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Relationships of the Sardinian isolate of tomato yellow leaf curl geminivirus with its whitefly vector *Bemisia tabaci* Gen.*

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Abstract

Relationships of an Italian isolate of tomato yellow leaf curl geminivirus from Sardinia (TYLCV-S) with its whitefly vector *Bemisia tabaci* were studied by means of experimental transmissions from tomato to tomato plants. TYLCV-S was confirmed to be transmitted in a persistent, circulative manner. The minimum latent period in the vector was between 17 and 20 h from the beginning of the acquisition access period (AAP). The maximum retention of infectivity was 8 days from the end of the AAP. Both acquisition and inoculation feeding times influenced the detected proportion of infective insects, with patterns well described by an exponential model. Acquisition was more efficient than inoculation. Males were significantly less efficient vectors than females. Nymphs were as efficient as adults in acquiring the virus. The length of AAP influenced both the retention of infectivity, and the pattern of transmission in serial transfer transmission tests with individual females. No significant difference in transmission efficiency was detected between two colonies of *B. tabaci*, one inducing typical silverleaf symptoms on squash, the other inducing only mild symptoms with more than 50 whiteflies per plant. The phenomenon of periodic acquisition was not unequivocally proved for TYLCV-S.

Introduction

Tomato yellow leaf curl geminivirus (TYLCV), causal agent of a severe disease of tomato, was identified in Israel [Cohen and Harpaz, 1964], first isolated in 1988 [Czosnek et al., 1988] and later identified in other Countries in the Mediterranean Region, in Western Africa and in South-Eastern Asia [Czosnek et al., 1990]. In Italy, the virus was first isolated in 1988 [Luisoni et al., 1989, Gallitelli et al., 1991] in Sardinia, and, almost contemporarily, identified in Sicily [Credi et al., 1989]. The Sardinian isolate was cloned and sequenced, and found to have 77% nucleotide identity with the TYLCV from Israel [Kheyr-Pour et al., 1991].

In its relationships with the whitefly vector, *Bemisia tabaci* Gen., TYLCV has shown some peculiarities, such as different vectoring efficiency of males and females [Cohen and Nitzani, 1966], discontinuity of transmission, and periodical rather than continual acquisition of the virus from infected plants by the vector [Cohen and Harpaz, 1964].

We report here some transmission properties of a Sardinian isolate of TYLCV (TYLCV-S) [Gallitelli et al., 1991], such as minimum latent period in the vector, efficiency of acquisition and

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inoculation, maximum retention of infectivity. We have also repeated with TYLCV-S the experiment of alternating acquisition and inoculation access periods, on which Cohen and Harpaz [1964] based their hypothesis of periodic acquisition of TYLCV by *B. tabaci*.

Materials and methods

Collection and maintenance of the virus and the whitefly vector

TYLCV was originally isolated from infected tomato plants (*Lycopersicon esculentum* Mill.) collected from glasshouses in Sardinia in the autumn 1988 and maintained in the glasshouse by transmission with *B. tabaci* to tomato cv Marmande. Experimentally infected plants served as virus source for transmission studies.

All the plants used were sown in steam-sterilised soil and grown in an insect-proof glasshouse. Tomato plants cv Marmande at the four-leaf stage were used as test plants throughout the study.

Two TYLCV-free colonies of *B. tabaci*, from Sardinia and Liguria, were established on cucumber (*Cucumis sativus* L.) cv Marketer, known to be immune to the virus [Crespi et al., 1991], and maintained in a growth chamber at 26 ± 1 °C, with a photoperiod of 16:8 (light:dark) h. The Ligurian colony induced typical silverleaf symptoms when feeding on zucchini squash (*Cucurbita pepo* L.) [Costa and Brown, 1991], while the Sardinian colony induced only mild silvering with over 50 individuals per plant under the same light conditions [Cohen et al., 1992].

Transmission tests

All transmission experiments were done using the Sardinian colony of *B. tabaci*, unless otherwise specified.

