manhattan, Kans., where experimental work was conducted, is shaded by the adjoining building so that direct sunlight

FIGURE 1.—Alfalfa seedling that has become dormant while growing in the greenhouse in December and January. *A*, Short internodes of the lateral shoots and the terminal bud. *B*, Apical bud, a potential feeding site for first-instar aphids; natural size.

can reach the plants only from 10 a. m. to 4 p. m. At that time of the year even the most susceptible alfalfa plants became so unattractive and unsuitable to the pea aphid that it was barely able to maintain itself on them. Although moisture and temperature favorable to growth were maintained and the plants remained green, they entered a period of dormancy and, although stem internodes formed, they ceased to elongate (fig. 1). Cross sections of these short internodes when stained with phloroglucinol and hydrochloric acid gave

violet-red and cherry-red colors in the phloem, indicating the presence of lignin and pentose sugar, and when stained with ruthenium red, showed an acid condition (fig. 2, *A*). Alfalfa that is immune to attack under favorable growing conditions stained in this manner in the fourth and fifth internodes.

Experiments in the greenhouse under light and temperature conditions suitable for active growth showed that when the water sup-

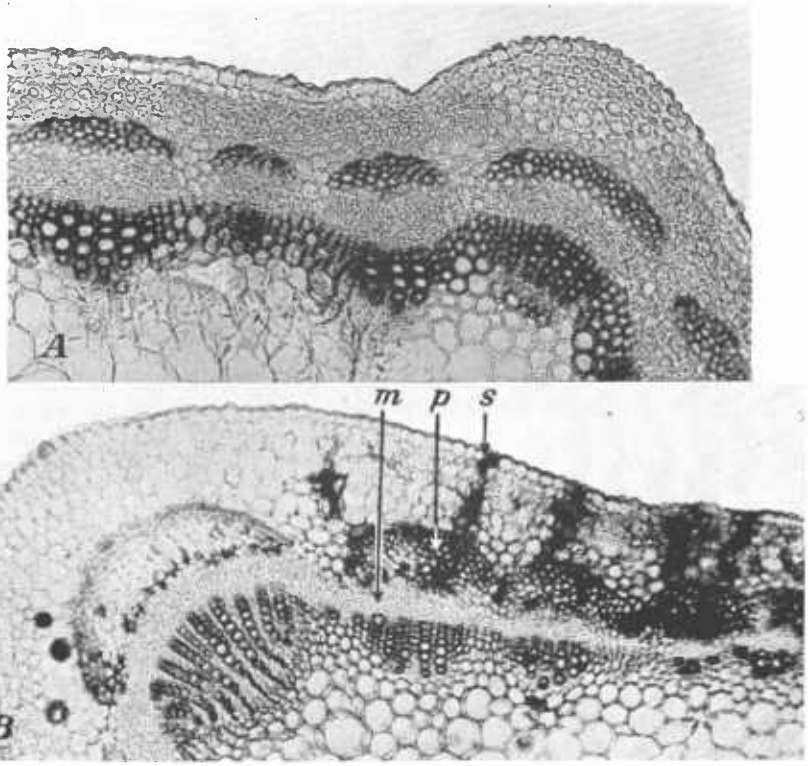

FIGURE 2.—Cross section of stem of dormant alfalfa plant, which is resistant to the pea aphid. *A*, Darker areas show the location of lignin and pentose sugar in the pericycle, outer phloem, and rays. *B*, Area where aphids have been obtaining food in the outer phloem; *b*, Outer phloem; *m*, metaphloem (the stained area ceases abruptly at the edge of the metaphloem); *s*, area penetrated by aphid's setae, stained, × 110.

ply of a plant was deficient for about 2 days the aphids left the feeding place. The outer phloem tissue of a section of a stem grown under these conditions, when given microchemical tests, indicated the presence of lignin, pentose sugar, and an acid condition (fig. 2, *B*). The tissue stained red to violet with phloroglucinol and hydrochloric acid. When the plant was watered, inducing new growth to develop, the aphids returned and fed on the new growth.

