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Influence of Amino Acids on Cassava Biotype *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) When Feeding on an Artificial System

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Abstract: An artificial feeding system for the cassava biotype whitefly *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) was developed and used to determine whether *B. tabaci* can successfully survive and oviposit eggs when feeding on amino acid diets. Sucrose 20% (W/V) plus each of 20 protein amino acids at four concentration levels (mg mL^{-1}) were investigated. Adult whiteflies showed at least 80% survival over 96 h when feeding on sucrose 20% or sucrose 20% plus each of 19 amino acids except valine on at least one concentration. *B. tabaci* oviposited at least 10 eggs when feeding on diets containing sucrose 20% plus either of asparagine, glutamine, serine, glutamic acid, alanine, aspartic acid, proline or glycine on at least one concentration. Survival of at least 80% and oviposition of at least 10 eggs occurred on feeding diets containing sucrose 20% plus either of glutamine, serine, alanine, aspartic acid, proline or glycine on at least one concentration. Important applications of the feeding system and these results are discussed.

Key words: *Bemisia tabaci*, survival, oviposition, diets

Introduction

Bemisia tabaci is an important insect pest affecting many crop species. The non-cassava biotype is polyphagous but does not colonize cassava (*Manihot esculenta* Crantz) whereas the cassava biotype is more restricted to cassava and some *Solanum* spp. (Legg *et al*., 1994). Artificial diets have been investigated for *Bemisia argentifolii* Bellows and Perring (Homoptera: Aleyrodidae) (Davidson *et al*., 2000), also referred to as B biotype of *B. tabaci*, but no such work has been conducted for cassava *B. tabaci*. The objective of this study was to develop a successful artificial feeding system for cassava biotype *B. tabaci* survival and secondly, to determine whether the whiteflies will oviposit eggs when feeding on the artificial system. Sucrose was included in the diet because simple sucrose diets have been used in studies involving other phloem sap sucking insects (Sogowa, 1974; Kim *et al*., 1985; Davidson *et al*., 1996). To improve the possibility of oviposition, 20 protein amino acids were investigated. The amino acids were selected because various studies seem to suggest the influence of amino acids on insect performance (Douglas and Prosser, 1992; Dorschner, 1993; Simpson *et al*., 1995; Fujita and Mitsuhashi, 1995).

Materials and Methods

Experiments were carried out in 2001 and the study period extended over a three year period. Circular and open ended Perspex cylinders of 2.5 cm external diameter, 2.0 cm inside diameter and 2.5 cm height, were used to prepare diet cylinders. One layer of parafilm was thinly stretched over one end of each cylinder. To the parafilm layer, the diet solution 200 µL was applied. A second layer of

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parafilm was placed over the first layer, so the diet solution was sandwiched between the two layers of parafilm. Care was taken to prevent the trapping of any air bubbles between the two layers of parafilm. With the use of an electric pooter, 10 adults (two days old, sex ratio 1:1) were introduced into the diet cylinders. Whiteflies were obtained from Namulonge, Uganda. After introduction of the whiteflies, each diet cylinder was covered with a green paper disc. The diet cylinders were randomly placed onto yellow coloured water sensitive papers impregnated with bromophenol blue, to detect honeydew droplets. In initial experiments the diet consisted of sucrose 20% (W/V) solution. Subsequently, whitefly survival and oviposition were recorded in a series of 20 experiments examining 80 diets (sucrose 20% plus each of the 20 amino acids at four concentration levels). The concentration range for each amino acid included its average concentration found in cassava var. Ebwanaterata (Stevenson unpublished data). Four to six replicates were used in the experiments. All experiments were maintained under conditions (27-29° C; 50-60% R.H; 14:10 h. L: D).

Results

Bemisia tabaci survival on sucrose 20% was 86% over 96 h and they also produced honeydew droplets indicating feeding activity. Whiteflies did not oviposit eggs when feeding on sucrose 20% solution.

