

Bemisia tabaci biotype Q determined for the first time on poinsettia crops in Finland and Sweden

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We surveyed 13 Finnish and 13 Swedish poinsettia greenhouses in 2006 for *Bemisia tabaci* biotype Q (currently *B. tabaci* Mediterranean) and determined the biotype status of *B. tabaci* populations with PCR followed by an RFLP assay. We collected approximately 100 adult whiteflies from yellow sticky traps or from plants and stored them in 80% ethanol until DNA extraction. Total nucleic acids of two to five whiteflies per sample were extracted using FTA cards or a modified Chelex method. We showed that biotype Q was present in 10 out of 13 whitefly samples from Finnish poinsettia greenhouses and in 12 out of the 13 Swedish poinsettia greenhouses sampled. We also determined the whitefly biotypes from yellow sticky traps. The common occurrence of insecticide-resistance-prone biotype Q whiteflies on poinsettia imported into Finland and Sweden, both protected zones for *B. tabaci*, emphasizes the importance of preserving the quarantine status of the pest to prevent permanent establishment. Nonetheless, the increasing occurrence of a resistance-prone biotype on imported plant material will complicate preservation of the protected zone status if solely insecticides are used to control the pest.

Introduction

Greenhouses are ecological islands vulnerable to invasions of alien species (Kiritani 2006) and can serve as stepping stones for alien species to invade the outdoor environment (e.g. Vierbergen 2002). Two alien polyphagous whitefly species inhabit Finnish greenhouses: the greenhouse whitefly *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae), which has occurred here since 1919 (Vappula 1962), and *Bemisia tabaci* (Vänninen *et al.* 2011), currently a quarantine species to be eradicated if it enters places of production (Ministry of Agriculture and Forestry 2008).

Modelling suggests, however, that the latter species would establish in Finland if its quarantine status were to be suspended (J. Heikkilä, MTT Agrifood Research Finland, pers. comm.). Previously, *B. tabaci* was considered to be a complex species consisting of more than 20 biotypes (Perring 2001), of which biotypes B and Q were the most common and invasive. The most recent taxonomy separates *B. tabaci* into 24 cryptic species (Dinsdale *et al.* 2010, De Barro *et al.* 2011). Of these, biotype B (currently *B. tabaci* Middle East–Asia Minor 1, or MEAM1) spread globally in 1986–1992 (Bedford *et al.* 1994), whereas biotype Q (*B. tabaci* Mediter-

ranean, or Med) started spreading within Europe in the 1990s (Perring 2001, Rauch & Nauen 2003), reached other continents in the 2000s (e.g. Chu *et al.* 2006, Ueda & Brown 2006, Hsieh *et al.* 2007, McKenzie *et al.* 2009, Dennehy *et al.* 2010, Grille *et al.* 2011), possibly due to increasing resistance to neonicotinoids and other insecticides (Fernández *et al.* 2009, Dennehy *et al.* 2010, Luo *et al.* 2010, Wang *et al.* 2010, Sun *et al.* 2013). Following its introduction to new areas, *B. tabaci* Med is replacing the previously common *B. tabaci* MEAM1 (e.g. Chu *et al.* 2010, Pan *et al.* 2011), and multiple introductions from several sources to a particular area can contribute to increased genetic diversity potentially leading to improved adaptation to new environments (Chu *et al.* 2014).

Up to 20 *B. tabaci* outbreaks per year occurred in Finnish ornamental greenhouses in 1995–2005, but in 2006–2008 the number of outbreaks increased to 50–120 per year (summarized in Heikkilä 2008). This increase prompted the need to look into the taxonomic status of *B. tabaci* introduced into Finland and Sweden, both countries having official status as protected zones from the pest. More frequent introductions of pesticide-resistant alien species in ornamental plants are likely to increase the risk of their spreading to greenhouse vegetable crops where *B. tabaci* are considered a risk due to their capacity for vectoring plant viruses (Jones 2003).

