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Viral infection of tobacco plants improves performance of *Bemisia tabaci* but more so for an invasive than for an indigenous biotype of the whitefly*

Jian LIU[1], Meng LI[1], Jun-min LI[1], Chang-jun HUANG[2], Xue-ping ZHOU[2], Fang-cheng XU[3], Shu-sheng LIU[†‡1]

(*[1]Ministry of Agriculture Key Laboratory of Molecular Biology of Crop Pathogens and Insects, Institute of Insect Sciences, Zhejiang University, Hangzhou 310029, China*)

(*[2]Institute of Biotechnology, Zhejiang University, Hangzhou 310029, China*)

(*[3]Wenzhou Academy of Agricultural Sciences, Wenzhou 325006, China*)

[†]E-mail: shshliu@zju.edu.cn

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Abstract: The ecological effects of plant-virus-vector interactions on invasion of alien plant viral vectors have been rarely investigated. We examined the transmission of *Tomato yellow leaf curl China virus* (TYLCCNV) by the invasive Q biotype and the indigenous ZHJ2 biotype of the whitefly *Bemisia tabaci*, a plant viral vector, as well as the influence of TYLCCNV-infection of plants on the performance of the two whitefly biotypes. Both whitefly biotypes were able to acquire viruses from infected plants and retained them in their bodies, but were unable to transmit them to either tobacco or tomato plants. However, when the Q biotype fed on tobacco plants infected with TYLCCNV, its fecundity and longevity were increased by 7- and 1-fold, respectively, compared to those of the Q biotype fed on uninfected tobacco plants. When the ZHJ2 biotype fed on virus-infected plants, its fecundity and longevity were increased by only 2- and 0.5-fold, respectively. These data show that the Q biotype acquired higher beneficial effects from TYLCCNV-infection of tobacco plants than the ZHJ2 biotype. Thus, the Q biotype whitefly may have advantages in its invasion and displacement of the indigenous ZHJ2 biotype.

Key words: *Bemisia tabaci*, Biotypes, Begomovirus, Vector-virus interaction, Biological invasion
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1 Introduction

Insects and the plant viruses they vector have complex interactions that can be direct, plant-mediated, or both, and the ecological effects of the interactions are highly variable depending on the species (Colvin *et al*., 2006; Belliure *et al*., 2005; Stout *et al*., 2006; Hogenhout *et al*., 2008). For example, viral infection of a host plant has been shown to exert positive, neutral, or negative effects on the performance of the vector (Belliure *et al*., 2005; Stout *et al*., 2006; Jiu *et al*., 2007). Both correlative and manipulative studies in the field have shown that plant-virus-vector interactions are among the primary determinants for the population dynamics of the vector herbivores and the epidemiology of plant virus diseases (Stout *et al*., 2006). Accordingly, these interactions can be expected to affect the invasion process of alien plant virus vectors when the invasive and indigenous species show niche overlap but differ in the interactions (Jiu *et al*., 2007). However, experimental studies on plant-virus-vector interactions in the context of biological invasions have rarely been conducted (Colvin *et al*., 2006; Jiu *et al*., 2007).

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is an insect species complex

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‡ Corresponding author
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including many morphologically indistinguishable but genetically diverse groups (Boykin *et al*., 2007), some of which have been named as "biotypes" (Perring, 2001). Two major recent events associated with *B. tabaci* have been the widespread invasion of the B biotype of the whitefly [also known as the silverleaf whitefly *Bemisia argentifolii* (Perring *et al*., 1993)] from its presumed origin in the Mediterranean-Asia Minor region to much of the rest of the world in the past 20–25 years (Brown *et al*., 1995; Boykin *et al*., 2007; Liu S.S. *et al*., 2007), and the rapid, widespread invasion of the Q biotype from its presumed origin in the Mediterranean region to other parts of the world from 2003 to 2008 (Horowitz *et al*., 2003; Chu *et al*., 2006; Ueda and Brown, 2006; Martinez-Carrillo and Brown, 2007; Mckenzie *et al*., 2009). In many regions of the world, epidemics of plant diseases caused by begomoviruses transmitted exclusively by *B. tabaci* have been occurring soon after the invasion of the B and Q biotypes of the whitefly (Varma and Malathi, 2003; Seal *et al*., 2006; Hogenhout *et al*., 2008).

