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A new silverleaf-inducing biotype Ms of *Bemisia tabaci* (Hemiptera: Aleyrodidae) indigenous to the islands of the south-west Indian Ocean

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Abstract

Following the first detection of tomato yellow leaf curl virus (TYLCV) from Réunion (700 km east of Madagascar) in 1997 and the upsurge of *Bemisia tabaci* (Gennadius) on vegetable crops, two genetic types of *B. tabaci* were distinguished using RAPD–PCR and cytochrome oxidase I (COI) gene sequence comparisons. One type was assigned to biotype B and the other was genetically dissimilar to the populations described elsewhere and was named Ms, after the Mascarenes Archipelago. This new genetic type forms a distinct group that is sister to two other groups, one to which the B biotype is a member and one to which the Q biotype belongs. The Ms biotype is thought to be indigenous to the region as it was also detected in Mauritius, the Seychelles and Madagascar. Both B and Ms populations of *B. tabaci* induced silverleaf symptoms on *Cucurbita* sp., and were able to acquire and transmit TYLCV. Taken together these results indicate that the Ms genetic type should be considered a new biotype of *B. tabaci*.

Introduction

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a widely distributed species throughout the tropical and subtropical regions of the world. Morphologically indistinguishable populations of *B. tabaci* exhibiting measurably different biological traits such as host range have been described and these are referred to as host races or biotypes (Bird & Maramorosch, 1978). In the past two decades, *B. tabaci* has become a major problem both as a pest and as vector of plant viruses, especially geminiviruses (Markham *et al*., 1996). During this period one particularly polyphagous and highly fecund population, named biotype B, was encountered in the USA (Costa & Brown, 1991). This biotype is characterized by both induction of silverleaf symptoms on *Cucurbita* spp. (Cucurbitaceae) and biochemically by a unique and homogeneous esterase profile (Bedford *et al*., 1994). Further analyses using random amplified polymorphism DNA–polymerase chain reaction (RAPD–PCR) (De Barro & Driver, 1997; Guirao *et al*., 1997; Banks *et al*., 2001), or specific gene sequences such as cytochrome oxidase I (COI), mitochondrial 16S rDNA (Frohlich *et al*., 1999) or ribosomal intergenic transcribed spacer 1 (De Barro *et al*., 2000), showed that biotype B is not restricted to northern America, but is also present in South America, Africa, Asia, Australia and Europe. Though biotype B appears to have originated from the north-east Africa/Middle East/Arabian peninsular region, international trade of ornamental plants has apparently favoured its current worldwide dispersal (Brown *et al*., 1995). Interestingly, wherever biotype B was introduced, at least one other

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biotype was detected that is generally considered to be indigenous (De Barro *et al*., 1998; Frohlich *et al*., 1999; Banks *et al*., 2001; Moya *et al*., 2001).

Réunion is a French island situated in the Mascarenes archipelago, in the south-western part of the Indian Ocean (700 km east of Madagascar). *Bemisia tabaci* was first described from Réunion in 1938 (Bourriquet, 1938) and later by Luziau (1953). There were no further reports until 1997, when tomato yellow leaf curl virus (TYLCV) infections of tomato crops were detected for the first time (Peterschmitt *et al*., 1999). Since then, *B. tabaci* has been found on most vegetable crops, ornamentals and weeds in all the tomato-producing areas of the island. It was not known if the emergence or re-emergence of *B. tabaci* in Réunion was due to the introduction of a new strain of *B. tabaci* or to the upsurge of a local population due to changing agricultural practices. Tomato is the major vegetable crop grown on Réunion with an annual production of 9000 tons in 2002. Yield losses due to TYLCV have reached 85% on the most susceptible cultivars (Reynaud *et al*., 2003). The aim of this work was to identify the populations of *B. tabaci* in Réunion.

