

# Impacts of cucurbit chlorotic yellows virus (CCYV) on biological characteristics of its vector *Bemisia tabaci*

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## Research Article

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# Abstract

It is known that plant viruses, to facilitate their transmission, can change the phenotypes and defense pathways of the host plants and the performance of their vectors. Cucurbit chlorotic yellows virus (CCYV), a newly reported virus occurring on cucurbit plants and many other plant species, is transmitted specifically by Middle East-Minor Asia 1 (B biotype) and Mediterranean (Q biotype) cryptic species of whitefly, *Bemisia tabaci* (Gennadius), in a semipersistent manner. This study evaluated the direct and indirect effects of CCYV on *B. tabaci* biology to better understand the plant-virus-vector interaction. By using CCYV-*B. tabaci*-cucumber as the model, we investigated whether or how a semipersistent plant virus impacts the biology of its whitefly vectors. CCYV mRNA were detectable in nymphs from 1st to 4th instars and adults of *B. tabaci* with different titers. Female nymph duration and female adult longevity greatly extended on CCYV-infected plants, but male nymph duration and male adult longevity were not significantly influenced. In addition, on CCYV-infected plants, the body length and oviposition of adult *B. tabaci* increased, but the hatching rates of eggs and survival rates of different stages were not affected. Most interestingly, the sex ratio (male:female) significantly reduced to 0.506:1 in whitefly populations on CCYV-infected plants, while the ratio remained about 0.979:1 on healthy plants. These results indicated that CCYV can significantly impact the biological characteristics of its vector *B. tabaci* through the host plants. It is speculated that CCYV and *B. tabaci* have established a typical mutualist relationship mediated by host plants.

# Introduction

The plant viruses have developed very specific relationships with insect vectors in the long course of coevolution. Approximately 80% of the plant viruses depend on insect vectors for transmission [1, 2]. More and more researches have proved that plant viruses can regulate the growth, mating, immunity, feeding, reproduction and other behaviors of vector insects. Studies have shown that after carrying tobacco curly shoot virus or tomato yellow leaf curl China virus, the fecundity and longevity of *B. tabaci* were significantly increased [3, 4], and the feeding behaviors were promoted in *B. tabaci* when carrying tomato yellow leaf curl virus (TYLCV) [5]. The activities of protective enzymes and detoxifying metabolic enzymes in non-vector brown planthopper *Nilaparvata lugens* and white-backed planthopper *Sogatella furcifera* were significantly increased when feed on rice black streak dwarf virus-infested rice plants, indicating that the viruses may change the metabolic process and affect the immune system of their non-vectors [6, 7]. The nymphal development and adult longevity of *S. furcifera* carrying southern rice black-streaked dwarf virus were significantly extended, and the females laid fewer eggs after feeding on the infected rice plants; but in BPH, there was no effect of the virus on the development or longevity [8–10]. The nymphs of *Laodelphax striatellus* were significantly prolonged after being infected by rice stripe virus, and the egg development were impaired and the incubation rate dropped significantly, while the weight of female gain and phloem ingestion time during feeding increased [11–13]. *Frankliniella occidentalis* carrying tomato spotted wilt orthotospovirus (TSWV) significantly extended its developmental period and mating time, and produced more progeny, most of which were males with

stronger virulent ability [14]. The incubation period was significantly shortened and pupated faster on virus-infected plants. These results show a mutualistic relationship between *F. occidentalis* and TSWV [15]. Thus, different transmission types of viruses have different effects on the vectors.

*Bemisia tabaci* (Gennadius) (*Hemiptera, Aleyrodidae*) is one of the most important agricultural pests and the most efficient vectors for the transmission of plant viruses in the world [16]. According to statistics, *B. tabaci* can transmit 212 viruses from 5 families and 5 genera [17–19], and some of these viruses cause serious damage and economic losses to agricultural production.

Cucurbit chlorotic yellows virus (CCYV) (genus: *Crinivirus*, family: *Closteroviridae*), as an emergent plant virus, was firstly identified in melon (*Cucumis melo*) in Japan in 2004 [20], which was composed of two single-stranded RNA, and transmitted specifically by *B. tabaci* in a semipersistent manner [21, 22]. CCYV can systematically infect melon plants such as watermelon, luffa, pumpkin and non-melon plants such as beet, quinoa, datura, and *Nicotiana benthamiana* [20], causing chlorotic leaf spots and complete yellowing of leaves. CCYV seriously affected the yield and quality of melons in most parts of China and many Asian countries [21]. In our previous study, we found that CCYV had direct and indirect effects on the feeding behavior of *B. tabaci*, and the degree of influence depends on the biological type (cryptic species) and sex of the insects [23, 24]. However, there have been no reports about the effect of CCYV on the biological characteristics of *B. tabaci*. In this study, the effects of CCYV on the growth, development, reproduction and other biological characteristics of *B. tabaci* were studied in order to provide evidences for the in-depth study of the interaction between *B. tabaci* and CCYV and probably a new idea for the implementation of virus prevention and control strategies.

## Materials And Methods

### The plants and insects

The colony of *B. tabaci* Mediterranean (MED, Q biotype) was maintained on cucumber plants (*Cucumis sativus* L.cv. Bojie-107) in cages (60 cm×60 cm×80 cm) in the greenhouse at 28±1°C, L:D=16 h:8 h and 75±1% relative humidity. The genetic purity of *B. tabaci* Q biotype cultures was monitored every 3 generations using the random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) technique combined with the sequencing of *mtCO1* gene [25]. To obtain CCYV-infected plant cultures, cucumber plants at 2 true-leaf stage were inoculated with *Agrobacterium tumefaciens*-mediated CCYV clones [26]. Plants of cucumber were kept under above-mentioned conditions.

### Quantification of CCYV in cucumber plants and whiteflies

Whiteflies of the same colony were fed on CCYV-infected cucumber plants in a clip cage for 48 h, and were collected in group of 10 individuals of each stage of 1<sup>st</sup> to 4<sup>th</sup> instar nymphs and adults for RNA extraction. Total RNA of whiteflies or infected cucumber plants (100 mg) was extracted using TRIzol® Reagent (Invitrogen Carlsbad, CA, USA) following the manufacturer's instructions. RNA concentration and purity were measured in a NanoDrop™ spectrophotometer (Thermo Scientific Wilmington, DE, USA) and

stored at -80°C for subsequent analysis. Total RNA (1 µg) from each sample was reverse transcribed to generate the first-strand cDNA using the PrimeScript® RT reagent Kit (Takara, Dalian, China).

