European Journal of Plant Pathology (2006) 114:245–253
DOI 10.1007/s10658-005-5400-9

Characterisation of a quantitative resistance to vector transmission of *Tomato yellow leaf curl virus* in *Lycopersicon pimpinellifolium*

H. Delatte*, H. Holota, B. Reynaud and J. Dintinger
*UMR 53 PVBMT CIRAD-Université de la Réunion, Pôle de Protection des Plantes Ligne Paradis, 97410, Saint Pierre, La Réunion, France; *Author for correspondence (Phone: +262-262-499235; Fax: +262-262-499293; E-mail: delatte@cirad.fr)*

Accepted 10 November 2005

Key words: *Begomovirus*, *Bemisia tabaci*, disease incidence and severity, graft inoculation, resistance to transmission, whitefly-mediated inoculation, susceptibility

Abstract

Two wild genotypes from the same species *Lycopersicon pimpinellifolium*, WVA106 (susceptible) and INRA-Hirsute (so-called 'resistant'), were compared with respect to their reaction to *Tomato yellow leaf curl virus* isolate Réunion (TYLCV-Mld[RE]), using both whitefly-mediated inoculation and graft inoculation. Disease incidence and symptom severity were scored. Presence and quantification of viral DNA were assessed by dot blot hybridisation. Upon insect inoculation, accession INRA-Hirsute showed a moderate resistance against TYLCV that was overcome by a high inoculation pressure obtained by increasing the cumulative number of inoculative whiteflies. Temporal analyses of the disease progress in relation to this criterion exhibited that the protection was quantitative, mainly reducing the TYLCV-Mld[RE] incidence by at maximum 50% at low inoculation pressure. When graft inoculated, the final TYLCV-Mld[RE] disease incidence was 100% in both susceptible and resistant genotypes with severe symptoms, suggesting a reduction of virus transmission by a vector resistance as a possible mechanism. Implications of using such type of resistance in breeding programmes are discussed.

Introduction

In tropical and subtropical tomato production zones, *Bemisia tabaci* (Hemiptera: Aleyrodidae) is an important insect pest provoking direct feeding damages and, above all, considerable indirect damages as a vector of numerous geminiviruses (Jones, 2003). In recent years, the Old World monopartite begomovirus *Tomato yellow leaf curl virus* (TYLCV) (*Geminiviridae*) has spread worldwide (Nakhla et al., 1994; Pico et al., 1996; Momol et al., 1999; Navas-Castillo et al., 1999; Moriones and Navas-Castillo, 2000). It has also reached Réunion island (Peterschmitt et al., 1999) situated in the southwestern part of the Indian Ocean, where it has become the first limiting factor for production both in the fields and in greenhouses. Since few inoculative whiteflies may be enough for transmitting the virus to a large number of plants, chemical controls as well as IPM strategies employed for controlling the vector have not been successful in decreasing the incidence of TYLCV on cultivated tomatoes (Reynaud et al., 2003). A more effective solution for a sustainable control of TYLCV is to develop virus resistant or less susceptible varieties.

No resistance to TYLCV was found in cultivated *Lycopersicon esculentum* (Laterrot, 1989; Pico et al., 1999b; Pilowsky and Cohen, 2000) and, during the last decades, considerable efforts

have been made by breeding programmes to develop TYLCV-resistant cultivars by transferring resistance from wild types of *Lycopersicon* into cultivated tomatoes (for a review, see Lapidot and Friedmann, 2002). However, progress in breeding for TYLCV resistance has been slow because of the complex genetics of resistance, which probably explains why the cultivars and breeding lines are most often not as protected as wild species. Moreover, most cultivars have been developed focusing on resistance or a lesser susceptibility to TYLCV and to no other begomoviruses (Lapidot and Friedmann, 2002). Nevertheless, TYLCV-resistant cultivars showing delayed symptoms and reduced accumulation of the virus are being released to farmers. Host resistance to whitefly vectors is another important factor that could slow virus epidemics by reducing transmission of the virus. Resistance to the vector was assumed to be present in TYLCV-resistant *L. chilense* LA 1969, when tested against *Tomato yellow mosaic virus* (ToYMV), a bipartite virus (Piven et al., 1995). According to Pico et al. (2001), a 'resistance' to TYLCV reported for *L. hirsutum* LA 1777 was also due to resistance to the vector. Such type of insect resistance might also be present in *L. pimpinellifolium* INRA-Hirsute, which was found to be partially protected against TYLCV as well as to the bipartite *Potato yellow mosaic virus* (PYMV) upon vector inoculation, but susceptible to both viruses upon graft-inoculation (unpublished data). These results strongly suggested the presence of a non-virus specific resistance to the vector in INRA-Hirsute, and are in agreement with previous studies (Pico et al., 2001). However, very few studies on identifying resistance to *B. tabaci* have been done to date and there is no evidence that such a resistance to whitefly vectors can effectively reduce the spread of begomoviruses. As *L. pimpinellifolium* is a species easy to use for gene transfer due to a very close genetic relationship with cultivated *L. esculentum*, INRA-Hirsute is a useful source to introgress TYLCV protection into commercial tomato cultivars. The main objective of this study was to quantify the effect of the moderately protected INRA-Hirsute genotype, in comparison with a highly susceptible *L. pimpinellifolium* genotype on the TYLCV-Mld[RE] disease progress. Vector and graft inoculation were applied.