All acquisition and inoculation access feeding periods took place in a growth chamber at 26 ± 1 °C with a photoperiod of 16:8 (light:dark) h.

For acquisition access feedings, adult males and females from TYLCV-free colonies were used, except where specified. Insects were confined in cages large enough to contain one tomato plant showing clear symptoms of TYLCV, infected 4–5 weeks in advance.

For inoculation access feeding, adult whiteflies were placed on test plants in glass tubes (6 cm ø, 12 cm high), topped with a nylon screen, except where specified.

At the end of the inoculation access periods, the test plants were sprayed with acephate (Orthene) plus buprofezin (Applaud), then transferred to an insect-proof glasshouse and observed for symptoms. In a few dubious cases, the plants were tested for TYLCV presence by dot-blot analysis using a TYLCV-S-specific digoxygenin-labelled DNA probe [Crespi et al., 1991].

Effect of length of inoculation access period (IAP)

To determine the effect of IAP on transmission rate, whiteflies reared on TYLCV-infected plants were allowed access to healthy plants, in groups of 15, for 0.25, 0.5, 1, 2, 4, 8, and 48 h (1 insect per plant was used for the 48-h IAP). In three different experiments, totals of 9, 9, 21, 9, 12, 12 and 50 test plants were used for the times tested.

Effect of length of acquisition access period (AAP)

To determine the effect of AAP on transmission rate, whiteflies were caged on infected plants for 0.5, 1, 2, 4, 14, 17 and 24 h, and then transferred, in groups of six (up to 2 h of AAP) or three insects (from 4 h onward), to test plants for 48–71.5 h (AAP + IAP = 72 h). In 4 experiments, totals of 18, 18, 30, 30, 8, 12 and 22 test plants were used for the times tested.

Analysis of results

All the infectivity results obtained with more than one insect per plant were reduced to the probability of disease transmission by a single vector (p) using the maximum likelihood estimator of p, $\hat{p} = 1 - Q^{1/k}$, where Q is the observed fraction of non infected plants and k is the number of insects used per plant, assuming that the vectors act independently [Swallow, 1985].

To describe the effect of the inoculation access on the transmission by a single vector, a model was used which implies that the growth rate of infectivity (dp/dt) increases linearly with the proportion of insects which have not yet expressed infectivity, i.e. $dp/dt = h \cdot (A - p)$, where A is the maximum proportion of infective insects, attained

for $t \rightarrow \infty$, and h is a proportionality constant. The solution of the differential equation is

$$p(t) = A - B \cdot e^{-ht}$$

where B is the exponential of the integration constant.

A similar model was used to describe the effect of increasing acquisition access periods.

Parameters were estimated by nonlinear fitting, giving to each $\hat{p}$ a weight inversely proportional to its mean squared error (MSE), calculated according to Swallow [1985], using the software Genstat 5 rel. 2 (NAG Ltd., Oxford, UK). An approximate standard error (SE) of h was calculated from the SE of e^{-h} estimated by the non linear fitting.

Minimum latent period in the vector

To determine the minimum latent period in the vector, adult insects were caged on infected plants for 14-h AAP, then transferred, for inoculation access, in groups of five, to test plants every 3 h up to 26 h from the beginning of AAP. Some insects were allowed to feed on test plant for 58 h from the end of AAP (AAP + IAP = 72 h) to determine the maximum infectivity rate.

Acquisition by nymphs

Leaves of TYLCV-infected tomato plants, colonised by *B. tabaci* pupae were cut and placed in petri dishes; as soon as the adults emerged, they were transferred to healthy tomato plants in groups of five, for a IAP of 17 h.

Transmission efficiency of male and female B. tabaci

To compare the vectoring efficiency of male and female *B. tabaci*, insects were either reared on TYLCV-infected plants or given a 24-h AAP and then tested individually for 48 h. At the end of the IAP, the survival of insects was recorded. Only those insects surviving to the end of the IAP were considered.