Primers were designed based on coding sequences of CCYV coat protein (CP) by using primer premier 5 software and the nucleotide sequence in GenBank (Accession No: HM581658.1). The primers used are shown in Table 1. Subsequent primer-blast searches showed that they had a high specificity towards CCYV. PCR products were connected with pMD18-T vector to construct standard recombinant plasmid. Six gradients ( $3.40 \times 10^3$ - $3.40 \times 10^8$  copies/µL) of standard recombinant plasmids were set up as a template for real-time qRT-PCR, with three replicates for each concentration, meanwhile blank control and negative control were set up. Amplification reactions were performed as follows: 94°C for 2 min, 40 cycles of 94°C for 15 s, 60°C for 20 s, 72°C for 20s. According to the standard curve automatically generated by the instrument, the correlation coefficient  $R^2=0.9984$ , the amplification efficiency  $E=95\%$ , and the standard curve equation is  $Y=-3.3396\lg X+27.8480$  (Figure S1).  $C_t$  value of each sample was detected by qRT-PCR, three replicates were examined, and the absolute quantification of CCYV mRNA molecules in cucumber plants or *B. tabaci* were calculated.

**Table 1 PCR primers for CCYV detection.** Note: Primers were designed based on coding sequences of CCYV coat protein (CP) by using primer premier 5 software and the nucleotide sequence in GenBank (Accession No: HM581658.1).

### Impacts of CCYV on biology of *B. tabaci*

A couple of 3-d adults were placed with a clip cage on a leaf of healthy cucumber plant for oviposition. After 24 hours, the adults were transferred to CCYV-infected cucumber plant for another 24 hours, then the adults were removed from plants. Thirty eggs on each leaf were marked under the super-depth microscope (Keyence digital microscope VHX-600E) and other eggs were removed. Three replicates were used for each treatment. Observations were taken every day under the super-depth microscope until all eggs hatched and 1st-instar nymphs were fixed. Locations of the nymphs were marked. The egg hatching rates ( $P_0$ ), nymph survival rates ( $P_n$ ), and sex ratio ( $P$ ) of newly emerging adults were calculated with the following equations: **see equations 1, 2, and 3 in the supplementary files.**

Where  $N_1$  is the number of 1<sup>st</sup>-instar nymphs;  $K$  is a constant of 30;  $P_n$  is the survival rates of each instar nymphs ( $n=1, 2, 3, 4$ );  $M$  is the number of males and  $F$  is the number of females. The nymph duration and adult longevity were recorded separately. Sizes of each individual of adults were measured.

In another set of experiments, a couple of newly emerging adults were placed with a clip cage on a leaf of healthy or CCYV-infected cucumber plant. The insects were moved to a new plant every 24 hours. Eggs on all leaves were counted, and dead male adults were replaced with new males until the female adults died. Ovipositional capacity was calculated. For this sets of experiments, 40 female adults were used.

### Data statistics

IBM SPSS Statistics 21.0 was used to conduct data analyses. Comparisons in body size, oviposition, nymph duration, adult longevity as well as sex ratio of insects on healthy and CCYV-infected cucumber plants were made using Independent-Samples *t*-test; one-way ANOVA, LSD test was used to analyze fertility rate, nymph survival rates and the amount of CCYV mRNA molecules among all instar nymphs and adults. Significant differences were tested at the 0.05 or 0.01 level. All data were expressed as Mean±SE of three independent experiments.

## Results

### Detection of CCYV in cucumber

The cucumber plants at 2 true-leaf stage were inoculated with *Agrobacterium tumefaciens*-mediated CCYV clones. At 25 days post-infiltration, leaves of *C. sativus* plants agroinfiltrated developed yellowing symptoms, typical of CCYV infection in plants (Fig. 1A), whereas no symptoms were observed on healthy leaves. Analysis by RT-PCR using the primers is specific to the CP coding sequence (Table 1). All samples displayed amplification products of the expected sizes (Fig. 1B). The amplification products were sequenced, which verified CCYV infection. qRT-PCR was used to detect the absolute quantification of CCYV mRNA molecules in healthy and CCYV-infected *C. sativus*. The results showed that the amount of CCYV mRNA molecules were only found in leaves of CCYV-infected *C. sativus* with 87114.56 copies, while nothing was found in healthy *C. sativus* (Fig. 1C).

Figure 1 **Determination of CCYV infection after agroinoculation in cucumber plant *C. sativus*. A.** Symptoms displayed on the systemic leaves of *C. sativus* at 25 days post-agroinoculation. Yellowing was observed on the systemic leaves of *C. sativus*. **B.** RT-PCR detection of CCYV in the systemic leaves of *C. sativus*. Lane 1 and lane 2, healthy *C. sativus*; Lane 3 and lane 4, CCYV-infected *C. sativus*; Lane 5, positive control; Lane 6, negative control. **C.** Absolute quantification of CCYV mRNA molecules in healthy and CCYV-infected *C. sativus*. Mean ± SE of three independent experiments.

## Absolute quantification of CCYV mRNA molecules in individual whiteflies

We used qRT-PCR to detect the absolute quantification of CCYV mRNA molecules of individual *B. tabaci* in different stages (1st to 4th instar and adult) fed on CCYV-infected cucumber plants for 48 h. The results showed that the amount of CCYV mRNA molecules were detected in all instars of nymphs as well as adults of *B. tabaci*, with 21360.08 copies in adults, followed by 1424.54 copies in the 2nd -instar nymphs, and 112.34 copies in 4th -instar nymphs (Fig. 2).

Figure 2 **Absolute quantification of CCYV mRNA molecules in individual whiteflies of *B. tabaci* obtained by amplifying portions of the CP genes using qRT-PCR.** Whiteflies of the same colony were fed on CCYV-infected cucumber plants for 48 h, and were collected in group of 10 individuals of each stage of 1st to 4th instar nymphs and adults for RNA extraction. Total RNA (1 µg) from each sample was reverse

transcribed to generate the first-strand cDNA for qRT-PCR. Mean  $\pm$  SE of three independent experiments is shown.  $P < 0.05$  (one-way ANOVA, LSD test).

## Effects of CCYV on nymph duration and adult longevity of *B. tabaci*

*B. tabaci* nymph duration and adult longevity were shown in Fig. 3. The female and male nymphs of *B. tabaci* on healthy cucumber plants were  $13.80 \pm 0.27$  d and  $13.94 \pm 0.34$  d respectively. The developmental duration of female nymphs on CCYV-infected cucumber plants were  $15.79 \pm 0.31$  d, and that of male nymphs was  $14.36 \pm 0.36$  d. These results showed that CCYV significantly extended the developmental duration of female nymph ( $P < 0.01$ ), but had no significant effect on duration of male nymph ( $P = 0.391$ ) (Fig. 3A).