Materials and methods

Plant material

The less susceptible accession *L. pimpinellifolium* 'INRA-Hirsute' was compared to the highly susceptible one *L. pimpinellifolium* 'WVA106'. Seeds of both varieties were supplied by INRA-URPV of Guadeloupe and further multiplied in La Réunion. Seedlings were grown during the hot season (November 2003 to May 2004) in an insect-proof greenhouse under normal daylight conditions (~13 h photoperiod) with temperatures ranging from 24 to 32 °C.

Virus source

The virus isolate used in this study was the TYLCV-Mld[RE] originating from Réunion (Peterschmitt et al., 1999; Delatte et al., 2005a). This field isolate was maintained in susceptible *L. esculentum* cv. Farmer (Know You Seed). These infected tomato plants were used as the source of virus.

Virus transmission

Adults of *B. tabaci* used in whitefly-mediated inoculation were obtained from a population of Réunion that was started from nymphs collected on cabbage (*Brassica oleracea*) and subsequently reared on the same species under laboratory conditions: 12 h photoperiod (white and red fluorescent tubes), 25 ± 2 °C temperature, and 60% ± 10% relative humidity. This population was identified as belonging to the B biotype using cytochrome oxidase (COI) markers (Delatte et al., 2005c). *Bemisia tabaci* nymphs were transferred at the L4 stage from cabbage plants to TYLCV-infected tomato plants of susceptible cv. Farmer placed in $1.0 \times 1.0 \times 1.5$ m insect-proof cages, in a greenhouse. Whiteflies were kept feeding on infected plants until their use for transmission tests.

In graft inoculation, 30 day-old seedlings of each accession were side graft-inoculated with a stem from TYLCV-infected source plants of tomato cv. Farmer as a scion.

Experimental design

Three trials were conducted during different seasons in 2003 and 2004 for whitefly-mediated

inoculation. For each trial, a split plot design was laid out with three blocks. Three main plots per block corresponded to three inoculation access periods (IAP: 6, 24, and 96 h), and two subplots per main plot corresponded to the two tested accessions INRA-Hirsute and WVA106. Within each trial, the blocks corresponded to the insect-proof cages placed in an insect-proof greenhouse. Inside each cage, each subplot consisted of 20–30 two-leaf stage plantlets for each accession and a large amount of insects that previously had fed on infected tomato plants. The three IAPs were carried out in the same cage removing the plantlets from whitefly exposure after 6, 24 or 96 h. Before plant removal, the mean number of feeding insects was estimated for each IAP to determine the efficiency of inoculation (see *cniwh* later). The transmission efficiency (proportion of inoculative insects) was determined on a sample of 100 whiteflies collected from the insect-proof cages and representing the whole population used in the trial. Whiteflies from this sample were confined individually in small PVC tube cages containing a susceptible tomato seedling at the two-leaf stage, and were maintained in a greenhouse. After a 72 h IAP, plants were sprayed with an insecticide (Confidor®, Bayer, Basel, Switzerland), and placed in an insect-proof greenhouse to allow symptom development. The occurrence of leaf symptoms was recorded 21 days later and a TAS-ELISA (Adgen, UK) was performed to assess the virus presence.

A single trial was conducted for graft inoculation with two repetitions of 20–30 plants per accession placed in an insect-proof greenhouse.

Symptom assessment

After estimating the number of feeding insects per plantlet, they were removed from the cages, sprayed with an insecticide (Confidor®, Bayer, Basel, Switzerland), repotted, then placed in an insect-proof greenhouse. Plants were sprayed with the same insecticide once a week to avoid secondary infestations, and provided with fertilisation. Symptoms were recorded every 3.5 days on individual plants for up to 31.5 days post-inoculation (dpi) on the three last fully expanded leaves of each plant using a scale established for rating TYLCV symptom severity as follows: 1, no symptom, to 10, dead plant, with numbers 1–9 corresponding to the 0–4 scale of Lapidot and Friedmann (2002).

