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Bemisia tabaci Q carrying *tomato yellow leaf curl virus* strongly suppresses host plant defenses

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The concurrence of *tomato yellow leaf curl virus* (TYLCV) with the spread of its vector *Bemisia tabaci* Q rather than B in China suggests a more mutualistic relationship between TYLCV and Q. Here, we investigated the hypothesis that viruliferous B and Q have different effects on plant defenses. We found the fecundity of nonviruliferous B, nonviruliferous Q, viruliferous Q and viruliferous B was 11.080, 12.060, 10.760, and 11.220 respectively on plants previously attacked by the other biotype, however, on their respective noninfested control leaves fecundity was 12.000, 10.880, 9.760, and 8.020 respectively. Only viruliferous B had higher fecundity on viruliferous Q-infested plants than on control plants. The longevity of viruliferous B showed the same phenomenon. At 1 d infestion, the jasmonic acid content in leaves noninfested and in leaves infested with nonviruliferous B, nonviruliferous Q, viruliferous B and viruliferous Q was 407.000, 281.333, 301.333, 266.667 and 134.000 ng/g FW, respectively. The JA content was lowest in viruliferous Q-infested leaves. The proteinase inhibitor activity and expression of JA-related upstream gene *LOX* and downstream gene *PI II* showed the same trend. The substantial suppression of host defenses by Q carrying TYLCV probably enhances the spread of Q and TYLCV in China.

About 80% of plant viruses depend on insect vectors for their transmission[1,2], and recent research showed that plant viruses, like other pathogens and parasites, can induce changes in their hosts or vectors that can enhance their transmission[3,4]. The three-way interaction between virus, plant, and insect vector is complex. Plant viruses can directly and indirectly (via the host plant) modify the growth and development of their vectors. At the same time, feeding by viruliferous vectors can affect plant defense responses, inverse, plant also affects viruliferous vectors[5,6]. Both the plant virus and the vector may benefit from such changes from plants.

The whitefly *Bemisia tabaci* (Gennadius) is a phloem-feeding pest that causes serious damage by its direct feeding and by its transmission of plant viruses[7]. *B. tabaci* is a species complex consisting of morphologically indistinguishable biotypes that differ in feeding behavior, endosymbiont communities, insecticide resistance, virus transmission, or other properties[8–12]. *B. tabaci* B and Q are the two most invasive and harmful whiteflies; they have invaded nearly 60 countries and have caused massive agricultural losses during the past two decades[13].

Tomato yellow leaf curl virus (TYLCV) is a single-stranded DNA plant virus in the genus Begomovirus, family Geminiviridae. It is transmitted by *B. tabaci* in a persistent and circulative manner. TYLCV has recently become a serious threat to tomato production in many countries[14–16]. In China, TYLCV was not detected until *B. tabaci* Q became established in 2005, even though *B. tabaci* B is an important vector of TYLCV and has been found in China since the mid-1990's[10,17].

The concurrence of the spread of TYLCV with the invasion of *B. tabaci* Q rather than B suggests that the relationship between TYLCV and *B. tabaci* is more mutualistic for Q than B[6,10,12,18]. Our recent research showed that TYLCV indirectly benefits *B. tabaci* Q[6,18] but directly and indirectly harms *B. tabaci* B[6]. In this context, indirect benefit or harm is mediated by the host plant.

Plant-mediated interactions between plant pathogens and herbivorous arthropods are potentially important determinants of the population dynamics of both the pathogens and the arthropods in managed and natural ecosystems[19]. Plant defenses including those involving jasmonic acid (JA) and salicylic acid (SA) play key regulatory roles in the interaction of insects and their vectored viruses[20]. Proteinase inhibitor (PI) and other defense-related proteins are also inducible during insect feeding[21,22], and their related gene expression such as *PI II* in resisting insect herbivores have been well demonstrated[23–25]. Zhang et al. (2004)[24] showed that the expression of *PI II* gene is caused

