FIRST RECORD OF *BEMISIA TABACI* BIOTYPE Q IN NEW ZEALAND

I.A.W. SCOTT[1], P.J. WORKMAN[2], G.M. DRAYTON[1] and G.M. BURNIP[3]

[1]*Crop & Food Research, Canterbury Agriculture & Science Centre, Private Bag 4704, Christchurch*
[2]*Crop & Food Research, Mount Albert Research Centre, Private Bag 92169, Auckland*
[3]*Biosecurity New Zealand, Ministry of Agriculture & Forestry, PO Box 24, Lincoln*

Corresponding author: scotti@crop.cri.nz

ABSTRACT

In January 2006 a grower reported failure to control whitefly on greenhouse capsicums in Karaka, South Auckland, despite high release rates of the biological control agent, *Encarsia formosa.* The whitefly was identified morphologically as *Bemisia tabaci* and capsicum represents a new host record for this species in New Zealand. *Bemisia tabaci* is polyphagous, and 24 biotypes are currently recognised worldwide. Biotypes B and Q have attracted international attention in the past two decades because of their rapid global spread. They are morphologically indistinguishable. DNA sequence analyses of samples collected in a survey in 2006 from capsicums and poinsettias revealed the presence of *B. tabaci* biotype Q in New Zealand. In a subsequent survey commissioned by Biosecurity New Zealand, 12 sites were visited between 27 June and 30 August 2006. *Bemisia tabaci* was only detected at one site but on multiple hosts. Sequences were identical to biotype Q sequences detected during the preliminary survey and to *B. tabaci* biotype Q samples from overseas.
Keywords: *Bemisia tabaci,* biotype Q, greenhouse, cytochrome oxidase I.

INTRODUCTION

The sweet potato whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is an important pest of a wide variety of vegetable, ornamental and other crops worldwide (Jones 2003; Ma et al. 2007). It is reported to be a vector of up to 111 important plant viruses (Jones 2003) and several of these are concerns for New Zealand's biosecurity.

Bemisia tabaci comprises twenty-four biotypes (A through T) (Perring 2001) that are primarily differentiated on genetic information that appears related to geographical separation rather than host plant associations (e.g. Brown & Idris 2005; de Barro et al. 2006; de la Rua et al. 2006). Two polyphagous biotypes, B and Q, have attracted considerable attention in the past two decades because of their rapid, global spread. Major outbreaks and invasions have occurred on all continents except Antarctica (de Barro et al. 2006). The Q biotype has exhibited an unusually rapid rate of dispersal. For example, Q was first recorded in the USA in March 2005 (Brown et al. 2005) and has since (to October 2006) spread to greenhouse cropping systems in 22 of the continental United States (Anon. 2007).

Both B and Q biotypes are of concern to growers because of known resistance to a range of insecticides, including organophosphates, carbamates and pyrethroids. Additionally, biotype Q is resistant, or has reduced susceptibility, to a number of neonicotinoids and insect growth regulators. The displacement of biotype B by biotype Q in many

Mediterranean countries is likely to be due to greater insecticide resistance in the latter, but biotype Q can be out-competed by biotype B under insecticide-free conditions (Pascual & Callejas 2004; Horowitz et al. 2005). The B and Q biotypes are morphologically indistinguishable and molecular diagnostic tools provide the only reliable means to discriminate between them (Perring 2001; de Barro et al. 2005). However, the taxonomy of *B. tabaci* remains confused and a global review of this species is desirable.

Bemisia tabaci was first detected in New Zealand in May 1991 on a poinsettia pot plant in the administration staff office of the DSIR Entomology Division, Mount Albert Research Centre, Auckland. The pot plant was purchased a year previously, so the arrival date of *B. tabaci* in New Zealand was perhaps 1990 or earlier (N.A. Martin, pers. comm.). A survey by MAF in 1991 showed that *B. tabaci* was widespread in the North Island but not in the South Island. At this time it was identified as simply *Bemisia tabaci*, as many of the currently recognised biotypes had either not been described or were not widely recognised. It was later presumed to be a mixed population of biotypes A and B, because of host range and the global spread of these biotypes at the time. The MAF survey recorded *B. tabaci* from poinsettia, begonia, gerbera, hibiscus, lisianthus and geranium (G.M. Burnip, pers. comm.). Additionally, molecular diagnoses of other samples of *B. tabaci* taken in the 1990s indicated that both biotypes A and B were present in New Zealand at that time (P. de Barro, pers. comm.).