Quantification of viral DNA accumulation

Viral DNA accumulation was quantified in the third upper leaf of the fifteen first plants of trials by dot-blot hybridisation performed every 3.5 days during the first 14 dpi. For graft experiments, day 0 was defined to start 96 h after grafting, to be consistent with the IAP 96 h of the vector inoculation. A small amount of leaves (0.05 g) was used at each sampling and ground in 0.5 ml of 0.4 M NaOH, then 10 μl were dotted on nylon membranes (Hybond N+; Amersham Pharmacia, Freiburg, Germany) (Lapidot et al., 1997). Dots of 5, 10, 25 and 50 ng of full length TYLCV-Mld[RE] (in 0.4 M NaOH), respectively [amplified from the TYLCV-Mld[RE] clone (Delatte et al., 2005b), accession no. AJ865337] plus two negative controls (non-inoculated plants) and one positive plant were added on each membrane. The full length TYLCV-Mld[RE] was used as a probe and labelled with the ECL kit; probe labelling, hybridisation and detection were performed according to the manufacturer's instructions (Amersham Pharmacia, Freiburg, Germany). Pictures of the films obtained from the membranes were analysed with the software Koadarray 2.2 (Koada Technology, UK) in order to quantify viral DNA.

Variable description

The variables were calculated on a subplot basis, disease incidence (inc_u) was defined as the percentage of plants exhibiting symptoms at the uth dpi, and disease severity (sev_u) was defined as the mean disease score of the plants exhibiting symptoms at the uth dpi. To integrate these variables over time, we calculated the area under the disease progress curve (AUDPC) (Jeger and Viljanen-Robinson, 2001), called inc_a for the disease incidence, and sev_a for the disease severity. $\mathrm{inc}_a = \sum_{i=1}^{n-1}\{([\mathrm{inc}_i + \mathrm{inc}_{i+1}]/2)(t_{i+1} - t_i)\}/(t_n - t_1)$, in which inc_i was the percentage of plants exhibiting symptoms at the ith rating date and $\mathrm{sev}_a = \sum_{i=1}^{n-1}\{([\mathrm{sev}_i + \mathrm{sev}_{i+1}]/2)(t_{i+1} - t_i)\}(t_n - t_1)$, in which sev_i was the mean disease score of the plants exhibiting symptoms at the ith rating date;

Table 1. Estimation of the cumulative number of inoculative whiteflies × hours (*cniwh*) in *Tomato yellow leaf curl virus* (TYLCV) trials

	IAP (h)[a]			
	6	24	96	Trial mean
WVA106				
Trial 1	61 (27)[b]	225 (53)	1802 (524)	696 (317)
Trial 2	31 (6)	111 (19)	255 (29)	132 (34)
Trial 3	22 (12)	101 (35)	314 (132)	145 (59)
IAP mean	38 (11)	146 (28)	790 (297)	
Genotype mean				325 (116)
INRA-Hirsute				
Trial 1	53 (7)	312 (93)	1125 (323)	497 (188)
Trial 2	24 (6)	83 (24)	234 (15)	114 (32)
Trial 3	19 (5)	85 (11)	219 (82)	108 (38)
IAP mean	32 (6)	160 (47)	526 (178)	
Genotype mean				239 (72)
General mean				282 (68)

[a]IAP: Inoculation access period (total time in hours during which the seedlings were in the cages with viruliferous insects).
[b]Means over all cages (standard error).

t_i was the time post-inoculation at the ith observation; n was the number of dates at which disease was recorded.

For estimating the inoculation pressure, we used the cumulative number of inoculative whiteflies *cniwh*, according to the formula: $cniwh_{ijk} = r_i \sum_{n=1}^{k} \bar{x}_{ijn} \cdot t_n$ where $cniwh_{ijk}$ is the cumulative number of inoculative whiteflies × hours at the kth IAP (k = 1 for 6 h, 2 for 24 h, and 3 for 96 h) in the jth cage of the ith trial; $\bar{x}_{ijn}$, the mean number of whiteflies per plant (visually estimated) in the jth cage of the ith trial during t_n hours; t_n, the number of hours corresponding to 1st IAP (6 h, n = 1), the difference between 2nd and 1st IAP (18 h, n = 2), or the difference between 3rd and 2nd IAP (72 h, n = 3); and r_i, the proportion of inoculative whiteflies overall estimated on the ith trial (Dintinger et al., 2005).

Statistics

An analysis of variance between groups (ANOVA) was performed to determine the effects of trial, IAP, and genotype on inoculation pressure estimated by *cniwh*. ANOVA tests were performed for each trial individually and across trials to determine the effect of genotype on TYLCV incidence and severity, taking into account IAP and cage effects. The variables analysed were inc_a and sev_a for each IAP. Mean values of the two genotypes were separated by Student–Newman–Keuls (SNK) multiple-range test (α = 0.05). An ANOVA was also performed for the grafting trial. The effect of genotype on virus concentration also was determined at 3.5, 7, 10.5, and 14 dpi for vector-inoculation trials 2 and 3, as well as for graft-inoculation trial. All analyses were conducted using PROC GLM (SAS 8.01, SAS Institute, Inc., Cary, NC).