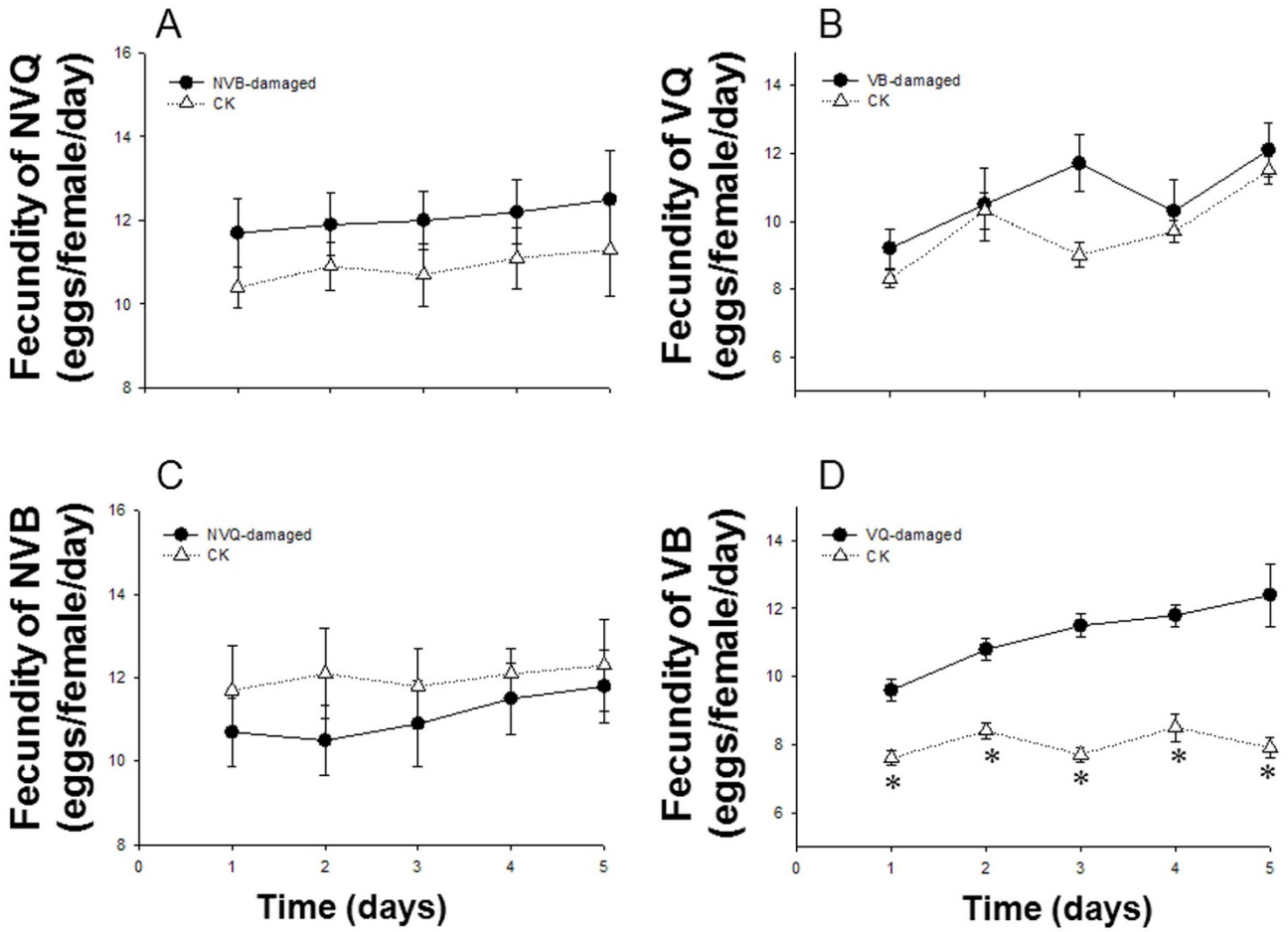

Figure 1 | Fecundity of *Bemisia tabaci* B and Q on tomato plants previously infested with nonviruliferous or viruliferous B and Q and on noninfested control plants (CK). The virus was TYLCV. (A). Fecundity of nonviruliferous Q (NVQ) on plants previously exposed to nonviruliferous B (NVB-damaged) and on noninfested plants (CK). (B). Fecundity of nonviruliferous B (NVB) on plants previously exposed to nonviruliferous Q (NVQ-damaged) and on noninfested plants (CK). (C). Fecundity of viruliferous Q (VQ) on plants previously exposed to viruliferous B (VB-damaged) and on noninfested plants (CK). (D). Fecundity of viruliferous B (VB) on plants previously exposed to viruliferous Q (VQ-damaged) and on noninfested plants (CK). Values are means ± SE. Asterisks indicate significant differences ($P < 0.05$).

by JA as a result of injury. Another study demonstrated that *LOX* gene is also involved in wound-induced JA biosynthesis such as aphids-infeston[25]. The prevailing view is that the SA pathway induces resistance against biotrophic pathogens and some phloem feeders, whereas the JA pathway induces resistance against chewing herbivores, some phloem-feeding insects, and necrotrophic pathogens[26]. Few studies, however, have investigated the role of JA or SA when a plant is simultaneously inoculated with an insect vector and virus[5,27]. Our latest study showed that SA content was always higher in leaves infested with viruliferous B than with viruliferous Q[5]. Furthermore, the relative gene expression associated with SA signaling was increased by the feeding of viruliferous B but not by the feeding of viruliferous Q[5]. Zhang et al. (2012)[27] demonstrated that co-infection of the begomovirus *tomato yellow leaf curl China virus* (TYLCCNV) and its betasatellite can repress JA-regulated defenses of tobacco against invasive whiteflies and accelerate population increases of the insects. Our recent study also showed that the interactions between tomato plant, TYLCV, and *B. tabaci* Q can reduce JA- and PI-associated plant defense[28]. However, the comprehensive understanding of plant-mediated interaction between viruliferous B and Q is still limited.

