Characterization of Biotype T of *Bemisia tabaci* Associated with *Euphorbia characias* in Sicily

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The T biotype of *Bemisia tabaci* (Gennadius, 1889), a population found colonizing *Euphorbia characias* L. plants on the Nebrodi-Peloritani mountains in Sicily, was biologically characterized. The minimum development time was 29.7 days at 28°C. Based on the regression of 1/day *vs* T, the rate of development was calculated as 0.00206, the theoretical lower temperature threshold for development as 9.3°C, and the sum of effective temperatures as 485.1. At 25°C, egg-to-adult development was significantly shorter on *Datura stramonium* (30.1 days) than on either *Euphorbia pulcherrima* or *Euphorbia characias* (35.6 and 35.4 days, respectively). The fourth instar nymphs grown on *D. stramonium* had the typical oval outline and seven pairs of dorsal setae located on cone-like processes, often barely visible. The fourth instar nymphs and their pupal cases grown on *E. characias* had the outline deformed by the presence of hairs on the lower surface of the leaf. The pupae on *D. stramonium* were significantly larger (both longer and wider) than those reared on *E. characias*; on both host plants, female pupae were significantly larger than male ones. Analysis of variance showed that width of females on *D. stramonium* was significantly larger than the width of those reared on *E. characias*. Attempts at courtship between T- and Q-biotypes were observed, but adults from different biotypes were never seen mating. Only males were obtained from the seven heterologous crossing attempts, either way, whereas homologous, control breeding produced males and females. The T biotype was able to transmit *Tomato yellow leaf curl Sardinia begomovirus* (TYLCSV) from *D. stramonium* to *D. stramonium*, from tomato to tomato and from tomato to *D. stramonium*. Attempts to transmit TYLCSV from *D. stramonium* to tomato were unsuccessful. The transmission efficiency was significantly lower when tomato was the test plant. The diverse biology and ecology of the T biotype confirm that it is genetically different from most Mediterranean biotypes.
KEY WORDS: *Bemisia tabaci* biotype T; *Euphorbia*; whitefly; Sicily; virus vector; geminivirus.[†]

INTRODUCTION

Bemisia tabaci (Gennadius, 1889) (Hemiptera: Aleyrodidae) is a pest of food, fiber and ornamental crops throughout the world and is the known vector of at least 111 plant viruses (24). The worldwide distribution and the huge number of host-plant species result in a high biological and genetic variability, so that *B. tabaci* is considered a "species complex" (7), with a number of populations identified, also defined as host races or biotypes (33). The

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different biotypes vary considerably in their ability to colonize different host plants (3,8) and to induce physiological disorders in some hosts (11), in their rates of development (41) and virus transmission efficiencies (3,42).

The presence of *B. tabaci* in Italy has been recorded since the end of the 19th Century (37), but the whitefly was not considered an important agricultural pest until the early 1980s (31). The pest role of *B. tabaci* increased when *Tomato yellow leaf curl Sardinia virus* (TYLCSV; Geminiviridae: Begomovirus), specifically transmitted by the sweetpotato whitefly, appeared in Italy (26,35). Since then, both the B biotype of *B. tabaci* and the indigenous Q biotype have been recorded in several parts of Italy on a number of host species (5,14). During a survey in Sicily, a population of *B. tabaci* was found colonizing *Euphorbia characias* L. plants at about 1000 m above sea level (a.s.l.). According to analysis of the sequence of the cytochrome oxidase *I* (COI) mitocondrial gene (39), the population is unrelated to other Mediterranean biotypes for which this analysis is available, but related to the Indian clade of *B. tabaci,* in which the M biotype from Turkey has been placed by esterase electrophoretic pattern (3) and RAPDS (19). It has an esterase electrophoretic pattern distinct from both the B and Q biotypes, and is restricted to *E. characias*. The population has been recognized as a new biotype, named T (38). The aim of the present work was the biological characterization of the T biotype of *B. tabaci*, expected to be somewhat tolerant to low temperatures and somehow reproductively isolated, given the peculiar biotope where the biotype lives (38). Temperature-development relationships, reproductive isolation, morphology, morphometry and vectoring ability of T biotype were investigated.

