Characterization of Amino Acids and Carbohydrates Found in Whitefly Honeydew As the First Step Toward Biological Control

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ABSTRACT

A Florida strain of sweet potato whitefly, Bemisia tabaci (Gennadius), was found to have an expanded range which includes several new food crops. To determine why, we examined how it processes plant nutrients. The amino acid and carbohydrate content of phloem sap of poinsettia and pumpkin and of honeydew produced by the Florida strain and a strain from Arizona feeding on both plants were analyzed. Poinsettia phloem sap contained 15 amino acids; 14 of these were in pumpkin phloem sap. Almost all the same amino acids were in the honeydews produced by the two strains on the two hosts. Approximately half of the amino acids found in the honeydew were at concentrations which were significantly lower than concentrations in the phloem sap. Honeydew from both hosts contain six additional amino acids. The major one was glutamine which may be used to expel nitrogen. Carbohydrates in phloem sap and honeydew were common transport sugars, like sucrose. Both honeydews contained trehalulose, a disaccharide not previously associated with insects. Both strains processed phloem sap and honeydew from both plants in the same manner, but the Florida strain produced significantly larger quantities of honeydew; it is therefore assumed to process more phloem sap. Since this strain has access to more phloem sap it also has access to more of the amino acids which are in short supply in the phloem sap of some plants allowing it to broaden its range.

INTRODUCTION

The sweet potato whitefly, *Bemisia tabaci* (Gennadius) has been a serious pest of vegetables and cotton in the Southwest since the 1981 growing season. In 1988 it was found feeding on poinsettias, *Euphorbia pulcherrima* (Willd.), in Arizona. It is felt that these insects are part of a population which originated in Florida where it has broadened its host range.

We wanted to examine the amino acid and carbohydrate content of the phloem sap of poinsettia and pumpkin, *Cucurbita maxima* Ducheshe, (a typical field host of *B. tabaci*). We wanted to compare this information with information concerning the content of whitefly honeydew (excrement). In doing so, we hoped to find clues concerning why the Florida strain was able to broaden its host range. We also needed to find the exact composition of whitefly honeydew because we have found that the spores of two entomogenous fungi, *Verticillium leucanii* and *Aschersonia aleyrodes*, will germinate on honeydew in the absence of insects. We are working to devise a system where we can apply a medium of artificial honeydew and fungal spores to ornamental plants to curtail whitefly populations.

METHODS AND MATERIALS

The Florida strain of *B. tabaci* was maintained on poinsettia while the Arizona strain was kept on pumpkin.

Honeydew was collected from the following combinations: 1) honeydew produced by the Florida strain of *B. tabaci* feeding on poinsettia; 2) honeydew produced by the Florida strain of *B. tabaci* feeding on pumpkin; 3) honeydew produced by the Arizona strain of *B. tabaci* feeding on pumpkin; and 4) honeydew produced by the Arizona strain of *B. tabaci* feeding on poinsettia.

Honeydew was collected by placing 40 whiteflies on the undersides of leaves in 5.0 cm diameter plastic petri dish clip cages for 48 h. Honeydew droplets fell onto the floor of the clip cages. Immediately after removal from the plants, the dishes and their contents were frozen at -70°C, before being processed for identification of amino acid and carbohydrate components.

The dry mass of the honeydew produced by each combination was also measured. Before their use as clip cages, petri dishes were weighed with a microbalance. After 40 whiteflies had deposited their honeydew for a 48 h period, the petri dishes were placed in a drying oven at 40°C and reweighed at 0.5 h intervals, until no additional discernible weight loss was observed.

Phloem sap was collected by cutting petioles and allowing them to bleed into an agent which prevented the sap from forming proteinaceous clots. This material, along with honeydew samples, was prepared for injection into an HPLC for carbohydrate and purine analysis. Similar samples were subjected to a Beckman Amino Acid Analyzer.