Results

Degree of infectibility

With the highly susceptible *L. pimpinellifolium* genotype WVA106, whitefly-mediated inoculations were successful with ~100% of the plants infected at 14 dpi with an inoculation access period (IAP) of 24 or 96 h, and ~90% with an IAP of 6 h. No plant escaped from infection when graft-inoculated, as shown by 100% of the plants infected on WVA106 at 14 dpi. ANOVA on *cniwh* showed a significant effect of trial ($P < 0.0001$), IAP ($P < 0.0001$), cage ($P = 0.0076$), and interaction trial × IAP ($P < 0.0001$). The proportion of whiteflies transmitting the virus (proportion of inoculative whiteflies estimated on a sample of 100 individuals from the insect population in each trial) ranged from 28% to 35%, depending on the trial. Nevertheless, the *cniwh* estimated values were

on average five times lower in trials 2 and 3 than in trial 1, due to a much larger number of whiteflies mass-released in this last one (Table 1).

Time course analyses

AUDPC values of incidence (inc_a) and severity (sev_a) were used to analyse progression of TYLCV-Mld[RE] disease in relation to time and to quantify disease protection of INRA-Hirsute after vector- and graft-inoculation (Figure 1). After vector-inoculation, the ANOVA indicated a significant effect of the plant genotype ($P < 0.0001$) and IAP ($P < 0.0001$) for incidence as well as severity over the three trials. Significant effects of the trial ($P = 0.007$ for inc_a, $P = 0.027$ for sev_a), cage ($P = 0.008$ for inc_a), and interaction between trial and genotype ($P = 0.016$ for inc_a) were also found. Mean values of virus incidence for INRA-Hirsute were found to increase significantly with IAP (Table 2, Figure 1a, c, and e), suggesting an inherited quantitative type of protection against to TYLCV-Mld[RE] in this genotype. The efficiency of this protection was strongly reduced by an intense inoculation pressure, corresponding to a 96 h IAP. Mean values of incidence for tomato INRA-Hirsute at IAP 96 h, were equivalent to the mean values for WVA106 at IAP 6 h (about 70%, Table 2). The virus susceptibility of both genotypes was confirmed by the graft-inoculation trial, resulting in 100% of infected plants in genotype INRA-Hirsute not differing from the susceptible WVA106 (Figure 1g). Mean values of severity were found to be high for both genotypes too, irrespective of the inoculation pressure (Figure 1b, d, and f), although slight but significant differences were observed (Table 2). However, the reduced susceptibility of INRA-Hirsute was shown to result in a reduced TYLCV disease incidence after whitefly inoculation, whereas no significant difference was found after graft-inoculation (Figure 1h).

Quantification of viral DNA accumulation

The average TYLCV DNA concentration in grafted plants was almost 10 times higher in that of vector-inoculated plants with a 96 h IAP at 3.5 dpi (Figure 2). Subsequently, this difference decreased with time and reversed, indicating that accumulation of virus was delayed when plants were vector-inoculated. TYLCV DNA accumulation detected at 3.5, 7, 10.5, and 14 dpi in INRA-Hirsute and WVA106 was not significantly different between both techniques of inoculation.

Discussion

Since all cultivars of tomato *Lycopersicon esculentum* are susceptible to TYLCV, breeding programmes have been based on the introgression of resistance genes from wild *Lycopersicon* accessions. One of the difficulties in selecting a source for resistance to TYLCV in wild plants is given by the fact that in the natural situation, the disease might occur with varying degrees of incidence as well as of severity. Variable rating of disease expression is often caused by difficulties in visual scoring, but also by a method of TYLCV detection, which does not always provide accurate quantification of viral DNA (Pico et al., 1998, 1999a). Discrepancies observed between studies also may be due to the varying host responses against the different TYLCV isolates used in the experiments, and differences in the method of inoculation (Pico et al., 2001). Thus, combined and standardized methods of TYLCV inoculations are necessary to screen wild accessions for TYLCV resistance. Furthermore, multiple criteria such as visual scoring (incidence and severity) and reliable viral DNA quantification are necessary to evaluate a resistance.

The so-called TYLCV resistance in *L. pimpinellifolium* INRA-Hirsute was described as quantitatively inherited with some dominance (Kasrawi and Mansour, 1994), involving a QTL on chromosome 6 with major effect, and with probably other chromosomal regions that may play a role (Chagué et al., 1997). Some studies also reported the accession *L. pimpinellifolium* INRA-Hirsute to be 'partially resistant' to both the vector and the virus (Zakay et al., 1991; Kasrawi and Mansour 1994; Pico et al., 2001). Nevertheless, no comparison between inoculation with and without vector in the same experiment was carried out to support this hypothesis. In our study, we tried to characterize this reduced susceptibility to TYLCV-Mld[RE] by graft- and vector-inoculation in an attempt to dissociate the two types of reaction. We also quantified more accurately the response of INRA-Hirsute to TYLCV-Mld[RE] inoculation by assessing the effect of the inoculum pressure on

