TOMATO YELLOW LEAF CURL VIRUS DISEASE

Tomato Yellow Leaf Curl Virus Disease

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CONTENTS

PART I

WORLDWIDE EXPANSION OF TYLCV

CHAPTER 1

APPEARANCE AND EXPANSION OF TYLCV: A HISTORICAL POINT OF VIEW

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1. INTRODUCTION

In 1959, the Israeli Ministry of Agriculture urged farmers in the Jordan Valley to replace the tasty but soft tomato "Marmande" with the long–shelf life variety "Money Maker," which was more suitable for export. A month after transplanting (August), most of the tomato plants in the region were affected by a disease of unknown etiology. Symptoms included severe stunting of plant growth, erect shoots, and markedly smaller and misshaped leaflets. The leaflets that appeared immediately after infection were cupped down and inward, and subsequently developing leaves were strikingly chlorotic and showed an upward curling of the leaflet margins. When young plants were infected, they barely produced any marketable fruits (Cohen & Nitzany, 1960). The growers' first reaction was to blame the change in tomato variety and they demanded compensation from the Ministry of Agriculture. Dr. F. E. Nitzany, head of the Virology Laboratory at the Volcani Center, Agricultural Research Organization (ARO), Israel, was asked to determine the causal agent of the disease and find solutions to the problem. A field survey revealed that most of the tomato plots in the area had been completely destroyed, and that the disease was accompanied by large populations of whiteflies. The whitefly population had built up in the nearby cotton fields, a crop which was being grown on a commercial scale for the first time in Israel. Soon enough, the suspicion that the whiteflies were the vector of the disease was confirmed, following controlled transmission experiments in the laboratory. Moreover, the "Marmande" tomato was found to be as susceptible as "Money Maker" to the disease, which was found to be viral in nature (Cohen & Nitzany, 1960). The virus was named *Tomato yellow leaf curl virus* (TYLCV) by the late Professor I. Harpaz of the Hebrew University (Cohen & Harpaz, 1964). Interestingly, similar disease symptoms had first been

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observed on tomatoes grown in the Jordan Valley as early as 1929, as well as in subsequent years (Avidov, 1944). The outbreaks of TYLCV disease were always accompanied by large populations of whiteflies (Cohen & Berlinger, 1986). However, the geminate shape of the viral capsid was first observed in 1980 (Russo et al., 1980), and it was only in 1988 that the virus was isolated (Czosnek et al., 1988). It took another 3 years to clone and sequence the virus, and to demonstrate that the genome of TYLCV is composed of only one single-stranded (ss) DNA molecule (Navot et al., 1991).

The first evidence of economic damage to vegetable crops caused by the whitefly *Bemisia tabaci* (Gennadius) in Israel was recorded in 1931 (Avidov, 1944). Since 1935, it has been a permanent pest, mainly in the Jordan Valley. Avidov concluded that the *Bemisia* whitefly can raise as many as 15 generations per year in the Jordan Valley, due to the favorable climate in the area (Avidov, 1944). The silvering of squashes caused by *Bemisia*, which was observed as early as 1963 (Baery & Kapoller, 1963), and the very wide host range of this insect indicate that the B (or silverleaf) biotype has been present in this region for a long time.

2. VIRUS–VECTOR INTERACTIONS

2.1. Acquisition and transmission

In 1960, the first steps were taken toward controlling the TYLCV epidemic. The virus–vector relationship was studied by testing the transmission efficiency of TYLCV by whiteflies. Following 48 h of acquisition access feeding on infected tomato, only 5% of the male whiteflies transmitted the virus by transmission feeding of a single insect per test plant. However, female whiteflies were able to transmit the virus with 32% efficiency, sixfold better than their male counterparts. Transmission feeding with 1, 3, 5, 10, and 15 viruliferous female whiteflies per plant yielded transmission rates of 32%, 83%, 84%, 86%, and 100%, respectively (Cohen & Nitzany, 1966).

It was found that the virus is circulative and persistent in the insect (Cohen & Nitzany, 1966). Once the whitefly vector feeds on an infected host plant and acquires the virus, viral transmission can occur within hours, and may continue for the life span of the vector. Acquisition and transmission thresholds were found to be between 15 and 30 min. However, at least 4 h were required to obtain high infection rates. The latent period was found to be from 21 to 24 h. In tests carried out with whiteflies having a life span of 20–50 days, following 48 h of acquisition feeding, only 2 out of 39 female whiteflies retained the virus for 20 days. Shorter acquisition feedings resulted in shorter virus-retention periods. TYLCV transmission efficiency by its vector declines with time; most of the females failed to transmit the virus for more than 10 days after acquisition (Cohen & Nitzany, 1966). Besides acquisition by adults, it was found that the virus is also acquired by the whitefly larval stages. Following feeding on an

infected plant, 28% of the emerging adults were able to transmit the virus (Cohen & Nitzany, 1966).

To test for virus transmission from viruliferous females to their progeny (transovarial transmission), viruliferous whiteflies were allowed to lay eggs on cotton plants, which are immune to TYLCV. Upon emergence from the pupal stage, the adult offspring were immediately transferred to TYLCV-susceptible plants for a 48 h transmission feeding. Out of 360 female offspring tested, none was found to transmit the virus. Thus it was concluded that TYLCV is not transmitted to the whitefly progeny.

The issue of whether TYLCV is transmitted transovarially to the whitefly progeny came up again for debate 30 years later, when different findings were published. Using molecular tools as well as PCR amplification (which were unavailable back in the 1960s), it was demonstrated that TYLCV DNA is transmitted transovarially to the progeny of viruliferous whiteflies (Ghanim et al., 1998). This was confirmed in an independent study by Polston et al. (2001) who also found that progeny of viruliferous whiteflies indeed contain TYLCV DNA. In another study, Bosco et al. (2004) demonstrated that DNA of *Tomato yellow leaf curl Sardinia virus* (TYLCSV) is transmitted to the whitefly progeny, whereas DNA of TYLCV is not. However, while according to one study (Ghanim et al., 1998), the TYLCV-carrying whitefly progeny were able to transmit the virus to test plants, in other studies (Polston et al., 2001; Bosco et al., 2004), the whitefly progeny, although containing TYLCV DNA, were unable to transmit the virus, supporting the original results obtained in the late 1960s (Cohen & Nitzany, 1966).