RESULTS

Amino Acid Analysis

Tables 1 and 2 show the results (presented as relative percents) of the amino acid analysis of the two phloem saps and the honeydews produced by the two whitefly strains. The phloem sap of poinsettia contained a total of 15 different amino acids; with the exception of proline, the same amino acids were found in pumpkin phloem sap. Glycine and alanine were the predominant amino acids in both plants (accounting for almost half the amino acids present in the honeydews from poinsettia and as much as 45% of those from pumpkin).

For the most part, the amino acids in the phloem sap of poinsettia were also found in the honeydew of the whiteflies feeding on that host, regardless of strain. The one exception was that the honeydew produced by the Florida strain did not contain methionine. For seven amino acids, the concentrations in the honeydew were significantly lower than concentrations in the phloem sap ($P < 0.01$): alanine, citrulline, valine, leucine, phenylalanine, lysine, histidine and arginine. In no case was an amino acid found at a higher concentration in the honeydew than in the phloem sap. Also, the amino acid concentrations in the honeydew produced by the two whitefly strains showed no significant difference ($P > 0.01$).

As an example, the concentration of alanine in the honeydew produced by both the Florida and Arizona strains was significantly below the levels found in the phloem sap of poinsettia ($P < 0.01$). Concentrations of alanine in the two honeydews were not significantly different from one another ($P > 0.01$).

With the exception of proline, the same amino acids were found in pumpkin phloem sap as in poinsettia. Of the 14 amino acids associated with pumpkins, six were concentrated in the honeydew at significantly lower levels than those in the phloem sap ($P < 0.01$). These, with the exception of histidine and arginine, were the same amino acids exhibiting reduced concentrations in the honeydew produced by whiteflies feeding on poinsettia. Again, the concentrations were not significantly different in the pumpkin honeydew produced by the two strains ($P > 0.01$).

In addition to the 15 amino acids found in poinsettia phloem sap and the 14 in pumpkin phloem sap, six additional amino acids were found in the honeydew produced by the two whitefly strains. The predominant additional amino acid produced by both strains feeding on both hosts was glutamine; this accounted for as much

as 54% of the amino acids. Others present in honeydew but not in phloem sap were aspartic acid, threonine, serine, asparagine and glutamic acid. For these six, no significant differences in concentration were found in honeydew produced by either strain ($P > 0.01$).

In addition, both strains of whitefly feeding on poinsettia produce honeydew containing proline, which was not found in the phloem sap.

Carbohydrate analysis

The predominant carbohydrate found in the phloem sap of poinsettia was sucrose (74%) (Table 3). Its two constituent monosaccharides, glucose and fructose, accounted for 6% and 20%, respectively, of the carbohydrates present. Whiteflies of both strains feeding on poinsettia converted a small portion (< 10%) of the carbohydrates from the phloem to the trisaccharide melezitose (Table 3). The honeydew produced by the Arizona strain contained a small portion (approximately 4% of the total carbohydrates) of stachyose. They converted a large portion of the carbohydrates found in phloem sap to the disaccharide, trehalulose, which is composed of glucose and fructose connected by a 1-1 carbon linkage. These conversions were largely at the expense of sucrose, since its percentage was the only one to be decreased significantly ($P < 0.01$); the levels of glucose and fructose remaining unchanged.

The same carbohydrates found in poinsettia phloem sap were in the phloem sap of pumpkin. We also detected raffinose and galactose (Table 4). The two whitefly strains metabolized the carbohydrates found in pumpkin phloem sap (Table 4). Levels of the trisaccharide raffinose were reduced significantly, while levels of the tetrasaccharide stachyose were unchanged ($P > 0.01$). Levels of the one monosaccharide, glucose, were reduced significantly in the honeydew produced by both strains ($P < 0.01$), while levels of fructose remained stable ($P > 0.01$). Levels of sucrose were also unchanged. Galactose, a component of raffinose, increased significantly ($P < 0.01$), probably at the expense of the trisaccharide. Trehalulose, which was not found in the pumpkin phloem sap, was present in the honeydew produced by both strains at low levels (i.e., < 10%).

Honeydew Weight

In terms of total dry weight of the honeydew produced by the four feeding combinations, the Florida strain feeding on poinsettia produced significantly more than any other combination ($p < 0.01$) (Table 5). It produced almost five times the amount produced by the Arizona strain feeding on the same plant.