In Kansas alfalfa planted in the field in August usually had only a light infestation of *Macrosiphum pisi* by the latter part of September, or during October and November. Observations indicated that a young alfalfa seedling will support an adult aphid on its cotyledons,

the aphid will produce young, and after the plant has developed trifoliate leaves aphids will develop on the apical bud (fig. 1, *B*). In fields of old alfalfa only occasional infestations were present in the fall of the year, depending on the time of cutting in relation to the date of the first frost. Although pea aphid infestations were usually present in the fall, large populations did not develop on either the seedlings or the old plants. According to Peltier and Tysdal (*11*), there was a sharp drop in the moisture content of the tissues of all alfalfa varieties tested by them in October, and it might continue to drop until April. This is the reaction of an alfalfa plant approaching a state of dormancy and resistance to cold, and in this condition it evidently is also resistant to the pea aphid.

In order to determine the biochemical condition of such plants grown in the field, stems were taken and fixed for sectioning on November 3, 1942, from alfalfa plants that had been susceptible to aphid attack in the spring but that did not support an aphid population in the fall. Microchemical tests of the internodes, where aphids would normally feed, indicated the presence of acid substances. Ruthenium red and methylene blue were the indicators used. Other tests with phloroglucinol and with diphenylamine indicated the presence of pentose sugar in the outer phloem. Figure 2 illustrates the reaction to microchemical staining of a dormant alfalfa stem. The plants upon which aphids were feeding in the greenhouse were similarly tested but gave negative reactions to stains for acid and pentose sugar. This fact indicated that the relatively light infestations in the fall were due to unfavorable food conditions resulting from the biochemical reactions of the plant to low temperature and a deficiency of moisture.

It may be inferred from the greenhouse and field observations described above that light, temperature, and moisture, when insufficient to meet the minimum requirements for plant growth, tend to induce dormancy in alfalfa and inhibit the feeding of aphids. Under such conditions the deposition of lignin in the cell walls of the pericycle produces a sclerenchymatous condition which is associated with resistance.

AVAILABILITY OF FOOD IN SUSCEPTIBLE AND RESISTANT ALFALFA PLANTS

It was commonly observed that the aphids reacted differently on individual alfalfa plants growing in the same flat or plot under favorable conditions of temperature, moisture, and light. Aphids were placed on resistant plants growing in the same flat in the greenhouse with susceptible plants. The following day the aphids had left the resistant plants and were found on the susceptible plants. By growing thousands of plants under identical conditions in the greenhouse, it was possible to eliminate the most susceptible ones and at the same time to locate those having high immunity. These observations indicated that the resistance or susceptibility of the alfalfa plant to pea aphid attack might be directly related to the abundance or scarcity of suitable food materials in the plant tissues.

The pea aphid feeds by imbibing its food in liquid form through a tubelike beak. It inserts the stylets or setae of its mouth parts between the cells of the cortex, through the endodermis into the outer phloem. *A*, *B*, and *C*, of figure 3 are cross sections of alfalfa stems