In China, at least six genetic groups of *B. tabaci* have been recorded in the last 10 years, including the introduced B and Q biotypes and the indigenous ZHJ1, ZHJ2, ZHJ3, and Cv genotypes/biotypes (Luo *et al*., 2002; Qiu *et al*., 2003; Wu *et al*., 2003; Zang *et al*., 2006; Xu, 2009). Crossing experiments conducted between B and Q, B and ZHJ1, B and ZHJ2, and ZHJ1 and ZHJ2 demonstrated that these genotypes/biotypes are incompatible in reproduction (Zang and Liu, 2007; Luan, 2008; Xu, 2009). Circumstantial evidence and field sampling data indicated that the B biotype entered China in the mid-late 1990s, spread quickly in this country, and by 2005–2006 had become the predominant or the only biotype of *B. tabaci* in many regions of China (Luo *et al*., 2002; Qiu *et al*., 2003; Li and Hu, 2005; Chu *et al*., 2006). The Q biotype was reported for the first time from China in Yunnan Province in 2003 (Chu *et al*., 2005; 2006), and since then it has been reported to occur in high numbers in many areas of the country (Chu *et al*., 2006; 2007; Xu *et al*., 2006; Xu, 2009).

Regular field surveys have shown that the B and Q biotypes have been displacing indigenous biotypes of the whitefly in the process of invasion (Liu S.S. *et al*., 2007; Xu, 2009). During the same period from the late 1990s to present, diseases caused by begomoviruses have been increasing rapidly and epidemics have been frequently recorded from tobacco, tomato, pumpkin, papaya, and other crops (Zhou *et al*., 2003b; Li *et al*., 2004; Liu Y. *et al*., 2007). One of the major causative agents of virus diseases in tobacco in southwest China is *Tomato yellow leaf curl China virus* (TYLCCNV) (Cui *et al*., 2004; Li *et al*., 2004; Liu Y. *et al*., 2007).

In a recent field sampling, we found that some TYLCCNV-infected tobacco plants were infested by both the B and Q biotypes of *B. tabaci*. In a previous study, we showed that both the B biotype and the indigenous ZHJ1 biotype of the whitefly were efficient vectors of TYLCCNV on tobacco, but TYLCCNV-infection of tobacco plants offered significant benefits only to the B biotype (Jiu *et al*., 2006; 2007). In this study, we conducted laboratory experiments to examine the acquisition and transmission of TYLCCNV by the invasive Q biotype and the indigenous ZHJ2 biotype, and to compare the effects of TYLCCNV-infection of tobacco plants on the performance of these two biotypes of the whitefly. Our objectives were to determine whether the two biotypes would interact differently in association with this virus-plant system. We found that the Q biotype was unable to transmit TYLCCNV to tobacco, and the ZHJ2 biotype was also a very poor vector of this virus; however, both biotypes of the whitefly were able to improve their performance on virus-infected tobacco plants but the benefits acquired by the Q biotype were much greater than those by the ZHJ2 biotype. These results were discussed in the context of the complexity of plant-virus-vector interactions and the possible channels from which the invasive Q biotype is able to take advantages in these interactions for its invasion and displacement of indigenous competitors even when it is not a vector of the begomovirus in question.

2 Materials and methods

2.1 Whiteflies

Two biotypes of the whitefly species complex were used. The Q biotype (mtCO1 sequence GenBank accession No. DQ473394) was first collected from capsicum *Capsicum annuum* Linn. in Hangzhou [30°18′17″ N, 120°17′46″ E, 10 m above sea level (asl)], China, in October 2006. The ZHJ2 biotype

(mtCO1 sequence GenBank accession No. AJ867557) was first collected from sweet potato *Dioscorea esculenta* in Jiande (29°19′35″ N, 119°19′41″ E, 99 m asl), China, in September 2004. These two biotypes belong to *B. tabaci* (Mediterranean) and *B. tabaci* (Asia II) genetic groups, respectively (Boykin *et al.*, 2007). Cultures of the two biotypes were maintained on cotton *Gossypium hirsutum* L. (cv. Zhemian 1793) plants in whitefly-proof cages in separate climate chambers at (26±1) °C, a photoperiod of 14 h light: 10 h darkness, and (65±10)% relative humidity. Every 3–5 generations, random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique (De Barro and Driver, 1997; Zang *et al.*, 2006) was used to monitor the purity of the cultures, and only pure cultures of each of the two biotypes were used for experiments.