Materials and methods

Insect and plant material

Bemisia tabaci individuals were collected from annual poinsettia *Euphorbia heterophylla* L. (Euphorbiaceae), tomato *Lycopersicon esculentum* Mill. (Solanaceae), eggplant *Solanum melongena* L. (Solanaceae), cabbage *Brassica oleracea* L. (Brassicaceae), sweet potato *Ipomoea batatas* L. (Convolvulaceae), cucumber *Cucumis* sp. L. (Cucurbitaceae), cotton-leaved jatropha *Jatropha gossypiifolia* L. (Euphorbiaceae), bean *Vigna* sp. L. (Fabaceae), *Chromolaena odorata* King & Robins. (Asteraceae), and cotton *Gossypium* sp. L. (Malvaceae) (table 1). At the CIRAD laboratory on Réunion, adults were allowed to emerge from whitefly pupae (generation 0) that had been previously observed under a stereomicroscope to select only whiteflies of the species *B. tabaci* using the key in Martin (1987). Except for whitefly sample no. 18 (table 1) collected from sweet potato and tested only at generation 0, isofemale lines were established from the other samples as follows. Single adult females (generation 0) were placed on an uninfested plant and confined inside insect-proof cages. A single female progeny (generation 1) was then collected and used to establish generation 2 and likewise generation 3. Each of these lines was reared on plants of the same species as the host plant on which the nymphs were collected originally, except for lines 4, 5 and 6 (table 1), which were reared on tomato. Adult whiteflies were collected at generations 1, 2 or 3 for DNA analysis. They were placed in micro-tubes filled with ethanol (90%) and stored at $-20°C$ until processing. After selection, lines 2, 4, 6, 11, 12, 15 and 17 were maintained for biological tests. Insects were reared in a climate chamber with a 12:12 h photoperiod, a temperature of $25 \pm 2°C$ and relative humidity of $60 \pm 10\%$. Adult *B. tabaci* were also collected from the nearby islands of Madagascar, Mauritius, the Seychelles and Mayotte and preserved in ethanol. Ethanol-preserved reference biotype A individuals from Colombia and biotype B from France were supplied by Nicolas Sauvion (Institut National de Recherche Agronomique, Guadeloupe). Adults of *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) were obtained from Réunion following the approach described above for *B. tabaci*.

DNA extraction and RAPD–PCR analyses

Insects were individually ground for 20 s in 5 µl of extraction buffer, containing: 50 mM KCl, 10 mM Tris-base, pH 8, 0.45% Nonidet P-40, 0.45% Tween 20, and 500 µg ml^{-1} proteinase K. Extraction buffer (20 µl) was added to the crude extract and was briefly centrifuged. The extract was incubated at 65°C for 1 h, submitted to a short centrifugation, incubated at 100°C for 15 min, and again briefly centrifuged. A volume of 35 µl of pure water was added to this extract which was then stored at $-20°C$ until use. The RAPD–PCR reactions were carried out in a volume of 25 µl consisting of 5 µl of DNA and 20 µl of PCR mix. The PCR reaction mix contained 2.5 mM of each dNTPs, 80 mM $MgCl_2$, 5 u of *Taq* DNA polymerase and 20 µM of primer. Amplification was done using a Perkin-Elmer thermocycler using the following parameters: 5 min at 94°C, 2 min at 40°C, and 3 min at 72°C, followed by 29 cycles of 1 min at 94°C, 1.5 min at 40°C, and 2 min at 72°C. Quantities of 10 µl of the amplification products were loaded on a 1.4% agarose gel, migrated for 3 h at 120 volts and stained with a solution of 1 µg ml^{-1} ethidium bromide. Eight 10-mer oligonucleotides were used as primers. Three of these (F12, H9, H16) were according to De Barro & Driver (1997) while the others were chosen at random: B1: CAGAACTCGG, B2: ACCATCGGCA, B3: ATCCCTGAAG, G1: ATTGTTCCTT and P1: TGACACATAG. Three sets of whiteflies, sets 1, 2 and 3, were amplified with these RAPD primers (table 1). For each set of whiteflies and each primer, the RAPD–PCR products were generated in the same experiment and resolved on the same gel. Individuals of biotype A from Colombia, biotype B from France as well as *T. vaporariorum* from Réunion were used as controls in each set. For whiteflies of set 2 (table 1, fig. 1), the presence or absence of bands in the RAPD patterns was recorded and a matrix of distances was constructed using the coefficient of Sokal & Michener (1958). Two programs were used for the construction of a neighbour-joining tree (Saitou & Nei, 1987): numerical taxonomy and multivariate analysis system (NTSYS) and Darwin (Perrier *et al*., 1999). Bootstraps were generated using Darwin.