MATERIALS AND METHODS

Field surveys and whitefly rearing *Bemisia tabaci* biotype T (38), originally collected in year 2000 on *E. characias* plants in the area of Sella dei Mandrazzi (Messina province) on the Nebrodi-Peloritani mountains in Sicily (approximate coordinates: 37° 58' N, 15° 08' E; 600 – 1000 m a.s.l.), were maintained on *E. characias* plants in a climate-controlled chamber (25±1°C; 60–90% r.h.; 16L:8D photoperiod). These rearing conditions were used throughout the work, unless otherwise specified. Insects from the same colony were also reared on *Datura stramonium* and *Euphorbia pulcherrima* (Willd. ex Klotzsch) (poinsettia). A colony of *B. tabaci* Q biotype, originally collected on melon plants at Oristano (Sardinia Region), was reared on cucumber (*Cucumis sativus* L.) under the same conditions.

Further sampling of whiteflies on *E. characias* was carried out at various sites in the same area at the beginning of March 2001.

Development rate and time To determine the relationship between development rate and temperature, three *E. characias* plants for each experimental temperature were infested with adult whiteflies (sex ratio 1:1), which were allowed to oviposit for 24 h at 25°C and then removed. Plants were then placed within Plexiglas and nylon cages in climate-controlled chambers at 16, 19, 22, 25, 28 and 31°C (± 0.5°C as indicated by the equipment, ±1°C as measured by the temperature recorder) and examined for emergence of adults. Newly emerged adults were counted daily and removed. The theoretical lower temperature threshold (LDT) for egg-to-adult development (the temperature at which the development rate equals zero, T0) was estimated by weighted linear regression of the inverse of days (1/day) *vs* rearing temperature (T) (20). The sum of effective temperatures (SET) was

estimated from the reciprocal of the slope of the regression line. Development time on a day-degree (°D) time scale was computed as °D = days · (T–T0) for T>T0 (16).

The development time at 25°C was also estimated on *E. pulcherrima* and *D. stramonium*, in the same way.

Sex ratio and parthenogenesis The sex ratio was determined at 25°C on the progeny of four couples reared, one couple per plant, on either *E. characias* or *D. stramonium,* counting and removing the newly emerged adults almost daily. Parthenogenesis was checked by rearing virgin females on *E. characias* plants, either individually or in groups. Virgin females were obtained from single fourth-instar nymphs isolated in glass tubes, with the leaf piece they were attached to, until emergence.

Morphometry and morphology Samples of T biotype fourth-instar nymphs were obtained from *E. characias* and *D. stramonium* by cutting small pieces of leaves hosting whiteflies. Length and width of pupae, recognized by the disappearance of mycetomes (25), were measured under a stereomicroscope at × 25 total magnification, with × 10 micrometric eyepiece. Measures were rounded to the smallest eyepiece division, *i.e.*, 40 μm. Each fourth-instar nymph on its leaf piece was isolated in a glass tube on wet filter paper until the emergence of the adult for sex identification.

For comparison, whiteflies of the Q biotype were reared on *D. stramonium* plants, and fourth-instar nymphs treated as T biotype samples.

Leaf pieces with one or more pupae or fresh exuviae were cut from rearing plants, both *E. characias* and *D. stramonium,* dehydrated by 1-h passages in 70% ethanol, absolute ethanol, and cyclohexane, and dried on absorbent paper in a vacuum desiccator with silica gel as desiccant at room temperature. Specimens were glued to aluminum stubs by two-sided sticky adhesive tape and then coated using a sputter coater model E 5000 C-PS3 during two consecutive treatments (60 s each, 10 mA, 0.1 Torr). Observations were made under SEM (Cambridge 200) at 13 KV, magnification ranging from 100 to 150.

Crossing experiments Attempts were made to cross biotype T with biotype Q, the latter being indigenous to the Mediterranean Region and spreading in Sicily (38). Virgin females and males from both biotypes were obtained from single fourth-instar nymphs isolated in glass tubes, with the leaf piece they were attached to, until emergence. The adults were then reared separately on *D. stramonium*, the only experimental host plant shared by the two biotypes. Males of one biotype were then transferred to the cage where females of the other biotype were being reared. Insects were observed for courtship and mating with a magnifying lens for 1 h each of the 5 days following the transfer, and then removed. Plants were kept until the emergence of the last adult or for 2 months in case no whiteflies were noticed in the cage.

Vectoring ability In order to test vectoring ability of the *E. characias* biotype, adult whiteflies were allowed to feed on TYLCSV-infected plants for 24 h, after which insects were transferred in small clip cages to test plants for 72 h of inoculation access. Virus source and test plants were either tomato (*Lycopersicon esculentum* Mill.) cv. 'Marmande' or *D. stramonium.* Source plants were experimentally infected by insects; test plants were exposed to viruliferous whiteflies at the fourth leaf stage. Insects were transferred either individually or in groups of three. At the end of the inoculation access, the test plants were freed from insects by removing the cages, observed for symptoms in an insect-proof greenhouse, and tested by dot-blot hybridization assay (2) in a few dubious cases. From the same virus sources, control transmission tests were done using *B. tabaci* of the B biotype.