On healthy cucumber, the developmental duration of *B. tabaci* female adults were  $12.32 \pm 0.20$  d, while that of male adults were  $13.89 \pm 0.22$  d. And on CCYV-infected cucumber plants, longevity of the female adult *B. tabaci* was  $13.87 \pm 0.26$  d, and that of male adults was  $14.53 \pm 0.36$  d. The results showed that CCYV significantly extended the female adult longevity of *B. tabaci* ( $P < 0.01$ ), but had no significant effect on longevity of male adult *B. tabaci* ( $P = 0.136$ ) (Fig. 3B).

Figure 3 **Impact of CCYV on the nymph duration (A) and adult longevity (B) of *B. tabaci***. Health: non-viruliferous whitefly; CCYV: viruliferous whitefly; nymph duration include stages from 1st-instar to 4th-instar. For each treatment, 40 samples were analyzed. Mean  $\pm$  SE is shown. \*  $P < 0.05$ ; \*\*  $P < 0.01$  (independent-sample *t* test).

## Effects of CCYV on body length and oviposition of adult *B. tabaci*

As shown in Fig. 4A, the body length of *B. tabaci* female and male adults on healthy cucumber plants was  $1066.30 \pm 5.04$   $\mu\text{m}$  and  $895.70 \pm 4.13$   $\mu\text{m}$ , respectively. The body length of female and male *B. tabaci* was  $1091.02 \pm 4.05$   $\mu\text{m}$  and  $913.52 \pm 3.18$   $\mu\text{m}$  respectively when feeding on cucumber plants infected with CCYV. Independent Sample *t*-test showed that CCYV could significantly increase the body length of female and male adults ( $P < 0.01$ ). Number of eggs laid by females on healthy plants were  $105.03 \pm 4.13$ , while and on the plants infected with CCYV, the number of oviposition of individual female adults were  $125.22 \pm 3.31$  (Fig. 4B). Independent Sample *t*-test results showed that CCYV could significantly increase oviposition of female adults of *B. tabaci* ( $P < 0.01$ ).

Figure 4 **Impact of CCYV on the body length (A) and oviposition (B) of *B. tabaci***. Health: non-viruliferous whitefly; CCYV: viruliferous whitefly; 177 and 135 samples were analyzed for the non-viruliferous females and males, respectively; 248 and 152 samples were analyzed for the viruliferous females and males, respectively; 58 samples were analyzed for the oviposition. Mean  $\pm$  SE is shown. \*  $P < 0.05$ ; \*\*  $P < 0.01$  (independent-sample *t* test).

# Effects of CCYV on hatching rate and nymphae survival rates of *B. tabaci*

The egg hatching rate and nymph survival rates of *B. tabaci* at various instars on healthy and virulent cucumbers were shown in Table 2. The egg hatching rate of *B. tabaci* on healthy cucumber plants was  $91.10 \pm 2.20\%$ , and that on CCYV-infected cucumber plants was  $85.53 \pm 3.99\%$ . Independent Sample *t*-test shown that CCYV had no significant effect on the egg hatching rate of *B. tabaci* ( $P = 0.184$ ). The survival rates from 1st to 4th -instar nymphs on healthy cucumber plants were  $98.25 \pm 1.75\%$ ,  $99.25 \pm 0.75\%$ ,  $100 \pm 0.00\%$  and  $100 \pm 0.00\%$ , respectively, and the survival rates on CCYV-infected cucumber plants were  $97.57 \pm 1.59\%$ ,  $96.00 \pm 2.12\%$ ,  $100 \pm 0.00\%$ , and  $99.25 \pm 0.75\%$ , respectively. The nymph survival rates of *B. tabaci* on healthy cucumber plants were all higher than those on cucumber plants infected with CCYV, but the difference did not reach to a significant level ( $P > 0.05$ ).

Table 2  
Fertility rates and nymph survival rates of *B. tabaci* on healthy and CCYV-infected cucumber plants

Developmental stage	Healthy plants	CCYV -infected plants	Sig.
egg	$91.10 \pm 2.20a$	$85.53 \pm 3.99a$	0.184
1st -instar	$98.25 \pm 1.75a$	$97.57 \pm 1.59a$	0.831
2nd -instar	$99.25 \pm 0.75a$	$96.00 \pm 2.12a$	0.109
3rd -instar	$100 \pm 0.00a$	$100 \pm 0.00a$	—
4th -instar	$100 \pm 0.00a$	$99.25 \pm 0.75a$	0.109
egg + nymphs	$88.84 \pm 1.60a$	$79.52 \pm 2.09a$	0.184

Table 2 Fertility rates and nymph survival rates of *B. tabaci* on healthy and CCYV-infected cucumber plants. Note: Mean  $\pm$  SE of three independent experiments is shown, and same letters in the same line indicate that survival rate of the *B. tabaci* on CCYV-infected and healthy cucumber plants was not significantly different at the 0.05 level. (one-way ANOVA, LSD test).

## Effect of CCYV on sex ratio of *B. tabaci*

The effect of CCYV on the sex ratio of *B. tabaci* is shown in Fig. 5. On the healthy cucumber plants, the sex ratio was 0.979:1, but on the cucumber plants infected with CCYV, the sex ratio was 0.506:1. *B. tabaci* had a higher percentage of females on the CCYV-infected cucumber plants (66.40%) than on the healthy plants (50.53%) ( $P < 0.05$ ).

Figure 5 The sex ratio of *B. tabaci* on the healthy cucumbers (Health) and CCYV-infected cucumbers (CCYV). Thirty eggs from a couple of 3-d adults on a leaf of healthy cucumber plant and CCYV-infected

cucumber plant were marked under the super-depth microscope. The sex ratio of newly emerging adults were calculated, Mean  $\pm$  SE of three independent experiments is shown. \*  $P < 0.05$ ; \*\*  $P < 0.01$  (independent-sample t test).

## Discussion

Vector-borne pathogens can alter the phenotypes of their hosts and vectors in ways that influence the frequency and nature of interactions between them, with significant implications for the transmission and spread of diseases [27]. Plant viruses can manipulate vector insects by directly influencing the feeding behavior. For example, *B. tabaci* carrying CCYV increased non-phloem probing and phloem salivation [24]. *B. tabaci* with TYLCV spend more time in phloem salivating and ingesting sap [28]. Plant viruses can also affect behaviors of vector insects indirectly via regulate host plant metabolism. For instance, terpenoid synthesis is suppressed in begomovirus-infected plants, leading to reduced plant resistance or modulate plant volatile production to influence vector behavior[29–31]. Although CCYV was present in the whiteflies, the differential biological characteristics obtained in this study could result from virus-infected plants, but not the direct effect of virus itself.