Analyses of molecular variance, F-statistics of genetic differentiation

Genetic variation and the F-statistics for the different host plant lines of *B. tabaci* within set 2 were carried out by AMOVA using Arlequin (Excoffier *et al*., 1992). The run was performed without the data of the control individuals for biotype A and *T. vaporariorum*. Males and females were used, as no differences have been shown between the two (Moya *et al*., 2001). Differences between the B and the non-B group (see below) (F_{CT}), among host plant populations within groups (F_{SC}) and within host plants (F_{ST}) were assessed. The genetic distance was estimated as the number of fragment differences between haplotypes. The contribution of the three partitions to the total variance, as well as the three F-statistics were tested statistically by randomization tests

Table 1. Geographic origin and host plant of *Bemisia tabaci* populations investigated in this study and summary of the results of the molecular and biological tests.

Code[1]	Geographic origin	Host plant	RAPD[2]			COI (EMBL accession no.)[3]	Transmission rate (no. of repetitions)[4]	SSL[5]
			Set 1	Set 2	Set 3			
Bemisia tabaci								
1	Réunion/St Gilles les Hauts	Eggplant	1B[2]	2B				
2	Réunion/St Gilles les Hauts	Eggplant		2B	6B	B (AJ550176) B (AJ550177)	48 (290)	
3	Réunion/St Gilles les Hauts	Eggplant		1B				
4	Réunion/Petite Ile	Eggplant		1B			36 (100)	
5	Réunion/Etang Salé les Hauts	Eggplant		1B				
6	Réunion/Ravine des Cabris	Cucumber		1B			18 (139)	
7	Réunion/St Gilles les Hauts	Tomato		1B				
8	Réunion/St Gilles les Hauts	Tomato	1B					
9	Réunion/St Gilles les Hauts	Cabbage	1B	1B				
10	Réunion/Bassin Martin	Cabbage	1B					
11	Réunion/Bassin Martin	Cabbage		2B	4B		53 (325)	SSL
12	Réunion/Ligne Paradis	Cotton	1B	1B	4B	B (AJ550174) B (AJ550175)	14 (176)	SSL
13	Réunion/Ligne Paradis	Cotton	1B	2B				
14	Réunion/Petite Ile	Annual poinsettia	1B	1B				
15	Réunion/Gol les Hauts	Annual poinsettia		2Ms	4Ms	Ms (AJ550178) Ms (AJ550179)	43 (205)	SSL
16	Réunion /Saint Joseph	Cotton-leaved Jatropha	1B					
17	Réunion/Bois d'Olive	Sweet potato	1Ms	1Ms		Ms (AJ550180)	37 (235)	SSL
18	Réunion/Bois d'Olive	Sweet potato	1Ms			Ms (AJ550181)		
19	France/Antibes Control B biotype	Eggplant	**1B**	**1B**	**1B**	B (AJ550169) B (AJ550170)		
20	Madagascar/Tulear	Tomato				Ms (AJ550171)		
21	Mauritius/Mahebourg	Bean				Ms (AJ550172)		
22	Mayotte/Cambani	Tomato				B (AJ550173)		
23	Seychelles/Mahe	Jatropha				Ms (AJ550182)		
24	Colombia/Cali Control A biotype	*Chromolaena odorata*	**1A**	**1A**	**1A**	A (AJ550167) A (AJ550168)		
Trialeurodes vaporariorum								
25	Réunion/Bassin Martin	Tomato	1Tv	1Tv	1Tv			
26	Réunion/Piton Hyacinthe	Tomato				0 (AJ550183)		

[1] Codes 1–17 correspond to *Bemisia tabaci* isofemale lines derived from a series of field and greenhouse collections. Codes 18, 25 and 26 correspond to whiteflies obtained from field collected third or fourth instar nymphs. Codes 20–23 correspond to adults collected in the field during this study. Codes 19 and 24 were adults received from the Institut National de Recherche Agronomique, Guadeloupe and used as controls for biotypes B and A, respectively.
[2] Number of whiteflies tested followed by the biotype identification according to RAPD patterns. Biotype identification are in bold for individuals used as control for B and A biotypes (populations 19 and 24, respectively). Tv, RAPD pattern obtained with a *T. vaporariorum* population.
[3] Biotype identification based on a 370-nucleotide fragment of the *B. tabaci* mitochondrial COI gene.
[4] Mean percentages of the number of tomato plants infected with tomato yellow leaf curl virus following transmission with different *B. tabaci* populations from Réunion.
[5] Symptoms of silverleaf.

based on 3024 permutations. The population pairwise F_{ST} was then estimated.