2.2. Periodic acquisition

While studying virus–vector interactions, a unique phenomenon, which was termed "periodic acquisition," was observed (Cohen & Harpaz, 1964). It was found that following TYLCV acquisition, viruliferous whiteflies progressively lose infectivity and about 10 days after completion of the acquisition feeding period, most of the insects are no longer able to transmit the virus. However, during that period, the vector is unable to compensate for its steadily decreasing viral-transmission capacity by reacquiring the virus from the infected source plant. That is, another cycle of acquisition feeding, while the vector can still transmit the virus (albeit at a decreasing efficiency), does not restore the transmission capability to its original efficiency. The vector must first completely lose its transmission ability before it can reacquire the virus (Cohen & Harpaz, 1964). A proteinaceous factor which appeared to be related to the phenomenon was found in homogenates of insects, and was termed periodic acquisition-related factor (PARF). This factor, via membrane feeding to nonviruliferous whiteflies, inhibited acquisition, transmission, and retention of TYLCV by the whiteflies (Cohen, 1967, 1969; Marco et al., 1972). Unfortunately, research into the mechanism underlying this phenomenon was never completed. Therefore,

whether this is an active antiviral mechanism or a temporary blockage of the salivary glands by degradation products of the viral capsid protein remains a mystery.

The long latent period of 21 h, the phenomenon of periodic acquisition, and the relatively long and efficient inoculation period of about 4 h suggest that the use of a fast-killing insecticide could effectively control the spread of TYLCV. Indeed, soon after the epidemics broke out, it was demonstrated that spraying with the cyclodiane "Andrin" solved the problem (Cohen et al., 1963). However, the whiteflies soon developed resistance to the insecticide and research shifted to cultural crop management and sanitation.

3. THE USE OF YELLOW MULCH TO PROTECT CROPS

In 1940, while working in the Jordan Valley, a researcher named K. M. Mendel observed that mulching of summer tomato nurseries with sawdust accelerates seedling growth (Avidov, 1944). This growth acceleration was attributed to the finding that the soil temperature under the mulch was cooler by 8–10°C than the temperature of bare soil. However, it was also noticed that the whitefly population on the mulched seedlings was much lower than on nonmulched seedlings (Avidov, 1944). Avidov first thought that the smell of the resin secreted from the sawdust repelled the insects. However, the same controlling effect was achieved by mulching the seedlings with straw and the scent-effect theory was rejected. Avidov also found that during the day, the temperature immediately above the sawdust mulch sometimes reached 47–51°C (temperatures that were later found to be lethal to whiteflies in a dry climate). He therefore concluded that the repelling effect of the sawdust mulch occurs by creating "an atmosphere of death" on its surface which repels the whiteflies (Avidov, 1944).

In an attempt to better understand the effect of straw mulching on whiteflies, the possible effect of whitewashing seedbed soil on whiteflies was also studied (Avidov, 1944). It was found that, 8 days after sprouting, the average number of whitefly eggs per seedling for whitewashed soil was 18.5, compared to 60 whitefly eggs per seedling in nonmulched soil. The same maximum soil surface temperature was recorded for the whitewashed soil (44°C) and the nonmulched control plot (45°C). These findings suggested that soil surface temperature is not the only factor involved in the mulch-based whitefly-controlling mechanism (Avidov, 1944).

3.1. How does it work?

Following Avidov's observations, Nitzany et al. (1964) demonstrated that, indeed, straw mulch can reduce the spread of another whitefly-borne virus, the semipersistent *Cucumber vein yellowing virus* (CVYV). Mulching cucumber seedlings with straw markedly reduced the whitefly population and, as a consequence, delayed CVYV spread for about 10 days. The straw mulch also increased

yield and vegetative development of the cucumber plants (Nitzany et al., 1964). Subsequent to Nitzany's work from 1964, straw mulch was used to control the spread of TYLCV (Cohen et al., 1974). The mulch was very effective in preventing the spread of the virus and the whitefly populations for the first 18 days following germination. However, it was important to extend the duration of the mulch's controlling effect beyond the first 18 days after germination, and the described putative mechanism underlying this effect was therefore reevaluated. In 1962, Mound demonstrated that yellow color attracts whiteflies (Mound, 1962). It was suggested that yellow radiation, which induces vegetative behavior, may be a component of the insect host–selection mechanism (Mound, 1962). This raised the possibility that yellow color also contributes to the controlling effect of the mulch. Thus, using an aphid flight chamber, the effect of straw on whitefly dispersal was studied (Cohen et al., 1974). It was found that nearly three times more whiteflies were attracted to sticky cardboard plates covered with straw compared to those covered with tomato leaves. Moreover, the number of whiteflies attracted to fresh straw was double the number of whiteflies attracted to old straw which had first been exposed to field conditions for 25 days (Cohen et al., 1974). It should be noted that the yellow color of fresh straw is much more intense than that of old straw, the latter fading with exposure to intense solar radiation.

The correlation between the mulch controlling effect and its attractiveness to whiteflies was demonstrated by testing the effects of four different-colored mulches on whiteflies: straw, and three different-colored polyethylene sheets – yellow, silver, and blue (Cohen & Melamed-Madjar, 1978). All four mulches reduced the spread of TYLCV compared to the nonmulched control, with the yellow mulch being the most effective. Moreover, the yellow mulch was the most attractive to whiteflies, in both an aphid flight chamber and the field. In the latter experiments, sticky traps consisting of Petri dishes covered with different-colored polyethylene sheets or with cropped straw were used. The traps were placed on same-colored mulch treatments. Indeed, 77 whiteflies were trapped on the yellow mulch, while only 39 whiteflies (nearly half) were trapped on the silver mulch, 23 whiteflies were trapped on the blue mulch, and 11 whiteflies were trapped on the straw mulch (Cohen & Melamed-Madjar, 1978). Once again, these results clearly demonstrated that the whiteflies were attracted to the yellow color of the mulch.

3.2. Effect of temperature

To study the role of temperature in the controlling ability of the yellow mulch the following experiments were carried out. Four temperature-controlled heating plates (each 10 cm in diameter) were attached to the floor of a flight chamber, 20 cm apart (Cohen, 1982). Yellow-painted Petri dishes covered with glue on the upper side were placed on the heating plates. The temperature of two opposing plates was set to 25°C, and that of the other two to 50°C. In each

experiment, 200 whiteflies were introduced into the flight chamber from the top; the number of insects adhering to the traps was counted 1 h later. After seven repeats, no significant differences were found in the attraction of the whiteflies to yellow traps heated to 50°C (total of 559 whiteflies) or to 25°C (total of 538 whiteflies) (Cohen, 1982). This indicated that high temperature does not repel the whiteflies, as it had been previously suggested (Avidov, 1944).