Recently a whitefly sample was collected from glasshouse capsicums in Karaka, South Auckland, in May 2006 and morphologically identified as *B. tabaci*. Capsicum represented a new host record for this species in New Zealand and concerns were raised that this might be a new *B. tabaci* biotype. As a result Biosecurity New Zealand (BNZ) commissioned a survey to ascertain which strain or strains of *B. tabaci* are present in New Zealand.

METHODS

Preliminary samples

Bemisia tabaci specimens from the original site at Karaka, South Auckland, were collected and puparia identified morphologically by microscopic examination of the ligular (Martin 1999). In addition, whitefly samples were collected from poinsettia at a private residence in Auckland and a glasshouse operation in Hillsborough, Christchurch, and morphologically identified as *B. tabaci.* Samples (adults, puparia and nymphs) from all three sites were preserved in 100% propylene glycol for molecular diagnosis.

Main survey and rationale

Eight glasshouse producers and four botanical gardens were visited during a BNZ-commissioned survey between 27 June and 30 August 2006. Access to a further glasshouse operation was denied but samples had been collected on a previous visit (see footnote to Table 1). Sites were selected for the following reasons:

1. As *B. tabaci* biotype Q appears to be of Mediterranean origin and has an optimal developmental temperature of 25–28°C, most of the selected survey sites were in the warmer areas of the country (i.e. the Northland and Auckland regions).
2. Poinsettia nurseries were targeted because poinsettias are a preferred host of biotype Q worldwide and were found to be a preferred host of *B. tabaci* in New Zealand during the 1991 MAF survey. Few garden centres were surveyed as it is more efficient to survey the nurseries at the point of production and because there are no poinsettias in garden centres during winter.
3. Other nurseries where *B. tabaci* had been identified in the 1991 survey were also targeted. In addition, botanical gardens in the main centres (Auckland, Christchurch, Hamilton and Wellington) were surveyed because they house a wide range of plants, including subtropical or tropical plants that are potential hosts of *B. tabaci*.

Table 1 indicates localities visited and hosts sampled during the BNZ-commissioned survey of 2006.

All material was collected into 100% propylene glycol for molecular analysis.

Molecular diagnosis

Samples for molecular diagnosis were catalogued and designated unique codes based on sample site and host plant. DNA was extracted from individuals (adults, nymph and puparia) using a modified CTAB protocol. The mitochondrial target, cytochrome oxidase subunit I (COI) was amplified by PCR using generic insect primers (C1-J-2195 and TL2-N-3014; Simon et al. 1994). Products were purified (Promega Wizard SV gel and PCR Clean-Up System) and quantified for sequencing (Macrogen, South Korea). Appropriate positive and "no DNA" negative controls were used.

Due to some anomalous results from analysis of the preliminary survey samples, two sets of new primers (TvapF 5'-GGCATTATTTCTCATCTTATTAGTGCT-3' and WFrev 5'-GTGAYTAAGRGMTGGYTTATT-3'; BtabF 5'-GAGGCTGGAAAATTAGAGGTAT-3'and BtabR 5'-AACTGTGATTAAGRGCTGGTTTATTA-3') were designed to preferentially amplify COI in the whitefly species, *Trialeurodes vaporariorum* and *B. tabaci*, that were expected to be sampled during the survey. In order to be certain *B. tabaci* had been detected, all DNA samples were first screened with the *T. vaporariorum* markers. Samples that failed to amplify were then screened using the *B. tabaci* primers. Appropriate positive and negative controls consisting of DNA from previously identified *T. vaporariorum* and *B. tabaci* as well as "no DNA" negative controls were used for these PCR experiments.

Sequences were edited using Sequencher (GeneCodes Corporation) and translated into amino acid sequences to determine if the appropriate mitochondrial target had been amplified; stop codons would indicate that sequences were not mitochondrial but nuclear in origin. Following editing, sequences were aligned using the software ClustalX for visual comparison. Individual sequences (~780-800 bp) were used to interrogate the GenBank, EMBL (European Molecular Biology) and DDBJ (DNA Data Bank of Japan) databases using the BLAST search facility hosted by the National Centre for Biotechnology Information.