Material and methods

We collected whitefly samples from a total of 26 commercial poinsettia greenhouses in different parts of Finland and Sweden. Inspectors of the Finnish Food Safety Authority collected whitefly samples in Finland during September–November 2006 and sent them to MTT Agri-food Research Finland for biotype determination. The Swedish Plant Protection Service provided samples from Sweden. The poinsettia cuttings with the intercepted *B. tabaci* had been imported to Finland from Denmark, the Netherlands and Kenya. In 2008, we obtained additional samples of *B. tabaci* from a Finnish greenhouse where the poinsettia plants were imported from Germany.

In 2006, we carried out taxonomic determinations on samples of adult *B. tabaci* from the 26 greenhouses either collected from poinsettia plants and preserved in 80% ethanol until DNA extraction, or on adults collected from yellow sticky traps. There were approximately 50 immigrant *B. tabaci* adults from Finland and 50 from Sweden. The number of adults was low in most greenhouses at the time of sampling due to the infestations having only recently begun. The sticky traps were sent to MTT, where two to five adults, or their parts, were pooled from the traps from each greenhouse, removed with an insect needle, transferred to a FTA Card (Whatman) and crushed on the card with a glass rod. Total nucleic acids from two to five *B. tabaci* adults crushed on a FTA Card were purified according to the protocol recommended by the manufacturer (Whatman). We extracted total nucleic acids from two *B. tabaci* individuals stored in 80% ethanol using the Chelex method (Walsh *et al.* 1991) modified by De la Rua *et al.* (2006).

We used primers C1-J-2195 and L2-N-3014 for the PCR reaction, which amplified a fragment (816 bp) of the mitochondrial cytochrome oxidase 1 (*mtCO1*) gene as described by Frohlich *et al.* (1999). PCR amplification and cycling parameters were calculated and performed according to the instructions of puReTaq Ready-To-Go PCR beads (GE Healthcare UK Limited). The final volume of the PCR mixture was 50 µl. For each reaction, 1 µl of each primer (40 µM), 45 µl of sterile water and 3 µl of template DNA were added to a tube containing a PCR bead. For FTA Card samples the amount of sterile water was 48 µl. PCR amplification was performed using a PTC-200 DNA engine (MJ Research, Inc. Watertown, USA). The annealing temperature for primers C1J-2195 and L2-N-3014 was 52 °C, as recommended by Khasdan *et al.* (2005). Aliquots of the PCR products were electrophoresed in a 1% agarose gel at 90 V for 1 h in 1 × Tris-acetate-EDTA buffer (TAE) to determine the amplified PCR product. Gels were stained with ethidium bromide and viewed under UV light.

PCR products amplified with primer pairs C1-J-2195 and L2-N-3014 were digested with restriction endonuclease (*Vsp*I) (Promega)

(Khasdan *et al.* 2005) according to the manufacturer's instructions. Digested PCR products were electrophoresed in 1.5% agarose gel at 90 V for 1 h in 1 × TAE buffer.

In 2008, we carried out PCR-RFLP analyses on ten adult *B. tabaci* individuals imported from Germany collected from poinsettia sticklings using a FTA Card (Whatman) for DNA extraction. They were analysed as described above except that each individual was analysed separately.

Results and discussion

Amplification of *B. tabaci* biotype specific band (816 bp) from the *mtCOI* gene and characteristic band sizes from enzymatic digestion of the amplified fragment (Khasdan *et al.* 2005) confirmed our hypothesis that biotype Q *B. tabaci* was present in Finland in the autumns of 2006 and 2008 on imported poinsettias (Table 1). We also detected biotype Q in 12 of the 13 poinsettia greenhouses representing different parts of Sweden in 2006. Restriction analysis confirmed that the PCR products amplified with primers C1-J-2195 and L2-N-3014 (Frohlich *et al.* 1999) originated from biotype Q because the fragments (500 and 300 bp) after digestion were typical for

the biotype (Khasdan *et al.* 2005, Grille *et al.* 2011).