Statistical analysis All statistical calculations were implemented using Genstat 4.2 (VSN International Ltd., Oxford, UK) (32).

TABLE 1. Development (dev.) time for the T biotype of *Bemisia tabaci* on *Euphorbia characias* in days as well as in day-degrees (°D) at the experimental temperatures

Exp. temp. (°C)	Adults obtained (no.)	Mean dev. time (days±SD)	& Range[z]	Mean dev. rate (1/day±SD)	Mean°D (±SD)	& Range
16	122	77.5±5.3	68–87	0.01297±0.00090	519.7±35.6	456.2–583.6
19	382	48.1±4.9	41–57	0.02088±0.00148	467.3±34.2	398.0–553.4
22	449	37.3±3.8	29–49	0.02705±0.00256	474.1±47.0	368.5–622.7
25	98	35.4±3.3	28–46	0.02853±0.00265	555.4±52.3	439.8–722.6
28	72	29.7±3.2	25–38	0.03402±0.00360	556.2±60.4	467.7–710.9
31	60	35.7±3.9	29–44	0.02839±0.00320	773.9±85.6	629.5–955.2

[z]Range indicates the days of the first and the last emergence.

TABLE 2. A Length and width (in μm) of male and female pupae of the T biotype reared on either *Datura stramonium* or *Euphorbia characias* plants. B Length and width of pupae of the T- and Q-biotypes reared on *D. stramonium*

A T biotype

Host plant	Sex	n	Mean length[z] ±SD	Mean width[y] ±SD
D. stramonium	F	14	874.3±41.1	637.1±39.9
	M	28	734.3±38.1	494.3±33.0
E. characias	F	28	762.9±26.5	520.0±32.7
	M	56	657.9±38.8	442.9±27.3

[z]LSD=13.9; [y]LSD=11.7.

B T- and Q-biotypes on *D. stramonium*

Biotype	Sex	n	Mean length[x] ±SD	Mean width[w] ±SD
T	F	14	874.3±41.1	637.1±39.9
	M	28	734.3±38.1	494.3±33.0
Q	F	28	862.9±35.2	611.4±34.2
	M	56	730.0±33.5	504.3±31.1

[x]LSD=13.3; [w]LSD=12.8.

RESULTS

Field surveys and whitefly rearing At the beginning of March only nymphs, from first- to fourth-instar, were observed and collected on *E. characias* at all sites in the Sella dei Mandrazzi area. At 1000 m a.s.l. the population of immature stages showed the highest proportion of first-instar, and no fourth-instar nymphs were observed. At lower altitudes, the proportion of fourth-instar nymphs gradually increased and first-instar ones were totally absent.

Continuous breeding of T biotype whiteflies was successful only on *E. characias*. Nevertheless, *D. stramonium* proved to be suitable for rearing whiteflies for a few generations. In spite of repeated attempts to rear T biotype on poinsettia, only in one case was a low number of progeny obtained.

TABLE 3. Heterologous crossing attempts between biotypes T and Q on *Datura stramonium* plants (H1. . .H7) and homologous controls on the same host (C1. . .C2)

Exp.	Biotype T		Biotype Q		Progeny	
	Female	Male	Female	Male	Female	Male
H1		19	13		0	19
H2	6			10	0	0
H3		4	2		0	91
H4	13			26	0	0
H5		1	2		0	14
H6		1	1		0	3
H7		4	5		0	91
C1	24	26			13	75
C2			1	3	45	15

TABLE 4. Vectoring ability of *Bemisia tabaci,* T biotype, as determined by transmission tests of *Tomato yellow leaf curl Sardinia begomovirus*

Biotype	Insects per plant	Source species	Test species	Positive (no.)	Tested (no.)	*P*
B	3	Datura	Datura	7	14	0.50
T	3	Datura	Datura	12	25	$0.48^{a,b,c}$
T	3	Datura	Tomato	0	16	$0.00^{a,d}$
T	3	Tomato	Datura	17	18	$0.94^{b,d,e}$
T	3	Tomato	Tomato	4	28	$0.14^{c,e,f}$
B	3	Tomato	Tomato	19	24	0.79^{f}
T	1	Tomato	Tomato	1	25	0.04^{g}
B	1	Tomato	Tomato	10	25	0.40^{g}

P having the same superscript differ very significantly in the χ^2-test ($P<0.01$; $df=1$).

