Studies on the Feeding Habits of *Lygus disponi* LINNAVUORI
(Hemiptera : Miridae) and the Injury to Its Host Plant
IV. Amino Acids and Sugars in the Injured Tissue of
Sugar Beet Leaf¹

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The amino acid and sugar composition of sugar beet leaf tissue injured by *Lygus disponi* were quantitatively and semiquantitatively analyzed. Thirteen amino acids and five unidentified ninhydrin positive compounds were detected in the tissue. Concentrations of all thirteen amino acids increased remarkably in injured tissue, while the composition pattern of individual amino acids was found to be markedly different from that of uninjured tissue. Five kinds of sugars (including an unidentified sugar) were detected in injured tissue and all of these with the exception of glucose were more abundant in injured rather than uninjured tissue. The unidentified sugar was not detected in uninjured tissue. The relationship between the alteration of the pattern of these compounds in the tissue and the injury (leaf malformations) is discussed.

INTRODUCTION

There have been a great number of investigations on the morphology and histology of injury caused by mirids, but only a very few concerned with the physiology of the injury. The leaves of sugar beet plants damaged by *Lygus disponi* show various types of malformation including distortion, bending and wrinkling (HORI, 1967). HORI (1971) ascribed these malformations in part to *Lygus*'s peculiar feeding site, i.e., the intrafascicular cambium in the petiole and leaf vein. STRONG (1970) investigated the physiology of plant injury caused by *Lygus hesperus* and concluded that injury is correlated to peculiar feeding sites (regions in which plant hormones are produced), the feeding size and the tissue destruction by salivary polygalacturonase. In addition to these causes, HORI (1973) inferred that the injury might be attributed to the continuous hypersensitive reaction brought about by quinones produced in the plant tissue as the result of the damage. Alterations in the pattern of amino acids and other compounds in plant tissue may possibly take part in the disturbance of various metabolic processes by which malformations might originate. The present study is concerned with alterations in the pattern of amino acids and sugars in injured tissue, and is part of a basic research study on the physiological mechanisms of injury caused by *L. disponi*.

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MATERIALS AND METHODS

Preparation of the solution. The test plants used were prepared according to the method described in the previous report (HORI, 1973). The test samples, petioles and leaf veins, were collected individually from injured parts of damaged plants and corresponding parts of the uninjured plants in similar amounts of fresh weight. These samples were extracted in distilled water (5 ml per 1.399 g of fresh weight) by grinding with a mortar and pestle in quartz sand. After refrigerated centrifugation at 25,000 g, 3.0 ml of a 5 % trichloroacetic acid solution was added to the supernatant in order to precipitate proteins. The solution was again centrifuged and the resulting supernatant was lyophilized. The solid matter was washed several times with ethylether to remove trichloroacetic acid and then re-lyophilized. The remnant solid was dissolved in distilled water and diluted to a suitable concentration to permit amino acid analysis.

Quantitative analysis of amino acids. Amino acids were analyzed on a NEC Amino Acid Analyzer 6AUS. The analyses were performed by using 0.8 ml of the test solution, which was prepared by dissolving the extracted lyophilized solid in 25 ml or 12.5 ml of distilled water for injured tissue and uninjured tissue samples, respectively.

Semiquantitative analysis of sugars. Analyses were performed by means of descending paper chromatography, using the same test solution as that for the analysis of amino acids. Fifteen μ l aliquots of the test solution and 5 μ l aliquots of standard solutions (each containing glucose, fructose, mannose, ribose, sorbose, arabinose, xylose, ramnose, raffinose, sucrose, lactose, maltose, deoxyribose, galacturonic acid or glucuronic acid) were spotted adjacently on the paper and dried at room temperature. Following development with a solvent system of phenol and water (4 : 1, v/v), the paper sheet was dried with a hair dryer, sprayed with aniline hydrogenphthalate in water-saturated *n*-butanol, and heated for several minutes at 110 °C for color development. The identification of sugars was performed by comparison of the test solution chromatogram with that of standard solutions. Amounts of various sugars contained in the injured and uninjured tissues were compared semiquantitatively according to the area and density of respective color spots.

RESULTS

Amino acids in injured and uninjured tissues

Thirteen kinds of amino acids and five kinds of unidentified ninhydrin positive compounds were detected in both injured and uninjured tissue. When measured per fresh weight or protein, all amino acids were far more plentiful in the injured than in the uninjured tissue. When measured per dry weight, the contents of aspartic acid, threonine, serine, glutamic acid and glycine were more abundant in uninjured rather than in injured tissue, whereas alanine, phenylalanine and lysine were only slightly more abundant, histidine moderately abundant, and valine, leucine, isoleucine and arginine far more abundant in the latter than in the former (Table 1). Three kinds of unidentified ninhydrin positive compounds (A, C and E) were more abundant per fresh weight in the injured rather than uninjured tissue, while the contrary was found for the two other compounds (B and D) (Table 2).