In another experiment, the combined effect of color and heat was studied. A similar experimental design was used except that, in this case, the yellow traps were not covered with glue, so the attracted whiteflies that landed on the traps could then fly away. The number of dead whiteflies found on each yellow trap was recorded 1 h after their release into the chamber. This time, the results showed significant differences between the treatments; significantly more dead whiteflies were found in the high-temperature plates. Thus, following a total of seven different experiments, no dead whiteflies were found on the plates heated to 25°C, compared with 203 dead whiteflies found on the plates heated to 35°C (Cohen, 1982). These results also contradicted the earlier hypothesis that whiteflies are repelled by high temperature. The controlling effect of yellow mulch therefore appears to be due to a combination of the whitefly attraction to the yellow color of the mulch and its consequent death due to dehydration induced by the high temperature of the mulch. It should be noted that the typical Israeli climate is semiarid – high temperature and low humidity. Moreover, in the tomato-growing regions, soil temperatures exceeding 30°C are quite common. Thus, the use of yellow plastic mulch to protect vegetable crops from whiteflies and whitefly-borne viruses has become common practice in Israeli agriculture (Zaks, 1997).

4. TYLCV EPIDEMIOLOGY

4.1. Wild hosts

In a series of studies aimed at finding ways to control viral spread, a search for the virus inoculum sources in the hot valleys of Israel was performed (Cohen et al., 1988). The surveys were carried out by collecting seeds or cuttings of plants and weeds (mainly the perennials) common to the Jordan Valley region. The samples (seedlings or cuttings) were inoculated with TYLCV to determine which species is susceptible to the virus and which could serve as a potential host. Plants that were found to be susceptible to the virus were tested again for the presence of TYLCV in another set of samples brought from the field. *Cynanchum acutum* was found to be the only natural perennial host of TYLCV. This weed is concentrated along the western bank of the Jordan River (where it covers large areas), a few kilometers east of the main tomato production region at the time. During the winter months (December–February), only the subterranean parts of the plant survive. The plants start growing again in the spring, reaching full vegetation in August–September, concomitant with the

increase in the whitefly population and the tomato-transplanting period. Since this host was concentrated at some distance from the tomato-growing areas, it was important to determine whether whiteflies could cross this distance. Therefore, an area of about 100 m^2, fully covered with *C. acutum* plants and a large population of whiteflies, was dusted with "Fire Orange," a daylight-fluorescent dust, using a mechanical hand duster (Cohen et al., 1988). This dust persisted on the whiteflies for at least 9 days. Whitefly movement was recorded by positioning yellow sticky traps at various distances from the dusted plants, and these traps were monitored weekly for the appearance of fluorescent whiteflies. Indeed, 1 week after the release, fluorescent whiteflies were found in the tomato fields, at a distance of 7 km from the dusting site.

4.2. Viruliferous whiteflies

Most interesting results were obtained when the percentage of viruliferous whiteflies in the general whitefly population was studied during the peak population period (September–November in our case), at which time the infection rate of nonprotected tomato plants reaches 90–100% (Cohen et al., 1988). Whiteflies were collected in the field from different hosts using a cordless rechargeable vacuum cleaner adapted to collect insects into a plastic cylinder (Cohen et al., 1989). The insects were released into a cage with a glass top and were then collected in groups of 20 into small clip cages. The clip cages were placed on the leaves of healthy tomato test plants (one clip cage per plant) and the whiteflies were allowed to feed for 48 h. Following this inoculation access period (IAP), the clip cages were removed, and the test plants were sprayed and monitored for the development of disease symptoms. Only 5.4% of the whitefly population collected on *C. acutum* was viruliferous, compared with 3.2% of the whiteflies collected from a tomato field. One explanation for the relatively low percentage of viruliferous whiteflies within this field population may be the aforementioned periodic acquisition effect.

4.3. Crop-free period

The Arava region of Israel is a 200 km long, 5–10 km wide arid region extending from the Dead Sea to the Red Sea. The climatic conditions during the winter, and moderate temperatures combined with intense solar radiation due to lack of clouds, make this region ideal for growing vegetable crops. The lack of water in the region is overcome by a pipeline from the north and the use of local wells. In 1982–1986, severe viral epidemics occurred in the Arava, threatening the future of vegetable crop cultivation in the region. The major viruses were found to be *Zucchini yellow mosaic virus* (ZYMV) and *Cucumber mosaic virus* (CMV) in cucurbits, *Potato virus Y* (PVY) in pepper, and TYLCV in tomato.

In Israel, TYLCV is widespread mainly in the late summer and autumn, due to the peaking whitefly population during that period (September–November).

The tomato season in the Arava region begins in mid-August. At that time, no infected wild hosts of TYLCV, such as the annual *Malva parviflora* or the perennial *C. acutum*, are found in the region. To determine whether the virus is already present at the beginning of the tomato season in the Arava region, tomato trap plants were distributed in the fields of the Arava and left for a week. Then the plants were collected, sprayed, and kept in an insect-proof greenhouse where the appearance of TYLCV-induced symptoms was monitored. No virus was found in the tomato trap plants dispersed weekly from June to the beginning of the tomato season in August. These results indicated that TYLCV is not endemic to the Arava region, but rather wasa being introduced every year by an influx of whiteflies from the western parts of Israel. Unfortunately, there is no direct evidence for this hypothesis. However, whiteflies have been trapped in mid-August in the northern, desert part of the Arava at a distance of approximately 20 km from the nearest cultivated fields, which may indicate that the whiteflies are dispersed over great distances.

During June and July, local vector populations were found to be relatively low and the natural sources of TYLCV were scarce. Cultivated fields were found to be the major source of whiteflies in this region. Therefore, in order to reduce whitefly-transmitted viral epidemics (such as TYLCV), a vegetable crop-free period for those months was suggested. Indeed, following the implementation of a 2-month crop-free period in 1986, 20 years ago, there has been no TYLCV or any other vegetable virus epidemic in the Arava region (Ucko et al., 1998).

5. BREEDING FOR TYLCV RESISTANCE

Genetic resistance in the host plant is an ideal defense against whitefly-transmitted (as well as other) viruses, since it requires no chemical input and/or plant seclusion and can potentially be stable and long-lasting. Thus, the best way to reduce TYLCV spread is by breeding tomatoes that are resistant or tolerant to the virus. Since all cultivars of tomato (*Solanum lycopersicum*) are extremely susceptible to TYLCV, wild tomato species have been screened for their response to the virus (Lapidot & Friedmann, 2002). The first attempts at breeding for TYLCV-resistant tomato plants were made in the early 1970s using *S. pimpinellifolium* accession LA 121 as the resistant source (Pilowsky & Cohen, 1974). After a few years of repeated tries to introgress the resistance into the domesticated tomato (*S. lycopersicum*), the resistance level of LA 121 was found to be insufficient and efforts were shifted to accessions of *S. peruvianum*, which was found to express a higher level of TYLCV resistance. Indeed, in 1986, the first commercial TYLCV-resistant tomato hybrid TY20 was released (Pilowsky & Cohen, 1990; Pilowsky et al., 1989). The breeding efforts continued, and led to the development of highly TYLCV-resistant lines which do not exhibit symptoms following inoculation with TYLCV (Friedmann et al., 1998; Lapidot et al., 1997). Moreover, it was demonstrated that tomato lines expressing a high level of TYLCV resistance serve as a poor inoculum source for the virus

(Lapidot et al., 2001). Today, due to the continuous breeding efforts of a number of research groups, including the Volcani group, elite commercial TYLCV-resistant tomato hybrids are available (Lapidot & Friedmann, 2002).

