Biological Characteristics and Esterase Patterns For Bemisia tabaci Populations, and the Association of Silverleaf Symptom Development in Squash With One Population

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Abstract

Biological characteristics (oviposition and survival rates) and esterase banding patterns were investigated to evaluate the extent of variation among three test populations of Bemisia tabaci Gennadius (Homoptera: Aleyrodidae). In terms of reproductive capabilities, whiteflies from the cotton (Gossypium hirsutum L.) and pumpkin (Cucurbita maxima Duchesne) populations performed similarly on the three host plant species tested. Both populations, which originated from the same wild-type field population, reproduced to higher levels on either cotton and pumpkin hosts than on a poinsettia (Euphorbia pulcherrima Willdenow) host. In contrast, whiteflies from the poinsettia population differed from cotton- and pumpkin-reared populations in that reproductive capabilities were relatively similar for the three host species tested. For whiteflies from pumpkin and cotton populations a similar and characteristic esterase banding pattern ("A-type") was observed, while whiteflies from the poinsettia population yielded a different banding pattern ("B-type"). In transmission studies, whiteflies from cotton or pumpkin sources did not induce silverleaf (SL) or white stem (WS) symptoms in Cucurbita spp. tested. In contrast, poinsettia population whiteflies were routinely associated with SL and WS symptom phenotypes which developed in Cucurbita spp. following exposure to whitefly adults. From these data, it is possible to correlate a specific esterase banding pattern (A or B) with reproductive capabilities and either the ability or inability to induce SL and WS symptoms.

Introduction

In the past decade the importance of the whitefly, *Bemisia tabaci* Gennadius, as serious pest in vegetable and fiber crops has increased on a worldwide basis. Recently, symptoms like those described for the squash silverleaf disorder (SSL) in *Cucurbita* spp. (Maynard and Cantliffe, 1989), were observed in field zucchini and pumpkin in Arizona (1989 and 1990 authors, pers. observ.). Although feeding by *B. tabaci* (Maynard and Cantliffe, 1989; Yokomi *et al.*, 1990) and the presence of two double-stranded RNAs (Bharathan *et al.*, 1990) have been associated with the disorder, the etiology of this disease is unknown.

Despite the unprecedented *B. tabaci* population levels which have been associated with increased virus incidence in the Southwestern U. S. during the past decade, SSL symptoms were not observed in Arizona until 1989. This observation suggests that either a recent change has occurred in endemic *B. tabaci* populations, or that an exotic population with different biological and/or genetic characteristics possibly affiliated with the SSL disorder has become prevalent.

B. tabaci populations have been described which utilize certain host plant species more efficiently for reproduction than others (Costa, 1978; Bird and Sanchez, 1971; Mound, 1983), and/or which differ in the ability to transmit several plant viruses (Bird and Sanchez, 1971). The terms "biotype" or "race" were used by investigators to refer to particular populations.

Electrophoretic analysis of isozymes has been successfully used to distinguish different genera of whiteflies (Prabhaker *et al.*, 1987). Different isozyme patterns have recently been observed for *B. tabaci* populations collected from different host plant species (Burban *et al.*, 1989, Costa and Brown, 1990) and/or from different geographic areas (Wool *et al.*, 1989).

In this study, biological characteristics (oviposition and survival rates) and esterase banding patterns were investigated to evaluate the extent of variation (with respect to these characteristics) among three test populations of *B. tabaci*. Esterase banding patterns were also compared for whiteflies collected from field and greenhouse plant hosts in both Arizona and Florida.

Methods

Biological Studies. Three populations of *B. tabaci* were studied: (1) a population originally collected from field cotton in Phoenix AZ in 1983 and maintained by continuous serial transfer on pumpkin (*C. maxima*, cv. "Big Max") in the greenhouse, (2) a sub-colony derived from the pumpkin population which was reared continuously in the greenhouse on cotton (*Gossypium hirsutum* L. cv."DP70") for more than two years, and (3) a population obtained from greenhouse grown poinsettias (*Euphorbia pulcherrima* Willd.) in Tucson, Arizona, and reared continuously on poinsettia ("Glory V-14") for more than two years.

Groups of five adult female whiteflies were collected in a random manner from colonies of each of the three populations and confined in clip cages (Costa et al. 1990) to the lower leaf surfaces of each of three host species (cotton, 3-4 leaf stage; pumpkin, 2-3 leaf stage; and poinsettia 10-12 leaf stage). Test plants were transferred to and maintained in a growth chamber (27 C and L12:D12) for the duration of the experiment. After 72 h, clip cages and adult whiteflies were removed and the number of eggs deposited during 72 h was recorded. Approximately 3 wk later (after immatures had completed development), the number of empty pupal cases (characteristic of successful adult eclosion) was recorded, and the proportion of immatures which survived was calculated.