2.2 Virus and agroinoculation of plants

Clones of TYLCCNV with its satellite DNA molecules, also known as DNAβ, constructed previously (Cui *et al.*, 2004) were used as inocula. The *Agrobacterium tumefaciens* culture was grown in YEP medium containing kanamycin (50 mg/L) and rifampicin (50 mg/L) at 28 °C, and centrifuged at 200 r/min for 24 h. Then the culture was centrifuged and re-suspended in the mixture containing 10 mmol/L $MgCl_2$, 10 mmol/L MES, and 200 mmol/L acetosyringone, adjusted to the optical density at 600 nm (OD_{600}) of 0.8 to 1.0, and incubated at room temperature for 3 h. For agroinoculation, 0.2 ml of bacterial culture per plant was used for injection, by a 1-ml fine needle, into stem or petioles of tobacco *Nicotiana tabacum* L. cv. NC89 (Zhou *et al.*, 2003a; Cui *et al.*, 2004). The procedure to inoculate the plants with both TYLCCNV and DNAβ was based on previous observations that DNAβ is required for symptom induction of TYLCCNV in several plants including tobacco and tomato (Cui *et al.*, 2004).

2.3 Plants

Tobacco *N. tabacum* L. cv. NC89, tomato *Solanum lycopersicum* Mill. cv. Hezuo903, and cotton (cv. Zhemian 1793) were used. Both tobacco and tomato are host plants of TYLCCNV, while cotton is a non-host of the virus. We cultivated uninfected tobacco, tomato, and cotton plants in a potting mix in 11-cm plastic pots in greenhouse under natural lighting and ambient temperature. To obtain virus-infected tobacco, the plants at 3–4 true-leaf stage were inoculated with TYLCCNV and its DNAβ by agroinoculation as previously described (Zhou *et al.*, 2003a; Cui *et al.*, 2004). Uninfected and TYLCCNV-infected tobacco plants were cultivated to the 7–8 true-leaf stage when used in the experiments. Virus infection of test plants was judged by the typical symptoms caused by the virus and further confirmed by PCR using the procedure as described below. Cotton plants were also cultivated to the 7–8 true-leaf stage for experiments. All plants were watered every 3–4 d as necessary.

All experiments were conducted in large climatic rooms at (26±1) °C, (65±10)% relative humidity, and a photoperiod of 14 h light:10 h darkness. Rearing of whiteflies on plants was conducted in insect-proof cages or leaf clip cages where necessary. The ventilated clip cage was made using the material and method as described in Zang *et al.* (2005). Adult whiteflies were collected and transferred by a mouth aspirator.

2.4 Detection of TYLCCNV DNA

Nucleic acids from individual whiteflies and plants were extracted using the methods of Luo *et al.* (2002) and Xie *et al.* (2002), respectively. Detection of TYLCCNV and its associated DNAβ in individual whiteflies and plants was conducted using the primers and PCR procedures as described by Qian and Zhou (2005).

2.5 Acquisition of TYLCCNV by Q and ZHJ2 biotypes

Approximately 300 newly emerged (0–6 h post emergence) adults of the Q biotype or the ZHJ2 biotype were allowed to feed on TYLCCNV-infected tobacco plants with typical symptoms. Ten adults were randomly collected from the leaves of virus-infected plants at each of the 12 designated acquisition access periods (Table 1) and stored at −20 °C. Collections were assayed individually for detection of TYLCCNV DNA by PCR.

2.6 Retention of TYLCCNV by Q and ZHJ2 biotypes

Approximately 300 viruliferous Q biotype or ZHJ2 biotype whitefly adults were obtained by caging

newly emerged adults on TYLCCNV-infected tobacco plants for a 48-h acquisition feeding period. Then all the adults of a given biotype were transferred onto cotton plants, a non-host plant of TYLCCNV. Following the initial transfer, 10 live adults were collected randomly from the cotton plants at each of the 10 pre-designated dates, i.e., 0, 0.5, 1, 2, 3, 4, 5, 10, 20, 30 and 40 d, respectively, until all the adults had died, and during this period 10 dead individuals were also collected following their natural death. During the experiments, the adults remaining on the plants were transferred onto new cotton plants every 14 d to avoid new adults in the offspring emerging on the same plants. The insects collected were kept at −20 °C and subsequently assayed individually for detection of TYLCCNV DNA by PCR.

2.7 Transmission of TYLCCNV by Q and ZHJ2 biotypes

Viruliferous adults of the Q or ZHJ2 biotype whitefly were obtained by caging newly emerged adults on TYLCCNV-infected tobacco plants for a 48-h acquisition feeding period. The viruliferous adults were then collected and inoculated onto the top 2nd leaf of an uninfected tomato or tobacco plant (at the 3 true-leaf stage) in groups of 1, 5 or 10 for a 48-h period of inoculation feeding. The plants were then sprayed with acetamiprid [20% (w/v) soluble powder (SP), Kesheng Biochemicals Ltd., Nanjing, China] at a concentration of 20 mg/L to kill all the whiteflies and then kept in insect-proof cages for symptom development. Twenty plants were used for each of the six treatments of three levels of adult density for each of the two whitefly biotypes (Table 2). Virus infection of the plants was determined by both observation of TYLCCNV symptoms and detection of TYLCCNV DNA by PCR.