COI gene sequencing

An 817-nucleotide fragment of the mitochondrial COI gene was amplified for individuals of *B. tabaci* selected from lines representative of the different RAPD types and from non-Réunion populations (table 1), using the following primers: C1-J-2195 and L2-N-3014 (Frohlich *et al*., 1999). PCR assays were conducted using 2 μl of template DNA extracted as above, in a total reaction volume of 25 μl. The PCR reaction mix contained 2.5 mM of each dNTPs, 50 mM $MgCl_2$, 5 u of *Taq* DNA polymerase and 20 μM of each primer. Amplification was done with the following parameters: 2 min at 95°C, 1 min at 56°C, and 1 min at 72°C for 30 cycles. PCR products were fractionated on 0.9% agarose gels, and bands were visualized as described above. PCR products were cloned into a pGEMT-Easy vector (Promega, Wisconsin, USA) and sequenced with plasmid specific primers. In addition, sequences for *B. tabaci* from a range of countries were obtained from Genbank (fig. 2). Sequences were aligned with the optimal alignment method of DNAMAN (version 5.0; Lynnon BioSoft, Quebec, Canada). The phylogenetic tree was set up with a Jukes and Cantor distance matrix using the neighbour-joining method of DNAMAN (Saitou & Nei, 1987). A thousand bootstrap

Fig. 1. Unrooted neighbour-joining tree of 16 populations of whiteflies based on the similarity values of Sokal & Michener inferred from 67 RAPD polymorphic fragments amplified with seven primers, B1, B3, G1, P1, F12, H9 and H16. The codes given for each individual refer to table 1. Individuals labelled with the same names were repeats within the same line. Bootstrap values based on 1000 random replicates are indicated on branches for values over 40%.

Fig. 2. Rooted neighbour-joining tree showing the genetic distance among 370 nt cytochrome oxydase I fragments of *Bemisia tabaci*, either sequenced in this study or selected from GenBank. Sequences are identified with their accession number and the geographic origin of the tested whitefly. The accession numbers of the sequences generated in this study are underlined and followed by their code number (in brackets) given in table 1. Two *Trialeurodes vaporariorum* sequences were added to this comparison, one was obtained in this study and the other one was from Genbank. The scale measures the Jukes & Cantor distance between sequences. Numbers associated with nodes represent the percentage of 1000 bootstrap iterations supporting the nodes; only percentages $\geq 81\%$ are indicated.

iterations were performed. The molecular clock hypothesis was tested with the relative rate test (Takezaki *et al.*, 1995).

Silverleaf test

Cucurbita moschata seedlings at the one leaf stage minus the cotyledons were individually caged under a net into which one pair of whiteflies was introduced. Plants were observed for symptoms of silverleaf 21 days later. Fifteen pairs of each of the following *B. tabaci* populations were tested: lines 11 and 12 (B group) and lines 15 and 17 (non-B group) (table 1). Fifteen plants grown under the same conditions, without insects were used as controls. The extent of silverleaf symptoms was rated according to Yokomi *et al.* (1990).

Transmission test

Transmission tests were carried out with the following *B. tabaci* lines derived from different host species: lines 2, 4, 6, 11 and 12 of the B group and lines 15 and 17 of the non-B group (see below) (table 1). For each line, a group of individuals was placed on one TYLCV-infected tomato plant cv. Farmer (Know You Seed Co.) for a 3-day acquisition access period. After the acquisition access period, each whitefly was caged with one healthy tomato plant cv. Farmer inside a plastic cylinder (diameter 2 cm, height 8.5 cm) for a five-day inoculation access period. The top of each cylinder was covered with thin muslin netting to allow evaporation and airflow. Whiteflies were introduced into the cage through a 3 mm hole in the side of the cylinder, which was subsequently closed with a piece of filter paper to help absorb excess moisture. At the end of the inoculation access period, insects were removed and the plants were treated with imidacloprid (Confidor®, Bayer, Basel, Switzerland) to prevent any subsequent whitefly contamination and placed in an insect-proof cage for observations of TYLCV symptoms

for 18 to 21 days. By adding all the transmission tests performed during different experiments, a total of 200 insects was tested for each line. The inoculated plants were finally analysed using triple antibody sandwich–enzyme linked immunosorbent assay (TAS–ELISA, Adgen Ltd, UK) for virus detection. The protocol followed manufacturer recommendations. Absorbance values were recorded at 405 nm with a spectrophotometer every 15 min for 2 h. A transmission was considered to be successful when the absorbance (A) obtained from the inoculated plant was higher than the mean A of the negative controls (crude extracts of 10 plant samples from non-inoculated plants) + 3 times their standard deviation.