Development time Development times and rates of *B. tabaci* T biotype on *E. characias* are shown in Table 1. The minimum time was attained at 28°C. The only higher temperature tested (31°C) did not shorten the egg-to-adult cycle while resulting in a high mortality, as judged by the low number of adults emerged compared with at lower temperatures, while the conditions for oviposition were the same. At 28°C the egg-to-adult development time was 29.7 ± 3.2 days (mean ± SD) (median = 30).

The equation fitted to 1/day *vs* T between 16 and 28°C by weighted linear regression was: 1/day = 0.002061 (±0.000025) T – 0.019153 (± 0.000436) (see Fig. 1)
(percentage variance accounted for: 87.7; $df=1127$; $P<0.001$; residuals are normally distributed). This indicates a theoretical LDT of 9.3°C for abolishing egg-to-adult development and a SET equal to 485.1 (±SE: 479.8–490.6). Development times on a day-degree (°D) time scale calculated for each experimental temperature are shown in Table 1. Even excluding the obvious outlier at T=31°C, an apparent non-linear trend is visible, but the mean values calculated for each experimental temperature were reasonably close to the calculated SET.

At 25°C, egg-to-adult development took 30.1±2.2 days (n=86) on *D. stramonium*, 35.6±3.8 days (n=31) on poinsettia, and 35.4 ± 3.3 days (n=98) on *E. characias*. Data from the different host plants were compared by the Mann-Whitney *U* test because of the non-homogeneous variances ($\chi^2=20.63$ on $df=2$ by Bartlett's test for homogeneity of variance; $P<0.001$). There was no significant difference between the development times on *E. characias* and on *E. pulcherrima* ($U=1475.5$; $P=0.811$). The development time on

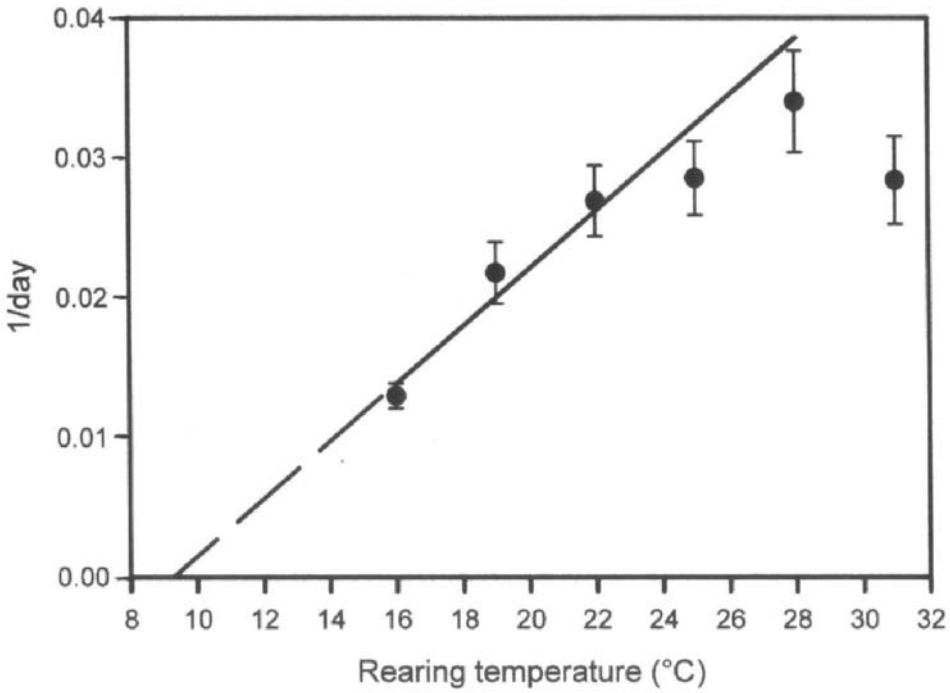

Fig. 1. Rate of development from egg to adult of *Bemisia tabaci* T biotype (1/day) in relation to the rearing temperature. The regression line is calculated by weighted linear regression, not considering the point at 31°C.

D. stramonium is significantly shorter than on either *Euphorbia* species ($P<0.001$ with $U=255.5$ and $U=746.5$ for *E. pulcherrima* and *E. characias*, respectively).