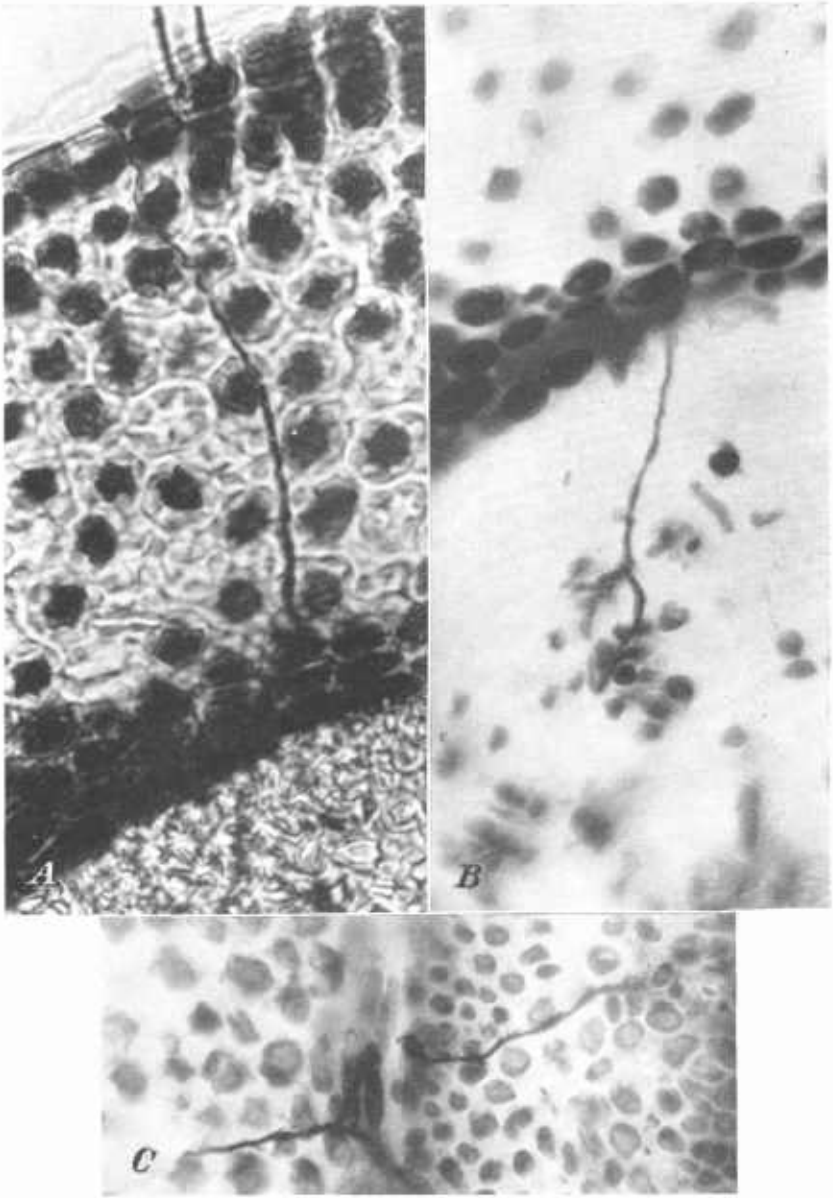

FIGURE 3.—Cross section of an alfalfa stem with setal tube of *Macrosiphum pisi* extending through the cortex and the epidermis and into the region of the outer phloem. *A*, The setal tube appears to branch at the distal end; *B*, lower part of section; *C*, first visible internode of a stem. Note the many barblike projections of the setal tube. Apparently the aphid was penetrating the cell walls in search of food. Aphids did not remain to feed on this internode, which indicates that food was not available. This internode was stained to determine the presence or absence of pectic substances. All × 650.

showing the paths made by the aphid's mouth parts through the tissues. It is believed that the mouth parts of an aphid, like those of some other insects, secrete a fluid which forms a tube, referred to as a setal tube (fig. 3, *C*). Studies of histological sections like those illustrated indicate that *Macrosiphum pisi* obtains its food from the phloem tissue of the alfalfa stem. Judging from the known structure and function of this tissue, it seems likely that *M. pisi* obtains its protein food from the liquid content of the sieve tubes of the phloem. Steele (*12, pp. 288–290*) stated that the main seat of protein and carbohydrate synthesis is in the green leaf and that these materials are transferred through the phloem tissue of the bark to the growing points of the plant, where they are resynthesized for tissue building and storage. It was believed, therefore, that the chemical character of the substances present in the tissue from which *M. pisi* evidently obtains its food might explain its ability or inability to subsist on certain individual plants and on different parts of a single stem.

An attempt to identify the kind of sugar in an alfalfa plant and in the gut of an aphid that had been feeding on it was made by means of a microchemical test for osazones according to a technique developed by S. H. Eckerson, of the Boyce Thompson Institute.[5] For this work phenylhydrazine hydrochloride and sodium acetate in glycerol were used. According to this method the reagents are prepared as follows: Phenylhydrazine hydrochloride is pulverized in a mortar, dissolved in glycerol, 1:10, filtered and kept in a brown bottle. Sodium acetate is dissolved in glycerol, 1:10, and kept in a brown bottle. For osazone formation, a drop of phenylhydrazine hydrochloride and a drop of sodium acetate are mixed on a slide, a section of tissue is placed in the reagent, and a cover glass is added.