Previous studies have shown that plant viruses can affect the insect vectors, but the degrees of influence of different virus-vector combinations are not identical. There have been many reports on alteration of physiology, molecular biology or feeding behaviors in insect vectors by persistently transmitted plant viruses, for example, *Begomovirus* on *B. tabaci*, but few or no studies are available on impact of semipersistent viruses on vectors [3–5, 14]. In our present study, we reported the effects of semi-persistent virus CCYV on the biological characteristics of the vector *B. tabaci*. Although the nymphs play no roles in virus transmission, their immobile stages (esp. 2nd to 4th instar) encounter the plant viruses when feeding on the plant. Nymphs can be affected, more or less, by virus particles taken with plant sap, and thereby may affect the status of the adults responsible for virus transmission. Therefore, this study comprehensively investigated the biological effects of CCYV on nymphs and adults of *B. tabaci*, with a view to fully obtaining the biological effects of the virus. The results indicated that all nymph instars can be infected with CCYV, and the viral mRNA accumulation varies with instars. The 2nd -instar has the highest viral mRNA accumulation (1424.54 copies) among the nymphs, followed by the 3rd -instar and the 1st -instar, and the 4th -instar (112.34 copies) has the lowest viral mRNA accumulation, which may be related to the behavior characteristics of each instar nymph. The 1st -instar nymphs have tentacles and feet and can crawl over a short distance to find a suitable feeding site and then settle down and start feeding. The tentacles and feet of the 2nd and 3rd -instar nymphs were degraded, and they had no crawling ability. They were fixed on the back of the leaves for feeding with the stylets [32]. The 4th -instar nymphs, also known as pseudo pupal stage, basically stopped feeding [33, 34], which may be the reason for the low viral mRNA accumulation of the 4th -instar nymphs. The viral mRNA accumulation of the adults was much greater than those of the nymphs, and the viral mRNA accumulation of the individual adult was up to 21360.08 copies. Adult *B. tabaci* is highly active and can even migrate over long distances with the assistance of air currents, becoming the main cause of the CCYV pandemic.

By comparing the development period of *B. tabaci*, it was found that CCYV could significantly extend the development period of female nymphs ( $P < 0.01$ ) and the longevity of female adults ( $P < 0.01$ ), but not significantly affect the development period of male nymphs ( $P = 0.391$ ) and the longevity of male adults ( $P = 0.136$ ). The influence of CCYV on the growth and development of females are much greater than that of males. This may be because females are larger than males and require more nutrients to reproduce, so females ingest more viruses than males, which in turn has a more significant impact on their growth and development. Longer development period and longevity means more possibility of virus transmission. Therefore, we speculate that females are more conducive to the transmission of CCYV virus than males.

Through a comparative analysis of the body length and oviposition of *B. tabaci*, we found that CCYV significantly increased the body length of female adults ( $P < 0.01$ ) and male adults ( $P < 0.01$ ), and increased the oviposition of individual female adult ( $P < 0.01$ ). It may be due to an extended developmental period and a higher intake of nutrients. The size of insect is an important factor affecting population development potential and community structure and function [35–37]. Relevant studies have shown that, compared with smaller individuals within the same species, larger insects often have advantages in reproduction, flight, competition, stress resistance and other aspects, contributing to the improvement of population fitness [38].

CCYV significantly increased the proportion of female adult from 50.53% on healthy plants to 66.40% on CCYV-infected cucumber plants. There are two reproductive modes of *B. tabaci*, including parthenogenesis and amphigenesis. The female offsprings of *B. tabaci* are all developed from fertilized eggs, while the male offsprings may come from fertilized eggs and parthenogenesis [39]. The increase of female proportion of whitefly may be due to the increase of body length caused by CCYV, which enables it to have comparative advantages in mating process and obtain more mating opportunities, so as to increase the proportion of female offspring by increasing the number of fertilized eggs, thus ensuring the reproduction of its offspring population.

## Conclusions

In conclusion, our results confirmed that CCYV could manipulate the growth and development of its vector, *B. tabaci*. We found that CCYV had more effects on female than male in development duration by increasing duration of female nymphs and adults. Interestingly, CCYV could significantly increase the body length and oviposition of *B. tabaci* and the ratio of females became higher on cucumber plants infected with CCYV, which will undoubtedly increase the population fitness and beneficial to its population reproduction, thus, it is beneficial to the transmission of CCYV. These results clearly indicated that the biological characteristics of *B. tabaci* Q biotypes changed greatly when infected with CCYV, and the effect on females is much greater than on males. Based on the above results, we can infer that CCYV and *B. tabaci* have a typical mutualism relationship and play an important role in *B. tabaci* outbreak mechanism. In this paper, the effects of semi-persistent viruses on the biological characteristics of vectors are studied, which will enrich people's understanding of the plant-virus-vector interaction.

# Abbreviations

CCYV: cucurbit chlorotic yellows virus; TSWV: tomato spotted wilt orthospovirus; TYLCV: tomato yellow leaf curl virus;

# Declarations

## Ethics approval

Not applicable.

## Consent to publication

All the authors consent to publish.

## Availability of data and material

All data generated or analysed during this study are included in this published article [and its supplementary information files].

## Competing interests

The authors declare that they have no competing interests.

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## Authors' Contributions

Conceptualization, FY and JL; methodology, HH and JL; software, HH and ZZ; validation, FY and JL; formal analysis, HH; investigation, HH, ZZ, XT and DS; resources, FY; data curation, HH, XT and DS; writing—original draft preparation, HH; writing—review and editing, all authors; supervision, FY; project administration, JL; funding acquisition, FY and JL. All authors read and approved the final manuscript.

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## Supplementary Materials

The following are available online , Figure S1: The amplification curve and standard curve of qRT-PCR.

