THE NATURE OF THE SHEATH MATERIAL IN THE FEEDING PUNCTURES PRODUCED BY THE POTATO LEAF HOPPER AND THE THREE-CORNERED ALFALFA HOPPER[1]

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INTRODUCTION

In a histological study of plant tissue injured by several species of leaf hoppers of the genus *Empoasca*, Smith and Poos (*19*)[4] showed that the feeding of each species was restricted to certain tissues, the mesophyll or the vascular bundle (phloem and xylem), and that the resulting injury to the host was correlated with the parts fed upon. The mesophyll-feeding species of *Empoasca* caused a stippling of the leaves, whereas the species feeding on the vascular tissue, *E. fabae* (Harris),[5] produced severe types of diseaselike injury on its various hosts. In the feeding punctures produced by all species, an amber-colored material that stained bright red with safranine was found either in the form of a cylindrical sheath about the punctures made by the insect's mouth parts or in aggregations at the ends of the punctures. This material was present in both new and old punctures. Smith and Poos suggested that its presence in the vascular cells might interfere with the process of translocation of plant materials and cause the plant to wilt when the xylem vessels were plugged or to become red or yellow when phloem tissue was fed upon.

In order to obtain further knowledge of the injury caused to plants by these insects, particularly by *Empoasca fabae*, it seemed desirable to determine the source of the sheath material and whether it is produced by the plant as a result of the puncture, by the leaf hopper alone, or by both the insect and the plant. The tests were made on sheaths surrounding feeding punctures made by adults of *E. fabae* and also, for comparative purposes, on those made by the adult three-cornered alfalfa hopper, *Stictocephala festina* (Say)[6]. The material was found in greater abundance in its feeding punctures than in those of *E. fabae*. The substances for which tests were made include those that are found in the cell walls and are formed by the plants, as well as additional substances.

REVIEW OF LITERATURE

The literature contains several references to sheaths in the feeding punctures of sucking insects, but investigators have differed in their conclusions as to the nature and source of this material. Prillieux

[1] Received for publication May 31, 1933; issued October, 1933. This paper presents the results of one phase of a cooperative study of leaf-hopper injury to forage crops undertaken by the Division of Cereal and Forage Insects, Bureau of Entomology, and the Division of Forage Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture.

[2] The writer is indebted to F. W. Poos for making possible the study of this problem; to H. O. Sampson, Mary E. Reid, and H. W. Johnson for advice and suggestions; and to J. W. Schrivener for assistance in preparing the insect-injured material used in the tests.

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[4] Reference is made by number (italic) to Literature Cited, p. 484.

[5] Order Homoptera, family Cicadellidae.

[6] Order Homoptera, family Membracidae.

(*17, p. 43*), in his studies of the feeding punctures made by *Puceron* (=*Eriosoma*) *lanigerum* Hausm. in apple in 1878, was apparently the first to describe the sheath in plant tissue around the proboscis of an insect; he believed that the sheath material was analogous to cellulose. Davidson (*4*) stated nearly a half century later that aphid sheaths were probably composed of callose or cellulose and tannin; and more recent tests by Painter (*15, pp. 234–237*) suggested the presence of callose, calcium pectate, and tannins in sheaths caused by punctures of the chinch bug, *Blissus leucopterus* (Say). These results indicate that the sheath was produced by the plant cells. Smith (*20, pp. 118, 120, 131*) believed that the sheath was made up of substances of both plant and animal origin, but he made no tests. King and Cook (*10, p. 8*) came to the same conclusion. Horsfall (*9, p. 9*), working with aphids, thought that the protein material and calcium pectate were laid down by the plant cells in response to the wound stimulus, although he stated (*9, p. 14*) that the protein material might possibly have been ejected by the aphid.

Büsgen (*1, pp. 43–44*), Withycombe (*22, p. 70–81*), Woods (*23, p. 20*), and Zweigelt (*24, p. 274*) concluded that the sheaths were of animal origin. Withycombe and Zweigelt based their conclusions on the fact that they observed the flow of the insect's saliva before or as it made the puncture and this saliva later hardened.

Other observations as to the specific nature of the sheath material are mentioned in the following paragraphs in connection with the substance for which the test was made.

