THE CHRISTMAS DISPERSAL OF *BEMISIA TABACI* (GENNADIUS) IN NEW ZEALAND

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ABSTRACT

In 2006, *Bemisia tabaci* biotype Q was identified in New Zealand. The Christmas trade in poinsettias is implicated in the recent, rapid dispersal of *B. tabaci* biotype Q through North America. Retail outlets were surveyed during December 2006 and 2007 to investigate whether this may be a pathway for dispersal in New Zealand. A PCR-based diagnostic tool confirmed the presence of *B. tabaci* biotype Q on poinsettias purchased in both Christchurch and Auckland. However, results indicated that abundance varied between the regions, years and propagation nurseries. In 2006, 169 and three adult whitefly were collected from a selection of retail outlets in Christchurch and Auckland, respectively, compared with 256 and 56 in 2007. Only *B. tabaci* biotype Q was identified from the samples collected from Christchurch, with *B. tabaci* biotype Q and relatively few *Trialeurodes vaporariorum* identified from the Auckland samples. **Keywords:** Sweet potato whitefly, *Bemisia tabaci*, Biotype Q, poinsettia, *Euphorbia pulcherrima*, dispersal.

INTRODUCTION

The sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is an important horticultural pest in many countries. This species can cause significant losses through direct feeding and the transmission of over 100 economically-important plant viruses (Jones 2003; de Barro et al. 2005). Numerous sweet potato whitefly biotypes have been identified, reflecting differences in host range, virus transmission and pesticide resistance attributes. Biotypes B and Q have attracted considerable attention because of their rapid global spread and their known resistance to selected organophosphate, carbamate and pyrethroid insecticides (de Barro et al. 2005). In addition, neonicotinoid resistance has been reported in selected biotype Q populations (Nauen et al. 2002).

In 1991, *B. tabaci* was first reported in New Zealand from poinsettia (*Euphorbia pulcherrima*) (N.A. Martin, pers. comm. in Scott et al. 2007). A subsequent Ministry of Agriculture and Forestry (MAF) survey determined that *B. tabaci* was widespread in the North Island, but was not present in the South Island (G.M. Burnip, pers. comm. in Scott et al. 2007). In the mid 1990s, molecular screening indicated that both biotypes A and B were present in New Zealand, which was consistent with the global spread of these biotypes at the time (P.J. de Barro, pers. comm. in Scott et al. 2007).

In May 2006, *B. tabaci* biotype Q was identified in New Zealand after samples were screened following their collection from a greenhouse capsicum crop in Karaka (South Auckland). This molecular identification was confirmed by the California State Department of Food and Agriculture in July 2007. A second MAF survey to determine the distribution of *B. tabaci* biotype Q was conducted in June 2006 and included eight greenhouses and four botanical gardens in the North and South Islands. Biotype Q was only located at the Hamilton botanical gardens from three plant species.

Dalton (2006) recently highlighted the issue of the movement of *B. tabaci* biotype Q around North America at Christmas time on infested poinsettia plants and the potential importance of this pathway as an annual source for the spread of this important pest. The current paper examines the importance of this pathway for the seasonal spread of sweet potato whitefly biotype Q within New Zealand.

METHODS

Purchase of poinsettia plants

Five retail outlets were selected in Christchurch and Auckland in December 2006. Outlets included garden centres, building supply stores and supermarkets. Five poinsettia plants were purchased from each outlet. Only two poinsettias were purchased from one Auckland outlet due to low stock levels. The plants were individually double-bagged outside each outlet to prevent the escape of adult whitefly and cross-contamination. The plants were stored overnight at 4°C.

The protocol described above was repeated in December 2007. An additional outlet was also visited in Christchurch. Low stock levels meant that fewer than five plants were purchased from two Christchurch outlets and three outlets in Auckland.

Whitefly collection

Each poinsettia plant was checked for adult whitefly and the number present were counted and collected into 96% ethanol. If the plant label stated the propagation nursery, this information was also noted.