Both the Sardinian and the Ligurian colonies were used to compare vectoring efficiency of males and females.

Proportions were compared by means of the chi square analysis [Fleiss, 1981].

Retention of infectivity of the vector

To determine the persistence of infectivity in the vector, two type of experiments were done. (1) About 100 male and female whiteflies were exposed to infected plants for 8, 14, 24 and 48 h in rearing cages, then the source plants were removed and two healthy tomato plants were exposed to the insects and changed every 24 h as long as there were live insects in the cage (16–17 days). (2) Female *B. tabaci* were given either a 6-h or a 24-h AAP on infected plants, then transferred individually at 24 h intervals to test plants to the end of their life.

Periodic acquisition

To test the phenomenon of periodic acquisition [Cohen and Harpaz, 1964] for the system TYLCV-S-Sardinian *B. tabaci*-tomato, female *B. tabaci* were given a 24-h AAP on infected plants, then transferred individually to test plants for a 24-h inoculation access; after this first IAP, the insects were randomly assigned to two groups, one transferred individually to new test plants every 24 h, the other alternating between 24-h AAP and 24-h IAP for their life.

Results

Effect of length of inoculation access period (IAP)

Figure 1 shows the estimated probabilities of whiteflies that transmitted TYLCV during IAPs of varying lengths. The minimum inoculation access time was between 0 and 15 min.

The effects of increasing IAPs are well described (94.9% of variance accounted for) by the fitted model

$$\hat{p}(t) = 0.2043 - 0.2027 \cdot e^{-0.0767 \cdot t}$$

Due to the SEs of the parameters (0.0255, 0.0251 and 0.0200 respectively) a second model was fitted with a constrained origin in (0,0). With this model, we obtained

$$\hat{p}(t) = 0.2019\ (1 - e^{-0.0827 \cdot t})$$

(95.7% variance accounted for; SEs of the parameters: 0.0223 and 0.0154 respectively). The

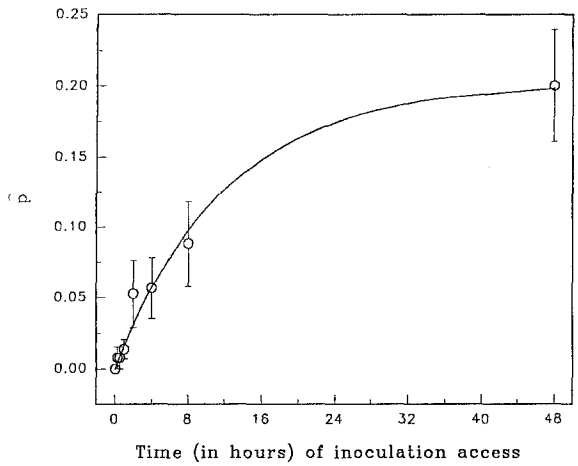

Fig. 1. Estimated probabilities ($\hat{p}$) of a single *B. tabaci* transmitting TYLCV during inoculation access periods (IAPs) of varying lengths. Plotted points are experimentally derived values for IAPs of 0.25, 0.5, 1, 2, 4, 8 and 48 h. The plotted curve is derived using an exponential model (see Materials and methods). Male and female whiteflies were reared on TYLCV-infected tomato plants and transferred to test plants in groups of 15 for all experimental IAPs except 48-h IAP, for which one insect per plant was used. Vertical bars represent rooted mean squared errors.