# References

1. Andretlink P, Fuchs M (2005) Transmission specificity of plant viruses by vectors. *Journal of Plant Pathology* 87:153–165.
2. Hohn T (2007) Plant virus transmission from the insect point of view. *Natl. Acad. Sci. USA* 104:17905–17906.
3. Jiu M, Zhou XP, Tong L, Xu J, Yang X, Wan FH, Liu SS (2007) Vector–virus mutualism accelerates population increase of an invasive whitefly. *PLoS ONE* 2:e182.
4. Guo JY, Ye GY, Dong SZ, Liu SS (2010) An invasive whitefly feeding on a virus–infected plant increased its egg production and realized fecundity. *PLoS ONE* 5:e11713.
5. Liu BM, Preisser EL, Chu D, Pan HP, Xie W, Zhang YJ (2013) Multiple forms of vector manipulation by a plant–infecting virus: *Bemisia tabaci* and *tomato yellow leaf curl virus*. *Journal of Virology* 87:4929–4937.
6. He XC, Xu HX, Zheng XS, Yang YJ, Gao GC, Pan JH, Lu ZX (2012) Ecological fitness of non–vector planthopper *Sogatella furcifera* on rice plants infected with rice black streaked dwarf virus. *Rice Science* 19:335–338.
7. Xu HX, He XC, Zheng XS, Yang YJ, Lu ZX (2014) Influence of rice black streaked dwarf virus on the ecological fitness of non–vector planthopper *Nilaparvata lugens* (Hemiptera: Delphacidae). *Insect Science* 21:507–514.
8. He XC, Xu HX, Gao GC, Zhou XJ, Zheng XS, Sun YJ, Yang YJ, Tian J, Lu ZX (2014) Virus–mediated chemical changes in rice plants impact the relationship between non–vector planthopper *Nilaparvata lugens* Stål and its egg parasitoid *Anagrus nilaparvatae* Pang et Wang. *PLoS ONE* 9:e105373.
9. Tu Z, Ling B, Xu DL, Zhang MX, Zhou GH (2013) Effects of southern rice black–streaked dwarf virus on the development and fecundity of its vector, *Sogatella furcifera*. *Virology Journal* 10:145–145.
10. Lei WB, Liu DF, Li P, Hou ML (2014) Interactive effects of southern rice black–streaked dwarf virus infection of host plant and vector on performance of the vector, *Sogatella furcifera* (Homoptera: Delphacidae). *Journal of Economic Entomology* 107:1721–1727.
11. He K, Guo JM, Li F, Lin KJ, Wang GR (2018) Impact of the rice stripe virus (RSV) on the biological, physiological and biochemical characteristics of the small brown planthopper, *Laodelphax striatellus* (Hemiptera: Delphacidae). *Chinese Journal of Applied Entomology* 55:87–95.
12. Li S, Wang SJ, Wang X, Li XL, Zi JY, Wong SK, Zhou YJ (2015) Rice stripe virus affects the viability of its vector offspring by changing developmental gene expression in embryos. *Scientific Reports* 5:7883.
13. Wan GJ, Jiang SL, Wang WJ (2015) Rice stripe virus counters reduced fecundity in its insect vector by modifying insect physiology, primary endosymbionts and feeding behavior. *Scientific Reports* 5:12527.

14. Wan YR, Hussain S, Merchant A, Xu BY, Xie W, Wang SL, Zhang YJ, Zhou XG, Wu QJ (2020) Tomato spotted wilt orthotospovirus influences the reproduction of its insect vector, western flower thrips, *Frankliniella occidentalis*, to facilitate transmission. *Pest Manag Sci* 76:2406-2414.
15. Maris PC, Joosten NN, Goldbach RW, Peters D (2004) Tomato spotted wilt virus infection improves host suitability for its vector *Frankliniella occidentalis*. *Phytopathology* 94:706–711.
16. De Barro PJ, Liu SS, Boykin LM, Dinsdale AB: *Bemisia tabaci* (2011) a statement of species status. *Annu Rev Entomol* 56:1–19.
17. Jones DR (2003) Plant viruses transmitted by whiteflies. *Eur J Plant Pathol* 109:195–219.
18. Bragard C, Caciagli P, Lemaire O, Lopez–Moya JJ, MacFarlane S, Peters D, Susi P, Torrance L (2013) Status and prospects of plant virus control through interference with vector transmission. *Annu Rev Phytopathol* 51:177–201.
19. Polston JE, De Barro PJ, Boykin LM (2014) Transmission specificities of plant viruses with the newly identified species of the *Bemisia tabaci* species complex. *Pest Manag Sci* 70:1547–52.
20. Gyoutoku Y, Okazaki S, Furuta A, Etoh T, Mizobe M, Kuno K, Hayashida S, Okuda M (2009) Chlorotic yellows disease of melon caused by cucurbit chlorotic yellows virus, a new crinivirus. *Jpn J Phytopathol* 75:109–111.
21. Okuda M, Okazaki S, Yamasaki S, Okuda S, Sugiyama M (2010) Host range and complete genome sequence *Cucurbit chlorotic yellows virus*, a new member the genus Crinivirus. *Phytopathology* 100:560–566.
22. Li JJ, Liang XZ, Wang XL, Shi Y, Gu QS, Kuo YW, Falk BW, Yan FM (2016) Direct evidence for the semipersistent transmission of cucurbit chlorotic yellows virus by a whitefly vector. *Scientific Reports* 6:36604.
23. Lu SH, Li JJ, Wang XL, Song DY, Bai RE, Shi Y, Gu QS, Kuo YW, Falk BW, Yan FM (2017) A semipersistent plant virus differentially manipulates feeding behaviors of different sexes and biotypes of its whitefly vector. *Viruses* 9(1), doi: 103390/v9010004.
24. Lu SH, Chen MS, Li JJ, Shi Y, Gu QS, Yan FM (2019) Changes in *Bemisia tabaci* feeding behaviors caused directly and indirectly by cucurbit chlorotic yellows virus. *Virology Journal* 16:1–14.
25. Chu D, Wan FH, Zhang YJ, Brown JK (2010) Chang in the biotype composition of *Bemisia tabaci* in Shandong province of China from 2005 to 2008. *Environmental Entomology* 39:1028–1036.
26. Shi Y, Shi YJ, Gu QS, Yan FM, Sun XY, Li HL, Chen LL, Sun BJ, Wang ZY (2016) Infectious clones of the crinivirus cucurbit chlorotic yellows virus are competent for plant systemic infection and vector transmission. *Journal of General Virology* 97:1458.
27. Mauck KE, De Moraes CM, Mescher MC (2010) Deceptive chemical signals induced by a plant virus attract insect vectors to inferior hosts. *Natl. Acad. Sci. USA* 107:3600–3655.
28. Moreno-Delafuente A, Garzo E, Moreno A, Fereres A (2013) A plant virus manipulates the behavior of its whitefly vector to enhance its transmission efficiency and spread. *PLoS ONE* 8:e61543.