We also determined biotype *B. tabaci* from samples collected from yellow sticky traps (Table 1). It was easier for the inspectors to find and collect adults from sticky traps than locate them on plants because whitefly numbers during sampling were low. The glue stock in the yellow sticky traps did not disturb the DNA extraction and PCR amplification when FTA Cards were used.

PCR-RFLP analyses confirmed the presence of biotype Q *B. tabaci* in the whiteflies collected in 2006 and 2008 in both Finland and Sweden. Results from the UK (also a protected zone country) with respect to *B. tabaci* interceptions, demonstrated that in recent years there has been a shift from biotype B to Q. Recent interceptions have been exclusively biotype Q (Powell *et al.* 2012). This suggests that in some places where production of poinsettia cuttings and sticklings takes place, biotype Q has replaced, or is in the process of replacing, biotype B.

Those places of production of plant material in countries that have a protected zone status for *B. tabaci* must, according to the law, ensure that their plant material is free of this pest (Cuthbertson 2013). Since this is clearly not the case, it can be assumed that pest control in the

Table 1. *Bemisia tabaci* biotypes determined on poinsettia crops in Finland in the autumn of 2006.

Sample code	Type of sample*	DNA isolation	PCR results 816 bp (B or Q)	Restriction analysis (Q)
1	Ethanol	Chelex	+	+
2	Ethanol	FTA card	+	+
3	Ethanol	Chelex	+	nt**
4	Ethanol	Chelex	+	nt
5	Y trap	FTA card	+	+
6	Y trap	FTA card	+	+
7	Y trap	FTA card	+	+
8	Ethanol	Chelex	+	nt
9	Y trap	FTA card	+	+
10	Y trap, Eth.	FTA card, Chel.	+	+
11	Y trap	FTA card	+	+
12	Y trap	FTA card	+	+
13	Ethanol	FTA card	+	+

* Ethanol = insects from plants stored in 80% ethanol before analysis. Y trap = insects from yellow sticky traps.

** nt = not tested.

country of immediate origin of the plant material has failed to some degree and that resistance to insecticides is the most likely reason. This emphasizes the importance of preserving the current quarantine status of *B. tabaci* in Finland to prevent it from becoming established permanently here. In Finland, year-round greenhouse tomato and cucumber crops represent ideal ecological islands for both pest species in terms of the day length of ≥ 16 hours (Blackmer *et al.* 2002, Chang & Zhu 2007), warm temperatures (Bonato *et al.* 2007), difficulties for the establishment of natural enemies during winter (Johansen *et al.* 2011), and resistance of both species to various insecticides (Elbert & Nauen 2001, Roditakis *et al.* 2009). Nonetheless the increasing occurrence of resistant and highly resistance-prone whiteflies in imported plant material will complicate preservation of the quarantine status. It is likely moreover that it will become increasingly difficult to eradicate the pest solely through application of insecticides. Therefore, additional means of management, particularly biological and mechanical control methods, should be incorporated into the eradication programme in the country for which the plant material is destined. The use of biological control could be particularly effective in mixed infestations of MEAM1 and Med as they usually differ in insecticide resistance. In the absence of the selection pressure by insecticides, resistant individuals have been shown to be outselected from the mixed population (Crowder *et al.* 2010). Consequently, it is easier to do late-season cleanup chemical control of the poinsettia crop when only individuals susceptible to chemicals remain on the plants. The success of such an approach depends on the time it takes for resistant individuals in the population to be out-selected which, in turn, may depend on the resistance mechanism and associated fitness costs of resistance. In the UK, Cuthbertson *et al.* (2012) have developed programs of integrated pest management against *B. tabaci* and showed that biorational insecticides and synthetic pesticides can be combined with microbiological control agents to successfully eradicate *B. tabaci* from poinsettia crops provided that treatments are started in the early stages of the crop. The next challenge is to apply IPM programs in bed-

ding plants in the springtime. *Bemisia tabaci* is increasingly being introduced to Finland on foreign plant material in the spring months. This creates a risk of the pest spreading from infested crops to outdoors and from there to other greenhouses unless timely eradication measures are available at the initial places of introduction.

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