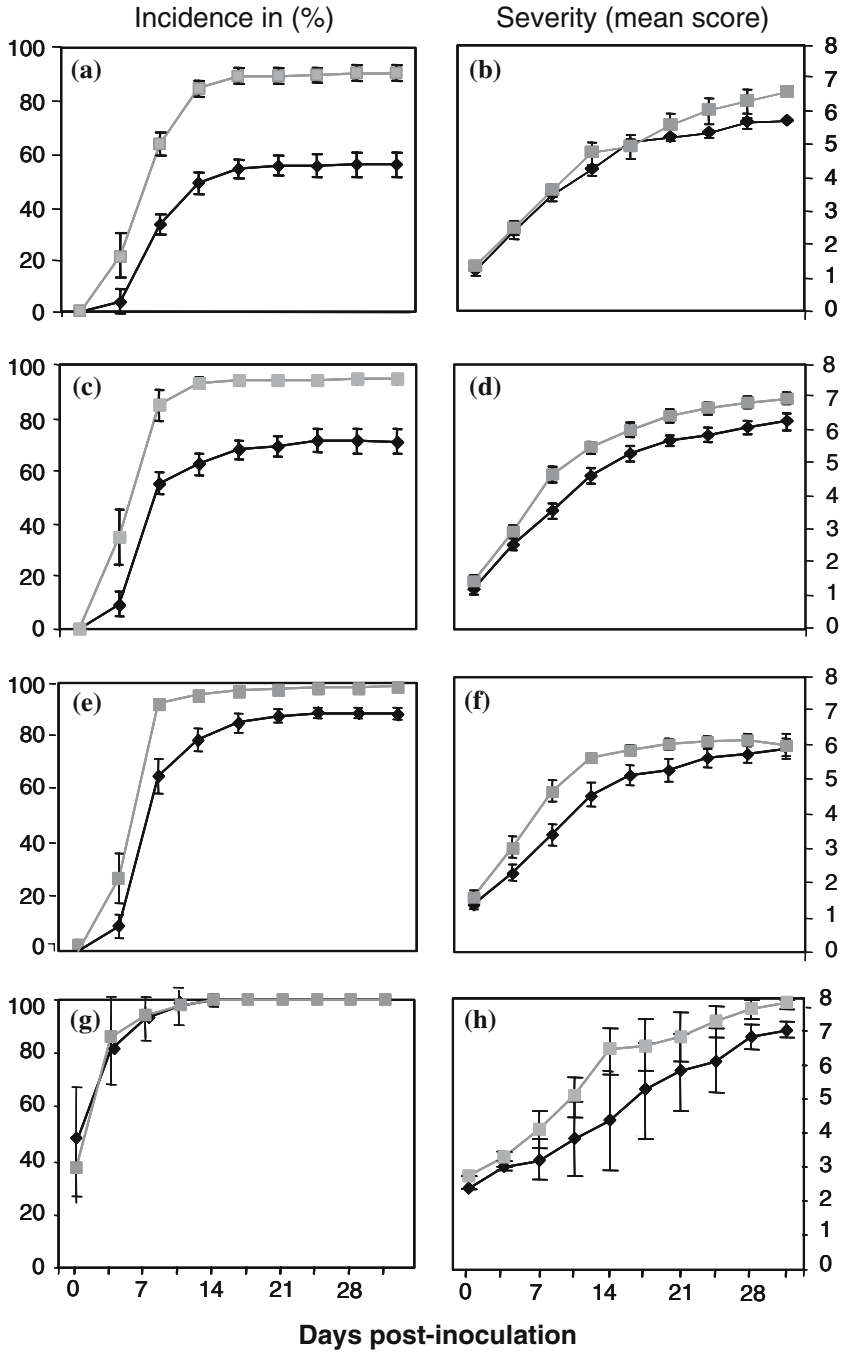

Figure 1. Mean values of disease incidence and severity following vector inoculation (a to f) and graft inoculation (g and h) of *Tomato yellow leaf curl virus* (TYLCV) vs. days post-inoculation (dpi), including trials 1 to 3. a–e and b–f correspond to IAPs of 6, 24 or 96 h, respectively. Wild accessions of *L. pimpinellifolium* INRA-Hirsute (♦) and WVA106 (■) were tested. Vertical bars represent the SE.

disease incidence and severity, and virus content, with increasing numbers of viruliferous whiteflies. When compared to the susceptible accession WVA106, INRA-Hirsute showed a slight protection against TYLCV-Mld[RE] infection events that may be overcome just by an intense

Table 2. Means of area under the disease progress curve (AUDPC) values per genotype and per inoculation access period (IAP) for the three trials after vector inoculation

