Biotype Q of *Bemisia tabaci* Identified in Israel

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The biotype status of samples of the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) collected from several field and greenhouse sites in Israel during 1999–2000 was determined by polyacrylamide gel electrophoresis (PAGE) for general esterases, and by RAPD-PCR using primers of arbitrary sequence. Results of this survey provide the first published evidence for the occurrence of the *B. tabaci* Q biotype, alongside the more widely distributed B biotype. Based on the collected samples, it appears that both the B and Q biotypes are present in Israel, and that field populations consist of a mixture of the two biotypes. A possible link between *B. tabaci* biotypes and insecticide resistance is discussed.
KEY WORDS: *Bemisia tabaci*; whitefly; biotypes; pyriproxyfen; insecticide resistance.

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), a serious pest in many agricultural systems (7), damages crops by feeding on phloem sap; in some cases this can result in >50% yield reduction. Also, honeydew excreted by the whitefly is a substrate for black sooty mould fungi that stain plant organs used for food and fiber. *B. tabaci* is also a vector of several important families of plant viruses.

As well as being of practical importance, *B. tabaci* is of considerable interest to evolutionary biologists owing to the extensive diversity that occurs in the *B. tabaci* complex (6). Biotypes have been distinguished largely on the basis of biochemical or molecular polymorphism. The biological significance of such biotypes is still unclear, but the biotypes are known to differ in some important characteristics, including host plant range and their capacity to cause plant disorders and/or transmit viral diseases. The B biotype was recognized in the late 1980s (11,12), following serious outbreaks of *B. tabaci* in the southwestern USA, and was proposed as a distinct species, *B. argentifolii*, in 1994 (3). The B biotype is widely distributed (4,6) and was reported to be the predominant biotype in Israeli *B. tabaci* populations (8). The second important biotype in southern Europe (the Q biotype) has generally been considered to be more restricted in range, occurring primarily in the Iberian Peninsula (2,14), Italy (Simón, B., pers. comm.) and Morocco (17). Reproductive incompatibility between some different *B. tabaci* biotypes is considered to contribute to maintaining their genetic isolation (1,8,12,13).

Following a survey of *B. tabaci* populations in Israel during 1999–2000, we report here the occurrence of the Q biotype in Israel, and present a provisional distribution of B and Q biotypes of *B. tabaci* at several locations in the country.

Two *B. tabaci* strains (the term 'strain' is used here to denote pyriproxyfen-resistant or -susceptible populations) were reared in a laboratory at The Volcani Center, near Bet Dagan, Israel. A susceptible standard strain (S) originated from cotton fields at Zora (near the Ayalon Valley) in 1987.

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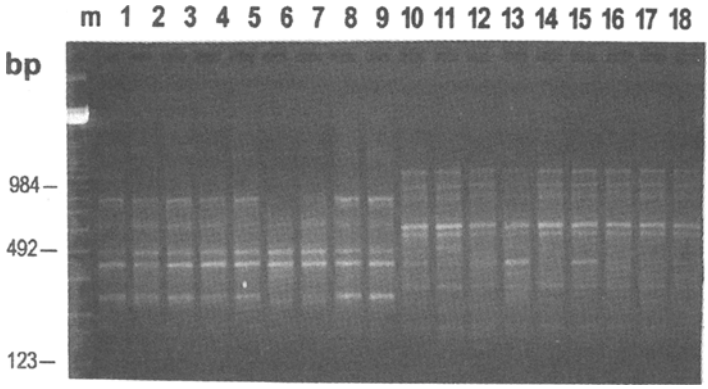

Fig. 1. Example of the amplification by RAPD-PCR of 18 individuals of *Bemisia tabaci* with primer OPE-06. Lane m: band size marker, ladder 123 bp. Lanes 1-2: samples from reference B biotype from Tenerife (Spain); lanes 3-9: samples of population Yesha-99; lanes 10-11: samples from reference Q biotype from Murcia (Spain); lanes 12-18: samples from population Pyri-R (see also Table 1).