Various workers have employed carbol fuchsin (*23, pp. 20–22*), safranine (*4, pp. 10, 19, 22*), and the haematoxylin stains (*15*) with counter stains for differentiating the sheaths from the plant tissue in histological studies. These stains, although good for permanent mounts, are not satisfactory for quick determinations in fresh material. Several color tests that appear to be desirable differential stains for the sheath material were found during the study reported in this paper.

TECHNIC

Microchemical tests that are described in several available textbooks were used in the present study, and references have been made to these books instead of to the scattered articles originally describing each test. In addition, macrochemical tests were modified for microchemical application. The imperfections of these modifications may account in part for certain inconsistencies in the results, particularly among the tests for the amino acids. Moreover, the negative results obtained with some of the reagents when others used for identification of the same substance were positive may have been due to the insolubility of the sheath in these reagents. Whenever a test was negative, the sheath material was positively identified by testing with Millon's reagent as discussed under the test for tyrosine.

The feeding punctures for study were procured by confining the leaf hoppers in small cages (*18*) to definite areas on petioles, stems, or leaf veins for periods of 1 or 2 days.[7] Alfalfa (*Medicago sativa*), apple (*Malus sylvestris*), cowpea (*Vigna sinensis*), Ladino clover

[7] Fife, in a paper (*5*) that appeared after the completion of the work reported in the present paper, described a feeding cage for use in studies on *Eutettix tenellus* (Baker) by means of which the insect secretion can be obtained apart from the plant tissue and thus interference by the latter in testing for solubility, crystal character, and optical properties can be eliminated.

(*Trifolium repens latum*), red clover (*T. pratense*) var. Tennessee, sweetclover (*Melilotus officinalis*), and potato (*Solanum tuberosum*) were used as host plants.

The tests were made from 1 to 77 days after the insects had been removed from the plants. The tissue was cut with a razor into slices from 1 to 3 cells thick, and these were placed on slides. The sections containing the natural amber or straw-colored sheaths were separated for study, and the tests observed by means of a microscope. Certain solubility tests and others that were prolonged were carried out in small vials, and the observations were made after the material had been transferred to slides.

RESULTS

The results of the tests for each substance, or group of substances, are presented in tables 1 to 3.

PLANT-RESERVE SUBSTANCES

As table 1 shows, the natural color of the sheath material was not changed by either of the dyes Scharlach R or Sudan III, or by osmic acid. The sheath material did not change when subjected to the color tests for tannin or when allowed to oxidize by exposure on a slide for several days. The negative tests in table 1 indicate the absence of plant-reserve substances in sheaths made by the potato leaf hopper and the alfalfa hopper.

So far as the writer knows, tannin is the only material in this group that has been mentioned by previous investigators in connection with sheath material in plant tissue. Petri (*16, p. 28*) stated that tannin was deposited about sheaths in feeding punctures by the grape phylloxera. Davidson (*4, p. 43*) made the same statement with respect to aphids. Painter, working with the chinch bug (*15, p. 234*), and Withycombe (*22, p. 81*) with the sugarcane froghopper (*Tomaspis saccharina* Dist.), said that tannins were always present. None of these investigators, however, mentioned the tests they used.

TABLE 1.—*Tests for plant-reserve substances in the sheath material surrounding punctures in plant tissue made by Empoasca fabae and Stictocephala festina*

Tested for presence of—	Test	Reaction	Reference
Fats and oils	Staining with Sudan III	Negative	Eckerson (*pp. 12–13*).[a]
	Staining with Scharlach R	do	Do.
	Staining with osmic acid	do	Chamberlain (*3, p. 80*).
	Solubility in chloroform	do	Do.
	Solubility in ether	do	Do.
	Solubility in carbon bisulphide	do	Do.
Waxes	Staining with Sudan III	do	Eckerson (*pp. 12–13*).[a]
	Staining with Scharlach R	do	Do.[a]
Gums, mucilage, gelatinized membranes.	Solubility in water	do	Chamberlain (*3, p. 82*).
Tannins	do	do	Gortner (*6, p. 597*).
	Reaction with potassium ferricyanide	do	Do.
	Reaction with 10 percent ferric chloride.	do	Eckerson (*p. 6*).[a]
	Reaction with gold chloride	do	Do.[a]
	Reaction with hydrochloric acid and osmic acid.	do	Do.[a]
	Reaction with ammonium molybdate-ammonium chloride.	do	Withycombe (*22*).
	Oxidation	do	Eckerson (*p. 6*).[a] Gortner (*7, p. 598*).