IAP	Genotype	Incidence	Severity
6 h	Hirsute INRA	0.44[d]	3.63[c]
	WVA 106	0.68[b]	4.07[b]
24 h	Hirsute INRA	0.58[c]	3.99[b]
	WVA 106	0.82[a]	4.68[a]
96 h	Hirsute INRA	0.71[b]	3.93[b]
	WVA 106	0.84[a]	4.47[a]

Values followed by the same letter (a to d) are not significantly different at $P = 0.05$ according to SNK test.

inoculation pressure corresponding to more than 20 inoculative whiteflies feeding on a single plant during 24 h. In contrast, the symptom severity seemed not to be reduced in INRA-Hirsute, as shown by disease progress curves, which are more or less similar for the two genotypes. These results are consistent with those obtained on plants graft-inoculated with TYLCV-Mld[RE], in which final disease incidence reached 100% accompanied by severe symptoms, irrespective of the genotype.

No significant difference was observed between both studied accessions when viral DNA was quantified by dot blot hybridization. However compared to vector inoculation, a very high transient level of virus DNA concentration was observed shortly after grafting at the beginning of the infection (3.5 dpi). This confirmed that graft inoculation delivered a larger amount of virus than vector inoculation. Furthermore, it indicates that the quantitative TYLCV protection of INRA-Hirsute has no or minor influence on virus accumulation in the plant was and is inefficient when a massive quantity of virus is inoculated into the phloem. This suggests the presence of resistance mechanisms to the insect vector in INRA-Hirsute, which reduce transmission and initial entry of the virus into plant cells. In a previous study with comparative tests of susceptibility for TYLCV-Mld[RE] and *Potato yellow mosaic virus* isolate Guadeloupe (PYMV-GA) of wild *Lycopersicon* species, INRA-Hirsute was found to be partially protected against both viruses when inoculated by viruliferous whiteflies, while it consistently developed symptoms when graft-inoculated (unpublished data). This strongly supports the hypothesis of a non-virus specific resistance to the vector reducing final disease incidence in the genotype of *L. pimpinellifolium* INRA-Hirsute. No difference in whitefly preference between WVA106 and INRA-Hirsute was found, as no significantly different numbers of individuals were counted on WVA106 and INRA-Hirsute (no significant effect of genotype on *cniwh* throughout the three experiments). Consequently, this reduced TYLCV susceptibility might result from a modified feeding behaviour of the vector on host plants, especially with respect to phloem acceptance.

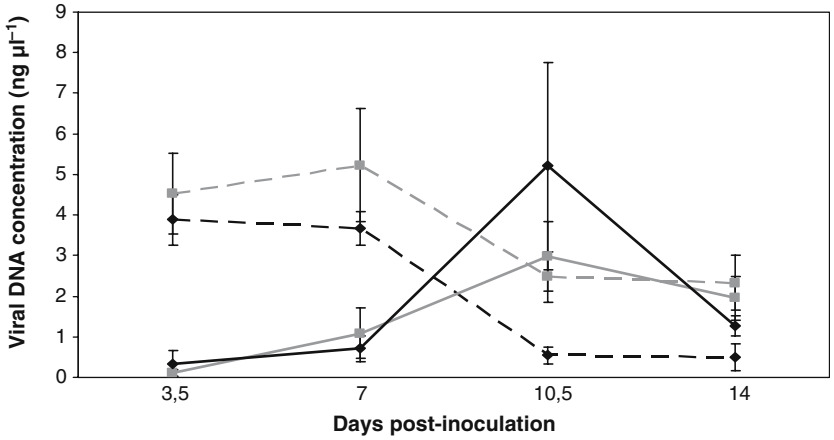

Figure 2. Tomato yellow leaf curl virus DNA concentration determined by dot blot hybridisation in relation to days post-inoculation. Grey lines represent virus concentrations in *L. pimpinellifolium* WVA106 (■) as determined by dot blot hybridization and image analysis. Dark lines show virus DNA concentrations of *L. pimpinellifolium* INRA-Hirsute (♦). Dotted lines represent average concentrations obtained after graft inoculation, and solid lines the average concentrations after massive whitefly inoculation (on plants after the inoculation access period of 96 h). Vertical bars represent the SE.

In their study, Kasrawi et al. (1988) reported INRA-Hirsute as partially resistant to the virus with only 50% of grafted plants presenting moderate symptoms. However, in our study we found 100% of infected plants with severe symptoms upon grafting. These discrepancies might be due to the environmental conditions and grafting procedures. Our results also differ slightly from the findings of Pico et al. (2001) who claimed the presence of resistance (i.e. reduced sensitivity) (Hull, 2002) to the virus itself in INRA-Hirsute. These authors reported 100% of those plants tested positive after stem-agroinoculation, similarly to what was found in our study after graft-inoculation. In contrast, in the same plants, average symptom rating at 30 dpi scored by these authors was twice as low than that scored under our conditions, and reduced TYLCV DNA accumulation was reported. However, the symptoms of the wild accessions tested were compared to *Lycopersicon esculentum*. Since symptoms in wild species are generally much weaker than in cultivated tomatoes, comparison of symptoms and susceptibility of *L. pimpinellifolium* INRA-Hirsute to those of WVA106 was a better estimator to evaluate the real value of INRA-Hirsute for breeding as a possible source of protection.