6. CONCLUDING REMARKS

TYLCV spread very rapidly from its origin in the Jordan Valley to other parts of Israel and neighboring countries in the eastern Mediterranean, such as Cyprus, Egypt, Jordan, Lebanon, Syria, and Turkey. However, over the last decade, the geographic range of TYLCV has greatly expanded to include the western Mediterranean, Japan, the Caribbean, and the southeastern United States (Polston and Anderson, 1997; Polston et al., 1999; Moriones & Navas-Castillo, 2000). Today, TYLCV is a limiting factor in tomato cultivation worldwide. The reasons for its vast spread and its establishment as a worldwide menace are discussed later in this book.

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CHAPTER 2

AN INSULAR ENVIRONMENT BEFORE AND AFTER TYLCV INTRODUCTION

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1. OVERVIEW

Tomato yellow leaf curl virus (TYLCV, genus *Begomovirus*, family *Geminiviradae*), vectored by the whitefly *Bemisia tabaci*, is one of the tomato infecting viruses which is inducing the most obvious symptoms. The severe growth reduction of the plants and the typical yellowing and curling of the leaves due to TYLCV infection is easily detected by farmers, even not being familiar with those symptoms. Therefore, it is expected that the introduction of TYLCV in a new environment is detected soon after the first infection of tomato plants. This was the case in 1997, when TYLCV was detected for the first time in Reunion, an island of the Indian Ocean at about 700 km east of Madagascar (Peterschmitt et al., 1999). One more reason for which it is thought that the delay between introduction and detection was short is that the local Plant Protection Services were aware of the TYLCV risk.

Subsequently to the first detection of TYLCV, the sampling of infected tomato plants and the collection of *B. tabaci* vectors over time gave us a unique opportunity to monitor the emergence and installation of a virus and its vector in an insular environment.

Firstly, we describe the situation before the arrival of TYLCV in Reunion and in the close environment of the South West Islands of the Indian Ocean (SWIO). Indigenous populations of *B. tabaci* were detected in all the islands whereas indigenous begomoviruses infecting tomato were detected in all of them but not in Reunion and Mauritius. Secondly, we describe the outbreak of TYLCV in 1997 in Reunion and the identification of the so-called cosmopolitan biotype B of *B. tabaci*. Thirdly, we describe the spread of TYLCV to the tomato production area within 2 years, and the evolution of TYLCV

H. Czosnek (ed.), Tomato Yellow Leaf Curl Virus Disease, 13–23.

populations and the distribution of *B. tabaci* populations after 1997. Finally, we discuss the risk of the simultaneous presence in the SWIO of the threatening TYLCV and the polyphagous biotype B.

2. THE SOUTH WEST ISLANDS OF THE INDIAN OCEAN BEFORE THE INTRODUCTION OF TYLCV

Originating from South America, tomato is now produced and consummated in all the tropical and subtropical regions. Interestingly, in most of these regions including the SWIO, tomato plants have revealed the presence of indigenous begomoviruses infecting the introduced tomato. Although indigenous populations of *B. tabaci* were detected in most of these tropical regions including SWIO, some of theses biotypes fed and reproduced on tomato to only a limited extent, minimizing transmission of begomoviruses to and from tomato plants (Polston & Anderson, 1997). In the New World where the indigenous biotype A populations did not readily feed on tomato, most of the tomato infecting begomoviruses were detected on tomato following the introduction of the polyphagous biotype B (Polston & Anderson, 1997; Ribeiro et al., 2003). In SWIO where indigenous populations of *B. tabaci* were identified, indigenous begomoviruses were transmitted to tomato by theses populations in natural conditions (Delatte et al., 2002).

Three species of begomoviruses indigenous of the SWIO were identified from tomato, one from Madagascar, *Tomato leaf curl Madagascar virus* (ToLCMGV), one from Mayotte, *Tomato leaf curl Mayotte virus* (ToLCYTV), and one from Seychelles, *Tomato leaf curl Seychelles virus* (ToLCSCV) (Delatte et al., 2005b: Lett et al., 2004). The symptoms induced on tomato by these viruses are similar to those induced by TYLCV but without yellowing. Sequence analysis revealed that these viruses had genome organizations of monopartite begomoviruses and that ToLCMGV, ToLCYTV, and ToLCSCV belong to the African begomoviruses but represent a distinct monophyletic group that we have tentatively named SWIO (Figure 1). All of the SWIO isolates examined were apparently complex recombinants. None of the sequences within the recombinant regions closely resembled that of any known non-SWIO begomovirus, suggesting an isolation of these virus populations. This is consistent with the geological history of this region where Madagascar and Seychelles, the continental derived islands, drifted away from the Gondwana about 130 million years ago (Figure 2). It is supposed that the progressive decrease of gene flow resulted in the differentiation between the populations of SWIO and those of the continents. Interestingly, no indigenous begomoviruses were detected on tomato in the two most eastern islands of the SWIO, namely Reunion and Mauritius (Mascarenes Islands). This may be explained by the recent volcanic origin of these islands which emerged from the Indian Ocean within the last 10 million years but also by the relatively important distance from Madagascar and the eastern dominant winds which both limited the possibility of viruliferous