Colonies and/or field populations were tested for the ability to induce white stem (WS)(Costa and Brown 1990) or silverleaf like symptoms in pumpkin and/or zucchini plants. Adult whiteflies were confined (15-20) to leaves for 72 h using clip cages as described above, and removed from plants using an aspirator. Plants were maintained in a greenhouse or growth chamber for 2-3 weeks and observed periodically during that time for symptom development.

Esterase Analysis. Samples of live adult whiteflies were collected from each of the three test populations and frozen immediately (-70 C). Individual adult whiteflies were homogenized in 12 microliters 0.1 M Tris-Borate-EDTA buffer, pH 7.0, containing 10% sucrose (Wool *et al.*, 1989). Samples were analyzed by polyacrylamide gel electrophoresis (PAGE) on 7% vertical native gels (0.75 mm thick) using a Tris-glycine, pH 8.3, running buffer (modified Laemmli, 1970). Esterase patterns were visualized in gels using a combination of alpha- and beta-naphthyl acetate as substrates and fast blue RR stain in 0.1 M phosphate buffer, pH 6.5 (Wool *et al.*, 1989).

In addition, esterase patterns for adult offspring of whiteflies originating from poinsettia population parents that had completed development on a pumpkin host, and conversely, offspring from pumpkin population parents which completed development on poinsettia, were analyzed electrophoretically for comparison with esterase banding patterns of the respective parent population. Adult whiteflies collected from fields and greenhouses in Arizona and Florida were also analyzed.

Results

Biological Studies. The mean number of offspring produced by five females in 72 hours by the pumpkin- and cotton-reared populations on either cotton or pumpkin hosts were not significantly different. Both of these populations produced significantly fewer offspring on poinsettia plants, however, while the poinsettia-reared population reproduced equally well on all three hosts (Table 1). Both whiteflies from the poinsettia-reared test population and offspring of poinsettia-reared parents that completed development on pumpkin, tested positive for production of SSL on zucchini and/or WS symptoms on pumpkin when exposed to susceptible plants (Table 2). Whiteflies collected from field cucumber (which apparently does not show SSL symptoms) also tested positive for the production of SSL symptoms in zucchini (Table 2). SSL or WS symptoms were not produced in zucchini or pumpkin by adult whiteflies from pumpkin-and cotton-reared populations, offspring of pumpkin-reared parents that completed development on poinsettia, or whiteflies collected from Arizona field cotton.

Esterase Analysis. PAGE analysis of whitefly adults yielded different esterase banding patterns for each of the three populations tested. Esterase banding patterns for whiteflies from the pumpkin and cotton populations were similar, but were not identical in all cases. Individuals from these populations consistently exhibited patterns resembling "A_1-A_6" types (pumpkin-type) (Figure 1). For these two populations, the most frequently encountered pattern consisted of two densely staining bands which migrated to the same location in the gel (Figure 1). Individuals from the poinsettia population routinely yielded a single band which migrated faster than either of the two densely staining bands characteristically associated with cotton or pumpkin individuals (Figure 1). For poinsettia individuals, a "B" type banding pattern was always observed (Table 2, Figure 1).

Offspring from the poinsettia population which were allowed to complete development on a pumpkin host showed an esterase banding pattern identical to that observed for the original parent (poinsettia) population. Likewise, progeny of pumpkin population whiteflies which completed their development on poinsettia plants had esterase banding patterns like those of the original parent (pumpkin) population.

Whiteflies collected from Arizona field cotton or tomato in 1989, and from field cotton in 1990 yielded pumpkin-type banding patterns (A_1-A_6) (Table 2). Whiteflies collected from Arizona field pumpkin in 1990, and which induced SSL in zucchini test plants, yielded esterase banding patterns characteristic of the poinsettia-type ("B") bands. Analysis of samples collected in 1990 from asymptomatic Arizona field cucumber also yielded the "B" type banding pattern. Samples of adults collected from both greenhouse poinsettia and field tomato in Florida, and analyzed in Arizona also exhibited the "B-type" banding pattern (Table 2).

Discussion

Whiteflies from the cotton and pumpkin populations both performed similarly in terms of reproductive capabilities, on the three host plant species tested (Table 1). Both populations reproduced to higher levels on either cotton or pumpkin than on poinsettia. These two populations originated from the same wild-type field population, and differed only in the host upon which they were reared in captivity. In contrast, whiteflies from the poinsettia population differed from cotton and pumpkin populations in that reproductive capabilities were nearly the same on all three host species tested. These results suggest relative differences in reproductive capabilities among these populations which could play an important role in host range adaptation of subsequent generations.