Purine Analysis

No uric acid or hypoxanthine were found in the honeydew produced by *B. tabaci* feeding on poinsettia. A very small percentage ($0.16 \pm 0.04\%$) of the sample was found to be xanthine.

DISCUSSION

All the amino acids found in the honeydew from the four feeding combinations have been observed in the excrement of various aphids. Usually, the amino acids in the honeydew are qualitatively the same as those found in the phloem sap of the host plant. There are reports, however, of homopterans producing additional amino acids.

The fact that basically the same amino acids were found in relatively reduced concentrations in the honeydew from both strains on both host plants indicates that the whiteflies are utilizing them in some manner. Homopterans commonly maintain prokaryotic bacteroids in their mycetocytes, which have been shown to synthesize several amino acids for their hosts. It seems unlikely, therefore that alanine, citrulline valine, leucine, phenylalanine lysine and histidine would all be essential to the whiteflies in the sense of the "rat 10 essential amino acids" (i.e., in their absence growth eventually ceases). It was known previously that the green peach aphid, *Myzus persicae* (Sulzer), has only three essential amino acids: methionine, isoleucine and histidine. When fed antibiotics, however, these aphids required several additional amino acids. Without a defined holidic diet,

however, it is impossible to determine which amino acids are essential for whiteflies. The nonessential amino acids undoubtedly provide components for the six or seven amino acids appearing in the honeydew which are not found in the phloem sap.

Because uric acid and hypoxanthine are absent, and because only trace amounts of xanthine are found in the honeydew, certain amino acids may be used to discharge excess nitrogen. While several may be involved, glutamine is a likely candidate; it was present in high concentrations in the honeydew, is relatively inexpensive energetically to produce (particularly in the presence of so much carbon) and has a high nitrogen to carbon ratio. This hypothesis will require further investigation.

The presence of high sucrose concentrations in the phloem sap of poinsettia was expected. This compound is commonly regarded as the main transport sugar in plants. The presence of its two constituents (glucose and fructose) is also not surprising, since stem walls are still metabolically active in this system; cell walls are known to contain invertases capable of hydrolyzing sucrose.

The presence of melezitose in the honeydew produced by whiteflies feeding on poinsettia could be anticipated, since this compound is commonly found in aphid honeydew. The two members of the raffinose family (raffinose and stachyose) are known to be widespread, particularly in the Cucurbitaceae where they serve as translocatable sugars; we could therefore expect to find them in pumpkin. The small amount of galactose in pumpkin phloem sap is likely the result of oligosaccharide catabolism by invertase. As with the amino acids, most of these carbohydrates have been reported in the honeydew of several aphids. The exception is galactose, but its presence is explainable.

The dramatic decline in the level of certain oligosaccharides (such as sucrose) in honeydew from poinsettia and the increase in others (like stachyose) in the honeydew from pumpkin are not easily explained; but the reasons are likely to relate to osmoregulation. We know that such a suggestion has been made concerning the presence of melezitose.

The most noteworthy discovery was that of the disaccharide trehalulose. Although it has been synthesized in the laboratory and appears to be produced by certain microorganisms, its production has never before been associated with members of Insecta. Earlier researchers found fructose to be a non-competitive inhibitor of the hydrolytic activity of isomaltulose synthase, an α-glucosidase that acts on sucrose. The presence of fructose apparently leads to unregulated trehalulose synthesis.

The bacteria *Erwinia* spp. are capable of synthesizing trehalulose when sucrose is available. It could be that isomaltulose synthase is in the bacteroid symbionts of *B. tabaci* mycetocytes. These bacteroids could be hydrolyzing sucrose by isomaltulose synthase to produce the trehalulose. The authors are working with others to characterize trehalulose more carefully and define its role, if any, for *B. tabaci*.