In our study, the viral DNA accumulation at 7 days post graft-inoculation was very high, showing the virulence of the virus isolate used. Lapidot et al. (1997) found a positive correlation between a 'resistance level', as evidenced by relative yield loss, and viral DNA accumulation for all 'highly resistant' accessions tested. In our experiments, TYLCV-Mld[RE] DNA accumulation in INRA-Hirsute and WVA106 was not significantly different from each other, irrespective of the inoculation techniques and the time after inoculation. This confirmed that INRA-Hirsute has probably no resistance to virus multiplication, and/or spread.

Nevertheless, in breeding programmes for protection against begomoviruses, host genotypes that can be infected but express reduced disease incidence may be discarded without full consideration for their epidemiological effects at the population level in the field. In this study, quantification of susceptibility for TYLCV-Mld[RE] showed that accession INRA-Hirsute, which possesses a moderate level of resistance, was effective in reducing the apparent infection rate of TYLCV epidemics. This type of host reaction that reduces the rate of epidemics has been reported for other viral pathosystems (Padgett et al., 1990; Buiel and Parlevliet 1995; Dintinger et al., 2005) and may be preferable over tolerant, but fully infectable plants as long as no resistant plants are available.

As a whole, the quantitative resistance found in INRA-Hirsute might affect the virus transmission efficiency via the vector, and therefore, could constitute a first barrier against the disease. Nevertheless further experiments should be done, such as using electrical penetration graph (EPG) technique to record the insect feeding behaviour and better characterize this resistance (Tjallingii 1988; Jiang et al., 1999, 2001). It should also be tested against a wider panel of begomoviruses. If confirmed as virus non-specific and affecting the vector, this quantitative insect resistance might be very useful in reducing the infection rate of begomovirus epidemics in tomato. In combination with resistances to TYLCV already used by breeders, and in conjunction with other control strategies such as insect-proof nets or UV-absorbing plastic sheets, a quantitative resistance to *B. tabaci* could contribute to a sustainable control of begomovirus disease.

Acknowledgements

We would like to thank F. Chiroleu for help in statistical analyses, I. Soustrade and N. Cassam for their advice, M. Grondin, E. Chapier, and F. Naze for their technical assistance. This work was funded by the 'Conseil Régional de la Réunion' and CIRAD. H. Delatte is a recipient of a sandwich PhD fellowship from Wageningen University, the Netherlands.

References

Buiel AAM and Parlevliet JE (1995) Epidemiology of peanut bud necrosis disease in groundnut in India. Recent studies on peanut bud necrosis disease: Proceedings of a meeting, ICRISAT, Asia Center, India.

Chagué V, Mercier JC, Guenard M, de Courcel A and Vedel F (1997) Identification of RAPD markers linked to a locus involved in quantitative resistance to TYLCV in tomato by Bulked Segregant Analysis. Theoretical and Applied Genetics 95: 671–677.

Delatte H, Holota H, Naze F, Peterschmitt M, Reynaud B and Lett JM (2005a) The presence of both recombinant and non

recombinant strains of *Tomato yellow leaf curl virus* on tomato in Réunion Island. Plant Pathology 54: 262.

Delatte H, Martin DP, Naze F, Golbach RW, Reynaud B, Peterschmitt M and Lett JM (2005b) South West Indian Ocean islands tomato begomovirus populations represent a new major monopartite begomovirus group. Journal of General Virology 86: 1533–1542.

Delatte H, Reynaud B, Granier M, Thornary L, Lett JM, Goldbach R and Peterschmitt M (2005c) A new silverleaf-inducing biotype Ms of *Bemisia tabaci* (Hemiptera: Aleyrodidae) indigenous of the islands of the south-west Indian Ocean. Bulletin of Entomological Research 95: 29–35.

Dintinger J, Boissot N, Chiroleu F, Hamon S and Reynaud B (2005) Evaluation of maize inbreds for maize stripe virus and maize mosaic virus resistance: Disease progress in relation to time and cumulative number of planthoppers. Phytopathology 95: 600–607.

Hull R (2002) Matthews' Plant Virology, Academic Press, London.

Jeger M and Viljanen-Robinson S (2001) The use of area under the disease progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. Theoretical and Applied Genetics 102: 32–40.

Jiang YX, Lei H, Collar JL, Martin B, Muniz M and Fereres A (1999) Probing and feeding behavior of two distinct biotypes of *Bemisia tabaci* (Homoptera: Aleyrodidae) on tomato plants. Entomologia Experimentalis et Applicata 92: 357–366.