Bemisia tabaci showed at least 80% survival over 96 h when feeding on sucrose 20% plus any of the amino acids except for valine, on at least one concentration. Amino acids on which better

Table 1: *B. tabaci* **survival % on four concentrations of 20 amino acids + sucrose 20% over 96 h**

Amino acid concentrations and *B. tabaci* survival %					Amino acid concentrations and *B. tabaci* survival %				
Amino acid		asparagine			Amino acid		cysteine		
Conc. (mg mL^{-1})	0.07	0.7	7.0	70	Conc. (mg mL^{-1})	0.004	0.04	0.4	4.0
B. tabaci survival %	95	90	65	70	*B. tabaci* survival %	90	95	82	0
Amino acid		glutamine			Amino acid		glycine		
Conc. (mg mL^{-1})	0.02	0.2	2.0	20	Conc. (mg mL^{-1})	0.003	0.03	0.3	3.0
B. tabaci survival %	90	85	82	78	*B. tabaci* survival %	88	78	85	90
Amino acid		serine			Amino acid		tyrosine		
Conc. (mg mL^{-1})	0.02	0.2	2.0	20	Conc. (mg mL^{-1})	0.03	0.3	3.0	30.0
B. tabaci survival %	85	90	95	95	*B. tabaci* survival %	78	82	78	85
Amino acid		glutamic acid			Amino acid		threonine		
Conc. (mg mL^{-1})	0.03	0.3	3.0	30	Conc. (mg mL^{-1})	0.002	0.02	0.2	2.0
B. tabaci survival %	72	92	5	5	*B. tabaci* survival %	78	85	82	98
Amino acid		alanine			Amino acid		lysine		
Conc. (mg mL^{-1})	0.003	0.03	0.3	3.0	Conc. (mg mL^{-1})	0.005	0.05	0.5	5.0
B. tabaci survival %	90	90	85	90	*B. tabaci* survival %	70	90	78	85
Amino acid		aspartic acid			Amino acid		phenylalanine		
Conc. (mg mL^{-1})	0.003	0.03	0.3	3.0	Conc. (mg mL^{-1})	0.0003	0.003	0.03	0.3
B. tabaci survival %	85	92	78	42	*B. tabaci* survival %	85	90	82	80
Amino acid		proline			Amino acid		isoleucine		
Conc. (mg mL^{-1})	0.002	0.02	0.2	2.0	Conc. (mg mL^{-1})	0.004	0.04	0.4	4.0
B. tabaci survival %	90	92	88	85	*B. tabaci* survival %	90	75	85	65
Amino acid		arginine			Amino acid		leucine		
Conc. (mg mL^{-1})	0.001	0.01	0.1	1.0	Conc. (mg mL^{-1})	0.003	0.03	0.3	3.0
B. tabaci survival %	88	90	75	65	*B. tabaci* survival %	60	65	85	75
Amino acid		methionine			Amino acid		valine		
Conc. (mg mL^{-1})	0.004	0.04	0.4	4.0	Conc. (mg mL^{-1})	0.002	0.02	0.2	2.0
B. tabaci survival %	75	90	82	62	*B. tabaci* survival %	55	78	70	75
Amino acid		histidine			Amino acid		tryptophan		
Conc. (mg mL^{-1})	0.005	0.05	0.5	5.0	Conc. (mg mL^{-1})	0.003	0.03	0.3	3.0
B. tabaci survival %	82	92	80	42	*B. tabaci* survival %	78	80	60	75

Table 2: *B. tabaci* oviposition on four concentrations of 20 amino acids + sucrose 20% over 96 h