All populations tested in the laboratory thus far, fall into one of two general categories: the pumpkin or "A-type", or the poinsettia or "B-type" esterase banding patterns. Analysis of field samples collected from cotton and tomato in 1989, and from cotton in 1990 indicate that all individuals tested have an "A-type" banding pattern like those observed for the pumpkin population. Based upon esterase banding patterns, these data suggest that some whitefly populations present in Arizona fields in 1989 and 1990 are apparently similar to those of previously collected from cotton in 1983. Likewise, it is apparent that rearing of many generations exclusively on a pumpkin host has not directly altered the esterase patterns of *B. tabaci* originating from field cotton. Electrophoretic analysis of adults from field pumpkin (symptomatic) and cucumber (asymptomatic) plants (Table 2) collected in 1990, suggest that field populations of whiteflies exhibiting the "B-type" banding pattern are now present in Arizona. Further, it seems unlikely that esterase banding patterns are due solely to host plant imparted qualities; thus other (as yet unidentified) factors such as pesticide exposure, or geographic barriers may be involved.

During the course of these studies, pumpkin plants exposed to whiteflies from the poinsettia test population developed vein clearing and stem whitening (WS) symptoms (Costa and Brown, 1990) strikingly reminiscent of the phenomena described for *Cucurbita* spp. affected by the SSL syndrome in Florida (Maynard and Cantliffe, 1989; Yokomi *et al.*, 1990), Puerto Rico (Segarracarmona *et al.*, 1990), and the Dominican Republic (J. K. Brown, pers. observ.) Whiteflies from the Arizona cotton and pumpkin test populations however, do not induce WS symptoms in pumpkin (Costa and Brown, 1990, 1991) (Table 2). Preliminary results of studies in which zucchini plants were exposed to adult whiteflies from the three test populations indicated that whiteflies from the poinsettia population, but not those from either the pumpkin or cotton population, induced SSL like symptoms in zucchini squash (Table 2) (Costa and Brown, 1991). These data suggest that not all *B. tabaci* populations in Arizona are capable of transmitting or inducing WS or SSL symptoms in pumpkin or zucchini, respectively under the conditions described here. Reports from Florida indicate that all populations of *B. tabaci* collected in the state are capable of producing SSL symptoms in *Cucurbita* species (Maynard and Cantliffe, 1989; Bharathan *et al.*, 1990; Yokomi *et al.*, 1990), while *B. tabaci* from a California-reared population tested were not (Bharathan *et al.*, 1990; R. Yokomi, pers. comm.).

From the information available to date, there appears to be a correlation between esterase banding pattern types and the ability or inability of the population to induce WS and SSL symptoms in *Cucurbita* species. All populations of whiteflies which have the "B-type" pattern have thus far, been associated with SSL symptoms in the field, or have produced WS or SSL symptoms in susceptible test hosts (Table 2). Whether there is a direct association between the silverleaf syndrome and whitefly populations with specific esterase banding patterns remains to be determined.

References

Bharathan, N., W. R. Graves, K. R. Narayanan, D. J. Schuster, H. H. Bryan & R. T. McMillan, Jr. 1990. Association of double-stranded RNA with whitefly-mediated silvering in squash. Plant Pathology 39: 530-538.

Bird, J. & J. Sanchez. 1971. Whitefly-transmitted viruses in Puerto Rico. J. of Agric. Univ. Puerto Rico 55:461-467

Burban, C., L. D. C. Fishpool & J. D. Abisgold. 1989. Characterization des populations de *Bemisia tabaci* en fonction des plantes hotes: recherche de marqueurs electrophoretiques et transferts d'hotes. IV International Plant Virus Epidemiology Workshop (1989). Montpellier, France Sept. 3-8. pp 267e-267h.

Costa, A. S. 1978. Failure of *Bemisia tabaci* to breed on cassava plants in Brazil (Homoptera:Aleyrodidae). Ciencia e Cultura 27: 388-390.

Costa, H. S. & J. K. Brown. 1990. Variability in biological characteristics, isozyme patterns, and virus transmission among populations of *Bemisia tabaci* in Arizona. Phytopathology 80: 888 (abstract).

Costa, H. S. & J. K. Brown. 1991. Variation in biological characteristics and esterase patterns among populations of *Bemisia tabaci*, and the possible association of one population with silverleaf symptom development. Entomologia exp. appl. (submitted)

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

Mound, L. A. 1983. Biology and identity of whitefly vectors of plant pathogens.In: R. T. Plumb & J. M. Thresh (eds) Plant Virus Epidemiology. The Spread and Control of Insect-borne Viruses. pp. 305- 313. Blackwell Scientific Pub. Oxford, U. K.