[a] ECKERSON, S. H. MICROCHEMISTRY. 30 p., n.d. Bot. Dept., Univ. Chicago. [Mimeographed.

Zweigelt (*24, pp. 275–276*), using methylene blue as a test, concluded that tannin was present and that the browning of the sheath on exposure was due to oxidation of the tannic acid present. He also stated that in plants poor in tannin there was no accumulation of this material about the sheaths. This observation is in agreement with the results obtained in the present studies and with those of Wells (*21, p. 280*), who found no tannins in sheaths made by *Pachypsylla mamma* Riley and *P. asteriscus* Riley.

CELL-WALL AND WOUND-RESPONSE SUBSTANCES

In the tests for cell-wall and wound-response substances (table 2) the sheath material did not change color when treated with the reagents giving color reactions for lignin and pentosans or with the solvents copper oxide-ammonia, hydrogen peroxide, or potassium chlorate. Nitric acid colored the sheath material a deep yellow. The chromic acid solution dissolved the heavy lignified walls of the xylem vessels and the sheath material in sections at about the same rate, but much more slowly than it dissolved the remaining plant cells. The similar solubility of lignified cell walls and sheath material in the same sections in chromic acid and their insolubility in copper oxide-ammonia do not necessarily indicate similarity between the two substances, however, for negative results were obtained with the other tests and the chitinous insect exoskeleton shows the same reactions.

Horsfall (*9, p. 9*), using the phloroglucinol test, found no lignin in the sheaths made by aphids.

In the present studies, all tests for callose in the sheath material except the copper oxide-ammonia test were negative. Although the tests employed for its detection are not given, the sheaths made by the grape phylloxera in grape (*16, p. 28*) and of *Macrosiphum rosarum* Walk. in rose (*4, p. 42*) are said to contain this substance, and there are indications that it is also present in the sheaths made by *Blissus leucopterus* in corn and sorghum (*15, p. 234*).

In the tests for cutin and suberin, which were all negative except the test for insolubility in copper oxide-ammonia, the appearance of the sheath material did not change except for the bleaching due to javelle water and Schultze's reagent (ceric acid test). Büsgen (*1, p. 44*) stated that the sheath was converted to a vacuolated mass in Schultze's reagent, but he attached no significance to the results.

All tests for cellulose in the sheath material were negative. In the presence of concentrated sulphuric acid, iodine-zinc chloride solution, hydrocellulose reaction, iodine, or iodine-potassium iodide this material turned brown. Büsgen (*1, p. 44*), using the hydrocellulose reaction, obtained a positive test for cellulose in the outer layer of the sheaths made by *Aphis brassicae* L. and *Coccus cacti* L., but Davidson, although he stated (*4, pp. 42, 52*) that sheaths contained cellulose, obtained negative results with this test and also with copper oxide-ammonia (Schweizer's cupra-ammonia) as a solvent (*4, p. 43*). Büsgen (*1, p. 44*) and Prillieux (*17, p. 43*), using zinc chloriodide, obtained negative tests for cellulose in sheaths. Millardet (*12, p. 8*) mentioned swellings of cellulose following the path of the stylets of the grape phylloxera, but evidently he made no chemical tests.

TABLE 2.—*Tests for plant-cell-wall and wound-response substances in the sheath material surrounding punctures made in plant tissue by Empoasca fabae and Stictocephala festina*