Sex ratio and parthenogenesis In the progeny of three couples reared on *E. characias,* 46, 31 and 30 females, and 31, 34 and 28 males were counted. The progeny of one couple reared on *D. stramonium* was represented by 29 females and 37 males. There was no significant difference among the experiments. The female-to-male global ratio was 1.0226: 0.9774, the value being not significantly different from a 1:1 ratio at the χ^2 test ($P=0.71$; $df=1$). Female-to-total (females + males) ratio, according to a more commonly used parameter, was therefore not significantly different from 0.5. When reared from a relatively large group of individuals (as in the experiment reported as C1 in Table 3), the female-to-total ratio was only 0.14 (95% confidence limits of the proportion are 0.12 and 0.24). Of 16 virgin females reared on *E. characias* plants, 207 males were obtained and no females.

Morphometry and morphology Mean length and width of pupae of the T biotype reared either on *D. stramonium* or *E. characias* are reported in Table 2A. The variances of the measurements did not differ significantly by Bartlett's test for the homogeneity of variances ($\chi^2=5.35$ and 3.84, respectively, both with 3 *df* and $P>0.14$) and there was no significant relationship between mean and variance, so that data did not need to be transformed for statistical analysis. As revealed by the analysis of variance, there was a significant

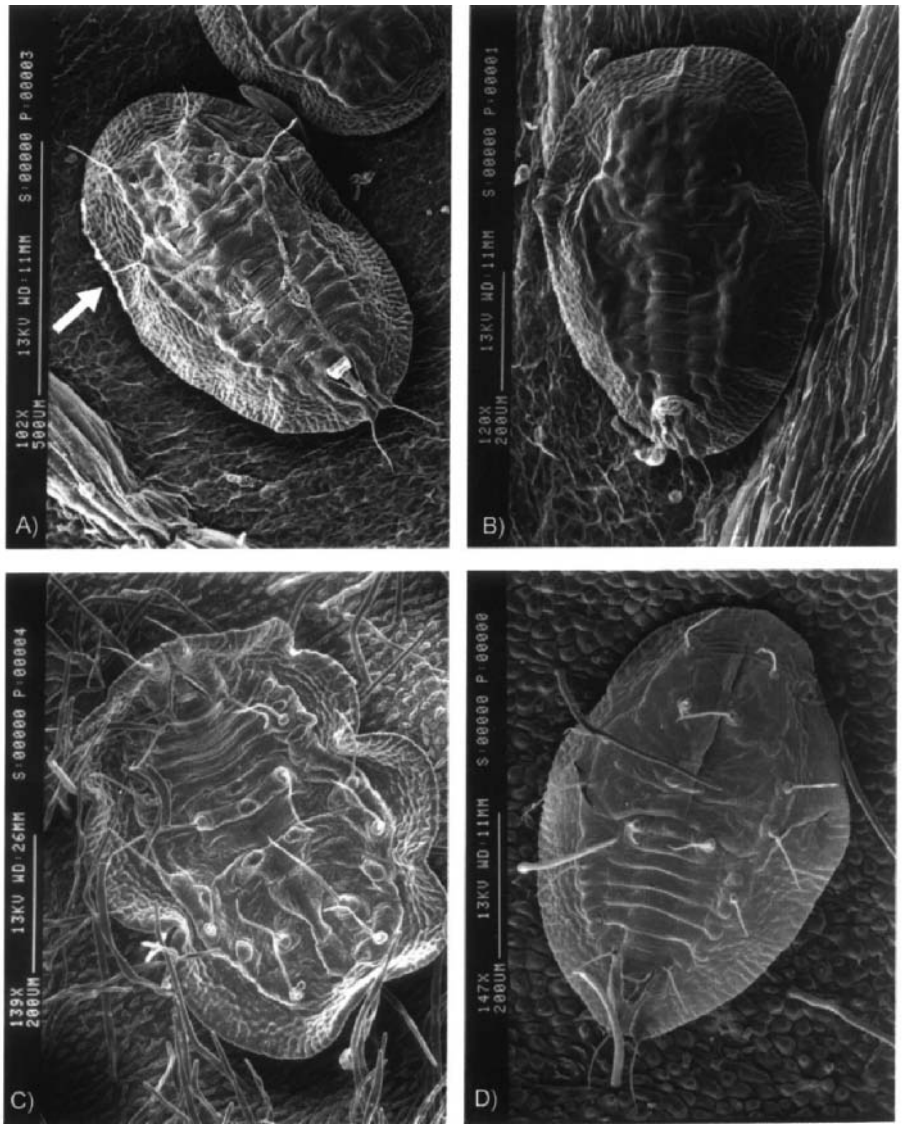

Fig. 2. SEM images of fourth-instar nymphs of *Bemisia tabaci* T biotype reared on *Datura stramonium* (A and B) or on *Euphorbia characias* (C and D). On *D. stramonium* the fourth-instar nymphs show an oval outline and seven pairs of dorsal setae located on cone-like processes. Variation in the number of setae was usual on fourth-instar nymphs (a variant with four pairs of setae in A; part of the waxy fringe, indicated by an arrow, is also visible). Setae are often lost and cone-like processes barely visible in most preparations from *D. stramonium* (B). The fourth-instar nymphs grown on *E. characias* (C and D) have seven pairs of easily distinguished dorsal setae, but their outline is deformed by the presence of hairs on the lower surface of the leaf.