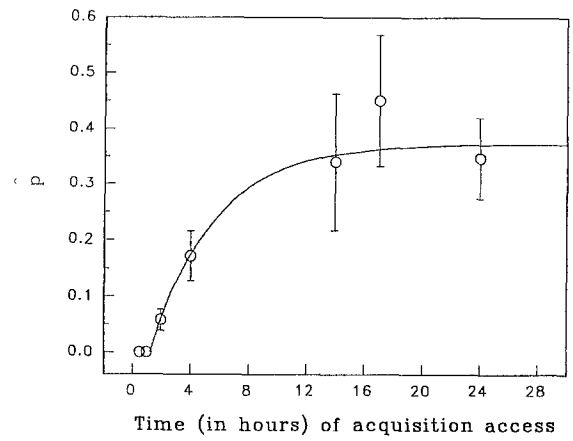

Fig. 2. Estimated probabilities ($\hat{p}$) of a single *B. tabaci* acquiring enough TYLCV to become infective, following acquisition access periods (AAPs) of various durations. Plotted points are experimentally derived values for AAPs of 0.5, 1, 2, 4, 14, 17 and 24 h. The plotted curve is derived using an exponential model (see Materials and methods). Male and female whiteflies were transferred to test plants in groups of six for 0.5, 1 and 2 h of AAP and in groups of three for the remaining AAPs. Vertical bars represent rooted mean squared errors.

fitted curve is represented by the continuous line in Fig. 1.

According to the model, the probability that a whitefly expresses infectivity within 24 h and 48 h of inoculation access is about 0.86 and 0.98 respectively.

Effect of length of acquisition access period (AAP)

Figure 2 shows the estimated probabilities of a single insect acquiring enough virus, within a given AAP, to become infective. The minimum access time for acquisition, experimentally determined, was between 1 and 2 h. Using the exponential model described in Materials and methods, we could fit the curve

$$\hat{p}(t) = 0.3706 - 0.4992 \cdot e^{-0.2337 \cdot t}$$

(SEs of the parameters are 0.0349, 0.0686 and 0.0743 respectively; 96% variance accounted for).

From this model, we estimated that in 24 h of AAP the probability that a whitefly acquires enough virus to become infective was 0.995.

Minimum latent period in the vector

None of the 100 insects tested was infective between 14 and 17 h from the beginning of AAP. Between 17 and 20 h, 20 and 23 h, 23 and 26 h, the estimated proportions, using 20, 20 and 14 test plants, were 0.01, 0.032 and 0.047 respectively. The maximum infectivity of the population used was 0.34, as determined by the longest IAP tested (58 h; 16 test plants).

Acquisition by nymphs

The newly emerged adults infected four plants out of eight ($\hat{p} = 0.1294$) with an IAP shorter than the minimum latent period.

Transmission efficiency of males and females

The vectoring efficiencies of male and female *B. tabaci* of the Sardinian colony, either reared on infected plants or allowed to feed on infected plants for 24 h, are summarised in Table 1a. Males were significantly less efficient vectors than females ($P < 0.05$). The Chi-square analysis showed a probable interaction ($0.05 < P < 0.1$) of sex and mode of acquisition to reduce vectoring efficiency of males.

Table 1. Vectoring efficiency of male and female *B. tabaci*, expressed as number of infective insects over number of tested insects. a) Insects from the Sardinian colony under different conditions of acquisition on TYLCV-infected plants and 48 h of inoculation access. b) Insects from the Ligurian and Sardinian colonies after 24-h acquisition and 48-h of inoculation access

	Females	Males
a)		
Reared on infected plants	35/68	18/43
24-h acquisition access	14/25	5/28
b)		
Sardinian colony	14/25	5/23
Ligurian colony	8/19	3/25

As shown in Table 1b, a difference in transmission rate between females and males after 24 h IAP was also present in the Ligurian colony, whereas there was no significant difference in vectoring efficiency between the two colonies.

Retention of infectivity

The infectivity of *B. tabaci*, tested daily on groups of about 100 individuals after AAPs of 8, 14, 24 and 48 h, lasted up to 160, 202, 192 and 196 h from the end of the AAP, respectively.

The infectivity expressed in serial passages of 24 h by individual female insects, following 6-h and 24-h AAPs, is shown in Fig. 3 together with the SE of the proportion of each passage.