The information obtained on specific carbohydrates and amino acids provides little help in answering the initial question about the expanded host range of the Florida strain of *B. tabaci,* since the same carbohydrates and amino acids are found in the honeydew of both strains and seem to be processed in much the same way. The question of why the Florida strain has been able to expand its host range may have a more simple answer - that it processes more phloem sap than the Arizona strain. This assumption is based on the fact that it produces significantly more honeydew. Because it processes more phloem sap, it has access to more of the amino acids which may be in short supply in a particular plant.

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Table 1. Amino acids found in the phloem sap of poinsettia and in the honeydew of two strains of *Bemisia tabaci* feeding on poinsettia, expressed as percent composition.

		Whitefly honeydew	
Amino acid	Poinsettia phloem sap	Florida strain	Arizona strain
Aspartic acid	0	1.9 ± 0.4a[zy]	2.8 ± 0.7a
Threonine	0	2.4 ± 0.5a	1.8 ± 1.3a
Serine	0	12.5 ± 3.6a	8.3 ± 1.2a
Asparagine	0	5.8 ± 1.0a	5.2 ± 0.5a
Glutamic acid	0	9.3 ± 2.0a	7.7 ± 1.9a
Glutamine	0	52.9 ± 11.0a	54.1 ± 13.5a
Proline	3.9 ± 0.5a	3.3 ± 0.2a	3.1 ± 1.0a
Glycine	27.4 ± 10.0a	7.4 ± 11.5a	7.6 ± 8.7a
Alanine	20.3 ± 3.4a	1.4 ± 0.8b	2.1 ± 1.3b
Citrulline	2.2 ± 1.2a	0.1 ± 1.5b	0.2 ± 0.1b
Valine	10.0 ± 4.2a	0.9 ± 0.4b	1.2 ± 0.3b
Cystine	0.7 ± 0.4a	0.3 ± 0.4a	0.3 ± 0.3a
Methionine	0.8 ± 0.2a	0 a	0.9 ± 1.3a
Isoleucine	2.6 ± 1.4a	0.2 ± 0.2a	0.6 ± 0.3a
Leucine	4.2 ± 0.8a	0.4 ± 0.4b	1.0 ± 0.1b
Tyrosine	1.8 ± 0.8a	trace a	trace a
Phenylalanine	4.4 ± 2.2a	0.2 ± 0.2b	0.4 ± 0.4b
Beta alanine	0.6 ± 2.0a	0.7 ± 0.4a	0.4 ± 0.3a
Lysine	4.4 ± 1.5a	0.1 ± 0.2b	0.5 ± 0.5b
Histidine	3.4 ± 1.3a	trace b	0.8 ± 1.3b
Arginine	13.2 ± 6.2a	0.2 ± 0.2b	0.9 ± 0.9b

[z]Mean ± the standard deviation

[y]Means in a row followed by the same letter are not significantly different according to a Student-Newman-Kuels test ($P < 0.01$).

Table 2. Amino acids found in the phloem sap of pumpkin and in the honeydew of two strains *Bemisia tabaci* feeding on pumpkin, expressed as percent composition.

Amino acid	Pumpkin phloem sap	Whitefly honeydew: Florida strain	Whitefly honeydew: Arizona strain
Aspartic acid	0	6.4 ± 4.6a[zy]	5.0 ± 0.4a
Threonine	0	1.3 ± 0.4a	2.2 ± 0.5a
Serine	0	6.8 ± 1.8a	8.5 ± 2.2a
Asparagine	0	4.7 ± 3.4a	5.5 ± 4.4a
Glutamic acid	0	10.1 ± 10.0a	6.6 ± 0.9a
Glutamine	0	45.0 ± 7.3a	20.2 ± 16.0a
Proline	0	4.6 ± 0.7a	5.7 ± 1.6a
Glycine	14.2 ± 3.2a	7.8 ± 4.0a	8.6 ± 1.8a
Alanine	28.7 ± 4.8a	2.3 ± 1.0b	6.2 ± 1.8b
Citrulline	1.8 ± 0.6a	0.2 ± 0.1b	0.8 ± 0.1b
Valine	9.7 ± 1.5a	1.6 ± 0.7b	3.5 ± 1.2b
Cystine	1.6 ± 0.6a	1.1 ± 0.5a	1.6 ± 0.9a
Methionine	2.2 ± 0.6a	1.2 ± 0.5a	1.6 ± 0.4a
Isoleucine	3.9 ± 1.0a	1.4 ± 0.9a	3.8 ± 2.2a
Leucine	10.1 ± 1.8a	1.1 ± 0.6b	4.0 ± 1.3b
Tyrosine	1.9 ± 0.4a	0.2 ± 0.2a	2.1 ± 3.6a
Phenylalanine	10.0 ± 2.6a	0.4 ± 0.3b	1.3 ± 0.8b
Beta alanine	0.7 ± 0.1a	1.1 ± 0.5a	0.7 ± 0.3a
Lysine	8.9 ± 1.1a	0.9 ± 0.4b	1.8 ± 0.6b
Histidine	1.6 ± 0.5a	0.2 ± 0.1a	0.6 ± 0.1a
Arginine	4.5 ± 0.4a	1.6 ± 0.4a	3.3 ± 0.9a