Each internode, beginning with the first one visible below the apical bud, was scraped to break the cell walls in the phloem and the sap was pressed into a drop of water on a slide. In order to remove the larger pieces of bark the water containing the sap thus obtained was then run onto another slide and tested for osazones. Figure 4, *A*, shows an osazone produced from sugar in the third visible internode. It is representative of the osazones obtained from the first and the fifth internodes, counting from the top of the stem. Apparently fewer osazones were produced from the fifth internode than from the third or the fourth. The sixth internode did not produce distinct osazones and none were obtained from the seventh or eighth internodes.

The internodes that produced osazones had not completed their growth in length, and these were the ones on which aphids would feed (fig. 5, *A*). Stems of both resistant and susceptible plants produced osazones.

Tests were made for osazones in the hindgut of *Macrosiphum pisi* and bodies resembling the osazones in alfalfa were found (fig. 4, *B*). A comparison of the osazones of sucrose and glucose made from commercial cane and corn sugars, respectively, is shown in figure 4, *C*, and *D*. There was a striking similarity between the sucrose osazones, those produced from sugar in alfalfa, and those found in the hindgut (fig. 4, *B*, *F*) and cornicles (fig. 4, *E*) of the aphid. Tests of

[5] ECKERSON, S. H. MICROCHEMISTRY. 36 pp. Dept. of Botany, Univ. of Chicago. [1920 (?).] [Processed.]