2.8 Fecundity and longevity of non-viruliferous adult whiteflies on virus-infected or uninfected plants

To compare the fecundity and longevity, initially non-viruliferous Q biotype and ZHJ2 biotype whiteflies completed development of immature stages on cotton, a non-host plant of the virus, and then were transferred to TYLCCNV-infected or uninfected tobacco plants. The experiments included two whitefly biotypes and two types of plants, making up four treatments (Table 3). In each of the four treatments, there were 33–39 replicates. In each of the replicates, one newly emerged female and one male adult whiteflies from the culture on cotton plants were placed on the lower surface of a plant leaf (the 4th to 6th from the top) enclosed in a clip cage. Every 7 d, the number of eggs laid by the female adult on the leaves was counted under a stereoscopic microscope, and the adults were transferred to new leaves, until deaths of the females. The survival of the female in each replicate was recorded daily to calculate longevity.

To analyze the performance of the two biotypes of whiteflies on the two types of tobacco plants, a two-factor analysis of variance (ANOVA) was performed. The factors included whitefly biotype (two levels) and plant status (two levels). The two response variables of fecundity and longevity were analyzed individually (Table 3). When an overall ANOVA indicated significant effects of the factors or their interactions at $P<0.05$, the means were compared by Fisher protected least significance difference (LSD) test. All statistical analyses in this paper were performed using the statistical software, STATISTICA version 6 (StatSoft Inc., 2003).

2.9 Life-history parameters of whiteflies on virus-infected and uninfected tobacco plants

Similar to above experiments, four treatments, composed of two whitefly biotypes and two types of plants, were conducted, with 10 replicates in each treatment. In each replicate of a treatment, approximately 20 adult whiteflies (10 females and 10 males, aged 7 d post emergence) were collected from the culture on cotton and transferred to a leaf (enclosed in a clip cage) of a tobacco plant. They were allowed to oviposit on the lower surface of the leaves for 24 h and then removed. From the 16th day onwards, newly emerged adults were collected and recorded twice daily (at 10:00 and 15:00) until all pupae had developed into adults or died. When all adults in each of the replicates had emerged, the leaf was examined under a stereomicroscope to count the numbers of dead eggs, nymphs, and pupae. From these recordings the percentage survival and development time from egg to adulthood were calculated.

Upon emergence of the adults, pairs of newly emerged adults in each treatment, one male and one female in each pair, were collected and transferred to

clip cages attached to the leaves (the 3rd to 6th leaves from the top) of the same type of plant. For the 2 Q biotype treatments, 20 replicates each were examined, while for the 2 ZHJ2 biotype treatments a lower number of replicates were examined due to low survival rates on tobacco (Table 4). Every 7 d, the number of eggs on the leaves was counted under a stereomicroscope, and the adults were transferred to new leaves, until the females died. The survival of the female in each replicate was recorded daily to calculate longevity.

For analysis of percentage survival, fecundity, and longevity for the two whitefly biotypes on the two types of plants, a two-factor ANOVA was conducted as described above. The data of percentage survival in each of the replicates in the four treatments were transformed by arcsine square root before ANOVA. The ANOVA on development time was based on mean values calculated from each individual that developed into adulthood in each of the four treatments.

2.10 Population increase of Q biotype whitefly on virus-infected and uninfected tobacco plants

Population increases of the Q biotype on virus-infected and uninfected tobacco plants, with 10 replicates each, were determined. For each replicate, 5 newly emerged female adult whiteflies and 5 male adult whiteflies were collected from the culture on cotton and released to a virus-infected or uninfected tobacco plant in a ventilated cage (55 cm×55 cm×55 cm). On the 28th day after inoculation, 5 replicates of each treatment were sampled to count the numbers of pupae and adults. The remaining 5 replicates in each treatment were sampled on the 56th day.

For each of the two sampling dates, mean numbers of whiteflies on the two types of plants were analyzed using one-way ANOVA. Ln(number+1) was preformed to transform the numbers of pupae and adults on each plant used for ANOVA.