Several studies investigated how damage by herbivore feeding affects subsequent herbivore feeding because feeding may alter plant defenses[29–31]. Sarmento et al. (2011b)[32], for example, showed that *Tetranychus evansi* had reduced performance on plants that were previously attacked by its congener *Tetranychus urticae*. During the invasion and spread of TYLCV and *B. tabaci* Q in China, *B. tabaci* B and Q and TYLCV usually coexist on the same host plants. However, no study has evaluated the role of JA in the interactions among *B. tabaci* B and Q, TYLCV, and the host plant.

In the current study, we compared the performance of *B. tabaci* B and Q on plants previously attacked by *B. tabaci* Q and B that were or were not carrying TYLCV, respectively. The viral load in viruliferous B and Q infected leaves was also compared. We also quantified the endogenous JA level and PI activity and JA-related gene expression in healthy tomato plants or plants infested by nonviruliferous and viruliferous *B. tabaci* B and Q. Our goals were to determine how plant defense responses were differently affected by viruliferous *B. tabaci* B vs. Q vectors and how those responses affect vector performance.

Results

Fecundity and longevity of viruliferous and nonviruliferous Q and B on plants previously exposed to viruliferous and nonviruliferous B and Q. Nonviruliferous Q laid an average number of 12.060 and 10.880 eggs per day on plants that were previously infested by nonviruliferous B and on noninfested control plants ($F_{1,\ 18} = 2.413$, $P = 0.138$) (Fig. 1A). Nonviruliferous B laid an average number of 11.080 and 12.000 eggs per day on plants that were previously infested by nonviruliferous Q and on noninfested control plants ($F_{1,\ 18} = 1.119$, $P = 0.304$) (Fig. 1C). Viruliferous Q laid an average number of 10.760 and 9.760 eggs per day on plants that were previously infested by