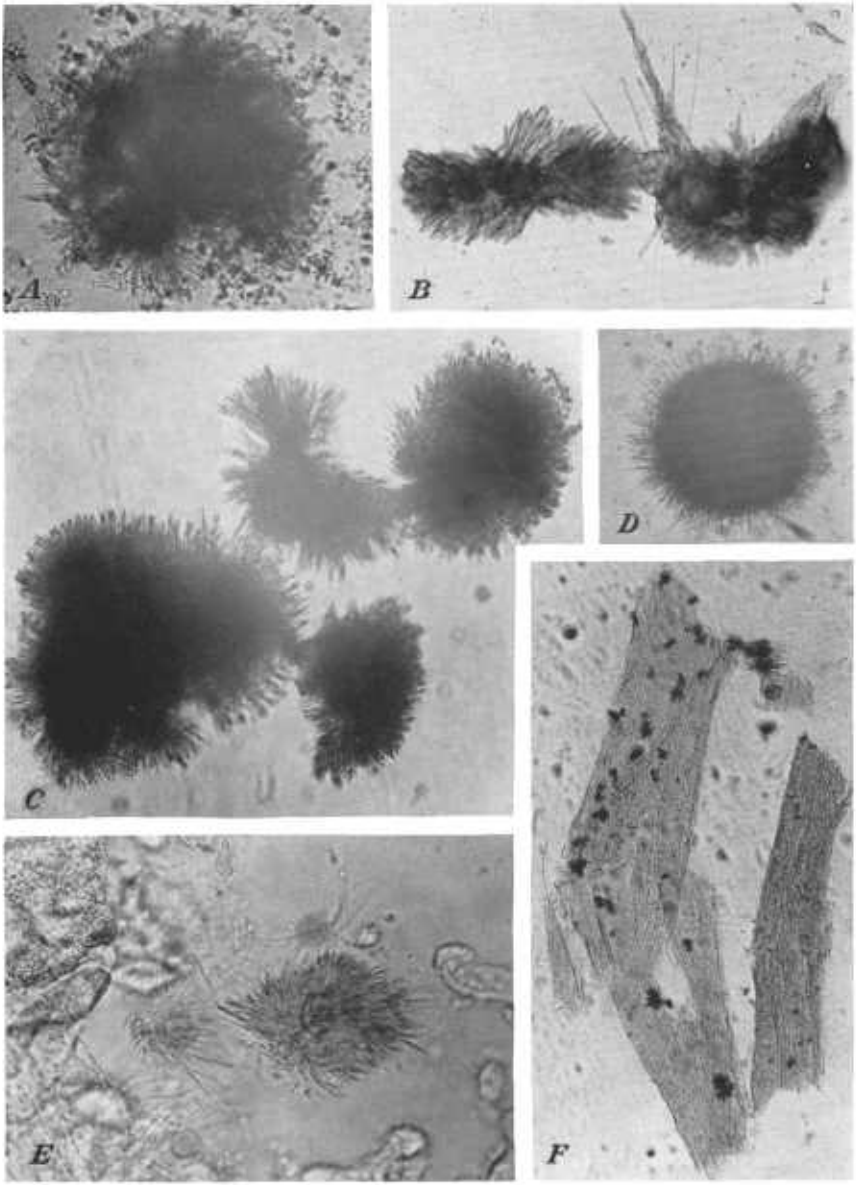

FIGURE 4.—Osazones obtained from an alfalfa stem and from the intestines of an aphid. (Note the individual crystals projecting from the mass.) *A*, Osazone from the third visible internode of a stem. × 650. *B*, Osazones from the hindgut of *Macrosiphum pisi*, × 650. *C*, Sucrose osazones made from commercial cane sugar, × 650. *D*, Glucose osazone made from commercial glucose. The crystals are much finer than those from sucrose, × 650. *E*, Osazones from the base of a cornicle of *M. pisi*, × 650. *F*, Section of hindgut of *M. pisi* showing osazones, × 110.

pea vine and a leaf upon which *M. pisi* had fed showed osazones resembling those from sucrose. The same type was produced in Austrian field peas, a host of the pea aphid. Osazones resembling those from glucose were not obtained from the aphids or from alfalfa. First-instar aphids just beginning to feed yielded no osazones when tested, but as they neared completion of the first instar when feeding on the