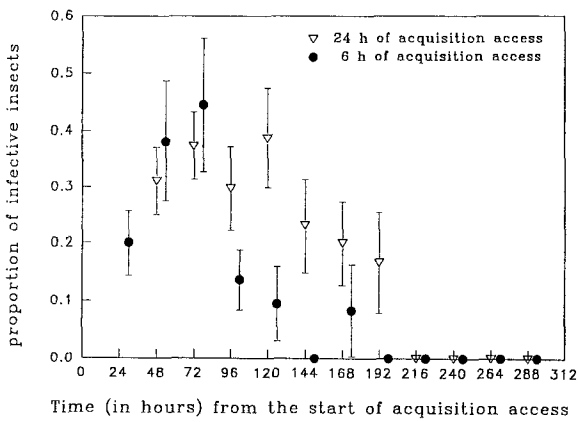

Fig. 3. Infectivity of female *B. tabaci* in serial transfer of 24 h of inoculation access. Insects were given either a 6-h (●) or a 24-h (▽) AAP on infected plants, then transferred individually at 24-h intervals to test plants to the end of their life. Vertical bars represent standard errors.

The overall rates of transmission (expressed as the number of insects which transmitted at least once over the number of insects tested at least once) were 0.4 (30/75) and 0.5125 (41/80) for insects with 6 h of AAP and 24 h of AAP respectively, in good agreement with the acquisition model.

The maximum retention of infectivity was between 150 and 174 h (7 days) for insects with 6-h AAP and between 168 and 192 h (8 days) for insects with 24-h AAP.

Periodic acquisition

The pattern of transmission of insects alternating between inoculation and acquisition accesses are shown in Fig. 4a, with the pattern of the control insects (not alternating) shown for comparison in Fig. 4b. This figure shows the presence of discontinuity in the transmission pattern of many whiteflies.

The proportions of infective insects of alternating group, and of the corresponding passages of the control group (odd passages), are shown in Fig. 5. Insects allowed multiple AAPs reached a higher rate of infectivity.

Discussion

TYLCV-S is circulative in the insect vector, using the criteria of the presence of a latent period and of the persistence of infectivity. This type of relationship is well known for TYLCV originally isolated in Israel (TYLCV-I hereafter) [Cohen and Haraz, 1964; Cohen and Nitzani, 1966], for the Jordanian isolate of TYLCV [Mansour and Al-Musa, 1992] and for other geminiviruses transmitted by *B. tabaci* [Duffus, 1987]. TYLCV-S had a minimum latent period slightly shorter (17 against 20 h), a much shorter retention of infectivity in the vector (8 days after the end of AAP against a maximum of 20 days), and a higher vectoring efficiency of male whiteflies compared to TYLCV-I [Cohen and Nitzani, 1966].

The retention of infectivity was shorter with a short acquisition access (8 h), but did not change when acquisition access was prolonged above 14 h. This could be due to the existence of a limit in the amount of virus that can be retained by the