2.11 Effect of virus acquisition on the fecundity of Q and ZHJ2 biotypes

To test whether TYLCCNV would directly influence the fecundity of Q and ZHJ2 biotypes, the number of eggs laid on the first 10 d was counted. Viruliferous Q and ZHJ2 biotype whiteflies were acquired by caging newly emerged adults that had completed development of immature stages on cotton and fed on TYLCCNV-infected tobacco plants for 48 h. Non-viruliferous Q and ZHJ2 biotype whiteflies were collected by caging newly emerged adults on healthy tobacco plants and fed for 48 h. In each replicate, a female adult and a male adult were then transferred onto a cotton plant and caged on a leaf in a clip-cage to feed for 10 d, and the number of eggs laid during this period (including those that had hatched) was counted under a stereoscopic microscope at the end of the 10 d.

For analysis of the numbers of eggs laid by non-viruliferous and viruliferous adults of each of the two whitefly biotypes on cotton plants, we conducted a two-factor ANOVA as described above. The factors included whitefly biotype (two levels) and whitefly status of virus-infection (two levels).

3 Results

3.1 Acquisition of TYLCCNV by Q and ZHJ2 biotypes

The whiteflies collected after various access periods were assessed for the presence of viral DNA by PCR (Table 1). The percentage of Q biotype adults with detectable TYLCCNV DNA increased with the

Table 1 Length of access period on TYLCCNV-infected tobacco plants by two biotypes of *Bemisia tabaci* adults and the percentages of adults with TYLCCNV DNA as detected by PCR

Duration of feeding (h)	Adults with TYLCCNV DNA (%)[a]	
	Q biotype	ZHJ2 biotype
0	0	0
0.25	0	0
0.5	0	0
1	10	0
1.5	10	0
2	20	30
3	30	40
4	10	40
6	40	40
8	45	60
10	90	60
12	80	40
24	100	70
48	100	100
72	100	100

[a] Ten adults of each biotype were examined at each duration of feeding

length of access period and reached 100% after a 24-h access period and thereafter consistently remained 100% (Table 1). For the ZHJ2 biotype, the percentage of adults with detectable TYLCCNV DNA also increased with the length of access period and reached 100% after a 48-h access period and thereafter consistently remained 100% (Table 1).

3.2 Retention of TYLCCNV in Q and ZHJ2 biotypes

For both Q and ZHJ2 biotypes, all adults after each of the durations of feeding on cotton plants (from 0 to 40 d for Q; from 0 to 30 d for ZHJ2) were found to be associated with TYLCCNV. The adults after their natural death were also detected to have the viral DNA. Thus, once the adults had acquired TYLCCNV, the viral DNA was always detected in all of them no matter how long the adults had fed on cotton plants.

3.3 Transmission of TYLCCNV by Q and ZHJ2 biotypes

None of the tomato or tobacco plants showed TYLCCNV symptoms 30 d post inoculation by the viruliferous Q biotype adults. For the 60 tomato plants and 60 tobacco plants inoculated by the ZHJ2 biotype, only 1 tobacco plant inoculated with 5 adults showed TYLCCNV symptoms (Table 2). These observations on virus infection of the plants were confirmed by PCR for TYLCCNV DNA.

Table 2 Transmission of TYLCCNV by the Q and ZHJ2 biotypes of *Bemisia tabaci* after a 48-h acquisition access period on TYLCCNV-infected tobacco plants and then a 48-h inoculation access period on healthy tomato or tobacco plants as a function of the number of vectors per plant

Biotype	Number of insects per plant	Transmission of TYLCCNV expressed as percentage of plants that became infected[a]	
		Tomato	Tobacco
Q	1	0	0
	5	0	0
	10	0	0
ZHJ2	1	0	0
	5	0	5
	10	0	0

[a] Twenty plants were tested in each of 12 treatments

3.4 Fecundity and longevity of non-viruliferous adult whiteflies on virus-infected or uninfected plants

Both biotype and plant status had a significant effect on fecundity and longevity of the whitefly, while biotype×status interaction had a significant effect on longevity, but not on fecundity (Table 3). When the Q biotype adults, which had developed on cotton from egg into adulthood, were transferred to TYLCCNV-infected tobacco plants, they had a higher level of fecundity and lived longer than they fed on uninfected plants. The fecundity and longevity of the ZHJ2 biotype on TYLCCNV-infected plants were also increased but the increases were not significant. On either uninfected or virus-infected plants, the Q biotype had much higher fecundity and longer longevity than the ZHJ2 biotype (Table 3).