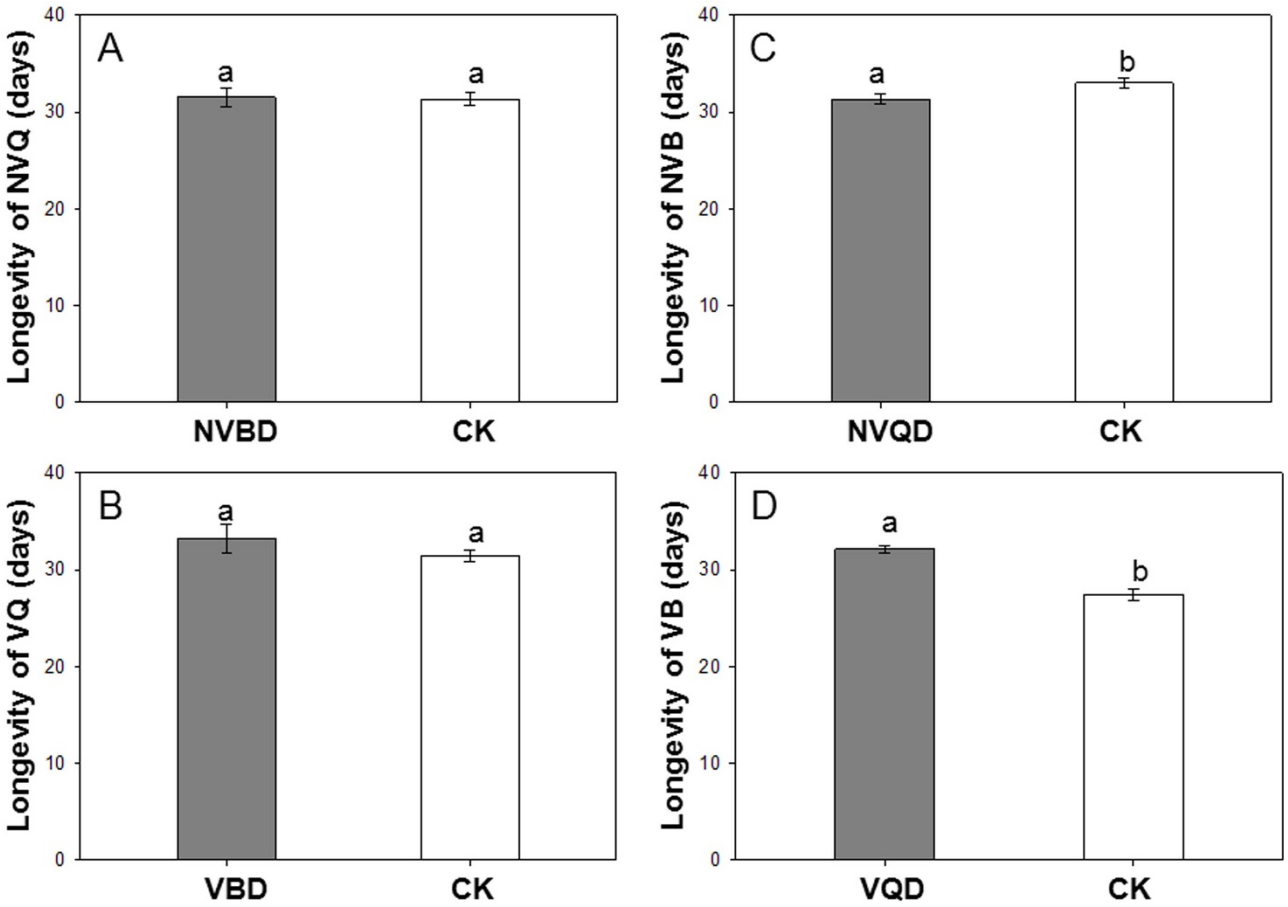

Figure 2 | Longevity of *Bemisia tabaci* B and Q on tomato plants previously infested with nonviruliferous or viruliferous B and Q and on noninfested control plants (CK). The virus was TYLCV. (A). Longevity of nonviruliferous Q (NVQ) on plants previously exposed to nonviruliferous B (NVBD) and on noninfested plants (CK). (B). Longevity of nonviruliferous B (NVB) on plants previously exposed to nonviruliferous Q (NVQD) and on noninfested plants (CK). (C). Longevity of viruliferous Q (VQ) on plants previously exposed to viruliferous B (VBD) and on noninfested plants (CK). (D). Longevity of viruliferous B (VB) on plants previously exposed to viruliferous Q (VQD) and on noninfested plants (CK). Values are means ± SE. Within each panel, different letters indicate significant differences ($P < 0.05$).

viruliferous B and on control plants ($F_{1,\ 18} = 2.468$, $P = 0.134$) (Fig. 1B). In contrast, viruliferous B laid an average number of 11.220 and 8.020 eggs per day on plants that were previously infested by viruliferous Q and on noninfested control plants ($F_{1,\ 18} = 80.616$, $P < 0.001$) (Fig. 1D). Only viruliferous B laid significantly more eggs on plants that were previously infested by viruliferous Q than on noninfested control plants.

The longevity of nonviruliferous Q was 31.500 and 31.300 days on plants previously infested by nonviruliferous B and on noninfested control plants ($F_{1,\ 18} = 1.220$, $P = 0.866$) (Fig. 2A). The longevity of viruliferous Q was 33.200 and 31.400 days on plants previously