difference in the overall dimension: (i) the pupae on *D. stramonium* were significantly larger (both longer and wider) than those reared on *E. characias* ($P<0.001$); and (ii) on both host plants, female pupae were significantly larger than male ones ($P<0.001$). The analysis

of variance of width revealed a significant interaction between plant host and insect sex, the width of females on *D. stramonium* being larger than that of those reared on *E. characias*. The differential influence of the host on the width of pupae was confirmed by analyzing the ratio of the two dimensions (ratio = length / width): there was no significant difference between ratios of males on the two hosts, but the ratio of females on *D. stramonium* was significantly greater than that of females reared on *E. characias* (t=2.91 on 40 *df*; P=0.003).

Measurements of the pupae of the Q biotype reared on *D. stramonium* are summarized in Table 2B. Females of the Q biotype were longer and wider than males (t=16.87 for length and t=14.39 for width, respectively, on 82 *df*; $P<0.001$ at the one-sided test for both). As seen in Table 2B and confirmed by analysis of variance, there were no significant differences between the measurements of the two biotypes reared on the common host *D. stramonium*, the only difference being linked to the sex of pupae.

The fourth-instar nymphs grown on *D. stramonium*, and their exuviae (Fig. 2A, B), had the typical aspect described in the literature (25), with an oval outline and seven pairs of dorsal setae located on cone-like processes. Variation in the number of setae was usual on fourth-instar nymphs (see a variant with four pairs of setae in Fig. 2A; part of the waxy fringe is also visible). Setae were often lost and cone-like processes barely visible in most preparations from *D. stramonium*. The fourth-instar nymphs grown on *E. characias* and their pupal cases (Fig. 2C, D) had seven pairs of dorsal setae, but their outline was deformed by the presence of hairs on the lower surface of the leaf. The nymphs were nevertheless vital and developed into adults.

Crossing experiments Attempts at courtship between T and Q biotypes were observed. Nevertheless, adults from different biotypes were never seen mating. Seven attempts at heterologous crossing were carried out as described in the Materials and Methods, together with two homologous crossings as controls. Details of the experiments and results are shown in Table 3. Only males were obtained from the heterologous crossing attempts, either way, whereas homologous breeding produced both males and females.

Vectoring ability Results of virus transmission tests are summarized in Table 4. The T biotype was able to transmit TYLCSV from both *D. stramonium* and tomato to homologous test plants. The transmission efficiency was comparable to that of the B biotype when transmitting from *D. stramonium* to *D. stramonium* (12 positive out of 25 tested and 7 out of 14, respectively), but was significantly lower when transmitting from tomato to tomato, independently of the number of vectors used per test plant (χ^2=9.44 and χ^2=11.39, both with 1 *df*, for transmission tests made with one and three insects per plant, respectively; $P<0.01$ in both cases). On tomato test plants, only ten insects of the T biotype out of 25 survived to the end of the inoculation access period (3 days), compared with 19 out of 25 of the B biotype. As shown in Table 4, transmission from tomato to *D. stramonium* plants by biotype T was very efficient, comparable to transmission of biotype B from tomato to tomato, whereas transmission with the T biotype from *D. stramonium* to tomato was unsuccessful, although the contemporary homologous transmissions succeeded.

DISCUSSION AND CONCLUSIONS

The biotype T *Bemisia tabaci*, originally isolated from *E. characias* in Sicily, and genetically similar to the Indian clade (38), is one of the nearly monophagous populations of the *B. tabaci* complex, like the biotypes E (*Asysthasia*), N (*Jatropha*), and the Cassava

biotype from the Ivory Coast (7). This notwithstanding, the T biotype could reproduce on *D. stramonium* under experimental conditions. Therefore, *D. stramonium* could serve as a common host plant, allowing comparisons and interbreeding experiments with the Q biotype.