[z]Mean ± the standard deviation
[y]Means in a row followed by the same letter are not significantly different (SNK) ($P < 0.01$).

Table 3. Carbohydrate components of the phloem sap of poinsettia and of the honeydew of two strains of *Bemisia tabaci* feeding on poinsettia (n = 4)

Carbohydrate	Poinsettia phloem sap	Whitefly honeydew: Florida strain	Whitefly honeydew: Arizona strain
Sucrose	74.5 ± 16.0%a[zy]	13.0 ± 1.9%b	11.0 ± 0.9%b
Glucose	6.0 ± 5.0%a	14.4 ± 1.9%a	12.4 ± 2.4%a
Fructose	19.5 ± 15.4%a	14.8 ± 0.5%a	17.7 ± 1.8%a
Stachyose	0a	0a	3.9 ± 2.6%a
Melezitose	0a	9.6 ± 1.1%b	6.1 ± 4.1%b
Trehalulose	0a	45.4 ± 4.9%b	50.8 ± 6.2%b

[z]Mean (± standard deviation) exuded per leaf over a six-hour period.
[y]Means in the same row followed by the same letter are not significantly different (SNK) ($P < 0.01$).

Table 4. Carbohydrate components of phloem sap of pumpkin and of the honeydew of two strains of Bemisia tabaci feeding on pumpkin (n=4).

Carbohydrate	Pumpkin phloem sap	Whitefly honeydew: Florida strain	Whitefly honeydew: Arizona strain
Sucrose	10.4 ± 4.7%a[zy]	4.7 ± 1.1%a	4.0 ± 0.9%a
Glucose	22.2 ± 2.8%a	8.2 ± 2.4%b	7.1 ± 3.0%b
Fructose	12.6 ± 1.6%a	12.8 ± 3.2%a	14.8 ± 0.5%a
Stachyose	12.6 ± 2.6%a	32.3 ± 14.7%a	35.0 ± 6.3%a
Raffinose	40.8 ± 5.9%a	21.5 ± 3.0%b	22.9 ± 1.5%b
Galactose	1.4 ± 1.1%a	5.5 ± 0.8%b	10.2 ± 1.6%c
Trehalulose	0a	14.8 ± 6.6%b	5.9 ± 3.6%ab

[z]Mean (± standard deviation) exuded per leaf over a six-hour period.
[y]Means in the same row followed by the same letter are not significantly different (SNK) ($P < 0.01$).

Table 5. Dry weight of honeydew produced by *Bemisia tabaci* strains feeding on different hosts. 40 insects fed for 48 h (n=4).

Whitefly/host combination	Mass[z] (μg)
Florida strain/poinsettia	17.2 ± 5.1a[y]
Florida strain/pumpkin	10.0 ± 2.7 b
Arizona strain/pumpkin	5.5 ± 1.9 b
Arizona strain/poinsettia	3.5 ± 2.4 b

[z]Mean plus or minus standard deviation.
[y]Numbers in the same column followed by the same letter are not significantly different from one another (SNK) ($P<0.01$).