infested by viruliferous B and on noninfested control plants ($F_{1,\ 18} = 6.787$, $P = 0.291$) (Fig. 2B). The longevity of nonviruliferous B was 31.300 and 33.000 days on plants previously infested by nonviruliferous Q and on noninfested control plants ($F_{1,\ 18} = 0.061$, $P = 0.029$) (Fig. 2C). The longevity of viruliferous B was 32.100 and 27.400 days on plants previously infested by viruliferous Q and on noninfested control plants ($F_{1,\ 18} = 2.866$, $P < 0.001$) (Fig. 2D). Nonviruliferous B lived shorter on plants previously infested by nonviruliferous Q than on noninfested control plants. On the contrary, viruliferous B lived longer on plants previously infested by viruliferous Q than on noninfested control plants.

Viral load in viruliferous whiteflies-infected and healthy tomato plants. After 7-day different biotypes inoculation, there wasn't significant TYLCV symptom on leaves. However, the viral load was highest in viruliferous Q-infected leaves than in leaves infested with viruliferous B ($F_{2,\ 33} = 82.824$, $P < 0.001$) (Fig. 3).

JA content in leaves infested by viruliferous and nonviruliferous B and Q. The JA content was highest in control leaves, lowest in leaves

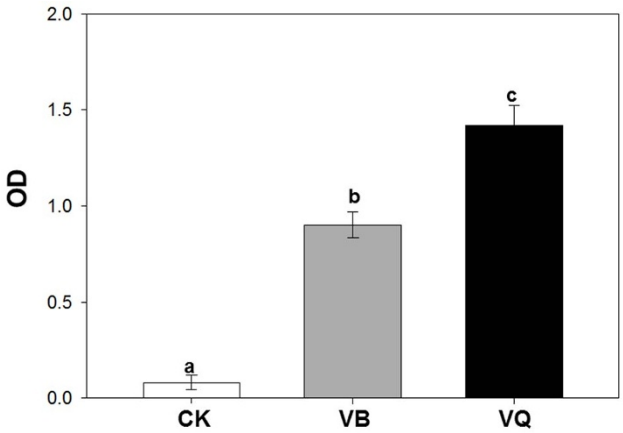

Figure 3 | Viral load in viruliferous whiteflies-infected and healthy tomato plants. VQ: Leaves were infested with viruliferous Q for 7 days; VB: Leaves were infested with viruliferous B for 7 days; CK: Leaves were not infested with whiteflies. Values are means ± SE. Different letters indicate significant differences ($P < 0.05$).