TABLE 1. Various populations of *Bemisia tabaci* collected in Israel, their biotype identification, and resistance to pyriproxyfen (see also the locations in Fig. 2)

Population no. in Fig. 2 map	Name	Collection date	Location	Biotype	Resistance to pyriproxyfen (RR of LC_{50})	Identification method: 1 = PAGE 2 = PCR
1	S[z]	1987	Zora	B	1	1,2
2	Yesha-99	VIII.1999	W. Negev	B	2	1,2
4	BD-00	VII.2000	Bet Dagan	B	4	2
2	Negev-00	VIII.2000	W. Negev	B	0.4	1,2
7	BS-00	VIII.2000	Bet She'an	B	9	1
2	Pyri-R[y]	1991	GH[x], W. Negev	Q	1,200	1,2
5	HC-00	VIII.2000	Carmel Coast	Q	637	1,2
8	GH-00	X.2000	Jordan Valley	Q	4	1,2
3	AV-99	VIII.1999	Ayalon Valley	Q(B)[w]	167	1,2
3	AV-00	VII.2000	Ayalon Valley	Q(B)	81	1
6	W-Gal	VIII.2000	W. Galilee	Q(B)	25	1

[z,y]S and Pyri-R strains are reared in the laboratory.
[x]GH, greenhouse.
LC_{50} of S strain: 0.043 (0.027–0.062) ppm.
[w]Q(B), a mixture of predominant Q and B biotypes.

The Pyri-R strain, highly resistant to the IGR (insect growth regulator) pyriproxyfen (15), was collected in a rose greenhouse in the western Negev in 1991 and was continuously pressurized with pyriproxyfen. Both strains have been maintained in isolation under standard controlled chamber conditions. The other *B. tabaci* populations were collected from field and greenhouse crops in various areas of Israel in 1999–2000. The whitefly collections were part of a nationwide program to monitor resistance to several important whitefly control agents including pyriproxyfen, acetamiprid and diafenthiuron. For the biotype determination which was carried

out in conjunction with bioassays to monitor resistance in each collection, a *B. tabaci* adult specimen was placed in phosphate buffer and a duplicate group was stored in 80% ethanol (for the biotype determinations).

Bioassays for determining resistance to pyriproxyfen (Sumitomo, Japan, obtained through Agan, Ashdod, Israel) were based upon methods described previously (15,16). Cotton seedlings (20–25 cm tall) were dipped in aqueous concentrations of the formulated insecticide, or in deionized water as the control. Twenty *B. tabaci* females confined in clip-on leaf cages were exposed to treated cotton seedlings for 48 h and kept under controlled growth chamber conditions of 26±2°C, 60% r.h., and a photoperiod of 14:10 (L:D). Egg lay was checked 2 days after treatment, and egg viability (egg hatch suppression) was determined 6–8 days later. Each bioassay used at least four concentrations, each with five to ten replicates. Resistance to pyriproxyfen was defined as resistance ratio (RR) values, determined as LC_{50} of a field strain divided by LC_{50} of the susceptible strain.

Esterase analyses were conducted at IACR-Rothamsted, UK. To determine *B. tabaci* biotypes, general esterases were analyzed by native polyacrylamide gel electrophoresis (PAGE), and non-specific esterase profiles were compared to reference ones for different biotypes (5,8,11,12). In each assay, *B. tabaci*-collected populations were compared with control populations that were used as standards; *B. tabaci* biotype B ('MCK') originated from the USA, and biotype Q (EL EJIDO) originated from Spain.

RAPD-PCR determinations were conducted in the laboratories of CIDA, Spain. In order to have adequate controls for the experiment, two additional populations (standards) were used: one was collected from Murcia (Spain) and the other from Tenerife (Canary Islands, Spain). Both populations have been fully characterized previously (14) as belonging to biotypes Q and B, respectively.

Extraction of DNA from individual adults and the PCR reactions followed a published protocol (9). Reactions were made in a thermocycler GeneAmp System 9600 (Perkin Elmer, Norwalk, CT, USA), programmed as follows: denaturation at 94°C for 1 min, annealing at 34°C for 1 min, and extension at 72°C for 1 min. PCR products were loaded into a 1.4% agarose gel in 1X TBE buffer, and electrophoresed at 5 V/cm for 2 h. Amplified DNA was visualized and photographed under UV light after staining the gel with a solution of 0.5 g ml^{-1} of ethidium bromide. The primers used were 10-mer oligonucleotides of arbitrary sequence, supplied by Operon Technologies (Alameda, CA, USA). After preliminary trials, 11 primers were selected and used in the PCR reactions: OPC-03, -04, -07, -15, -16; OPE-06; OPO-01, -02, -06, -08; and OPP-05. At least five individuals from each population were analyzed, along with the reference samples of B and Q biotypes.