Tested for presence of—	Test	Reaction	Reference
Lignin	Reaction with phloroglucinol-hydrochloric acid.	Negative	Eckerson (*pp. 10, 12*).[a]
	Reaction with orcinol-hydrochloric acid	do	Eckerson (*p. 10*).[a]
	Reaction with Schultze's maceration fluid followed by the hydrocellulose reaction.	do	Chamberlain (*3, pp. 80–81*).
	Insolubility in copper oxide-ammonia	Positive	Eckerson (*p. 12*).[a]
	Solubility in nitric acid	Negative	Do.[a]
	Solubility in hydrogen peroxide	do	Do.[a]
	Solubility in potassium chlorate	do	Do.[a]
	Solubility in 50 percent chromic acid	Positive	Eckerson (*pp. 12–13*).[a]
Pentosans	Reaction with phloroglucinol-hydrochloric acid.	Negative	Eckerson (*p. 10*).[a]
	Reaction with orcinol-hydrochloric acid	do	Do.[a]
	Hydrocellulose reaction	do	Chamberlain (*3, p. 81*).
	Reaction with zinc chloriodide	do	Do.
Callose	Insolubility in copper oxide-ammonia	Positive	Eckerson (*p. 11*).[a]
	Staining with resorcin blue	Negative	Do.[a]
	Staining with aniline blue	do	Do.[a]
	Staining with coralline and sodium bicarbonate.	do	Chamberlain (*3, p. 81*).
	Solubility in 1 percent alkali	do	Eckerson (*p. 11*).[a]
Cutin and suberin	Insolubility in copper oxide-ammonia	Positive	Eckerson (*p. 13*).[a]
	Staining with Sudan III	Negative	Eckerson (*p. 12*).[a]
	Staining with Scharlach R	do	Do.[a]
	Staining with alcannin in 50 percent alcohol.	do	Chamberlain (*3, p. 82*).
	Staining with javelle water and Grübler s cyanin.	do	Do.
	Ceric acid reaction	do	Eckerson (*p. 13*).[a]
	Phellonic acid reaction	do	Do.[a]
	Solubility in 3 percent alcoholic potassium hydroxide.	do	Do.[a]
Cellulose and hemicelluloses.	Hydrocellulose reaction	do	Eckerson (*p. 9*).[a]
	Reaction with zinc chloriodide	do	Hawk (*8, p. 48*).
	Solubility in copper oxide-ammonia	do	Chamberlain (*3, p. 80*).
	Solubility in concentrated sulphuric acid	do	Do.
	Staining with iodine solution	do	Hawk (*8, p. 48*).
	Staining with iodine-potassium iodide solution.	do	Eckerson (*p. 9*).[a]
Pectic compounds	Reaction with phloroglucinol and hydrochloric acid.	do	Gortner (*6, p. 585*).
	Reaction with zinc chloriodide	Positive	Chamberlain (*3, p. 81*).
	Solubility in Schultze's reagent	Negative	Chamberlain (*3, pp. 80, 137*).
	Solubility in ammonium oxalate	do	Gortner (*6, p. 587*).
	Solubility in 2 percent alkalies	do	Eckerson (*p. 12*).[a]
	Staining with ruthenium red	Positive	Eckerson (*pp. 11–12*).[a]
	Staining with methylene blue	do	Do.[a]
Starch, amyloid, saponarin, narceine, dextrin.	Staining with iodine solution	Negative	Eckerson (*p. 9*).[a]
	Staining with iodine-potassium iodide solution.	do	Do.[a]

[a] ECKERSON, S. H. (See footnote to table 1.)

In the tests for pectic compounds the sheath material turned brown, as do pectic compounds, in the presence of zinc chloriodide. Ruthenium red and methylene blue stained the sheath material with their respective colors, a positive test for pectic compounds. Eckerson's procedure was then applied to determine to which class of pectic compounds—pectin, pectose, or pectic acid—the staining was due. An acid-soluble substance behaving like pectose was indicated. Horsfall (*9, p. 9*) and Davidson (*4, p. 43*) obtained similar results by the same procedure in their studies of sheaths made by aphids, but they called the substance calcium pectate. Withycombe (*22, pp. 78–80*) obtained positive staining only occasionally with either ruthenium red or methylene blue after treating with hydrochloric acid. Petri (*16, p. 28*), Wells (*21, p. 280*), and Painter (*15, p. 234*) obtained a positive indication of calcium pectate in sheaths, but they did not give the tests they employed.