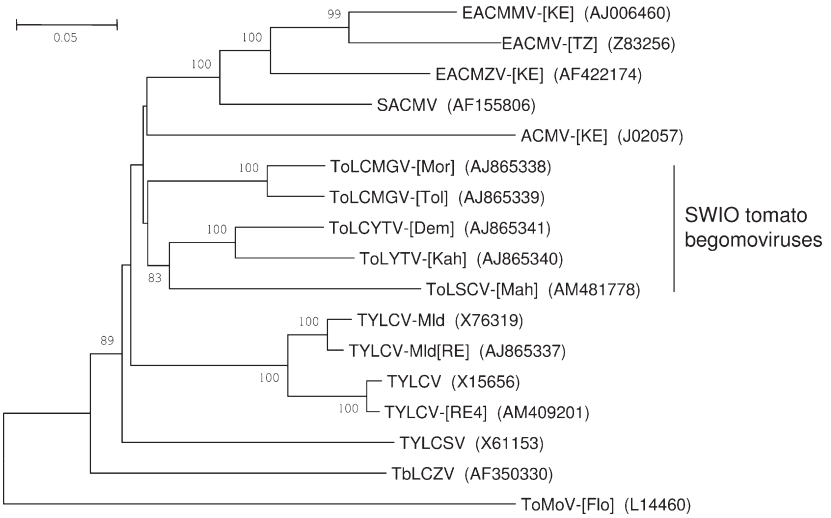

Figure 1. Neighbour joining tree indicating the relationships between the full-length DNA A sequences of tomato begomovirus isolates from the South West islands of the Indian Ocean and those of representative sampling of publicly available African and Mediterranean begomoviruses. The tree was constructed using Jukes–Cantor distances and rooted using ToMoV-[FL] as an outlyer. Numbers associated with the nodes indicate the percentage support for those nodes in 1,000 bootstrap replicates. Horizontal distances represent genetic distances, as indicated by the scale bar, whereas vertical distances are arbitrary. Nucleotide sequence database accession numbers of sequences used in this study: African cassava mosaic virus – [Kenya] (ACMV-[KE]), East African cassava mosaic virus – [Tanzania] (EACMV-[TZ]), East African cassava mosaic Malawi virus – [Kenya] (EACMMV-[KE]), East African cassava mosaic Zanzibar virus – [Kenya] (EACMZV-[KE]), South African cassava mosaic virus (SACMV), Tobacco leaf curl Zimbabwe virus (TbLCZV), Tomato leaf curl Madagascar virus – [Morondava] (ToLCMGV-[Mor]), ToLCMGV-[Toliary] (ToLCMGV-[Tol]), Tomato leaf curl Mayotte virus – [Dembeni] (ToLCYTV-[Dem]), ToLCYTV-[Kahani] (ToLCYTV-[Kah]), Tomato leaf curl Seychelles virus – [Mahé] (ToLCSCV-[Mah]), Tomato yellow leaf curl virus (TYLCV), Tomato yellow leaf curl virus – Mild (TYLCV-Mld), TYLCV– Mild[Reunion] (TYLCV-Mld[RE]), TYLCV-[Reunion4] (TYLCV-[RE4]), Tomato yellow leaf curl Sardinia virus (TYLCSV) and Tomato mottle virus – [Florida] (ToMoV-[FL]).

vectors to reach the Mascarenes. The risk of introduction due to human activities was also limited because of the distance and the relatively recent settings of permanent settlements in these islands, about 400 years ago. On the contrary, although the volcanic islands of Comoros emerged in the same period as the Mascarenes, it is apparently the shorter distance to Madagascar (300 km), the earlier permanent settlements and the dominant winds that have permitted the introduction of SWIO begomoviruses, either naturally through viruliferous vectors and/or through human activities.

Although no SWIO begomoviruses could be detected in the Mascarenes, *B. tabaci* was reported from Reunion on cassava as early as 1938 (Bouriquet,

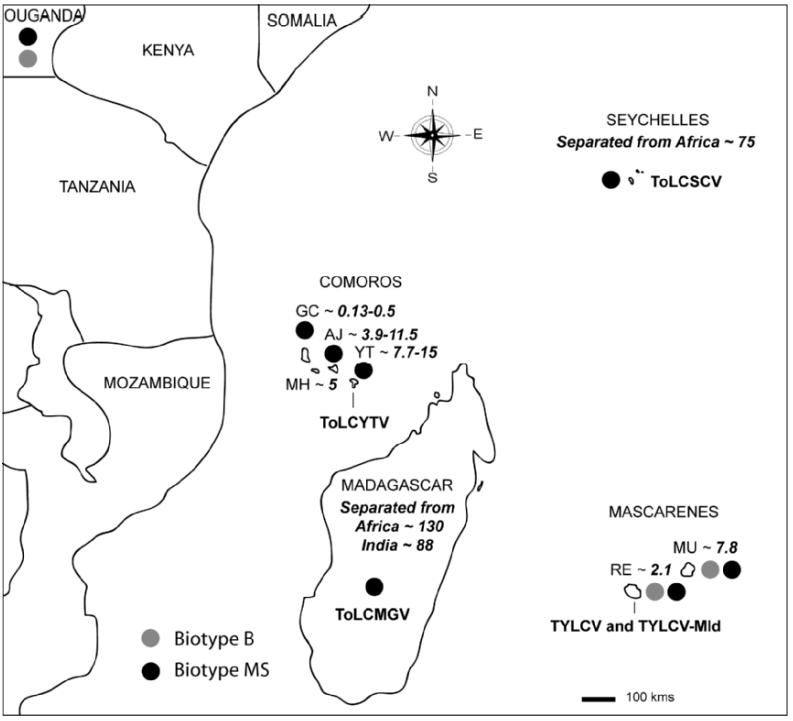

Figure 2. Map of the South West islands of the Indian Ocean showing their geological age indicated in million years (Warren et al. 2003). Besides Madagascar and Seychelles, which are continental-derived islands, the other islands are of volcanic origin: Grande Comore (GC), Mohéli (MH), Anjouan (AJ), Mayotte (YT), Reunion (RE), and Mauritius (MU). Distribution of tomato begomoviruses are indicated: Tomato leaf curl Madagascar virus (ToLCMGV), Tomato leaf curl Mayotte virus (ToLCYTV), Tomato leaf curl Seychelles virus (ToLCSCV), Tomato yellow leaf curl virus (TYLCV), and the mild strain of TYLCV (TYLCV-Mld). Distribution of *Bemisia tabaci* biotypes are also indicated: the indigenous biotype Ms and the exotic biotype B.