Table 3 Fecundity and longevity of two biotypes of non-viruliferous *Bemisia tabaci* that developed on cotton from egg to adulthood and were transferred upon emergence onto uninfected or virus-infected tobacco plants

Biotype	Status of host plants	n	Mean (±SE) number of eggs per female[a]	Mean (±SE) longevity (d)[b]
Q	Uninfected	38	83.9±15.3 b	16.6±2.0 b
	TYLCCNV-infected	33	144.8±19.6 a	31.0±2.6 a
ZHJ2	Uninfected	39	10.3±3.0 c	4.9±1.0 c
	TYLCCNV-infected	38	23.3±5.4 c	9.2±2.0 c

[a] Two-factor ANOVA: $F_{biotype}$=64.0, df=1, 144, P<0.001; F_{status}=9.2, df=1, 144, P<0.05; $F_{biotype×status}$=3.9, df=1, 144, P>0.05; [b] Two-factor ANOVA: $F_{biotype}$=74.2, df=1, 144, P<0.001; F_{status}=23.1, df=1, 144, P<0.001; $F_{biotype×status}$=6.7, df=1, 144, P<0.05. Means in the same column followed by the same letter do not differ significantly at P<0.05 level

3.5 Life-history parameters of whiteflies on virus-infected or uninfected tobacco plants

Survival in immature stages was significantly affected by whitefly biotype, but not by plant status or by biotype×status interaction, while development time from egg to adulthood and the number of eggs laid were significantly affected by both whitefly biotype and plant status as well as biotype×status interaction (Table 4). Longevity was also significantly affected by both whitefly biotype and plant status but not by the biotype×status interaction (Table 4). On both types of plants, the Q biotype had a much

higher survival for the immature stages and shorter development time than the ZHJ2 biotype. For each biotype, survival of whiteflies on TYLCCNV-infected plants was somewhat lower and development time was longer than that on uninfected plants. For the Q biotype, on virus-infected plants the mean number of eggs laid was 8 times and the longevity was twice that of uninfected plants. In contrast, fecundity and longevity of the ZHJ2 biotype on virus-infected plants did not increase significantly compared to those on uninfected plants (Table 4).

3.6 Population increase of Q biotype whiteflies on virus-infected or uninfected tobacco plants

Plants were grown to bear 9–10 leaves on the 28th day and to bear 12–13 leaves on the 56th day after the initiation of the whitefly cohorts. The population of the Q biotype reared on TYLCCNV-infected tobacco plants increased more rapidly than that reared on uninfected plants. By the 56th day, the mean numbers of pupae and adults on TYLCCNV-infected plants were 2.1 and 5.1 times higher than those on uninfected plants, respectively (Fig. 1).

3.7 Effect of virus acquisition on the fecundity of Q and ZHJ2 biotypes

The mean numbers of eggs laid in the first 10 d by the Q and ZHJ2 biotype whiteflies were not affected by biotype or virus-infection status of the whitefly (Table 5).

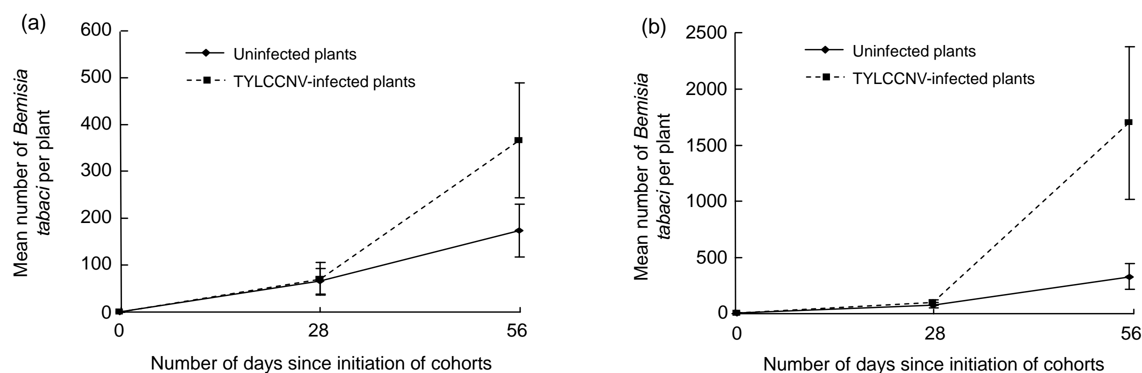

Fig. 1 Mean (±SE) numbers of pupae (a) and adults (b) per plant at two sampling dates (the 28th and 56th days) of two cohorts of the Q biotype *Bemisia tabaci* that were initiated on uninfected and TYLCCNV-infected tobacco plants

Factorial ANOVA statistics for analyzing ln(number+1) of whiteflies between uninfected and TYLCCNV-infected tobacco plants: number of pupae on the 28th day, $F_{1,8}$=0.1, P>0.05; number of adults on the 28th day, $F_{1,8}$=1.0, P>0.05; number of pupae on the 56th day, $F_{1,8}$=1.9, P>0.05; number of adults on the 56th day, $F_{1,8}$=13.4, P<0.05