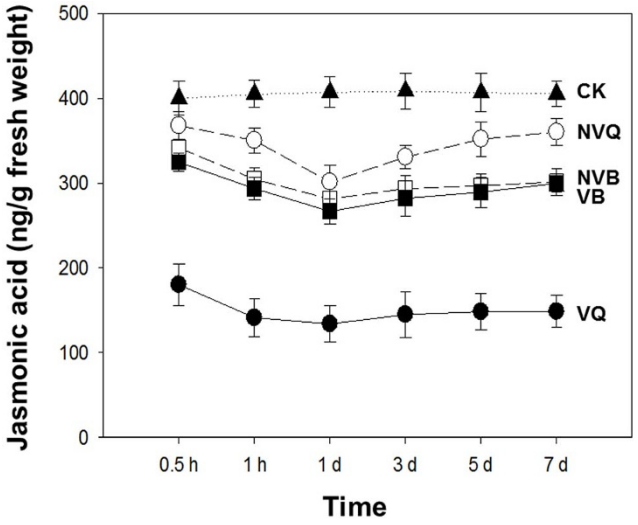

Figure 4 | JA content of tomato leaves without *Bemisia tabaci* infestation or after infestation (for 0.5 h to 7 d) with viruliferous or nonviruliferous *Bemisia tabaci* B and Q. The virus was TYLCV. NVQ: Leaves were infested with nonviruliferous Q; NVB: Leaves were infested with nonviruliferous B; VQ: Leaves were infested with viruliferous Q; VB: Leaves were infested with viruliferous B; CK: Leaves were not infested with whiteflies or virus. Values are means ± SE. Different letters indicate significant differences ($P < 0.05$).

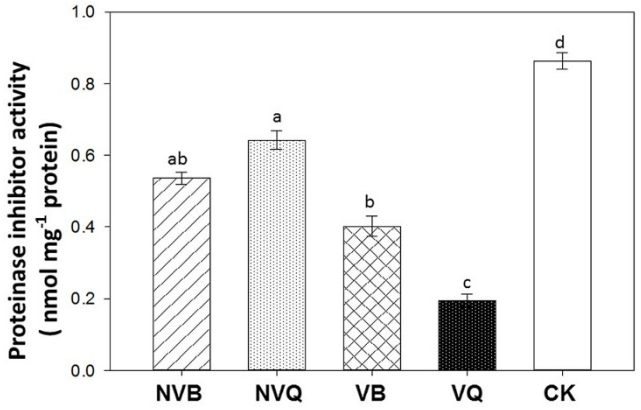

Figure 5 | Proteinase inhibitor activity in tomato leaves without *Bemisia tabaci* infestation or after infestation (for 1 day) with viruliferous or nonviruliferous *Bemisia tabaci* B and Q. The virus was TYLCV. NVB: non-viruliferous B; VB: viruliferous B; NVQ: nonviruliferous Q; VQ: viruliferous Q; CK: noninfested leaves. Values are means ± SE. Different letters indicate significant differences ($P < 0.05$).

infested with viruliferous Q, and intermediate in leaves infested with nonviruliferous B and Q whiteflies ($F_{4,\ 40} = 348.001$, $P < 0.001$) (Fig. 4). JA titers were much lower in leaves infested with viruliferous Q than with nonviruliferous Q. However, JA titers did not differ in leaves infested with viruliferous and nonviruliferous B (Fig. 4). For example, at 1 d infestion, the JA content in leaves infested with nonviruliferous B, nonviruliferous Q, viruliferous B and viruliferous Q was 281.333, 301.333, 266.667 and 134.000 ng/g FW, respectively.