29. Li R, Weldegergis BT, Li J, Jung C, Qu J, Sun Y, Qian H, Tee C, van Loon JJA, Dicke M (2014) Virulence factors of geminivirus interact with MYC2 to subvert plant resistance and promote vector performance. *The Plant Cell* 26:4991–5008.
30. Luan JB, Yao DM, Zhang T, Walling LL, Yang M, Wang YJ, Liu SS (2013) Suppression of terpenoid synthesis in plants by a virus promotes its mutualism with vectors. *Ecology Letters* 16:390–398.
31. Zhang T, Luan JB, Qi JF, Huang CJ, Li M, Zhou XP, Liu SS (2012) Begomovirus-whitefly mutualism is achieved through repression of plant defences by a virus pathogenicity factor. *Molecular Ecology* 21:1294–1304.
32. Yang NN, Zhang YJ, Yang X, Huang DY, Long T, Wan P (2016) Differential expression of the detoxification enzyme genes in different developmental stages of the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Acta Entomologica Sinica* 59:1166–1173.
33. Chandi RS, Kular JS (2014) Biological parameters of whitefly, *Bemisia tabaci* (Gennadius) on Bt and non-Bt cotton under Punjab conditions. *Journal of Experimental Zoology* 17:555–561.
34. Yan FM, Bai RE (2017) Whitefly Fauna of China. Zhengzhou. Henan Science and Technology Press 2:1–7.
35. Siemann E, Haarstad J, Tilman D (1996) Insect species diversity, abundance and body size relationships. *Nature* 380:704–706.
36. Whitman DW (2008) Body size in Orthoptera The significance of body size in the Orthoptera: A Review. *Journal of Orthoptera Research* 17:117–134.
37. Henri DC, Veen F (2011) Body size, life history and the structure of host–parasitoid networks. *Advances in Ecological Research* 45:135–180.
38. Huang YS, Zhang JY, Jiang, MX (2017) Effects of body size on the population biology of insects. *Acta Ecologica Sinica* 37:2158–2168.
39. Byrne DN, Bellows TS (1991) Whitefly biology. *Annual Review of Entomology* 36:431–457.

## Tables

**Table 1 PCR primers for CCYV detection**

Primers	Positions	Sequence (5′-3′)	Size (bp)
CCYV-F	548-567	GCGACCATCATCTACAGGCA	152
CCYV-R	679-699	CCGACTTGTTCCTTTCAGAGC	

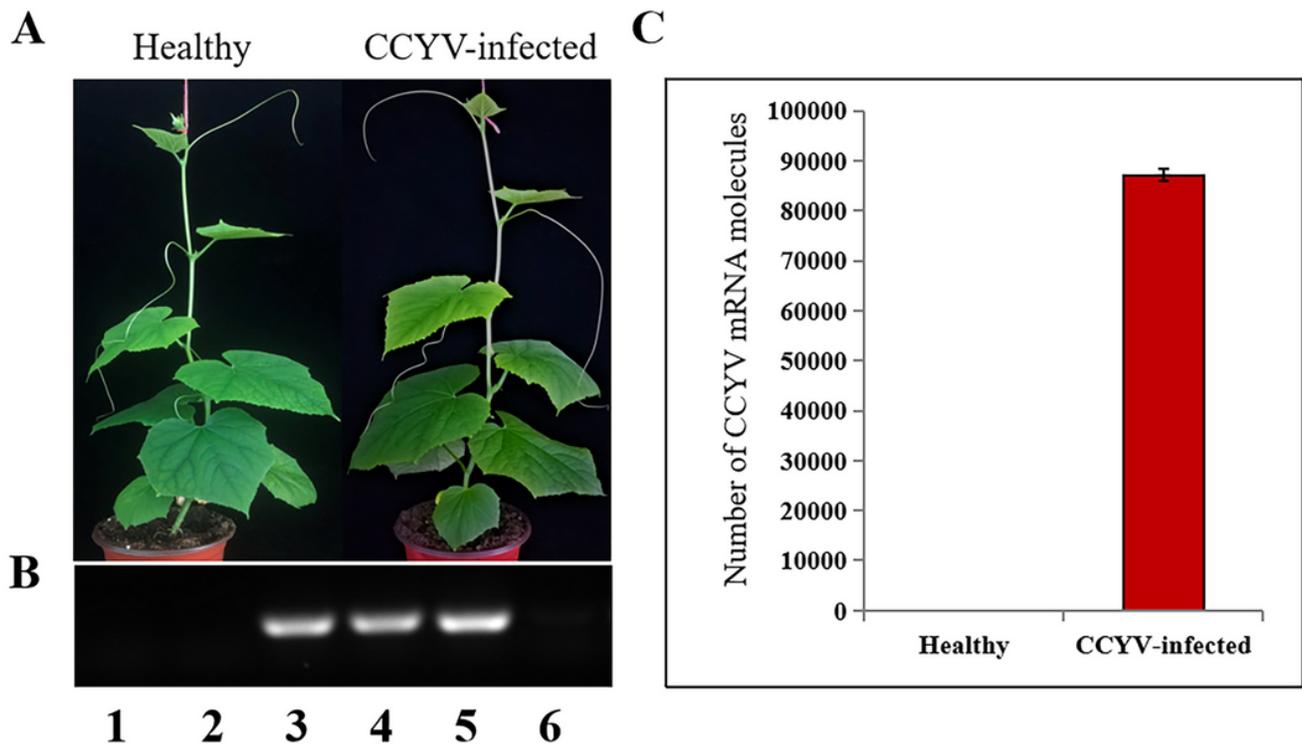
**Note:** Primers were designed based on coding sequences of CCYV coat protein (CP) by using primer premier 5 software and the nucleotide sequence in GenBank (Accession No: HM581658.1).

**Table 2 Fertility rates and nymph survival rates of *B. tabaci* on healthy and CCYV-infected cucumber plants**

Developmental stage	Healthy plants	CCYV -infected plants	Sig.
egg	91.10±2.20a	85.53±3.99a	0.184
1 <sup>st</sup> -instar	98.25±1.75a	97.57±1.59a	0.831
2 <sup>nd</sup> -instar	99.25±0.75a	96.00±2.12a	0.109
3 <sup>rd</sup> -instar	100±0.00a	100±0.00a	---
4 <sup>th</sup> -instar	100±0.00a	99.25±0.75a	0.109
egg+nymphs	88.84±1.60a	79.52±2.09a	0.184

**Note:** Mean±SE of three independent experiments is shown, and same letters in the same line indicate that survival rate of the *B. tabaci* on CCYV-infected and healthy cucumber plants was not significantly different at the 0.05 level. (one-way ANOVA, LSD test).