Table 4 Performance of two biotypes of *Bemisia tabaci* on uninfected or virus-infected tobacco plants

Biotype	Status of host plants	Immature stage			Adult stage		
		n[a]	Mean (±SE) survival (%)[c]	Mean (±SE) development time (d)[d]	n[b]	Mean (±SE) egg number per female[e]	Mean (±SE) longevity (d)[f]
Q	Uninfected	10 (526)	90.8±1.5 a	18.4±0.1 d	20	24.8±6.8 b	15.9±2.9 b
	TYLCCNV-infected	10 (462)	83.0±3.5 b	19.4±0.1 c	20	192.9±19.3 a	32.9±2.8 a
ZHJ2	Uninfected	12 (763)	8.1±2.5 c	22.8±0.2 b	13	10.8±4.9 b	8.3±2.5 b
	TYLCCNV-infected	10 (533)	5.4±1.7 c	25.1±0.2 a	18	43.9±9.7 b	13.9±2.8 b

[a] Number of replicates. The number of eggs in each replicate varied from 13 to 109 and the figures shown in parentheses indicate the total number of eggs at the beginning of each of the treatments; [b] Number of female adults in each of the treatments; [c] Two-factor ANOVA: $F_{biotype}$=434.8, df=1, 38, P<0.001; F_{status}=2.4, df=1, 38, P>0.05; $F_{biotype×status}$=0.4, df=1, 38, P>0.05; [d] Two-factor ANOVA: $F_{biotype}$=851.4, df=1, 983, P<0.001; F_{status}=89.1, df=1, 983, P<0.001; $F_{biotype×status}$=15.7, df=1, 983, P<0.001; [e] Two-factor ANOVA: $F_{biotype}$=39.9, df=1, 67, P<0.001; F_{status}=60.9, df=1, 67, P<0.001; $F_{biotype×status}$=27.4, df=1, 67, P<0.001; [f] Two-factor ANOVA: $F_{biotype}$=21.3, df=1, 67, P<0.001; F_{status}=15.4, df=1, 67, P<0.05; $F_{biotype×status}$=3.9, df=1, 67, P>0.05. Means in the same column followed by the same letter do not differ significantly at P<0.05 level

Table 5 Fecundity of non-viruliferous and viruliferous females of two biotypes of *Bemisia tabaci* on cotton plants

Biotype	Status of whitefly	*n*	Mean (±SE) number of eggs per female during the first 10 d[a]
Q	Non-viruliferous	25	40.0±3.9
	Viruliferous	22	45.3±4.1
ZHJ2	Non-viruliferous	26	28.7±4.3
	Viruliferous	30	39.2±5.5

[a] Two-factor ANOVA: $F_{biotype}$=3.4, df=1, 99, P>0.05; F_{status}=2.8, df=1, 99, P>0.05; $F_{biotype\times status}$=0.3, df=1, 99, P>0.05

4 Discussion

Both the Q and ZHJ2 biotypes of *B. tabaci* were able to efficiently acquire TYLCCNV from infected plants and retain the virus in their bodies for a long period of time (Table 1), but the Q biotype was not able to transmit the virus, and the ZHJ2 biotype was barely able to transmit the virus (Table 2). However, both biotypes of the whitefly were able to improve their performance on TYLCCNV-infected tobacco plants but the benefits acquired by the Q biotype were much greater than those by the ZHJ2 biotype (Tables 3 and 4; Fig. 1). These benefits may be acquired through indirect interactions with TYLCCNV via the tobacco plants, since TYLCCNV-infection of the whiteflies did not affect their fecundity (Table 5). These results present an example of the complexity that may exist in plant-virus-vector interactions and suggest that an invasive insect vector may be able to benefit from virus-infection of the host plant even when it is not a vector of the virus.