Both the PAGE and RAPD-PCR methods indicated that the B and Q biotypes were present, based on comparison with references for well-studied biotypes (Table 1, Figs. 1, 2). In comparisons using both methods, similar results were obtained. Thus, both methods are suitable for defining biotypes of *B. tabaci*. Of 11 populations collected in Israel, five contained only the B biotype and three contained only the Q biotype. Two samples from the Ayalon Valley and one from the western Galilee were a mixture of B and Q biotypes, with the majority being the Q biotype (approx. 80%).

It is of interest that a strain (Pyri-R) identified during this survey as the Q biotype had been collected as early as 1991 from a rose greenhouse in the western Negev (15). This strain, which has been characterized as highly resistant to the IGR pyriproxyfen, has since been maintained in isolation in the laboratory under strong selection pressure with pyriproxyfen (15,16). Among other *B. tabaci* populations collected recently (Table 1), four of five populations that were defined as the Q biotype were also resistant to pyriproxyfen, and all consisting of the B biotype were susceptible to this insecticide. Thus, it is possible that inherent levels of resistance to insecticides appear to differ in the B and Q biotypes; and these levels may have contributed to the dynamics and current distribution of B and Q biotypes in Israel. Other authors have hypothesized a link between establishment of invasive biotypes and insecticide resistance (10,12), but this needs to be investigated further.

Restrictions to interbreeding between different biotypes of *B. tabaci* (1,13; ARH, unpub. results), if confirmed, would limit the transfer of various genes between these biotypes.

Further study of biotype distribution and interactions among *B. tabaci* biotypes is needed to resolve differences in biochemical, physiological, and life-history traits that may affect their phenology, host plant specificity, insecticide resistance, and efficiency of virus transmission, in order to design effective crop protection strategies.

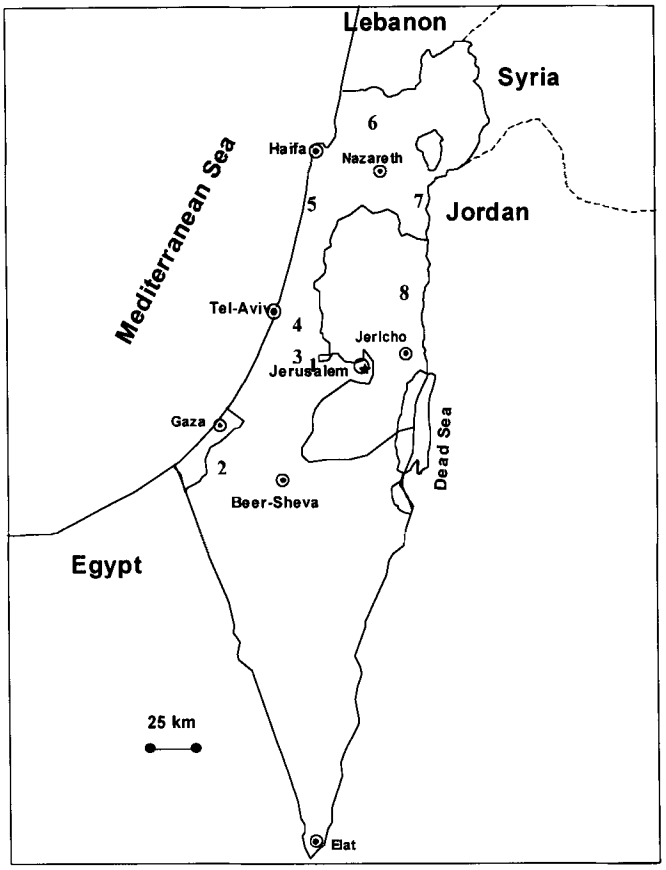

Fig. 2. Provisional distribution of *Bemisia tabaci* B and Q biotypes in Israel. Numbers on the map correspond to population numbers in Table 1: 1, S; 2, Pyri-R; 3, AV-99; 2, Yesha-99; 3, AV-00; 4, BD-00; 5, HC-00; 2, Negev-00; 6, W-Gal; 7-BS-00; 8, GH-00.