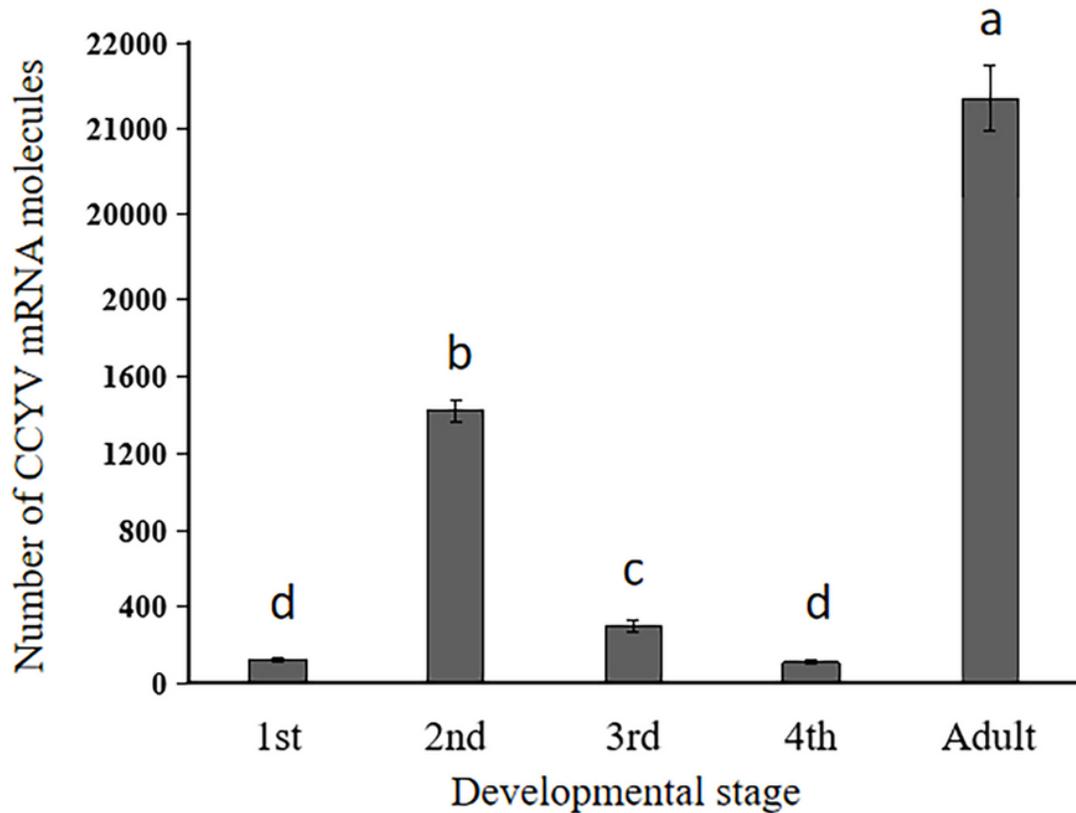
## Figures



**Figure 1**

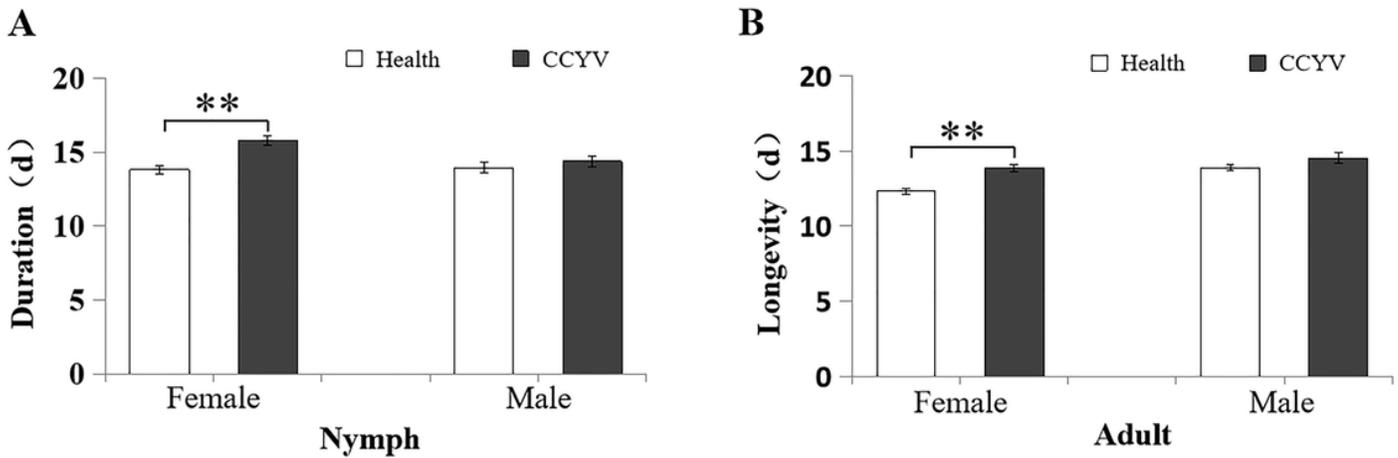
Determination of CCYV infection after agroinoculation in cucumber plant *C. sativus*. A. Symptoms displayed on the systemic leaves of *C. sativus* at 25 days post-agroinoculation. Yellowing was observed

on the systemic leaves of *C. sativus*. B. RT-PCR detection of CCYV in the systemic leaves of *C. sativus*. Lane 1 and lane 2, healthy *C. sativus*; Lane 3 and lane 4, CCYV-infected *C. sativus*; Lane 5, positive control; Lane 6, negative control. C. Absolute quantification of CCYV mRNA molecules in healthy and CCYV-infected *C. sativus*. Mean  $\pm$  SE of three independent experiments.