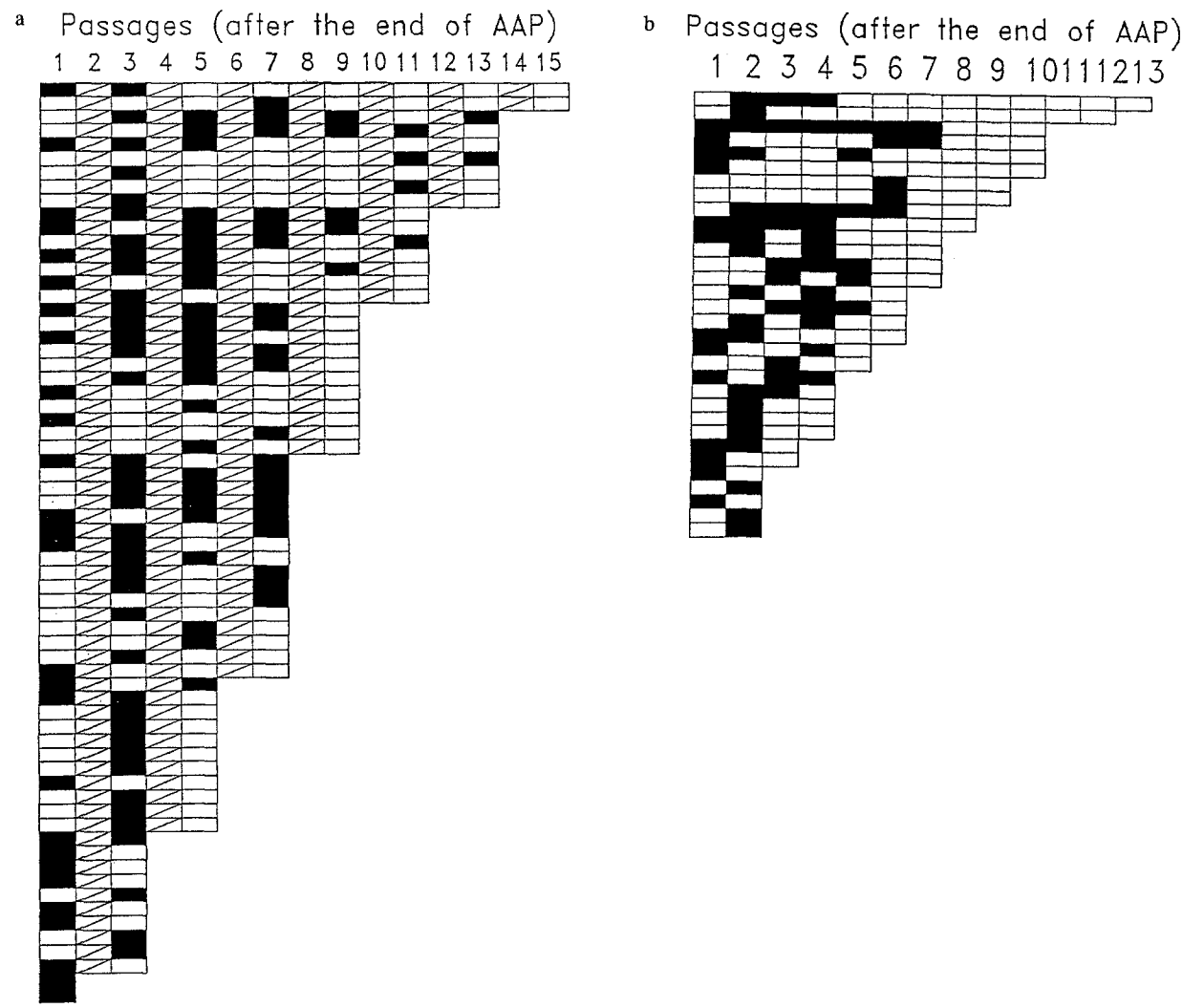

Fig. 4. Transmission patterns of individual female *B. tabaci*. Each line represent the life history of an insect after the acquisition access period (AAP). Filled rectangles indicate that the test plant has been infected. All insects were given a 24-h AAP to TYLCV-infected plants, then randomly assigned to two groups: one alternating between 24 h inoculation access (odd passages) and 24 h of access to TYLCV-infected plants (dashed rectangles) (Fig. 4a); the other serially transferred to test plants every 24 h (control) (Fig. 4b).

insects, as suggested by Zeidan and Czosnek [1991] for TYLCV-I.

The shorter persistence obtained in serial transfer transmission tests relatively to the comparable massive testing (24-h AAP) may have resulted either from behavioural differences of insects between the two tests (insects of the massive test could have disturbed each other and therefore fed for a globally shorter time, releasing less virus than insects tested individually) or from experimental conditions, which imposed a relatively small number of insects tested in the serial transfer tests.