Maynard, D. N. & D. J. Cantliffe. 1989. Squash silverleaf and tomato irregular ripening: new vegetable disorders in Florida. Fla. Coop. Ext. Ser., IFAS VC-37, 4 pp.

Prabhaker, N., D. L. Coudriet, & D. E. Meyerdirk. 1987. Discrimination of three whitefly species (Homoptera, Aleyrodidae) by electrophoresis of non-specific esterases. J. Appl. Entomol. 103: 447-451.

Segarracarmona, A. E., J. Bird & J. Escudero. 1990. Silvering of *Cucurbita moschata* (Duchesne) Poir associated with *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae) in Puerto Rico. J. of Agric. Univ. Puerto Rico. 74: 477-478.

Wool, D., D. Gerling, B. L. Nolt, L. M. Constantino, A. C. Bellotti & F. J. Morales. 1989. The use of electrophoresis for identification of adult whiteflies (Homoptera, Aleyrodidae) in Israel and Columbia. J. Appl. Entomology 107: 344-350.

Yokomi, R. K., K. A. Hoelmer, & L. S. Osborne. 1990. Relationship between the sweetpotato whitefly and the squash silverleaf disorder. Phytopathology 80: 895-900.

Table 1 Offspring production by whiteflies from three *Bemisia tabaci* populations on three host plant species. Mean number of offspring produced by five females in 72 hours.

	Host Plant Species		
	Cotton	**Pumpkin**	**Poinsettia**
Cotton whitefly population	51.6 ± 8.1a[a]	49.2 ± 10.5a	13.8 ± 3.8b
Pumpkin whitefly population	66.3 ± 6.3a	69.7 ± 10.0a	4.7 ± 1.6b
Poinsettia whitefly population	39.0 ± 4.5a	37.8 ± 14.8a	42.0 ± 6.1a

[a] Means in rows followed by the same letter are not significantly different ($P > 0.05$). (Tukey option in SAS, GLM procedure using square root transformed data, SAS institute 1985)

Table 2. Bemisia tabaci populations analyzed for esterase banding patterns and for the production of stem whitening and/or silverleaf symptom production in cucurbits.

Origin	Host Plant	Banding Pattern	Production of Symptoms	
			Pumpkin	Zucchini
Arizona 1990	Pumpkin colony population	A	(-)[a,b]	(-)[b]
Arizona 1990	Offspring of whiteflies from the pumpkin population which developed from egg to adult on a poinsettia host	A	(-)[a]	NT[e]
Arizona 1990	Cotton colony population	A	(-)[b]	(-)[b]
Arizona 1989	Field cotton	A	NT	NT
Arizona 1990	Field cotton	A	NT	(-)[b]
Arizona 1989	Field tomato	A	NT	NT
Arizona 1990	Poinsettia colony population	B	+[ab]	+[ab]
Arizona 1990	Offspring of whiteflies from the poinsettia population which developed from egg to adult on a pumpkin host	B	+[a]	NT
Arizona 1990	Field pumpkin showing symptom	B	+[c]	NT
Arizona 1990	Field cucumber	B	NT	+[b]
Florida 1989	Greenhouse poinsettia	B	+[d]	+[d]
Florida 1989	Field tomato	B	+[d]	+[d]

[a] Plants exposed to whiteflies in the greenhouse.
[b] Plants exposed to whiteflies in the growth chamber
[c] Field symptoms in pumpkin
[d] All Florida populations tested are reported to cause symptoms (Yokomi et al.1990; Bharathan et al.1990)
[e] Not tested

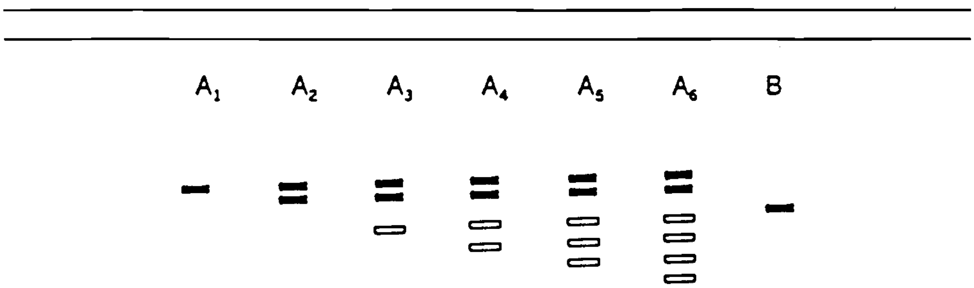

Figure 1. Diagram of esterase banding patterns associated with *Bemisia tabaci* test populations.