The sheath material did not appear to be reduced in quantity by the solvents for pectic compounds, and it reacted positively to Millon's reagent after such treatment, indicating that the sheath is composed largely of substances other than pectic compounds. If a pectic compound is present, its solubility indicates a pectoselike form. Tests performed on sheaths produced by leaf hoppers feeding from 1 to 22 days previously indicated that the material does not increase in quantity as time passes. If the material is pectose or some other plant substance and is not produced by the insect, it apparently dissolves and intermixes with the insect's secretion, which retains it on hardening. The absence of pectic compounds is evidenced by the staining of the sheath material on the outside of the epidermis at the point of puncture and also in the intercellular and intracellular spaces within the plant tissue.

PROTEINS AND PROTEINACEOUS SUBSTANCES

Certain color reactions characteristic of either amino acids or peptide linkages were employed in testing for proteinaceous substances (table 3). Some of these reactions are general protein reactions; others are specific for some particular amino acid.

The sheath material became pink to lilac in the biuret test, indicating the presence of derived proteins, such as proteoses or peptones. Büsgen (*1, p. 44*) obtained a positive biuret reaction in sheaths produced by *Aphis brassicae* and *Coccus cacti.*

A positive xanthoproteic reaction is due to the presence in the protein molecule of benzene nuclei with which the nitric acid forms modifications. The particular complexes of the protein molecule that are of special importance in this connection are those of tyrosine, tryptophane, and phenylalanine. According to Gortner (*6, p. 324*), the benzene nucleus of phenylalanine is not readily nitrated, and the xanthoproteic reaction is specific for aromatic nuclei that are easily nitrated (tryptophane or tyrosine). Sheath material gave positive color reactions for these two materials but did not dissolve. Horsfall (*9, p. 9*) obtained a positive reaction with this test for proteins in sheaths of aphids.

Sheath material, when subjected to the ninhydrin reaction, became a positive greenish-blue color, which indicated the presence of peptones, peptides, or amino acids containing a free carboxyl group and an alpha-amino group.

The negative response to the Molisch reaction indicates that the sheath does not contain a conjugated protein. The negative test with acetic acid-potassium ferrocyanide may have been due to the highly insoluble nature of the sheath material, since the reagent is intended for testing proteins in solution.

Sheath material developed an intense red color [8] when Sakaguchi's test for arginine, as given by Gortner (*6, pp. 325–326*), was made. Only compounds containing the free guanidine group react, but this group is free in most naturally occurring proteins. On sheath material the test is very rapid and it is easy to obtain the color contrast between the sheath and the plant parts. A positive test was obtained at room temperature in from 1 to 2 minutes and then the color faded. Gortner

[8] Since the sheath material was not in solution, 5 cc of water was added to the mixture of sodium hydroxide and alpha-naphthol to make up to the volume of 1 percent protein as recommended.

advises placing the material in the reagent at 2° to 4° C. (35.6° to 39.2° F.) for 40 minutes before examining for color, but slides which the writer prepared and placed on melting ice for 40 minutes showed no color upon examination, the color having faded before the end of this time.

TABLE 3.—*Tests for proteins, amino acids, mucin, and chitin in the sheath material surrounding punctures in plant tissue made by Empoasca fabae and Stictocephala festina*