Sequence data were also used to compile a multiple sequence alignment with reference data for *B. tabaci* biotypes A, B and Q obtained in other studies. This reference COI sequence dataset comprises 30 sequences (3xA, 10xB, 17xQ). It was edited to 730 bp and compiled to encompass both the geographic and genotypic range of *B. tabaci* biotype Q. PAUP* (Swofford 1998) was used to calculate genetic distance matrices (uncorrected *p* and General Time Reversible distances) from the alignment and these matrices were then used to generate phylogenetic trees.

RESULTS AND DISCUSSION

A total of 20 sequences were obtained from samples collected from the three sites sampled during the preliminary survey. All were of mitochondrial origin. Sequences obtained from the New Zealand sites were identical and following database interrogation were found to match, or nearly match, sequences from known *B. tabaci* biotype Q sequences from other countries. However, three COI sequences obtained from Karaka belonged to the braconid parasitoid, *Encarsia pergandiella,* and this necessitated the design of specific *T. vaporariorum* and *B. tabaci* primers.

Table 1 shows the results of molecular diagnoses performed in the study. A total of 384 whitefly samples (adults, nymphs, puparia) were collected from ten of the 13 sites visited during the main survey, including Anderson's Zealandia. *Bemisia tabaci* puparia were identified morphologically from two sites. At one of these sites (Hamilton Botanic Gardens), *B. tabaci* were found on several different hosts (*Abutilon* sp., *Duranta repens, Gossypium hirsutum, Hibiscus hawaiiai, Pelargonium* sp.). Voucher specimens were taken and a subsequent subsample of 160 individuals was selected to ensure that samples from all sites were diagnosed using the new specific markers. A total of 101 individuals were subsequently assigned to either *T. vaporariorum* or *B. tabaci.* The target DNA fragment failed to amplify from 59 whitefly individuals with either the *T. vaporariorum* or the

B. tabaci markers. This was likely due either to poor DNA yield (perhaps empty puparia), to individuals being parasitised (insufficient whitefly target DNA) or to individuals belonging to a whitefly species other than either *T. vaporariorum* or *B. tabaci*.

TABLE 1: Locality and host plants where white fly adults and puparia were found along with the total number of white fly sampled (N1) in the 2006 survey. The presence of *Bemisia tabaci* puparia from morphological identification is indicated. Also shown are the number of samples that were diagnosed with molecular markers (N2), and the presence of *B. tabaci* biotype Q and *Trialeurodes vaporariorum* (Tvap).

Property	Host plant	Whitefly adults / puparia (N1)	*B. tabaci* puparia	Molecular diagnosis N2/Q/Tvap
Hamilton Botanical	Cotton	+/+ (13)	+	8/3/4
Gardens	Pelargonium	+/+ (20)	–	5/0/1
	Duranta	+/+ (12)	+	8/0/7
	Hibiscus	+/+ (30)	+	15/3/1
	Abutilon	+/+ (18)	+	16/4/0
Gellert Nursery	Sow thistle	+/– (10)	–	9/0/6
	Ox tongue	+/– (2)	–	1/0/1
King's Plant Barn	Tamarillo	–/+ (2)	–	NA
	Geranium	+/+ (12)	–	3/0/2
	Gerbera	+/+ (56)	–	7/0/6
Auckland Botanical	Poinsettia	+/– (5)	–	2/0/0
Gardens	Abutilon	+/+ (6)	–	4/0/1
	Geranium	+/+ (6)	–	4/0/0
Knight's Nurseries	Poinsettia	–/–	–	NA
Anderson's Nursery	---	–/–	–	NA
Waipu Plants	Hibiscus	–/–	–	NA
Southern Paprika	Capsicum	+/+ (13)	–	6/0/4
	St. Nettle	+/– (13)	–	4/0/1
Gourmet Paprika	Capsicum	+/+[1] (8)	–	5/0/1
Under Glass	Tomato	+/+ (7)	–	5/0/5
Wellington	Lobelia	+/+ (26)	–	5/0/5
Botanical Gardens	Primula	+/+ (5)	-	3/0/3
Christchurch	Pelargonium	+/+ (44)	–	20/0/18
Botanical Gardens	Calceolaria	+/+ (26)	–	5/0/5
Anderson's	Poinsettia	+/+ (30)	+	15/10/0
Zealandia SI[2]	Gerbera	+/+ (20)	–	10/0/10

[1]Emerged.
[2]Access for survey denied 23 June; sampled previously (7 June 2006).