1938) and later in 1953 (Luziau, 1953). However there was no further report or detection of *B. tabaci* in Reunion before the outbreak of TYLCV in 1997. The suspicion of the existence of indigenous populations of *B. tabaci* in Reunion and in the SWIO was confirmed using cytochrome oxidase 1 (CO1) sequencing (Figure 3) (Delatte et al., 2005a). The SWIO populations formed a new distinct genetic group that is sister to two other groups, the B and Q biotypes. It was named Ms after the Mascarenes Archipelago. The Ms biotype was thought to be indigenous to the region as it was detected in all the SWIO. Ms populations of *B. tabaci* induced silverleaf symptoms on Cucurbita sp., and were able to acquire and transmit TYLCV. Adult individuals of the Ms biotype were detected on several families of plants, e.g., Convolvulaceae, Euphorbiaceae, Solanaceae, Fabaceae, Verbenaceae, Brassicaceae, Cucurbitacae, suggesting that

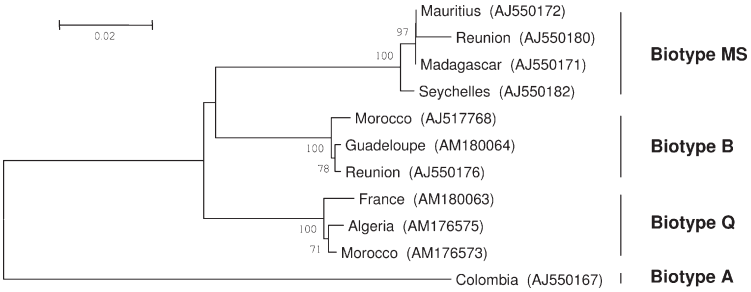

Figure 3. Rooted neighbour-joining tree showing the genetic distance among 816 nt cytochrome oxydase I fragments of *Bemisia tabaci*. Sequences are identified with their geographic origin followed by their Genbank accession number. The scale measures the Jukes–Cantor distance between sequences. Numbers associated with nodes represent the percentage of 1,000 bootstrap iterations supporting the nodes.

it is a polyphagous biotype. It has been estimated (on the basis of mitochondrial CO1 markers) that Ms biotype diverged from B and Q about 3 (±0.3) million years ago (Delatte et al., 2005b), much after the time of the continental separation of Madagascar from the African continent (about 130 million years). The expected African origin was confirmed by the detection of a polyphagous populations of *B. tabaci* from Uganda (genotype cluster Ug7) closely related to biotype Ms according to CO1 (98–99% identity) (Sseruwagi et al., 2005). The detection of seven other genotype clusters in Uganda beside the Ug7 populations, whereas only biotype Ms was detected in the SWIO, suggests that the *B. tabaci* populations of SWIO have originated from Africa following a founder effect.

3. THE OUTBREAK OF TYLCV IN REUNION IN 1997 AND THE DETECTION OF THE BIOTYPE B OF *B. TABACI*

In September 1997, typical TYLCV symptoms, namely, stunting, reduced leaf size, leaf curling, and yellow margins, were observed on tomato plants on a farm of the South of Reunion near Saint Pierre (Peterschmitt et al., 1999) (Figure 4). Diseased plants gave positive reactions by TAS-ELISA and an expected size product was obtained by PCR with degenerate primers designed to amplify a region of the A component of begomoviruses. The sequencing of this cloned PCR product and later of the complete cloned genome showed that plants were infected with a member of the Mild strain of TYLCV (TYLCV-Mld) (Delatte et al., 2005b). The alignment of complete genomes showed that the highest nucleotide identity was obtained with members of the TYLCV-Mld strain that were isolated elsewhere shortly before the 1997 outbreak in Reunion: TYLCV-Mld[JR:Shz] (99.1%) isolated after its first detection in 1996 in Japan, TYLCV-Mld[PT] (98.8%) isolated after its first detection in 1995 in Portugal,

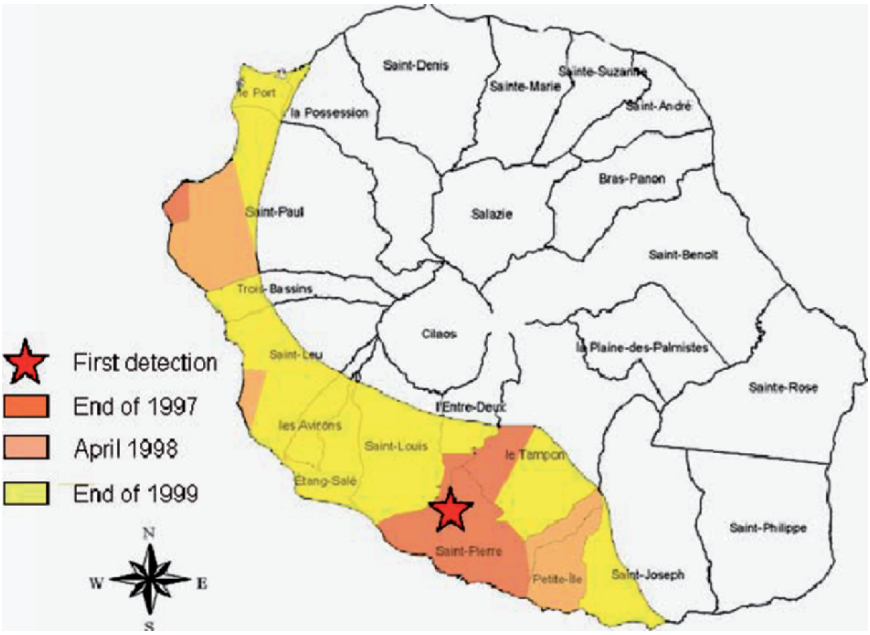

Figure 4. Map of Reunion Island showing the location of the first farm in which TYLCV was detected and its progressive spread to the whole tomato-growing area between 1997 and 1999.

and TYLCV-Mld[ES:72:97] (98.8%) isolated after its first detection in 1997 in Spain. The nucleotide identity was the lowest with TYLCV-Mld[IL] (97.8%), the type member of the strain, isolated before 1994 in Israel. It seems that closely related isolates were simultaneously spread to different regions of the world in the mid-1990s including Reunion. About 3 months after the first detection in September 1997, TYLCV was detected in 13 farms in the region of Saint Pierre and also in a small area near Saint Paul in the West of the island (Figure 4). Severe economic losses were observed, up to 85% in outdoor and/or protected tomato crops. Tomato is grown year round in Reunion and is the most grown vegetable crop. Farmer (Know You Seed), the most popular tomato cultivar grown in Reunion, was found to be highly susceptible to TYLCV.

As stated above, *B. tabaci* were not reported in Reunion between its second report in 1953 and the outbreak of TYLCV in 1997. It was only at the time of the TYLCV outbreak that *B. tabaci* has been observed on tomato crops, but population levels were low compared with those of the whitefly *Trialeurodes vaporariorum* Westwood. During the first 6 months of 1998, *B. tabaci* was also detected in plants occurring near infected crops: *Euphorbia heterophylla* L., *Lantana camara* Mold., *Solanum melongena* L., *S. nigrum* L., *Phaseolus vulgaris* L. *B. tabaci* individuals collected from these plant species and sequenced

in their CO1 gene were found to belong to two distinct biotypes. Some of them were of the indigenous biotype Ms but others clustered with individuals of the biotype B (Figure 3). The B biotype individuals of Reunion are not thought to be indigenous because, beside Mauritius where biotype B was detected in 1998 (Ganeshan & Abeeluck, 2000), biotype B individuals were not detected in the SWIO. We suppose that these biotype B individuals were recently introduced, maybe from Mediterranean countries together with the Mediterranean TYLCV-Mld.