Plant-virus-vector interactions, like plant-pathogen-insect interactions in general, show a high degree of variability (Stout *et al*., 2006). Reviews on case studies demonstrate that virus infection of host plants may have positive, neutral, or negative effects on the vector (Belliure *et al*., 2005; Stout *et al*., 2006; Jiu *et al*., 2007). Effort to investigate the factors responsible for the variability has been increasing, but so far no clear patterns have emerged. For example, Castle and Berger (1993) investigated the performance of the aphid *Myzus persicae* on potato plants infected with three types of viruses and found that the positive effects acquired by the aphid through feeding on virus-infected plants decreased with a decrease in the dependence of the virus on the aphid for transmission. However, Hodge and Powell (2008) did a similar investigation with the pea aphid *Acyrthosiphon pisum*, tic beans, and three types of viruses, and found that the performance of the aphid on virus-infected plants was not related to the dependence of the virus on the aphid for transmission. It is interesting to note that in the study by Castle and Berger (1993) the aphid acquired some benefits from the infection of the host plant even when the virus was not aphid-transmitted, an observation similar to those found in this study for the Q and ZHJ2 biotypes of whiteflies feeding on TYLCCNV-infected tobacco plants. These interactions between insects and the viruses that they do not vector may not be uncommon in view of the many studies on herbivores and phytopathogenic fungi, in which the pathogens have little or no dependence on the herbivores for infection, but fungus-infection of plants can improve the plant's suitability for the herbivores (Rostás *et al*., 2003; Stout *et al*., 2006).

Our results show that both the Q and ZHJ2 biotypes of *B. tabaci* were able to acquire TYLCCNV from infected tobacco plants and retain the virus in their bodies for the entire adult life, but were unable to transmit the virus to either tobacco or tomato (Table 2). The coat protein (CP) is the only begomoviral gene product that directly interacts with whitefly factors during the circulative transmission of the virus (Czosnek *et al*., 2002), and the GroEL homologue is necessary for the survival of begomoviruses in their insect vector (Morin *et al*., 1999). Changes of amino acid in the CP would result in efficient whitefly transmission of a non-transmissible geminivirus isolate (Wu *et al*., 1996; Höfer *et al*., 1997; Höhnle *et al*., 2001). Although the GroEL-CP interaction in the haemolymph is a necessary condition for circulative transmission, the non-transmissibility of *Abutilon mosaic virus* (AbMV) is not the result of a lack of binding to GroEL in the *B. tabaci* haemolymph, but is most likely associated with an inability to cross the gut wall into the haemolymph (Morin *et al*., 2000). The greenhouse whitefly *Trialeurodes vaporariorum* is capable of ingesting, but does not transmit begomoviruses, and for this whitefly-virus combination at least one barrier to transmission has been shown to occur at the gut-haemocoel interface (Czosnek *et al*., 2002). The mechanisms responsible for the

non-transmissibility of the Q and ZHJ2 biotypes of *B. tabaci* for TYLCCNV remain unclear.

Extensive field surveys of *B. tabaci* in Zhejiang, China, from 2004 to 2008 revealed rapid temporal changes of distribution of five genotypes/biotypes, i.e., B, Q, ZHJ1, ZHJ2, and ZHJ3 (Liu S.S. *et al*., 2007; Xu, 2009). The data of the field surveys indicate that the B biotype entered Zhejiang before 2003 and was spreading rapidly in the east region of Zhejiang and displacing the indigenous ZHJ1 and ZHJ3 biotypes. By 2006, they had become the predominant or the only biotype in many localities (Liu S.S. *et al*., 2007; Xu, 2009). The Q biotype entered Zhejiang in 2005, spread very rapidly thereafter, and has been displacing the B biotype in many localities of Zhejiang (Xu *et al*., 2006; Xu, 2009). However, by the end of 2008 the indigenous ZHJ2 still remained the predominant biotype in the west, more remote areas of Zhejiang (Xu, 2009). The B and Q biotypes are likely to continue their spread towards the west region of Zhejiang and have intensively competitive interactions with ZHJ2. Reports from the literature and GenBank indicate that ZHJ2 is indigenous to a wide geographic range in Asia. The ZHJ2 biotype from China, the K biotype from Pakistan, the P biotype from Nepal, and the UNK from India, Bangladesh and Italy show over 99% identity in CO1 sequence, demonstrating that they are of the same genetic group (Frohlich *et al*., 1999; De Barro *et al*., 2000; Kirk *et al*., 2000; Zang *et al*., 2006; Boykin *et al*., 2007). With the expected further spread of the B and Q biotypes in Asia in the years to come, these two invasive biotypes are likely to have competitive interactions with ZHJ2 in many areas of this geographic region. Because the B biotype is an efficient vector for TYLCCNV (Jiu *et al*., 2006), it can transmit the virus in the field and cause disease epidemics (Cui *et al*., 2004; Li *et al*., 2004; Jiu *et al*., 2007; Liu Y. *et al*., 2007). When the spread of the B and Q biotypes occurs with concurrent epidemics of plant diseases caused by TYLCCNV, the different indirect interactions with the virus between the Q and ZHJ2 biotypes, as revealed by this study, may offer advantage to the Q biotype in its competitive displacement of ZHJ2.

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