Bemisia tabaci biotype Q sequences (n=10) were detected in all samples from the Hamilton Botanic Gardens except for those collected from *Pelargonium* sp. and *D. repens.* These sequences were identical to each other and to those obtained from the preliminary survey. BLAST interrogation of databases showed that New Zealand s*B. tabaci* biotype Q sequence is identical to that reported by Ueda & Brown (2006) who recorded the first occurrence (2004) of the Q biotype in Japan (Genbank Accession ##AB204586-AB204588). In addition, the NZ biotype Q sequence is identical to sequences reported from both Korea (Genbank Accession ##DQ462583-DQ462585) and Greece (Tsagkarakou et al. 2007; Genbank Accession #DQ365874).

The phylogenetic tree derived from GTR distances using the neighbour-joining algorithm in PAUP* shows that *B. tabaci* COI sequences from New Zealand nest well within the group of biotype Q sequences from elsewhere in the world (Fig. 1). This

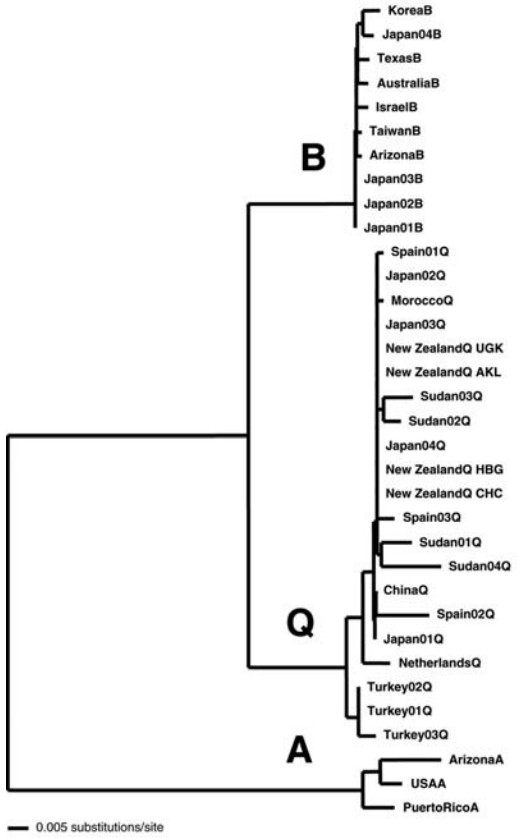

FIGURE 1: Neighbour-joining tree of *Bemisia tabaci* COI DNA sequences derived from GTR genetic distance matrix. The four New Zealand samples indicated are 'UGK'=ex. capsicum, "Under Glass", Karaka; 'AKL'=ex. poinsettia, Auckland; 'CHC'=ex. poinsettia, Anderson's Zealandia, Hillsborough; 'HBG'=ex. abutilon, hibiscus and cotton, Hamilton Botanic Gardens.

tree is the same as that generated from uncorrected *p* distances. The greatest genetic distance observed between recognised biotype Q sequences is 2.6% (Sudan04Q versus Turkey03Q). Genetic distances (GTR) between the NZ Q sequences and reference biotype Q sequences range up to 1.5%. In contrast, distances between B and Q sequences range from 4.9 to 6.7%, whereas distances between Q or B sequences with those from known biotype A range from 14.8 to 17.1%.

Molecular diagnosis of *B. tabaci* material collected in this study suggests that only biotype Q is present in New Zealand. Biotypes A and B were diagnosed in the 1990s using contemporary molecular techniques at a time when Q was unrecognised. It is considered that the detection methods described in this paper are more sensitive and that biotypes other than Q would have been detected if they were present in the samples. The small total number of whiteflies collected during the main survey was probably due to sampling during winter and the paucity of appropriate hosts.