4. THE EVOLUTION AFTER 1997

Although the number of farms in which infected tomato plants were detected outdoors and indoors had increased from 13 by the end of 1997, to 29 in April 1998 (Figure 4), TYLCV had apparently not spread to the whole tomato growing area (mainly on the Western leeward coast; the inner mountain areas and the Eastern coast are not convenient for tomato production). By the end of 1998 to the beginning of 1999, a survey showed that almost the whole tomato-growing area was infected with TYLCV, from Le Port in the North to Saint Joseph in the South and towards the inner island up to 900 m altitude (Figure 4). It was only in 2003 that TYLCV symptoms were observed in the eastern part of Reunion, near the Southeastern town of Saint Rose.

Prior to 1997, begomovirus-induced symptoms were never reported in Reunion. As the first tomato samples infected with TYLCV were most probably collected shortly after its introduction (see above), a unique opportunity was provided to analyse the evolution of TYLCV population almost from the initial inoculum in an isolated agroecosystem, apparently free of any other tomato-infecting begomovirus. A total of 111 samples were obtained from surveys conducted from 1997 to 2004 in the main tomato growing areas in the western part of Reunion. Genetic variation of TYLCV-Mld[RE] was monitored (Delatte et al., 2007). The very low diversity of the isolates observed in 1997 did not provide any evidence of multiple TYLCV introductions in Reunion. In addition, no other *Begomovirus* species or strains were detected during the studied period. The very low initial diversity was followed by a quasi-linear increase in genetic diversity across years. Analysis of population effective size indicated that TYLCV-Mld[RE] in Reunion was in expansion which is consistent with a founder effect due to the introduction of a small virus population in an insular environment. Surprisingly, one nucleotide substitution introducing a premature stop codon in the C4 ORF was observed in an increasing number of isolates in the population of TYLCV-Mld[RE] over time, contrasting with the other substitutions which were observed at lower frequencies. This substitution which shortens the C4 protein by four amino acids may have been selected during TYLCV-Mld[RE] evolution.

The 8-year sampling for the evolution studies was stopped in April 2004 when an isolate of the so-called recombinant TYLCV strain was detected in Saint

Gilles in the northwest region of Reunion (Delatte et al., 2005a, b). This new strain caused more severe yellow leaf curl symptoms than those usually observed with the Mild strain. Intraspecific competition between the two strains is under investigation. This new introduction illustrates how difficult it is to protect an environment from begomovirus infection even in an isolated island. We have recently shown that not only plants and whitefly vectors can be a mean of introduction but also the tomato fruit itself (Delatte et al., 2003).

Evolution studies of the vector populations showed evidence of introgression of the indigenous Ms population into the introduced B population. A multiple sampling survey conducted on the *B. tabaci* biotypes during 2 years (2001–2002) with microsatellite markers in Reunion revealed that biotype B was predominant on the island, with however proportions of the two biotypes varying according to geographic or ecological factors (Figure 5) (Delatte et al., 2006). The B biotype was found predominantly in the north, west, and south part on crops, corresponding to the tomato growing area and leeward dry coast. While, the biotype Ms predominated on weeds in the windward and humid coast, B and Ms biotypes coexist in sympatry throughout most of their geographical ranges. Interestingly, the genetic study revealed a third group of whiteflies genotype, intermediate between B and Ms biotypes (Figure 6). This

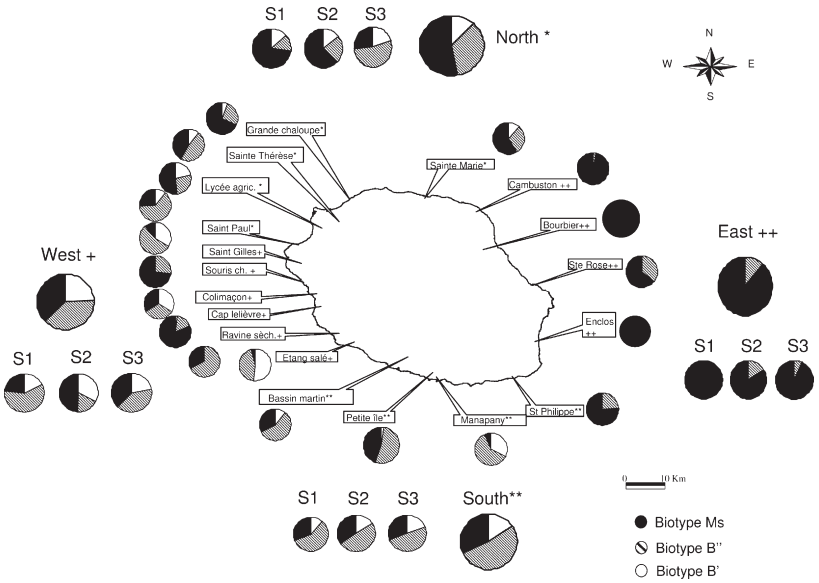

Figure 5. Map of Reunion Island with whitefly biotype B and Ms global repartition in absolute numbers per sector and sampling period (S1, February–March 2001; S2: September–October 2001; S3: February–March 2002). The different sites are represented individually with years grouped, for biotype Ms, groups B′ (pure biotype B) and B″ supposed to be a B form introgressed with Ms alleles (see Figure 6). The sampled sites are represented, and the symbols refer to the sectors they belong to.

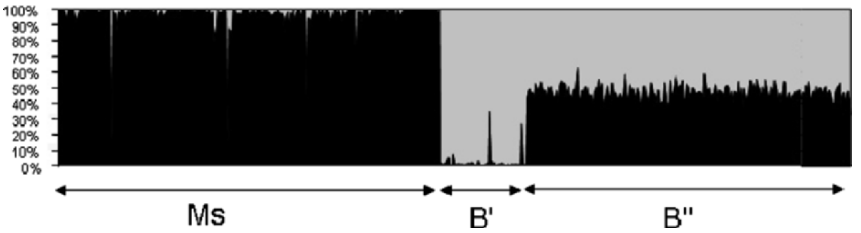

Figure 6. Genetic structure of *Bemisia tabaci* sampled over Reunion Island. Summary plot of estimates of Q (the estimated membership coefficient for each individual in each cluster) given by the software Structure v2.1 with the admixture option (Pritchard et al. 2000). Each of the 567 individuals is represented by a single vertical line broken into K populations (K = 2 in this case), with length proportional to the inferred proportion of B ancestry; individuals from the two subgroups B′ and B″ have been represented in different sections of the graph to emphasize their genetic differences.

group had asymmetrical and locus-specific introgressions between both B and Ms biotypes, especially detected within syntopic populations. This group was therefore proposed as being a hybrid group between B and Ms populations. However, there is no clinal geographical structure typical of classical hybrid zones. The biotypes situation on Reunion appears as a novel strategy of invasion, which does not refer to displacement of a population, to competition by interference for food, or to a complete eradication of one biotype, but rather to the introgression of one population into another. This might lead to the complete disappearance of the parental biotypes and the appearance of a fitter hybrid group of whitefly, or the coexistence of the three groups. More evolutionary time is needed to confirm the extent of the different populations, and know the long-term outcome of introgression in the field.