Molecular diagnosis

Genomic DNA was extracted from at least five individual whitefly adults from each plant using a modified CTAB method. Insects were initially homogenised in 200 µl of 2xCTAB (2% CTAB, 100 mM Tris-Cl pH8.0, 20 mM EDTA, 1.4 M NaCl) and incubated at 50°C for 30–60 min in the presence of Proteinase K (10 µl at 20 ng/µl). A chloroform extraction was then performed, after which the upper phase was removed to a clean tube. DNA was precipitated by the addition of 2.5 volumes of ice cold 100% ethanol in the presence of 0.1 volume of 3 M sodium acetate (pH 5.2) and incubation at -20°C for 60 min. DNA was pelleted by centrifugation and the pellet washed with 70% ethanol. The pellet was allowed to air-dry before resuspension in 30 µl of TE. DNA quality and quantity was then assessed by agarose gel electrophoresis against known standards. A fragment of the mitochondrial cytochrome oxidase subunit I was amplified using a stepdown PCR profile that comprised cycles of 30 s denaturation (95°C), 30 s annealing (70°C–45°C) and 30 s extension (72°C) steps. The annealing temperature was dropped in 5°C increments every two cycles until 45°C was reached, at which temperature 25 PCR cycles were done. A final extension was performed for 10 min to ensure fragment elongation. Scott et al. (2007) described two sets of primers (TvapF 5'-GGCATTATTTCTCATCTTATTAGTGCT-3' and Wfrev 5'-GTGAYTAAGRGMTGGYTTATT-3'; BtabF 5'-GAGGCTGGAAAATTAGAGGTAT-3' and BtabR 5'-AACTGTGATTAAGRGCTGGTTTATTA-3') for screening for the presence of the whitefly species, *Trialeurodes vaporariorum* and *B. tabaci*, which 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 (TvapF and Wfrev). Samples that failed to amplify were then screened using the *B. tabaci* primers (BtabF and BtabR). 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. Each PCR included appropriate negative (no DNA template), and *B. tabaci* or *T. vaporariorum* positive DNA controls. All DNA samples were screened using both primer sets. A selected number of PCR samples from each outlet that had been amplified with *B. tabaci* primers were purified (QIAquick PCR purification kit, Qiagen) and quantified (spectrophotometer, Nanodrop® ND-1000, Biolab) before DNA sequence analysis (Macrogen, South Korea) for DNA sequencing. The DNA sequences (~780–800 bp) were edited using Sequencher (GeneCodes Corporation). All sequences were checked for stop codons and were determined to be

of mitochondrial origin. Sequences were aligned using ClustalX software for visual comparison. Individual sequences were used to interrogate the GenBank, European Molecular Biology (EMBL) and DNA Data Bank of Japan (DDBJ) databases using the BLAST search facility hosted by the National Centre for Biotechnology Information (www.ncbi.nlm.nih.gov/BLAST/).

RESULTS

All Christchurch retail outlets had whitefly infestations in 2006 and 2007, with all but two plants being infested with at least one adult whitefly in 2006 and all plants being infested in 2007 (Table 1). In 2006, 169 adult whitefly were collected from all plants, with an overall mean of 6.8 adult whitefly per plant. In 2007, 256 adult whitefly were collected from all plants (excluding the data collected from the extra outlet sampled), and there was an overall mean of 12.2 adult whitefly per plant. In Auckland in 2006, only three adult whitefly were collected, resulting in a mean of 0.1 whitefly adults per plant (Table 1). However in 2007, 56 adult whitefly were collected, with a mean of 2.8 per plant.

TABLE 1: Total number of whitefly (range in parentheses) collected from five poinsettia plants per retail outlet (unless otherwise indicated in Outlet column) in Christchurch or Auckland in 2006 and 2007. The number of *B. tabaci* and *T. vaporariorum* individuals identified in the species diagnostic PCR test are indicated by “Bem/Tri”, while “Q” indicates results of DNA sequence analysis to confirm the biotype Q status of selected *B. tabaci* individuals.

Year	Christchurch				Auckland			
	Outlet ¹	Total whitefly	Bem/Tri	Q	Outlet ¹	Total whitefly	Bem/Tri	Q
2006	1	41 (2-15)	21/0	4/4	A	1 (0-1)	0/1	–
2007	1	118 (7-48)	21/0	6/6	A	23 (0-9)	13/1	6/6
2006	2	4 (0-2)	3/0	4/4	B	0	–	–
2007	2	45 (2-19)	17/0	7/7	B	5 (0-3)	5/0	2/2
2006	3	69 (5-24)	25/0	4/4	C	1 (0-1)	1/0	1/1
2007	3 (1)	4	2/0	2/2	C (4)	1 (0-1)	1/0	–
2006	4	20 (1-10)	14/0	4/4	D	1 (0-1)	1/0	1/1
2007	4	31 (3-10)	17/0	8/8	D (4)	27 (3-16)	10/1	6/6
2006	5	35 (0-27)	13/0	4/4	E (2)	0	–	–
2007	5	58 (2-31)	12/0	8/8	E (2)	0	–	–
2007	6 (3)	74 (17-37)	14/0	7/7	No sixth retail outlet			

¹Five poinsettia plants were purchased per retail outlet unless indicated in parentheses.

In 2006 approximately half (76/169 Christchurch, 2/3 Auckland) of the adult whitefly collected were screened by PCR to confirm their identification (Table 1). In 2007, 69/256 whitefly collected in Christchurch and 31/56 whitefly collected in Auckland were also screened.

PCR diagnosis using *B. tabaci*-specific primers indicated that all of the individuals sampled from retail outlets in Christchurch in both 2006 and 2007 were *B. tabaci*. In

comparison, 2/3 of the Auckland samples screened in 2006 were sweet potato whitefly and 29/31 in 2007. A COI amplicon was amplified for these three Auckland samples using the alternate species-specific markers and they were determined to be *T. vaporariorum*.

In 2006, all 22 sequences obtained from *B. tabaci* positive samples taken from Christchurch (n=20) and Auckland (n=2) retail outlets (Table 1) were confirmed as sweet potato whitefly biotype Q. Similarly in 2007, all 52 sequences (Christchurch (n=38) and Auckland (n=14)) were confirmed as sweet potato whitefly biotype Q.