Sugar in the injured and uninjured tissues

Five kinds of sugars (including an unidentified sugar) were detected in the leaf

Table 1. AMINO ACIDS CONTAINED IN UNINJURED SUGAR BEET TISSUE AND TISSUE INJURED BY *L. disponsi*

Amino acid	Injured tissue (mg per 100g) (fresh weight)	Uninjured tissue (mg per 100g) (fresh weight)	Relative value (Uninjured = 100)	Injured tissue (mg per 100g) (dry weight)	Uninjured tissue (mg per 100g) (dry weight)	Relative value (Uninjured = 100)	Relative value per protein (Uninjured = 100)
Aspartic acid	34.14	21.41	159	334.57	369.11	91	148
Threonine	63.06	42.06	150	617.99	725.11	85	139
Serine	18.41	12.94	142	180.42	223.09	81	132
Glutamic acid	132.89	84.56	157	1302.32	1457.81	89	146
Glycine	3.10	2.79	111	30.38	48.10	63	103
Alanine	25.30	10.58	239	247.94	182.40	136	221
Valine	11.18	1.96	570	109.56	33.79	324	527
Isoleucine	10.76	1.79	601	105.45	30.86	342	558
Leucine	15.04	2.61	576	147.39	45.00	328	533
Phenylalanine	46.29	22.72	204	453.64	391.69	116	189
Histidine	4.78	1.30	368	46.84	22.41	209	341
Lysine	6.76	2.50	270	66.25	43.10	154	250
Arginine	12.07	1.89	639	118.29	32.58	363	592
Total	383.78	209.11	184	3761.04	3605.05	104	169

Table 2. UNIDENTIFIED NINHYDRIN POSITIVE COMPOUNDS IN UNINJURED SUGAR BEET TISSUE AND TISSUE INJURED BY *L. disponsi*

Compound ^a	Relative value of amount of compound	
	Injured tissue	Uninjured tissue
A	200	100
B	50	100
C	178	100
D	77	100
E	200	100

^a (A) appeared just before aspartic acid, (B) appeared between histidine and lysine, (C) and (D) appeared between lysine and NH_3 and (E) appeared between NH_3 and arginine.

Table 3. SUGARS CONTAINED IN THE UNINJURED SUGAR BEET TISSUE AND TISSUE INJURED BY *L. disponsi*^a

Sugar	Injured tissue	Uninjured tissue
Glucose	+++	+++
Fructose	++	+
Ribose	++	+
Deoxyribose	+	±
Unidentified sugar ^b	+	—

^a +++, large amount; ++, moderate amount; +, small amount; ±, trace; —, absent.

^b The unidentified sugar was 0.16 in R_f value and pink in colour on the chromatopaper.

tissue (the petiole and leaf vein) of sugar beet. Glucose was detected in equal abundance in both injured as well as in the uninjured tissue. The injured tissue contained fructose and ribose in moderate amounts, while the uninjured tissue only contained these in small quantities. In the injured tissue a small quantity of deoxyribose was detected, while in the uninjured tissue only a trace amount was detected. An unidentified sugar (Rf value=0.16, pink in colour) was found only in the injured tissue (Table 3).

DISCUSSION

Some amino acids (especially those which are non-nutritional) fed to hemipterous bugs pass through the hemolymph to enter the salivary gland and are then ejected into the host plant (NUORTEVA, 1955, 1956; NUORTEVA and LAUREMA, 1961). It has also become clear that many aphids have various kinds of amino acids in their salivary gland (KLOFT, 1960; SCHÄLLER, 1968). The amino acids ejected into the host plant are capable of disturbing various metabolic processes in plant tissue (KLOFT, 1960) and act as a sort of phytotoxin (NUORTEVA, 1962). In some cases, they have been considered to take part in gall-formation (SCHÄLLER, 1968). Accordingly, the phytopathological degeneration of plant tissue injured by hemipterous bugs seems to be partly attributable to the alteration of the concentration and constituents of amino acids and other compounds in the tissue.

The amount of amino acids per fresh weight or protein increased remarkably in the tissue of sugar beet leaf when injured by *L. disponi*. SOGAWA (1971) found a similar increase of amino acids in the leaf blade of rice plants infested with the brown planthopper. According to the rate of increase or decrease per dry weight, amino acids can be divided into the following four groups: the first group contains aspartic acid, threonine, serine, glutamic acid and glycine which are more abundant in the uninjured than in injured tissue; the second group comprised of alanine, phenylalanine and lysine is present in uninjured tissue at 65 to 85% of the contents occurring in injured tissue; the third group includes only histidine which increases about twofold owing to the injury; the fourth group contains valine, leucine, isoleucine and arginine which increase more than threefold as the result of the injury. Thus the pattern of amino acids and sugars in the injured tissue differed compositionally and in concentration from that of the uninjured tissue (the alteration of the relative concentrations among different kinds of amino acids or sugars).

In view of these facts, it is apparent that the alteration of the pattern of amino acids and sugars in the tissue is in some way linked with the injury, the malformations, of sugar beet leaf by *L. disponi*. A question to be investigated in the future is whether the alteration in the pattern of amino acids and sugars is a direct consequence of mixing of amino acids contained in the saliva of the bugs in the tissue or whether it is brought about by the primary disturbance and/or imbalance of various metabolic processes in the plant tissue itself, owing to the mechanical stimulus by the stylets of the bugs and to phytotoxin and amino acids in their saliva. In any case, it may be presumed that the alteration of the pattern of amino acids and sugars gives rise to a secondary disturbance of the metabolism in the plant tissue, which then results in the injury (leaf malformations) in addition to the primary disturbance.

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