Results

RAPD–PCR analysis

Adults of *B. tabaci* whiteflies were tested with eight RAPD primers except individuals belonging to set 2 (table 1) which were not tested with primer B2. A total of 67 polymorphic bands were scored with individuals from set 2. A neighbour-joining tree (fig. 1) was derived from the Sokal & Michener distance matrix using the RAPD patterns obtained for set 2 minus line 7 which only amplified with primers B1, B3, and F12. A B-group including a control biotype B individual from France (population 19, table 1) and most of the lines from Réunion was supported with a 100% bootstrap value (fig. 1). A non-B group consisting of the annual poinsettia and sweet potato lines (coded 15 and 17 respectively) was supported with a 71% bootstrap value. Individuals tested in sets 1 and 3 exhibited either the B or non-B patterns that were identified in individuals of set 2 (table 1).

The results of the hierarchical analyses, including the results of the AMOVA test and their corresponding F-statistics of genetic differentiation are presented in table 2. Three sources of variation were taken into account: the difference between B and non-B groups (F_{CT}), differences among host plants within groups (F_{SC}), and differences within host plants (F_{ST}). Most of the haplotype diversity was found between B and non-B groups (57.3%). Diversity within the host plants was also high (36.3%). The difference among host plants within groups was small (6.4%). The high value obtained for F_{CT} (0.57), showed there was a high level of genetic differentiation between the B and non-B groups. The F_{ST} value (0.64) was also quite high, indicating a very high level of genetic differentiation for insect populations on the same host plants. A low value of 0.15 was observed for the F_{SC}, indicating that individuals among host plants and within the same group were genetically more homogeneous.

Table 2. Analysis of molecular variance, and F-statistics of genetic differentiation when *Bemisia tabaci* set 2 haplotypes are grouped hierarchically according to B and Ms groups, among host plant populations within groups and within host plants.

Source of variation	d.f.	Sum of squares	Variance	%	F-statistics
Between B and Ms groups	1	39.12	6.66	57.3	F_{CT}: 0.57
Among host plants within groups	5	29.86	0.75	6.4	F_{SC}: 0.15
Within host plants	11	46.41	4.22	36.3	F_{ST}: 0.64
Total	17	115.4	11.62		

COI gene sequence comparisons

The phylogenetic tree was estimated using a 370 bp portion of the COI sequence (fig. 2). Individuals from Réunion previously identified as belonging to the B biotype using RAPD, clustered (99% bootstrap support) with other B individuals obtained from Genbank. The individual from the island of Mayotte also belonged to the B group. Individuals belonging to the Q and related biotypes were sister to this group (83% bootstrap support). Non-B lines from Réunion (lines 15, 17 and 18) together with individuals from Madagascar, Mauritius and the Seychelles, formed a third sister group with 100% bootstrap support. This new group detected in the south-west Indian Ocean was named Ms after the Mascarenes archipelago. One of the two individuals of the B-type eggplant line 2 (AJ550177) was divergent from the other individuals of the B/B2 group with a mean distance of 0.031 ± 0.003. All the individuals analysed from the islands of the south-west Indian Ocean clustered in a large African–Mediterranean–Arabian group (86% bootstrap value) including the Ms genetic type, B biotype, Q biotype and Q-related African individuals. The mean distance between the B and Q groups (0.047 ± 0.005) was smaller than the distance between the Ms group and the group formed by the B and Q biotypes (0.069 ± 0.007). The molecular clock hypothesis was not rejected by the relative rate test (Takezaki *et al.*, 1995) and therefore a linearized tree (Nei & Kumar, 2000) was constructed. This allows for inferring nodes for which divergence time can be estimated based on Browers's estimation of 2.3% pairwise sequence divergence per million years (Brower 1994). The divergence between the Ms group and the group formed by B and Q biotypes on this basis was estimated at 3 ± 0.3 million years.

The remaining individuals formed the same Asian, American and African clusters that have been identified in previous studies (Frohlich *et al.*, 1999; De Barro *et al.*, 2000; Legg *et al.*, 2002). As expected the two individuals sequenced as the A biotype control clustered with the America group, and the *T. vaporariorum* individual from Réunion clustered with the *T. vaporariorum* from which the sequence was available from Genbank.