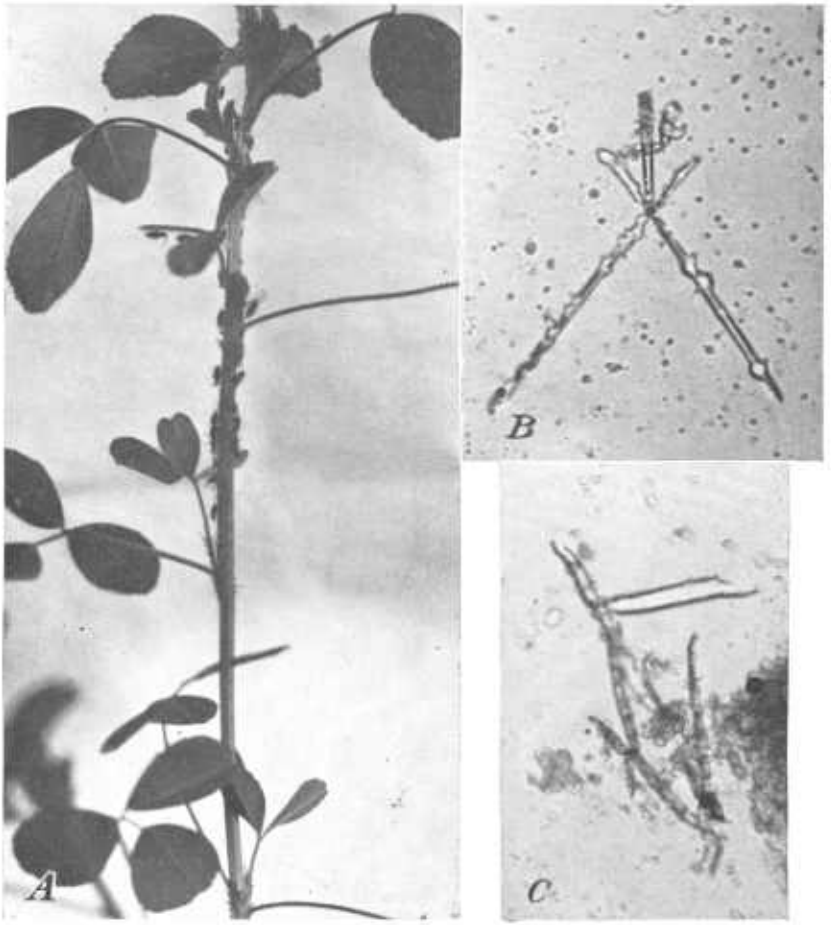

FIGURE 5.—*A*, Alfalfa stem, showing the distribution of aphids. The first and fifth internodes have no aphids on them, natural size. *B*, Osazones obtained from a first-instar *Macrosiphum pisi*, found feeding on an alfalfa steam, × 110. *C*, Osazones obtained from a mature alfalfa leaf, × 110.

stem it was possible to obtain osazones from the guts, as shown in figure 5, *B*.

In repeated tests of the apical leaf (covered with small aphids, as shown in figure 1, *B*) few or no osazones were found. An aphid larger than the second instar was seldom observed feeding on the apical leaf. Adult aphids were more often found on stems that produced osazones abundantly, that is, on internodes 2, 3, and 4 in alfalfa. Aphids were seldom seen feeding on a mature leaf. Mature

alfalfa leaves tested in this experiment usually gave negative reactions for osazones. Figure 5, *C*, shows a simple type of osazone obtained from a mature leaf. These osazones resemble those obtained from first-instar aphids.

Mumford and Hey (*8*) pointed out that a highly nitrogenous diet stimulates reproduction in insects, and some workers feel that immunity or susceptibility to insect attack is closely related to the protein value in the plant. According to Miller (*7*) and Meyer and Anderson (*6, p. 451*), protein synthesis is dependent on the "carbohydrate-nitrogen ratio." Since resistant plants produced sucrose in the stems, as did susceptible plants, the "carbohydrate-nitrogen ratio" would not be changed so far as protein synthesis is concerned.

Plant physiologists state that the apical bud during its formative period and early growth receives its protein and carbohydrates from other parts (leaves and stems) of the plant. At this period the "cell sap" has attained its highest proportion in the growing protoplast. As the internodes elongate the cell walls require additional carbohydrates for building material. Aphids feed on the apical bud only during the first instar. Tests of the apical bud show few or no sucrose osazones and, since larger aphids seek feeding areas where sucrose is abundant, it would seem that the availability of sucrose is the probable explanation for their shift to other feeding places, and that protein is not a factor of resistance in this case.

Dunnam and Clark (*2*) have reported a larger total number of young per aphid observed on cotton plants dusted with calcium arsenate than on plants not dusted. They observed that the acidity of the soil was reduced where the plants had been dusted with calcium arsenate.

In a controlled experiment, the writer limed one part of an alfalfa plot and left the rest untreated. Sweepings of the alfalfa on May 18, May 30, and June 23 showed twice as many aphids on the plants growing on the limed part of the plot as on the unlimed part. In a field near Manhattan, Kans., the heavy outbreak of the pea aphid in 1943 caused the death of the alfalfa tops in well-defined areas or spots. Soil samples taken from these areas in 1944 showed a pH value of 6.28, whereas samples of the soil taken 100 yards away from an area where no severe plant injury occurred showed a pH value of 5.86. Population counts in 1944, as determined by the number of aphids in 20 sweeps of an insect net, showed the population of aphids to be four times as great on an area with a soil having a pH value of 6.28 as on the other parts of the field where the soil pH was lower. These two observations indicate that aphid populations tend to be higher on plants growing in soil with a high pH value, or with additional available calcium. Parker and Truog (*10*) state that the greater part of the calcium taken up by the plant is probably used for the neutralization and precipitation of the acids in the plant sap.