It is likely that *B. tabaci* biotype Q is more widely distributed within New Zealand than indicated by the present study and this has important implications for management of *B. tabaci* in covered crops. Control failure with *Encarsia formosa* has already been observed and the potential for *E. pergandiella* as an alternative biological control agent is presently being evaluated. The identification of an effective natural enemy is especially important because it is likely that control of biotype Q with insecticides will be limited due to resistance.

A further survey of *Bemisia tabaci* was conducted during summer 2006-07, targeting retail outlets selling poinsettias over the Christmas period, as this plant species is known to be a preferred host. It seems likely that the Christmas trade in poinsettias could facilitate the spread of this pest throughout the country. Morphological and molecular diagnoses of the samples collected are currently underway.

ACKNOWLEDGEMENTS

David Teulon, Daniel Park and Tracy Williams reviewed earlier drafts, while Sue Zydenbos and David Logan made helpful comments following submission. Nicholas Martin and Peter Holder provided information relating to the first appearance of *Bemisia tabaci* in New Zealand and the 1991 MAF survey. This work was partially funded by New Zealand's Foundation for Research, Science and Technology through the Better Border Biosecurity (B3) Programme (www.b3nz.org).

REFERENCES

Anon. 2007. http://www.mrec.ifas.ufl.edu/LSO/BEMISIA/positive_states.htm (accessed 30 March 2007).

Brown JK, Dennehy TJ, Degain B, Rogan D, Harpold G, Byrne F, Nichols R 2005. First report of the Q biotype of *Bemisia tabaci* (Gennadius) in the USA and resistance to insecticides in an Arizona population. http://www.whitefly.org/whiteflyforum/forum_posts.asp?TID=32&PN=1 (accessed 7 April 2007).

Brown JK, Idris AM 2005. Genetic differentiation of whitefly *Bemisia tabaci* mitochondrial cytochrome oxidase I, and phylogeographic concordance with the coat protein of the plant virus genus *Begomovirus*. Annals of the Entomological Society of America 98(6): 827-837.

de Barro PJ, Trueman JWH, Frohlich DR 2005. *Bemisia argentifolii* is a race of *B. tabaci* (Hemiptera: Aleyrodidae): the molecular genetic differentiation of *B. tabaci* populations around the world. Bulletin of Entomological Research 95: 193-203.

de la Rua P, Simon B, Cifuentes D, Martinez-Mora C, Cenis JL 2006. New insights into the mitochondrial phylogeny of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) in the Mediterranean Basin. Journal of Zoological Systematics and Evolutionary Research 44(1): 25-33.

Horowitz AR, Kontsedalov, Khasdan V, Ishaaya I 2005. Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. Archives of Insect Biochemistry and Physiology 58: 216-225.

Jones DR 2003. Plant viruses transmitted by whiteflies. European Journal of Plant Pathology 109: 195-219.

Ma D, Gorman K, Devine G, Luo W, Denholm I 2007. The biotype and insecticide-resistance status of whiteflies, *Bemisia tabaci* (Hemiptera: Aleyrodidae), invading cropping systems in Xinjiang Uygur Autonomous Region, northwestern China. Crop Protection 26: 612-617.

Martin NA 1999. Whitefly Biology, Identification and Life Cycle. Crop & Food Research Broadsheet No. 91. Crop & Food Research, Lincoln, New Zealand. 8 pp.

Pascual S, Callejas C 2004. Intra- and interspecific competition between biotypes B and Q of *Bemisia tabaci* (Hemiptera: Aleyrodidae) from Spain. Bulletin of Entomological Research 94(4): 369-375.

Perring TM 2001. The *Bemisia tabaci* species complex. Crop Protection 20: 725-737.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87(6): 651-701.

Swofford D 1998. PAUP*: Phylogenetic analysis using parsimony (and other methods). Sinauer, Sunderland, Massachusetts.

Tsagkarakou A, Tseginopoulos CS, Gorman K, Lagnel J, Bedford ID 2007. Biotype status and genetic polymorphism of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Greece: mitochondrial DNA and microsatellites. Bulletin of Entomological Research 97: 29-40.

Ueda S, Brown JK 2006. First report of the Q Biotype of *Bemisia tabaci* in Japan by mitochondrial cytochrome oxidase I sequence analysis. Phytoparasitica 34(4): 405-411.