5. RISK ASSESSMENT FOR THE SOUTH WEST ISLANDS OF THE INDIAN OCEAN

The introduction of exotic begomoviruses into Reunion and exotic *B. tabaci* populations into Reunion and Mauritius is generating new risks for the SWIO that need to be assessed (Figure 2). On the vector side, there is a risk of spread of biotype B populations to the other SWIO where indigenous begomoviruses are infecting tomato. As biotype B was found to be dominant on vegetable crops compared to biotypes Ms (Delatte et al., 2006), the introduction of biotype B in these islands may increase the transmission of these viruses to and within tomato with an increased impact on tomato production. Introduction of biotype B may even induce emergence of so far weed infecting begomoviruses in cultivated crops. On the virus side, there is a risk of overlapping between the distribution areas of the indigenous begomoviruses and the exotic TYLCV either by the introduction of the indigenous begomoviruses into Reunion or the introduction of TYLCV into the islands infested with the indigenous begomoviruses.

Knowing the propensity of begomoviruses to recombine (Fauquet et al., 2005), emergence of new recombinant begomoviruses, possibly with increased virulence and modified host range, is expected. The natural recombinant detected between TYLCV and TYLCSV in Spain (Monci et al., 2002) demonstrated that the probability of such an occurrence is high, especially as the genetic distance between TYLCV and the SWIO indigenous ToLCVs is similar to the distance between TYLCV and TYLCSV (Figure 1).

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CHAPTER 3

THE *BEMISIA TABACI* COMPLEX: GENETIC AND PHENOTYPIC VARIATION AND RELEVANCE TO TYLCV–VECTOR INTERACTIONS

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1. OVERVIEW

The purpose of this review is to present an overview of "the biotype concept" in relation to the whitefly *Bemisia tabaci* (Gennadius) vector of *Tomato yellow leaf curl virus* (TYLCV), the plant virus, which is the topic of this volume. It seems an unlikely coincidence that this single species of whitefly, itself widely variable and plastic, is the arthropod vector of a widespread, dynamic suite of closely related viruses species that also diversify rapidly and adapt to human activities. This chapter will contextualize current scientific knowledge, and raise questions where understanding is lacking – or not yet congealed to reach a satisfactory conclusion. This will involve delineating the characteristics, processes, and concepts that unite or set apart the *B. tabaci* complex from other whitefly species, and other vector–virus complexes. Also discussed will be the characteristics that uniquely delimit variants or "biological types" of *B. tabaci* – recognizable both in terms of biological and/or genetic variability, which yield distinct consequences in agroecosystems – that would not prevail if such variability were absent or irrelevant. The review will also address how knowledge of different and shared characters among biotypes and less well-studied haplotypes (phenotypic variants), could assist in predicting whether a variant could become an invasive, or successful vector. And, how greater than expected genetic variation, together with phenotypic plasticity, influence virus–vector competency, virus dispersion, and virus host adaptation or host range shifts, and support diversification or emergence of begomoviral species. The unprecedented invasiveness of this insect pest and plant virus vector has contributed widely to the intrigue that has fostered the recent interest in this ancient whitefly species. As well, so do its fascinating biology, unresolved taxonomy, unprecedented (apparent)

H. Czosnek (ed.), Tomato Yellow Leaf Curl Virus Disease, 25–56.

interspecific variability, and extent of reproductive isolation. This review will present a historical perspective of the biotype concept, and describe the attributes of the *B. tabaci* complex relevant to its role as a vector and pest in agriculture. It also will provide examples of the best-studied biological types of *B. tabaci* and their significance to begomovirus disease outbreaks. A generalized sequence of events outlining the "history of the biotype concept" and the contributions of many to its legacy is provided in Table 1. It is particularly important to credit our many colleagues whom over the years have generously contributed whitefly and virus collections for molecular analysis. Without them, much of the work described here would not have been possible. It is regrettable that space limitations do not allow inclusion of a comprehensive chronology citing all that have made important contributions to this new field of study. Even so, every effort has been made to highlight key events and the contributions of as many as possible. This chapter is dedicated to Dr. Julio Bird, *Emeritus*, University of Puerto Rico, a priceless mentor and friend who continues mostly unknowingly through his insights and keen observations, to inspire "students of *B. tabaci*" around the world.

Table 1. Chronological history of the "biotype concept"

1889	P. Gennadius described *B. tabaci* (*Aleyrodes tabaci*)
1914	Quaintance and Baker established *Bemisia* as a genus (*inconspicua*)
1936	H. H. Storey reports outbreaks of virus-like disease in cassava in Africa; Takahashi synonomized *B. hibisci* with *B. tabaci*
1957–1977	In Puerto Rico J. Bird provides the first evidence for polyphagous (Sida race) and monophagous (Jatropha race) *B. tabaci*
1957	L. Russell synonymized nine additional species/two genera into the *B. tabaci* taxon (following the decisions to lump instead of split the species by two systematists before her)
1975	Costa and Russell (1975) reported that *B. tabaci* did not colonize cassava where it was native in Brazil, but noted that it readily colonized cassava plant in Africa
1978	Mound and Halsey further synonymized the species (total 23 species, 2 genera)
1980	Outbreak of the A biotype in the southwestern US deserts and NW Mexico; previously undescribed begomoviruses and criniviruses (Brown, 1990, 1994)
1980–1982	Geminiviruses are recognized as a new group of plant viruses containing ssDNA (Goodman, 1981; Hamilton et al., 1982)
1980–1981	First "suspect B" biotype documented, Hawaii (R. Gill, personal communication/Bernar Kumashiro, Bishop Museum, Honolulu)
1985–1990	Ornamentals in continental USA and Europe colonized by *B. tabaci* instead of *T. vaporariorum,* "the norm" (Alderman, 1987; Lindquist and Tayama, 1987)
1986–1987	Silverleaf and irregular ripening observed in Florida for first time (Schuster et al., 1990, 1991; Yokomi et al., 1990). Invasive B biotype not yet recognized

(continued)