Amino acid concentrations and *B. tabaci* oviposition					Amino acid concentrations and *B. tabaci* oviposition				
Amino acid		asparagine			Amino acid		cysteine		
Conc. (mg mL^{-1})	0.07	0.7	7.0	70	Conc. (mg mL^{-1})	0.004	0.04	0.4	4.0
No. of eggs	1	4	5	15	No. of eggs	1	0	5	0
Amino acid		glutamine			Amino acid		glycine		
Conc. (mg mL^{-1})	0.02	0.2	2.0	20	Conc. (mg mL^{-1})	0.003	0.03	0.3	3.0
No. of eggs	12	0	3	1	No. of eggs	10	1	0	6
Amino acid		serine			Amino acid		tyrosine		
Conc. (mg mL^{-1})	0.02	0.2	2.0	20	Conc. (mg mL^{-1})	0.03	0.3	3.0	30.0
No. of eggs	7	2	17	4	No. of eggs	0	4	0	0
Amino acid		glutamic acid			Amino acid		threonine		
Conc. (mg mL^{-1})	0.03	0.3	3.0	30	Conc. (mg mL^{-1})	0.002	0.02	0.2	2.0
No. of eggs	16	5	0	0	No. of eggs	0	0	0	0
Amino acid		alanine			Amino acid		lysine		
Conc. (mg mL^{-1})	0.003	0.03	0.3	3.0	Conc. (mg mL^{-1})	0.005	0.05	0.5	5.0
No. of eggs	7	21	5	10	No. of eggs	0	0	0	0
Amino acid		aspartic acid			Amino acid		phenylalanine		
Conc. (mg mL^{-1})	0.003	0.03	0.3	3.0	Conc. (mg mL^{-1})	0.0003	0.003	0.03	0.3
No. of eggs	29	17	0	2	No. of eggs	1	0	0	0
Amino acid		proline			Amino acid		isoleucine		
Conc. (mg mL^{-1})	0.002	0.02	0.2	2.0	Conc. (mg mL^{-1})	0.004	0.04	0.4	4.0
No. of eggs	11	16	8	29	No. of eggs	1	0	2	1
Amino acid		arginine			Amino acid		leucine		
Conc. (mg mL^{-1})	0.001	0.01	0.1	1.0	Conc. (mg mL^{-1})	0.003	0.03	0.3	3.0
No. of eggs	6	4	3	0	No. of eggs	0	0	0	0
Amino acid		methionine			Amino acid		valine		
Conc. (mg mL^{-1})	0.004	0.04	0.4	4.0	Conc. (mg mL^{-1})	0.002	0.02	0.2	2.0
No. of eggs	3	7	1	4	No. of eggs	0	0	0	0
Amino acid		histidine			Amino acid		tryptophan		
Conc. (mg mL^{-1})	0.005	0.05	0.5	5.0	Conc. (mg mL^{-1})	0.003	0.03	0.3	3.0
No. of eggs	1	4	0	0	No. of eggs	0	0	0	1

survival occurred at the lower concentration range included asparagine, glutamine, glutamic acid, aspartic acid, proline, arginine, histidine, cysteine, phenylalanine and tryptophan (Table 1).

Serine and leucine were amino acids on which survival was better on the higher concentrations. Survival was constant across the concentration range for alanine and tyrosine, while there was no particular trend of survival on glycine, threonine, lysine, isoleucine and valine. On methionine, survival was best on the median concentration range (Table 1).

Bemisia tabaci oviposited at least 10 eggs over 96 h, when feeding on sucrose 20% plus any of the following amino acids on at least one concentration: asparagine, glutamine, serine, glutamic acid, alanine, aspartic acid, proline and glycine (Table 2).

Bemisia tabaci oviposited more eggs when feeding on the lower concentration range of glutamic acid, aspartic acid, arginine, histidine and tyrosine. The situation was the reverse for asparagine, while there was no particular trend for the other amino acid diets on which *B. tabaci* oviposited eggs (Table 2). No eggs were oviposited where feeding occurred on diets containing threonine, lysine, leucine or valine, while oviposition was particularly low on diets containing phenylalanine, isoleucine or tryptophan (Table 2).

Bemisia tabaci showed at least 80% survival and oviposited at least 10 eggs over 96 h, when feeding on sucrose 20% plus any of the following amino acids on at least one concentration: glutamine, serine, alanine, aspartic acid, proline and glycine.

Discussion

The concentrations of amino acids on which *B. tabaci* showed at least 80% survival and oviposited at least 10 eggs were as follows: glutamine (0.02 mg mL^{-1}), serine (2.0), alanine (0.03, 3.0), aspartic acid (0.003, 0.03), proline (0.002, 0.02, 2.0) and glycine (0.003). This group of amino acids is dominated by the amino acids considered non-essential for higher animals. Like some aphid species performing well on diets containing non-essential amino acids (Douglas, 1993) the present results show that *B. tabaci* can also perform well on diets containing non-essential amino acids.