In 2006 and 2007, all poinsettia plants purchased in Christchurch were from the same nursery as the plants purchased in Auckland in 2006. Of the 20 poinsettia plants collected in Auckland in 2007, 15 were from the same nursery as 2006 with the remaining five from a second nursery. Of the total 56 adult whitefly sampled 54 (96%) were collected off poinsettia plants from the 2006 nursery.

DISCUSSION

Only *B. tabaci* biotype Q was identified from the material collected in this study. Biotypes A and B were diagnosed in the 1990s using then contemporary molecular techniques before biotype Q was recognised (P.J. de Barro, pers. comm. in Scott et al. 2007). The detection methods described in the current paper are more sensitive than those earlier techniques and biotypes other than Q would have been detected if they were present in the samples collected. The fates of biotypes A and B in New Zealand are currently unknown.

The presence of *B. tabaci* biotype Q on commercial poinsettia plants available for sale at Christmas in New Zealand is similar to that found in the United States and indicates a potential pathway for the spread of this pest insect in New Zealand. The poinsettia pathway was implicated in the spread of *B. tabaci* biotype B through the USA in the late 1980s and 1990s, an invasion that is estimated to have caused losses greater than US\$1 billion to cotton and vegetable growers in the US and Mexico (Dalton 2006). The same pathway is also implicated in the recent "second-wave" of biotype Q (Dalton 2006; Bethke et al. 2009). Since its initial detection in Arizona in December 2004, biotype Q has now spread to 24 of the continental United States and Hawaii (http://mrec.ifas.ufl.edu/LSO/BEMISIA/positive_states.htm). The appearance of biotype Q in the USA has been accompanied by reports, primarily from ornamental growers, of increasing problems in controlling whitefly. While insect growth regulators have been successfully used to control biotype B in the USA, biotype Q is unaffected or has reduced susceptibility to both insect growth regulators and neonicotinoids (Mackenzie et al. 2009). Both insecticide classes are vital for whitefly control in protected and open cropping systems (McKenzie et al. 2009). While the Q biotype has not been detected in the USA on hosts other than ornamental species, there are legitimate concerns that it will, like biotype B before, spread to the vegetable and cotton industries (Dalton 2006; Bethke et al. 2009). In New Zealand *B. tabaci* biotype Q has been reported from several different host plant species in the Hamilton botanical gardens and at least one commercial capsicum greenhouse in South Auckland (Scott et al. 2007).

The relevance of these findings must be considered in relation to the current distribution of *B. tabaci* biotype Q in New Zealand. While previous work has shown biotype Q to be present on other hosts in New Zealand (Scott et al. 2007), it is most commonly associated with poinsettias. It would therefore be prudent to reduce the seasonal spread of this insect species by ensuring that pest infestations on poinsettia plants, especially those plants that are spread around the country, are well managed. In 2006 and 2007, greater numbers of *B. tabaci* adults were found on poinsettia plants in Christchurch than in Auckland. Although this may indicate that this species is more common in Christchurch, this is unlikely due to differences in climate or levels of covered crop production between the two regions. Auckland poinsettia growers may have different whitefly management practices, due to an increased awareness or concern about the risk of spreading *B. tabaci* within New Zealand. Another factor could be that poinsettia plants from the two regions may have been harvested at different times during the chemical management programme,

resulting in differences in the level of infestation when the plants were purchased.

An additional factor needs to be considered that relates to the distribution and dispersal of *Bemisia tabaci* in New Zealand. Poinsettias are produced from mother stock, which is replenished from sources outside New Zealand providing a pathway for future entry of *B. tabaci* biotypes into New Zealand. Immature stages and eggs are difficult to detect because of their small size and location on the underside of leaves and the movement of poinsettia cuttings from one country to another is thought to be the most important pathway for the spread of *B. tabaci* globally (Bethke et al. 2009; Lemmetty & Vanninen 2008; DEFRA factsheet 2008).

Despite the current restricted distribution of *B. tabaci* biotype Q in New Zealand eradication of this insect will be challenging. The more pressing concern regarding *B. tabaci* biotype Q in New Zealand is the potential for developing increased resistance to insecticides coupled with the potential spread to non-ornamental hosts in other indoor and open cropping systems. Chemical control options are limited and research continues towards the identification of effective biological control agents. It remains important to monitor the seasonal proliferation of *B. tabaci* biotype Q on poinsettia plants to assess this pathway for spread within New Zealand and to provide an opportunity to track the development of insecticide resistance. However, it is more important to mitigate the potential effects that *B. tabaci* biotype Q may have on NZ horticulture. Attempts in the United States to manage the spread of *B. tabaci* biotype Q have been confounded by political interference and the inability of the USDA to restrict importation or movement of poinsettias (Dalton 2006). It is clear that a unified, national response is required from all sectors of NZ horticulture and the Ministry of Agriculture and Forestry in order that the problem does not escalate to a level comparable to that seen in the United States.

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