Jiang YX, Nombela G and Muniz M (2001) Analysis by DC-EPG of the resistance to *Bemisia tabaci* on an Mi-tomato line. Entomologia Experimentalis et Applicata 99: 295–302.

Jones D (2003) Plant viruses transmitted by whiteflies. European Journal of Plant Pathology 109: 195–219.

Kasrawi MA and Mansour A (1994) Genetics of resistance to tomato yellow leaf curl virus in tomato. Journal of Horticultural Science 69: 1095–1100.

Kasrawi MA, Suwwan MA and Mansour A (1988) Sources of resistance to tomato-yellow-leaf-curl-virus (TYLCV) in Lycopersicon species. Euphytica 37: 61–64.

Lapidot M, Friedman M, Lachman O, Yeheszkel A, Nahon S, Cohen S and Pilowsky M (1997) Comparison of resistance level to tomato yellow leaf curl virus among commercial cultivars and breeding lines. Plant Disease 81: 1425–1428.

Lapidot M and Friedmann M (2002) Breeding for resistance to whitefly-transmitted geminiviruses. Annals of Applied Biology 140: 109–127.

Laterrot H (1989) La tomate: Intérêt et utilisation des espèces sauvages pour la création variétale. P.H.M. – Revue horticole 295: 3–7.

Momol MT, Simone GW, Dankers W, Sprenkel K, Olson M, Momol EA, Polson JE and Hiebert E (1999) First report of tomato yellow leaf curl virus in tomato in South Georgia. Plant Disease 83: 487.

Moriones E and Navas-Castillo J (2000) Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. Virus Research 71: 123–134.

Nakhla MK, Maxwell DP, Martinez RT, Carvalho MG and Gilbertson RL (1994) Widespread occurrence of the eastern Mediterranean strain of tomato yellow leaf curl geminivirus in tomatoes in the Dominican Republic. Plant Disease 78: 926.

Navas-Castillo J, Sanchez-Campos S, Diaz JA, Saez-Alonso E and Moriones E (1999) Tomato yellow leaf curl virus – is causes a novel disease of common bean and severe epidemics in tomato in Spain. Plant Disease 83: 29–32.

Padgett GB, Nutter FW, Kuhn CW and All JN (1990) Quantification of disease resistance that reduces the rate of Tobacco etch virus epidemics in bell pepper. Phytopathology 80: 451–455.

Peterschmitt M, Granier M, Mekdoud R, Dalmon A, Gambin O, Vayssieres JF and Reynaud B (1999) First report of tomato yellow leaf curl virus in Réunion Island. Plant Disease 83: 303.

Pico B, Diez M-J and Nuez F (1996) Viral diseases causing the greatest economic losses to the tomato crop. II. The tomato yellow leaf curl virus – a review. Scientia Horticulturae 67: 151–196.

Pico B, Diez M and Nuez F (1998) Evaluation of whitefly-mediated inoculation techniques to screen *Lycopersicon esculentum* and wild relatives for resistance to tomato yellow leaf curl virus. Euphytica 101: 259–271.

Pico B, Diez MJ and Nuez F (1999a) Improved diagnostic techniques for tomato yellow leaf curl virus in tomato breeding programs. Plant Disease 83: 1006–1012.

Pico B, Ferriol M, Diez M and Nuez F (1999b) Developing tomato breeding lines resistant to tomato yellow leaf curl virus. Plant Breeding 118: 537–542.

Pico B, Ferriol M, Diez MJ and Vinals FN (2001) Agroinoculation methods to screen wild *Lycopersicon* for resistance to *Tomato yellow leaf curl virus*. Journal of Plant Pathology 83: 215–220.

Pilowsky M and Cohen S (2000) Screening additional wild tomatoes for resistance to the whitefly-borne tomato yellow leaf curl virus. Acta Physiologicae Plantarum 22: 351–353.

Piven NM, de Uzcategui RC and Infante HD (1995) Resistance to tomato yellow mosaic virus in species of *Lycopersicon*. Plant Disease 79: 590–594.

Reynaud B, Wuster G, Delatte H, Soustrade I, Lett JM, Gambin O and Peterschmitt M (2003) Les maladies à bégomovirus chez la tomate dans les départements français d'Outre-Mer. Phytoma – La Défense des Végétaux 562: 13–17.

Tjallingii WF (1988) Electrical recording of stylet penetration activities. In: Minks AK and Harrewijn P (eds.) Aphids: their Biology, Natural Enemies and Control. Vol. 2B (pp. 95–108). Elsevier, Amsterdam.

Zakay Y, Navot N, Zeidan M, Kedar N, Rabinowitch HD, Czosnek H and Zamir D (1991) Screening of *Lycopersicon* accessions for resistance to tomato yellow leaf curl virus: Presence of viral DNA and symptom development. Plant Disease 75: 279–281.