The presence of a minimum time (1 h) for acquisition could be explained by the time needed by the insects to settle on the source plant, as suggested by Getz et al. [1982], since for acquisition tests insects were transferred in large cages to old plants. This delay should influence all the AAPs tested. The model we used to describe the influence of AAP on infectivity rate differs from that of Getz et al. [1982] because it takes into

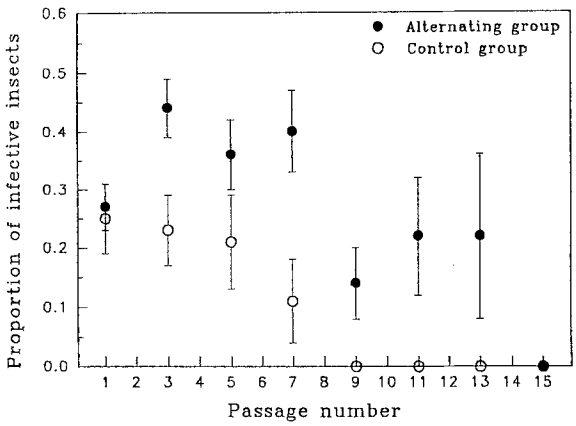

Fig. 5. Infectivity of female *B. tabaci* following a 24-h acquisition access period (AAP) on TYLCV-infected tomato plants. After the AAP, insects were randomly assigned to two groups: one alternating between 24 h inoculation access and 24 h of access to TYLCV-infected plants (●); the other serially transferred to test plants every 24 h (○). For the controls, only the results of the odd passages (for which a corresponding passage in the alternating group exists) are shown. Vertical bars represent standard errors of the proportions.

account this settling time (not constraining the origin in 0) and because it allows a maximum infectivity lower than 100%.

The same type of model was suitable to describe the influence of IAP on infectivity rate.

Comparing the *h* parameters (or shape parameters of the exponential) of the acquisition and inoculation models, we deduced that acquisition was a more efficient process than inoculation.

Female *B. tabaci* were more efficient vectors than males, with a possible influence of the acquisition mode on the proportion of infective males. The transmission efficiency of males was, nevertheless, much higher than that reported by Cohen and Nitzani [1966] for TYLCV-I.

Nymphs proved to be able to acquire TYLCV-S at a rate comparable to adults.

No significant difference in vectoring efficiency was found between the two colonies of whiteflies. Markham et al. [1992] detected difference in transmission of geminiviruses by whiteflies with different esterase patterns, pattern B being correlated with the ability to induce silver-leaf.

The transmission patterns of serial transfer tests showed marked differences for the two AAPs tested (6 and 24 h). Although the two groups of insects had parallel growth in transmission rates, the rate of loss of infectivity was much higher for the group with a short AAP.

There was also a high rate of discontinuity in the pattern of transmission, a phenomenon already evident in the pattern of transmission reported for TYLCV-I by Cohen and Harpaz [1964].

The results of serial transfer transmission tests suggest that there may be a limit to the rate at which the virus can be transferred to the salivary glands of the whitefly. The virus may accumulate in the body of the insect and then move into the salivary glands at a rate which is much slower than the accumulation rate, as suggested by Gildow and Rochow [1981] for luteoviruses and by Cohen et al. [1989] for squash leaf curl virus (SLCV) and melon leaf curl virus, two closely related geminiviruses.

The infectivity rate of the insects alternating between acquisition and inoculation showed a second peak of transmissions which rose from a minimum coincident with the loss of infectivity of the control group, as reported by Cohen and Harpaz [1964]. However, the analysis of the pattern of transmission by single insects indicates that there may be explanations to this result other than the periodic acquisition. For instance, for the insects which contributed to this second peak, a late single acquisition may be hypothesized, rather than a second acquisition after discharging. Should this be so, it would cast a shade of doubt on the periodic acquisition hypothesis, which, however, is still compatible with the overall data of the present study.