The influence of amino acids on phloem sap sucking insects has been reported in earlier studies. Dadd and Krieger (1968) reported the importance of aspartic acid, glutamine, serine and alanine, in the growth of the aphid, *Myzus persicae* (Sulzer). Threonine and a combination of glutamine/asparagine were positively correlated to the relative growth rate of *Brevicoryne brassicae* (L.), while the glutamine/asparagine combination were positively correlated to the relative growth rate of *M. persicae* (Van Emden and Bashford, 1971). Glycine and threonine ostensibly had positive effects on the aphid, *Drepanosiphum platanoides* (Schrank) since these dominated the amino acid composition in the phloem of the host plant, *Acer pseudoplanatus* L. during the September to October period where the nutritional quality of the host plant for this insect species was superior to that during the summer months of July to August (Douglas, 1993). Colvin *et al.* (1999) observed higher population levels of *B. tabaci* associated with increased levels of asparagine in cassava plants infected with the Ugandan variant of *East African cassava mosaic virus*.

On diets containing threonine, lysine, leucine or valine, *B. tabaci* did not oviposit eggs, while oviposition was low on tryptophan, phenylalanine or isoleucine diets. These amino acids may more play an important role in other behavioral and physiological functions, or may not be beneficial in artificial diets for *B. tabaci*. Dadd and Krieger (1968) reported isoleucine as essential for growth of *M. persica*, while a moderate reduction in weight occurred in the absence of lysine. These researchers also reported that, phenylalanine, leucine, valine and tryptophan were not essential for the growth of *M. persicae*. In this study, *B. tabaci* performance on diets containing valine in particular, was comparatively poor since survival was less than 80% and no oviposition occurred across the concentration range.

Performance of phloem sap sucking insects on natural host plants could be different from that on amino acid diets mimicking the phloem sap composition of the host plants (Sandstrom, 1994). Thus performance on the host plant cannot be used to predict the performance on diets mimicking phloem sap of the host plant. In studies on *B. tabaci*, Blackmer and Byrne (1999) observed positive correlation between essential amino acids and whitefly weight and a negative correlation with developmental time on *Cucumis melo* L. They also found positive correlation between essential amino acids and whitefly emergence. These findings showed that within the natural host plant the essential amino acids were the dominant amino acids influencing growth and development. In this work, whiteflies in general showed better survival and oviposition when feeding on non-essential amino acids in artificial diets.

From these findings the varying influences of amino acids within artificial diets and the host plant could be explained in terms of other nutritional and physiological factors present in the host plant and devoid in the artificial diets.

Bemisia tabaci oviposition while feeding on amino acid diets is of significance since it reflects the ability of these whiteflies to not only survive, but to reproduce as well. This is the first report of *B. tabaci* oviposition on parafilm when feeding on artificial diets. Thus these artificial diets serve the basis for the development of holidic diets for cassava biotype *B. tabaci* and the *B. tabaci* species complex as a whole. One important area for research will be a determination of the optimum concentration levels for each amino acid to be included in a complete mix in attempts to develop holidic diets. Such diets have hitherto, not yet been developed for *B. tabaci*.

With the present feeding system, 80% of *B. tabaci* adults survived for as long as 96 h on sucrose 20% or 48 of the amino acid diets. In other studies, adult *B. argentifolii* showed 85-90% survival over 48 h when feeding on diets containing 15% (W/V) sucrose (Davidson *et al*., 1996). These researchers evaluated the influence of various compounds on adult *B. argentifolii* for a 48 h period. The present feeding system can be used for similar type studies, but allows for a longer monitoring period. This is especially important in testing the influence of slow acting compounds.

Other artificial feeding systems developed for *B. argentifolii* were used to evaluate the development of juveniles (Jancovich *et al*., 1997; Davidson *et al*., 2000) Studies of adult whiteflies on artificial feeding systems as in the present study, are also important and can result in the identification of compounds with insecticidal properties (through bio-assay work) which can then be utilized in the development of biorational insecticides. This will be of significance in the management of the important adult/reproductive stage, responsible for the transmission of geminiviruses.

Finally, studies on whitefly virus acquisition can be undertaken with the present feeding system. Specifically, the ability of cassava biotype *B. tabaci* to acquire non-cassava Whitefly Transmissible Geminiviruses (WTGs) can be investigated. If this can be achieved it will be of significant importance since such studies cannot occur on natural infected non-cassava host plants because these are not colonized by the cassava biotype (Legg *et al*., 1994). Investigating the possibility of cassava biotype *B. tabaci* successfully acquiring and transmitting non-cassava WTGs will provide a much better understanding of the interactions of the species complex of *B. tabaci* with host plants and the Geminiviruses they transmit.

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