Tested for presence of—	Test	Reaction	Reference
General proteins	Biuret reaction	Positive	Gortner (*6, p. 323*).
	Ninhydrin reaction	do	Do.
	Xanthoproteic reaction	do	Mathews (*11, pp. 154–155*).
	Molisch reaction	Negative	Gortner (*6, p. 325*).
	Reaction with acetic acid-potassium ferrocyanide.	do	Hawk (*8, p. 103*).
Arginine	Reaction with Sakaguchi's test	Positive	Gortner (*6, pp. 325–326*).
Asparagine and glutamine.	Reaction with quinone solution	do	Eckerson (*p. 19*).[a]
	Reaction with copper acetate and alcohol	Negative	Do.[1]
	Reaction with Ehrlich's reagent	do	Gortner (*6, p. 325*).
	Reaction with Acree-Rosenheim test	do	Do.
Tryptophane	Reaction with Liebermann's test	Positive	Gortner (*6, p. 324*).
	Reaction with Adamkiewicz test	do	Gortner (*6, p. 325*).
	Benedict's modification of Hopkins-Cole reaction.	do	Hawk (*8, p. 98*). Matthews (*11, p. 981*).
	Reaction with Folin and Denis' reagent	Negative	Hawk (*8, p. 86*).
Tyrosine	Reaction with Millon's reagent	Positive	Eckerson (*p. 19*).[a] Mathews (*11, pp. 980–981*).
	Reaction with Folin and Denis' reagent	Negative	Hawk (*8, p. 86*).
	Reaction with Mörner's reagent formaldehyde-sulphuric acid.	do	Morrow (*13, p. 117*).
	Reaction with sodium molybdate-sulphuric acid test.	do	Eckerson (*p. 19*).[a]
Mucin	Staining with thionine blue	do	Mathews (*11, p. 335*).
	Reaction with alkali and Ehrlich's reagent.	do	Mathews (*11, p. 337*).
	Reaction with ferric chloride and hydrochloric acid.	do	Hawk (*8, p. 85*).
	Solubility in concentrated hydrochloric acid.	do	(b).
	Solubility in concentrated potassium hydroxide.	do	(b).
	Solubility in 0.5 percent sodium hydroxide	do	(b).
Chitin	Solubility in 50 percent chromic acid	do	Eckerson (*pp. 12–13*).[a]
	Chitosan reaction	Positive	Eckerson (*p. 13*).[a] Campbell (*2, pp. 404–405*).

[a] ECKERSON, S. H. (See footnote to table 1.)
[b] In tests made by the author, mucin, precipitated from saliva, dissolved in these reagents within 48 hours but sheath material did not.

Sheath material gave a cherry-red color reaction after about 15 minutes in the cold in the presence of quinone solution, indicating the presence of asparagine or glutamine. Chitinous walls of leaf-hopper legs did not give the color reaction, but the muscles and apodemes did. Other tests for differentiation of these amino acids as given by Eckerson [9] were negative. The highly insoluble nature of the sheath material probably prevented any of its components from going into solution and thus accounted for the negative results.

Three of the tests for tryptophane in sheath material were positive, i.e., Liebermann's, Adamkiewicz, and Benedict's modification of the Hopkins-Cole reaction. Benedict's modification of the Hopkins-Cole test produced a reddish-violet coloration of sheath material in plant sections after 10 to 15 minutes in the cold. This test seems to be excellent for color differentiation, since the sheath was the only

[9] ECKERSON, S. H. (See footnote to table 1.)

material in the plant section that took the color and the results were obtained quickly and without distortion of cell structure.

Although the Acree-Rosenheim test for tryptophane gave no color reaction when applied to sheaths or chitinous body walls of leaf hoppers, the vascular tissues in sections from badly injured stems gave positive violet to black colorations, in contrast to uninjured tissue. This indicates that proteins may accumulate, as well as carbohydrates, as previously indicated (*7*), owing to interference in translocation by the feeding of the insects.

In the tests for tyrosine the sheath material became brick red when treated with Millon's reagent. The test is quickly made and the contrast makes the reagent a useful one in detecting feeding punctures.

Büsgen (*1, p. 44*) was the first to report a positive test on sheath material with Millon's reagent and commented on its specific color reaction. Morstatt (*14, pp. 354–355*) was unable to obtain a positive color test with this reagent on sheaths of the scale insect *Diaspis fallax* Horvath. Zweigelt (*24, p. 275*) believed that the positive test obtained with Millon's reagent did not mean that no other substances were included, and Petri (*16, p. 9*, footnote) believed that the test indicated an albumin-tannic acid combination. In some of the sheaths of *Stictocephala festina* the tubular wall seemed to consist of two layers, an inner, dark brick-red, thin-walled layer of rather definite outline and an outer, lighter red accumulation less regular in outline. Painter (*15, p. 234*) observed the two layers in the sheath of the chinch bug. The first evidence of the plugging of xylem vessels by sheath material was obtained by the writer (*19, p. 278*) with this reagent. In tests with Millon's reagent on the chitinous parts of the bodies of *Empoasca*, a similar red color was observed in the veins of the wings and in the spines and walls of the legs. The similarity in the response of the insect exoskeleton and the sheath material secreted by the same insect is extremely interesting. This reaction is given by all organic compounds containing a monohydroxybenzene nucleus, including phenol and salicylic acid (*11, p. 154*). According to Gortner (*6, p. 324*), the red coloration is specific for tyrosine in protein substances.