When insect couples were reared separately on either *E. characias* or *D. stramonium* plants, the T biotype had a global sex ratio not significantly different from 1:1, that is, 0.5 if computed as ratio of females to total. The female-to-total ratio has been reported to vary from 0.51 to 0.61 for the A biotype (15), and 0.76 in *B. tabaci* B biotype obtained from a large number of adults (sex ratio of parents, 1:1) on poinsettia (16) at the same temperature. Under the same conditions, using relatively large numbers of adults with a sex ratio approx. 1:1, the T biotype had a female-to-total ratio of 0.14 on *D. stramonium,* indicating that different breeding conditions can significantly influence the global sex ratio. The parthenogenesis of the T biotype was arrhenotokous, as for most whiteflies (10), with unmated females producing male offspring only.

Development mean time of the *B. tabaci* T biotype on *E. characias* varied with temperature from 77.5 days to a minimum of 29.7 days, attained at 28°C. This is longer than reported by Wagner (40) for the B biotype on cotton (16.2 days) at about the same optimal temperature (27.6°C). The only higher temperature tested (31°C) did not shorten the egg-to-adult cycle. This phenomenon is well known in various biotypes of *B. tabaci* (5,15) and for *Bemisia argentifolii* (Bellows and Perring) on different host plants (30). A significant reduction of the mean development time on solanaceous hosts (30.1 days on *D. stramonium*, compared with 35. 4 and 35.6 days on *E. characias* and *E. pulcherrima,* respectively, at the same temperature) is known also for the B biotype, which, at 25°C, develops much faster on eggplant than on poinsettia (or cotton) (15), and it is also documented for the Q biotype on different solanaceous hosts, although to a lesser extent (28).

The slope of development rate from egg to adult of the T biotype on *E. characias* against temperature (0.002061 ± 0.000025) was sensibly lower than the value calculated for an Italian population of the B biotype reared on French beans (5), for the B- and Q-biotypes reared on sweet pepper (29), and for the poinsettia strain on *E. pulcherrima* (16), which is among the lowest reported for *B. tabaci* (15,30). This means that the development rate is relatively higher at a lower temperature, which is typical of cold-adapted populations (22). The theoretical LDT of 9.3°C for abolishing egg-to-adult development is also unusually low compared to the above-mentioned biotypes (13.9°C and 11.5°C, respectively, for the Italian B biotype (5) and the poinsettia strain (16)), but slightly higher than those reported by Muñiz and Nombela (29) for B- and Q-biotypes on sweet pepper, as calculated with the linear equation we also used. The LDT was quite close to the lower temperature threshold of the B biotype as calculated by the same authors (29) using a non-linear model. An attempt to use the same model on our data failed to converge, giving unreliable parameters, possibly because the temperature points we used were not well chosen or were insufficient for the four-parameter curve. The calculated SET was 485.1°D and this also is unusually high, when compared to data reported for development of *B. tabaci* on cotton (9,43), on poinsettia (16), and on French beans (5), for the B- and Q-biotypes on sweet pepper (29). These findings, together with the location of the population, indicate that the T biotype is very well cold-adapted, much more than the previously described Italian B biotype from Sardinia (5). The cold adaptation of the T biotype is confirmed by its distribution restricted

to relatively cold areas, where other *B. tabaci* biotypes are not present. Moreover, T biotype is able to overwinter under prolonged snow coverage, an unlikely performance for other biotypes. Recently, the T biotype has also been found in the Foresta Umbra, in the Gargano area of Apulia (approximate coordinates: 41° 41' N, 15° 58' E; 600–800 m a.s.l.), on *E. characias*, outside the northeastern boundary distribution of the other *B. tabaci* biotypes in outdoor conditions in Italy (5). Analysis of the mt CO *I* gene sequence confirms that this population belongs to T biotype (unpublished results).

Development time on a day-degree time scale calculated for each experimental temperature showed a non-linear trend (see Table 1), even excluding the obvious outlier at T=31°C, but the mean values calculated for each experimental temperature were reasonably close to the calculated SET (approximate 95% confidence limits for the mean values: 474–496). A non-linear trend for the SET calculated for each experimental temperature has already been noted by Hart *et al.* (20) in the case of the Syrphidae *Epysirphus balteatus*. Use of a quadratic model would also fit the °D value calculated for 31°C. It should be stressed therefore that the independence of °D from temperature is an approximate assumption, and should be used with caution. On the other hand, assuming such independency, that is, using the SET value (485°D) to predict the average time of development, would lead to an acceptable error, for environmental temperatures varying moderately, as it underestimates the effect of low and high temperatures, but it overestimates similarly the effect of the central temperatures, within the range of individual variations.