SOME PLANT PROCESSES THAT AFFECT THE FEEDING OF APHIDS

Gortner (*4*) and Gisvold and Rogers [6] point out that pectic substances are abundant in newly forming tissue. These substances include pectic acid, pectin (which serves to cement and support new tissue), pentosans, and pentose sugar, all of which aid in the main-

[6] Gisvold, O., and Rogers, C. H. THE CHEMISTRY OF PLANT CONSTITUENTS. Rev. ed., 392 pp. Minneapolis, Minn. 1941. [Processed.]

tenance of the water balance of the plant and precede the synthesis of other carbohydrates. According to Miller (*7, p. 19*), "The formation of pectic substances in plants appears to occur when metabolism and growth are at their maxima. As growth slows and as maturity is reached the production of pectic materials decreases and those present in the [cell] wall are slowly converted into other substances." In this process it is believed that lignin is synthesized from substances in the structures containing hemicellulose. The presence of lignin in the pericycle of an internode is an indication that the stem is resistant to the pea aphid at that internode (fig.2, *A*).

Norman (*9*) stated that the hexose → pentose transformation through the intermediate formation of uronic acid does not appear to be completed in the mannose series. However, a polygalacturonic acid containing some sugar units develops as pectin. It is during the period in which the hexose → pentose transformation takes place in the alfalfa plant that the condition of immunity develops. It has been observed that the first visible internode of a susceptible alfalfa plant growing under favorable conditions is not fed upon by the pea aphid. Microchemical tests of both susceptible and resistant plants revealed the presence of pectic substances in this internode. The second, third, and fourth internodes, where aphids normally feed on a susceptible plant, gave negative reactions for pectic substances and acidity, whereas the corresponding internodes of a resistant plant gave positive reactions. These results indicate that resistance in alfalfa is dependent on the character of the carbohydrates present and on the acidity of the sap. Plant chemists have shown that the acidity of the cell sap varies with differences in temperature, light, and moisture. In the tests performed in this study the continuously resistant plants maintained an acid condition throughout their growth, which prevented feeding by aphids.

According to Meyer and Anderson (*6, pp. 473–474*), there appears to be an optimum hydrogen-ion concentration for the activity of each enzyme, as well as upper and lower limits of hydrogen-ion concentration, beyond which the enzyme is inactive, or may actually be destroyed. Tests by Fife and Frampton (*3*) of the pH gradient in leaves of the sugar beet showed that the success of the beet leafhopper (*Eutettix tenellus*) in finding the phloem in the petiole is related to the hydrogen-ion concentration. They stated that the leafhopper prefers an alkaline food (pH 8.5) to one that is acid (pH 5.0) in reaction. In a study of cell-sap acidity and the incidence of a white fly, *Bemisia gossypiperda*, on cotton, Husain, Puri, and Trehan (*5*) showed that (1) there is a difference in the hydrogen-ion concentration of varieties of cotton plants, (2) the cell-sap acidity of the same plant differs with a change of seasons, (3) the pH gradient from top to bottom of the plant varies with the age of the plant, and (4) the attack of the white fly was less severe when the hydrogen-ion concentration was relatively low. From the results reported by these workers, and by the present author in this article, it seems logical to infer that the enzyme activity of the salivary secretion of the aphid is inhibited by the acid condition of the cell sap, and as a result the food in the cells cannot be digested.

Breeding tests have shown that resistant plants when cross-pollinated produce resistant progeny.

SUMMARY AND CONCLUSIONS

Temporary immunity to the feeding of the pea aphid (*Macrosiphum pisi*) on alfalfa is associated with an acid condition in the plant. The acidity may be caused by (1) a deficiency of water, (2) a deficiency of light for photosynthesis, (3) temperatures too low for rapid growth of alfalfa, and (4) temperatures sufficiently high to cause the formation of lignin.

Tests of the cell sap for osazones have shown that aphids feed most in the elongating internodes, where growth is rapid and where sucrose is most abundant. Since the synthesis of protein is dependent on a "carbohydrate-nitrogen ratio," it is believed that resistance of alfalfa to the pea aphid is correlated primarily with an acid condition and a scarcity or absence of sucrose, rather than with a scarcity of proteins in the plants.

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