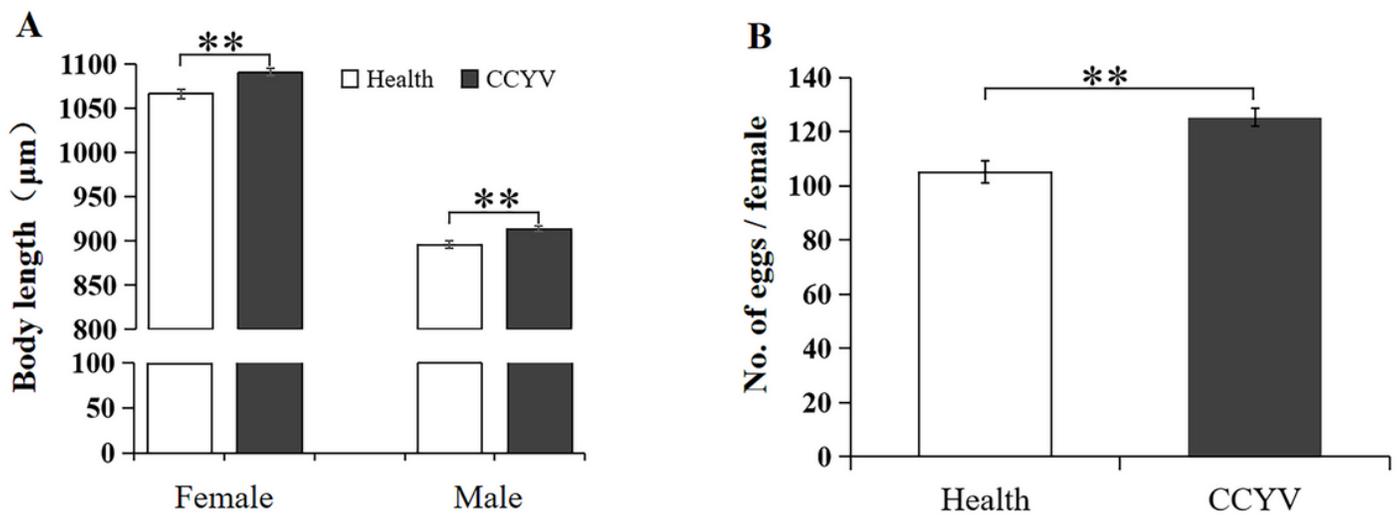
**Figure 2**

Absolute quantification of CCYV mRNA molecules in individual whiteflies of *B. tabaci* obtained by amplifying portions of the CP genes using qRT-PCR. Whiteflies of the same colony were fed on CCYV-infected cucumber plants for 48 h, and were collected in group of 10 individuals of each stage of 1st to 4th instar nymphs and adults for RNA extraction. Total RNA (1  $\mu$ g) from each sample was reverse transcribed to generate the first-strand cDNA for qRT-PCR. Mean  $\pm$  SE of three independent experiments is shown.  $P < 0.05$  (one-way ANOVA, LSD test).



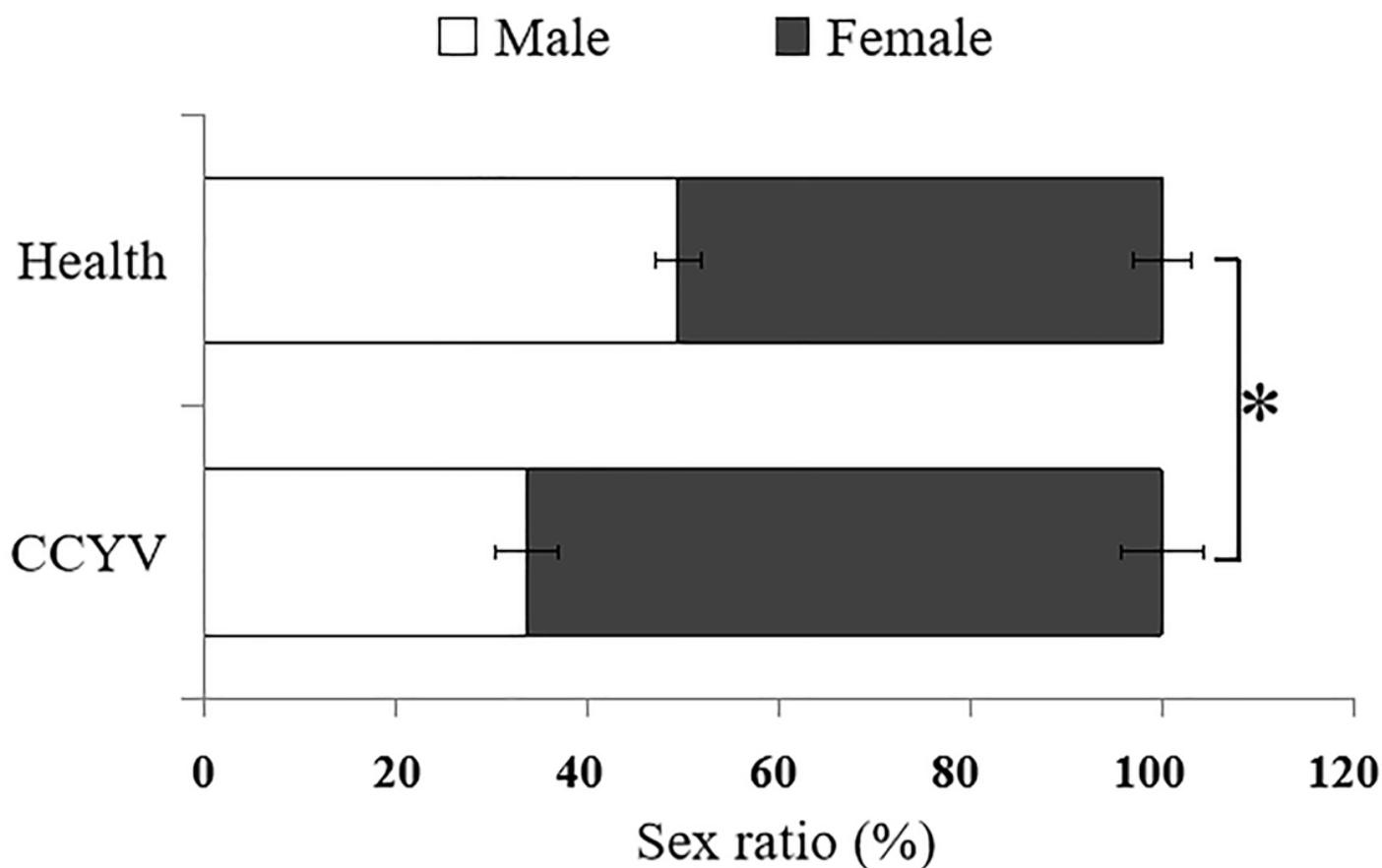
**Figure 3**

Impact of CCYV on the nymph duration (A) and adult longevity (B) of *B. tabaci*. Health: non-viruliferous whitewolf; CCYV: viruliferous whitewolf; nymph duration include stages from 1st-instar to 4th-instar. For each treatment, 40 samples were analyzed. Mean±SE is shown. \* P<0.05; \*\* P<0.01 (independent-sample t test).



**Figure 4**

Impact of CCYV on the body length (A) and oviposition (B) of *B. tabaci*. Health: non-viruliferous whitewolf; CCYV: viruliferous whitewolf; 177 and 135 samples were analyzed for the non-viruliferous females and males, respectively; 248 and 152 samples were analyzed for the viruliferous females and males, respectively; 58 samples were analyzed for the oviposition. Mean±SE is shown. \* P<0.05; \*\* P<0.01 (independent-sample t test).



**Figure 5**

The sex ratio of *B. tabaci* on the healthy cucumbers (Health) and CCYV-infected cucumbers (CCYV). Thirty eggs from a couple of 3-d adults on a leaf of healthy cucumber plant and CCYV-infected cucumber plant were marked under the super-depth microscope. The sex ratio of newly emerging adults were calculated, Mean $\pm$ SE of three independent experiments is shown. \*  $P < 0.05$ ; \*\*  $P < 0.01$  (independent-sample t test).

## Supplementary Files

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