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REFERENCES

1. Bedford, I.D., Briddon, R.W., Brown, J.K., Rosell, R.C. and Markham, P.G. (1994) Geminivirus transmission and biological characterisation of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. *Ann. Appl. Biol.* 125:311-325.

2. Beitia, F., Mayo, I., Robles-Chillida, E.M., Guirao, P. and Cenis, J.L. (1997) Current status of *Bemisia tabaci* (Gennadius) in Spain: The presence of biotypes of this species. *Bull. OILB/SROP* 20(4):99-107.
3. Bellows, T.S. Jr., Perring, T.M., Gill, R.J. and Headrick, D.H. (1994) Description of a species of *Bemisia* (Homoptera: Aleyrodidae). *Ann. Entomol. Soc. Am.* 87:195-206.
4. Brown, J.K. (2000) Molecular markers for the identification and global tracking of whitefly vector-begomovirus complexes. *Virus Res.* 71: 233-260.
5. Brown, J.K., Coats, S., Bedford, I.D., Markham, P.G., Bird, J. and Frohlich, D.R. (1995) Characterization and distribution of esterase electromorphs in the whitefly, *Bemisia tabaci* (Genn.) (Homoptera:Aleyrodidae). *Biochem. Gen.* 33:205-214.
6. Brown, J.K., Frohlich, D.R. and Rosell, R.C. (1995) The sweetpotato or silverleaf whiteflies: Biotypes of *Bemisia tabaci* or a species complex? *Annu. Rev. Entomol.* 40:511-534.
7. Byrne, D.N. and Bellows, T.S. (1991) Whitefly biology. *Annu. Rev. Entomol.* 36:431-457.
8. Byrne, F.J., Bedford, I.D., Devonshire, A.L. and Markham, P.G. (1995) Esterase variation and squash silverleaf induction in B-type *Bemisia tabaci* (Gennadius). *Bull. Entomol. Res.* 85:175-179.
9. Cenis, J.L., Perez, P. and Fereres, A. (1993) Identification of various aphids (Homoptera: Aphididae) species and clones by Random Amplified Polymorphic DNA (RAPD-PCR). *Ann. Entomol. Soc. Am.* 86:545–550.
10. Coats, S.A., Brown, J.K. and Hendrix, D.L. (1994) Biochemical characterization of biotype-specific esterases in the whitefly *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae). *Insect Biochem. Mol. Biol.* 24:723-728.
11. Costa, H.S. and Brown, J.K. (1991) Variation in biological characteristics and esterase patterns among populations of *Bemisia tabaci* (Genn.) and the association of one population with silverleaf symptom induction. *Entomol. Exp. Appl.* 61:211-219.
12. Costa, H.S., Brown, J.K., Sivasupramaniam, S. and Bird, J. (1993) Regional distribution, insecticide resistance, and reciprocal crosses between the 'A' and 'B' biotypes of *Bemisia tabaci. Insect Sci. Appl.* 14:255-266.
13. De Barro, P.J. and Hart, P.J. (2000) Mating interactions between two biotypes of the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Australia. *Bull. Entomol. Res.* 90:103-112.
14. Guirao, P., Beitia, F. and Cenis, J.L. (1997) Biotype determination of Spanish populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Bull. Entomol. Res.* 87:587-593.
15. Horowitz, A.R. and Ishaaya, I. (1994) Managing resistance to insect growth regulators in the sweetpotato whitefly (Homoptera: Aleyrodidae). *J. Econ. Entomol.* 87:866-871.
16. Ishaaya, I. and Horowitz, A.R. (1995) Pyriproxyfen, a novel insect growth regulator for controlling whiteflies: mechanisms and resistance management. *Pestic. Sci.* 43:227-232.
17. Monci, F., Navas-Castillo, J., Cenis, J.L., Lacasa, A., Benazoun, A. and Moriones, E. (2000) Spread of *Tomato yellow leaf curl virus* Sar from the Mediterranean Basin: presence in the Canary Islands and Morocco. *Plant Dis.* 84:490.