The absence of overwintering adults suggests that the T biotype cannot complete egg-to-adult development under natural conditions during winter. However, the different immature stages found at the different collection sites (from 600 to 1000 m a.s.l.) suggest that the T biotype could overwinter as egg and/or first instar. Field surveys and continuous breeding in the laboratory at 25°C exclude the presence of a dormant stage. In Israel (18) and southern California (12), all stages, including adult, were found during winter. The different overwintering mode of the T biotype seems related to the colder winter temperatures of the Peloritani-Nebrodi mountain area. Previous studies under controlled conditions showed that the adult is the most sensitive stage to cold in *B. tabaci* (5).

As expected (7,36), there were no morphological details to tell the T biotype from other *B. tabaci* biotypes. The dimensions (length and width) of pupae are also not different from those of the pupae of the Q biotype reared on the same host plant, whereas significant differences were detected within the B biotype when reared on either *D. stramonium* or *E. characias*. The host effect is also documented for two populations of *B. tabaci* from California (cotton and poinsettia) (4), although, in this case, the interaction between host plant and population is significant, meaning that both factors influence the length of fourth-instar nymphs. The *D. stramonium*-reared T biotype pupae are longer than reported for other populations (4,25), but the dimensions of pupae are strongly influenced by their density on the leaf surface (4), and we have not considered this factor. As reported for other populations (4,17), pupae of males and females were significantly different in both length and width within the T biotype (as well as within the Q biotype), although with some overlap. Therefore, extreme values of dimensions can be used to select males and females, in case of need, but dimensions *per se* did not allow total separation of sexes before emergence of adults in the T biotype, as is true for the cassava biotype (17). Furthermore, as we do not know the influence of genetics other than sex on the dimensions

of pupae, building populations from dimension-selected males and females can introduce uncontrolled selection pressure.

Attempts to cross the T biotype with the Q biotype in both combinations were unsuccessful, as only males were obtained from the heterologous crossing attempts, although in a few cases we observed courtship between members of heterologous couples. Also in inter-strain crosses between A and B biotypes, Perring *et al.* (34) observed courtship behavior, but none of the pairs mated. Even leaving out the controversial case of *B. argentifolii* (21), all kinds of reproductive interaction have been demonstrated in the *B. tabaci* species-complex, from total reciprocal mating incompatibility (27), to production of fertile hybrids, *e.g.* between *Jatropha* and Arizona biotypes (6), through some sort of mating isolation, with production of possibly sterile hybrids (13). Thus, unsuccessful mating between T- and Q-biotypes is not surprising. The limited number of experiments does not allow us to assume total mating incompatibility, but successful crossing must be rare or absent under experimental conditions, and is improbable under natural conditions because of the geographical and ecological isolation of the T biotype.

The last aspect of the biology of the T biotype we have considered is its ability to transmit TYLCSV, the main whitefly-borne begomovirus infecting tomato in Sicily, although not the only one (1). T biotype could transmit TYLCSV both from *D. stramonium* to *D. stramonium* and from tomato to tomato. Transmission efficiency was comparable to that of the B biotype when transmitting from *D. stramonium* to *D. stramonium*, but was significantly lower when transmitting from tomato to tomato. Whenever tomato was the test plant, transmission was very inefficient, regardless of the source plant. Differences in the efficiency of transmission of a plant virus by different *B. tabaci* biotypes are well known for geminiviruses (3) and criniviruses (42), and influences of the source and test plants on efficiency have also been reported (3,23). On the other hand, biotypes B and Q have comparable efficiencies in transmitting TYLCSV from tomato to tomato (14), and from tomato to *Solanum nigrum* and *D. stramonium*, from *D. stramonium* back to tomato (23). In our experiments, the low transmission efficiency of TYLCSV from tomato to tomato by the T biotype was linked to reduced survival on tomato plants. The S biotype is unable to survive on tomato long enough to acquire or transmit TYLCSV, although it could transmit the virus from *S. nigrum* to *S. nigrum* (23). Because of the failure to transmit TYLCSV from *Datura* (a possible intermediate host) to tomato, and because of the quasi-monophagy, the T biotype cannot be considered a threat to agriculture.

The presence of this biotype is probably not due to recent transfer associated with agricultural activity, because this biotype is restricted to a wild plant species growing in natural areas. We can speculate that the T biotype represents a residual population of the Indian clade, originally widespread in the Mediterranean area (the Indian region is considered the geographical origin of the species (7)), confined to *E. characias* by the spread of more fit Mediterranean biotypes.

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