References

Cohen S, Duffus JE and Liu HY (1989) Acquisition, interference, and retention of cucurbit leaf curl viruses in whiteflies. Phytopathology 79: 109–113

Cohen S, Duffus JE and Liu HY (1992) A new *Bemisia tabaci* biotype in the Southern United States and its role in silverleaf of squash and transmission of lettuce infectious yellows virus. Phytopathology 82: 86–90

Cohen S and Harpaz I (1964) Periodic, rather than continual acquisition of a new tomato virus by its vector, the tobacco whitefly (*Bemisia tabaci* Gennadius). Entomologia Experimentalis et Applicata 7: 155–166

Cohen S and Nitzani FE (1966) Transmission and host range of the tomato yellow leaf curl virus. Phytopathology 56: 1127–1131

Costa HS and Brown JK (1991) Variation in biological char-

acteristics and esterase patterns among populations of *Bemisia tabaci*, and the association of one population with silverleaf symptom induction. Entomologia Experimentalis et Applicata 61: 211–219

Credi R, Betti L and Canova A (1989) Association of a geminivirus with a severe disease of tomato in Sicily. Phytopathologia Mediterranea 28: 223–226

Crespi S, Accotto GP, Caciagli P and Gronenborn B (1991) Use of digoxygenin-labelled probes for detection and host-range studies of tomato yellow leaf curl geminivirus. Research in Virology 142: 283–288

Czosnek H, Ber R, Antignus Y, Cohen S, Navot N and Zamir D (1988) Isolation of tomato yellow leaf curl virus, a geminivirus. Phytopathology 78: 508–512

Czosnek H, Navot N and Laterrot H (1990) Geographical distribution of tomato yellow leaf curl virus. A first survey using a specific DNA probe. Phytopathologia Mediterranea 29: 1–6

Duffus JE (1987) Whitefly transmission of plant viruses. In: Harris KF (ed) Current topics in vector research vol 4 (pp. 73–91) Springer-Verlag, New York

Fleiss JL (1981) Statistical methods for rates and proportions, second edition. John Wiley & Sons, New York. xviii + 321 pp

Gallitelli D, Luisoni E, Martelli GP, Caciagli P, Milne RG, Accotto GP e Antignus Y (1991) L'accartocciamento fogliare giallo del pomodoro in Sardegna. Informatore Fitopatologico (7–8): 42–46

Getz WM, Sylvester ES and Richardson J (1982) Pea enation mosaic virus transmission by the pea aphid: a multiphase model of virus transmission. Phytopathology 72: 1145–1148

Gildow FE and Rochow WF (1981) Selectivity in transmission of plant pathogens by vectors: a luteovirus-aphid model. In: McKelvey JJ, Eldridge BF and Maramorosh K (eds) Vectors of disease agents – Interactions with plants, animals, and man (pp. 61–71) Praeger Publishers, New York

Kheyr-Pour A, Bendahmane M, Matzeit V, Accotto GP, Crespi S and Gronenborn B (1991) Tomato yellow leaf curl virus from Sardinia is a whitefly-transmitted monopartite geminivirus. Nucleic Acid Research 19(24): 6763–6769

Luisoni E, Milne RG, Caciagli P, Accotto GP, Conti M, Gallitelli D, Martelli GP, Antignus Y, Cohen S, Marras F, Idini G, Loche P, Piras S and Leoni S (1989) A geminivirus associated with a severe leaf curl disease of tomato in Sardinia. 6th Conference of the Vegetable Virus Working Group of ISHS, Asilomar, USA, August 27–31 1989, pp. 13–14

Mansour A and Al-Musa A (1992) Tomato yellow leaf curl virus: host range and virus-vector relationships. Plant Pathology 41: 122–125

Markham PG, Briddon RW and Bedford ID (1992) Transmission of geminiviruses by different populations of *Bemisia tabaci*. 5th International Plant Virus Epidemiology Workshop: Viruses, Vectors and the Environment, Valenzano (Bari), Italy, 27–31 July 1992, pp. 121–122

Swallow WH (1985) Group testing for estimating infection rates and probabilities of disease transmission. Phytopathology 75: 882–889

Zeidan M and Czosnek H (1991) Acquisition of tomato yellow leaf curl virus by the whitefly *Bemisia tabaci*. Journal of General Virology 72: 2607–2614