Sheath material and chitinous insect parts gave negative tests for tyrosine when treated with Folin and Denis' reagent, Mörner's reagent, or sodium molybdate-sulphuric acid. These materials became chestnut brown in the presence of the last two reagents. The vascular tissue in the badly injured sections gave positive blue color reactions when treated with Folin and Denis' reagent or with sodium molybdate-sulphuric acid. The greater brilliance of the blue color observed in treated sections of injured plants as compared with that seen in uninjured tissue of the same age suggests the possibility of local accumulation of proteins in addition to starch (*7*) as a result of insect feeding and the consequent interference with translocation of plant material.

In the tests for mucin in the sheath material, chitinous parts of leaf hoppers and chitosan produced from chitin gave negative reactions when treated with Ehrlich's reagent, ferric chloride and hydrochloric acid, or with thionine blue, as compared with mucin from saliva. These materials did not dissolve in concentrated hydrochloric acid, potassium hydroxide, or in 0.5 percent sodium carbonate, as did mucin.

Horsfall's test with thionine blue for mucin in sheaths of aphids was negative (*9, p. 9*).

Büsgen (*1, p. 44*) and Davidson (*4, p. 43*) stated that javelle water dissolved the sheaths of aphids. In the present study sheath material was dissolved in 1½ hours by several changes of javelle water.

Chitin is relatively insoluble in 50 percent chromic acid. In tests with this reagent on sheaths in plant sections, as indicated under the tests for lignin, all the cells except traces of the xylem tubes dissolved at the end of 22 minutes, but portions of the sheath walls remained. Chitinous walls on legs of leaf hoppers were apparently unchanged by a like treatment, but these parts are much larger than the thin walls of the sheath.

Sheath material, when tested for chitosan, gave a positive delicate violet color. Three percent acetic acid dissolved the sheath material after it had been changed by the chitosan procedure. Upon addition of 75 percent sulphuric acid the violet-colored sheath dissolved as the color faded; then, after a short interval, the cellulose walls of the plant cells exhibited the typical hydrocellulose reaction. With the exception of the test for chitosan sulphate crystals, the chitosan procedure was checked by carrying out the tests upon chitinous body parts of *Empoasca*. A flocculent material in the cells, particularly of the vascular bundle, gave a blue color with iodine-potassium iodide and often interfered with the observations on the sheaths. This was avoided by boiling the sections in 12 percent hydrochloric acid for 10 minutes before heating them in potassium hydroxide. The quantity of material that gave the test for chitosan was much less than that which gave a positive test with Millon's reagent.

These tests indicate that chitin is present in the sheaths of these insects, and, so far as the writer is aware, constitute the first evidence to be reported that this substance is secreted by the salivary glands of an insect.

SUMMARY AND CONCLUSIONS

A study has been made of the chemical nature of the sheaths found in feeding punctures produced by the potato leaf hopper (*Empoasca fabae*) and the three-cornered alfalfa hopper (*Stictocephala festina*). Tests were made for materials that might be derived from the surrounding plant cells and for materials that might be produced by the insects. The results are compared with those of previous workers.

In all tests, the sheaths made by the two species responded similarly.

All tests for plant-reserve, wound-response, and plant-cell-wall substances except the color tests for pectin compounds were negative.

Color tests for mucin were negative, but certain color tests for proteins and amino acids showed that the sheath material is largely proteinaceous.

The sheath material may contain chitin, as it responded similarly to chitinous exoskeleton in a number of tests.

In the course of this study four tests were found which differentiate sheath material from plant tissue by color, namely, Adamkiewicz, Sakaguchi, quinone solution, and Benedict's modification of the Hopkins-Cole reaction.

It appears that the sheath is largely of insect origin and contains no plant substance, with the possible exception of pectose. The highly insoluble sheath, persisting in the vascular tissue, probably interferes

with the normal translocation of plant materials and accounts for the typical plant injuries caused by the feeding of *Empoasca fabae* and *Stictocephala festina*.

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