Silverleaf test

All the tested *B. tabaci* lines from the B and the Ms groups induced symptoms of silverleaf on *Cucurbita moschata* (table 1). No significant differentiation of symptoms was noticed between lines.

TYLCV transmission test

The TYLCV was transmitted by all the *B. tabaci* lines tested (table 1). The B and non-B lines could not be discriminated using transmission rates which ranged from 14 to 53%.

Discussion

Two genetic types of *B. tabaci* were identified in Réunion with RAPD markers and COI sequences, the cosmopolitan B

biotype, and a so far undescribed genetic type, named Ms after the Mascarenes Archipelago. The Ms genetic type was also detected on Mauritius as well as from the neighbouring islands of the Seychelles and Madagascar. This genetic type was therefore considered to be indigenous to these islands. The phylogenetic tree comparing the COI sequences of the Ms genetic type to those of a panel of *B. tabaci* originating from a wide geographical range showed that the Ms genetic type belongs to a Mediterranean/Arabian Peninsular/African group of genotypes to which biotypes Q and B also belong (group MsQB, 86% bootstrap value). This is consistent with the link shown by others between the geographical origin and the phylogeny of *B. tabaci* (De Barro *et al*., 2000; Perring, 2001; Viscarret *et al*., 2003). The genetic distinction between the MsQB group of populations mainly detected on non-cassava species, and an African group of populations detected on cassava and *Asystasia gangetica* (L.) T. Anders (Acanthaceae) (96% bootstrap value) illustrates that both geographical isolation and host plant species may have contributed in this part of the world to the population structure of *B. tabaci* (Brown *et al*., 1995; Abdullahi *et al*., 2004).

Since Réunion is a volcanic island that appeared relatively recently, around 2 million years ago, the Ms genetic type probably originated elsewhere because its divergence from the group formed by the B and Q biotypes is estimated to be older, at 3 ± 0.3 million years. The divergence could have occurred in Madagascar where the Ms genetic type was also detected, because it is estimated that Madagascar drifted away from the continent about 165 million years ago. Extensive samplings in south-east African countries are needed to establish the origin of the Ms genetic type which has so far not been reported from the African mainland.

As expected, the B biotype populations induced typical symptoms of silverleaf. Ms populations were able to induce the same symptoms showing that non-B populations are also able to induce silverleafing (Bedford *et al*., 1994; Secker *et al*., 1998; De Barro *et al*., 2000; Perring, 2001) and therefore indicating that the silverleaf bioassay is not sufficiently diagnostic to conclusively identify the B biotype of *B. tabaci* (Secker *et al*., 1998). All the whitefly lines transmitted TYLCV. As the tested populations were collected and reared on different host species, host plant adaptation may have influenced transmission efficiency (Bedford *et al*., 1994; Brown *et al*., 1995) more than the genetic background of the B and Ms populations. Biological differences between B and Ms genetic types are however suspected because biotype B was detected in most of our samplings (table 1) whereas the Ms genetic type was found only at two out of ten sampling sites and on two out of eight host plant species. Moreover, the visual observations made while maintaining a B and an Ms population on cabbage under the same rearing conditions, showed that the B population always reached a high density whereas the Ms population remained at a low density.

Taken together, these data indicate that the Ms genetic type detected on the islands of the south-west Indian Ocean should be considered a new biotype because: (i) the Ms genetic type was detected in a specific and rather isolated geographic region of the world; (ii) the genetic distance between the Ms genetic type and its two closest biotypes B and Q was higher than the distance between B and Q; and (iii) the Ms genetic type produces silverleaf symptoms, in contrast to other reported non-B biotypes.

The absence of *B. tabaci* populations on vegetable crops before the first detection of TYLCV in 1997 suggests that the polyphagous biotype B was introduced simultaneously with TYLCV or shortly before. Moreover, the absence of any recent detection of infestations of *B. tabaci* prior to 1997 on Réunion, although it was reported there long ago, suggests that the indigenous Ms biotype has a low fecundity and/or a restricted host range. However, the Ms biotype was not found to be monophagous since it was collected on two host species in our samplings, and progeny could be reared on *C. moschata* and cabbage.

Future work will focus on the detection of the natural host range of the Ms biotype, its fecundity on these host plants, its ability to interbreed with biotype B, its geographical distribution and its possible presence in south-east Africa. The insularity of Réunion emphasizes the need for further studies on ecological, genetic and spatial interactions between the two biotypes.

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