PI activity in leaves infested by nonviruliferous and viruliferous B and Q. The proteinase inhibitor activity in leaves noninfested and in leaves infested with nonviruliferous B, nonviruliferous Q, viruliferous B and viruliferous Q was 0.536, 0.642, 0.401, 0.195 and 0.863 nmol mg^{-1} protein, respectively. PI activity was highest in the control leaves and was lower in leaves infested with viruliferous Q than with nonviruliferous Q ($F_{4,\ 40} = 62.567$, $P < 0.001$) (Fig. 5). PI activity tended to be lower in leaves that were infested with viruliferous B than with nonviruliferous B. PI activity was significantly lower in leaves infested with viruliferous Q than with viruliferous B (Fig. 5).

Gene expression in leaves infested by viruliferous and nonviruliferous B and Q. The relative expression of *LOX* was lower in leaves that were infested with viruliferous Q than with nonviruliferous Q and was lowest in the control ($F_{4,\ 10} = 11.830$, $P = 0.001$) (Fig. 6). *LOX* expression was significantly lower in leaves infested with viruliferous Q than with nonviruliferous B and Q.

The expression of *PI II* was highest in the control, lowest in leaves infested with viruliferous Q, and intermediate in leaves infested with nonviruliferous Q, nonviruliferous B, and viruliferous B ($F_{4,\ 10} = 21.695$, $P < 0.001$) (Fig. 6).

Discussion

Bemisia tabaci and its associated begomoviruses have caused serious economic losses in many parts of the world[33]. The indirect losses resulting from virus transmission far surpass the direct losses resulting from feeding. In recent years, *B. tabaci* Q has invaded China[34] and has now displaced B as the predominant *B. tabaci* in most parts of the country[17]. Concurrent with the spread of *B. tabaci* Q, TYLCV outbreaks have caused great damage to tomato production in many provinces in China[10]. In this study, we determined how the interaction between *B. tabaci* B and Q is affected by their virus status. The results showed that when plants were first damaged by nonviruliferous B and Q, the performance of the *B. tabaci* that subsequently infested the plants was not affected. However, the fecundity and longevity of viruliferous B were significantly higher on plants that had been previously infested by viruliferous Q than on noninfested plants. The fecundity and longevity of viruliferous Q were not affected on plants that were previously attacked by viruliferous B and on noninfested plants. In other words, the feeding by nonviruliferous *B. tabaci* did not affect the performance of *B. tabaci* that followed, but the feeding of viruliferous Q enhanced the performance of viruliferous B that followed. In addition, the viral load was significantly higher in viruliferous Q-infected leaves than in viruliferous B-infected leaves at 7 days after the initial inoculation. We concluded that the interactions between *B. tabaci* B and Q were differentially altered by TYLCV depending on which biotypes of *B. tabaci* initially infested the host plant. Most importantly, the results indicated that infestation by viruliferous *B. tabaci* Q reduced the plant's defense against subsequent infestation by *B. tabaci.*

Recent studies showed that the defense provided by the JA signaling pathway against phloem-feeding insects can be reduced by insect-vectored viruses[27,35,36]. For example, Lewsey et al. (2010)[36] indicated that infection of plants with *cucumber mosaic virus*, which is transmitted by the aphid *Myzus persicae*, strongly inhibited JA-regulated gene expression. Our recent study also showed that viruliferous Q reduced the JA content to a lower level than nonviruliferous Q[28]. Furthermore, our study determined that the endogenous JA content of leaves was greatly reduced by infestation with viruliferous Q but only moderately reduced by infestation with nonviruliferous Q, nonviruliferous B, or viruliferous B. We concluded that the co-infection of TYLCV and *B. tabaci* Q rather than B could reduce JA production and promote the spread of both Q and TYLCV.

Many reports documented antagonistic interactions between the SA and JA pathways[37,38]. For example, application of exogenous SA reduced the JA-dependent defense response[39–41]. Evidence also indicated that SA accumulation was induced by phloem-feeding insects[40,42]. Our previous study showed that feeding by viruliferous *B. tabaci* B induced higher levels of endogenous SA than feeding by nonviruliferous B but that feeding by viruliferous Q did not induce higher levels of endogenous SA than feeding by nonviruliferous Q[5,28].

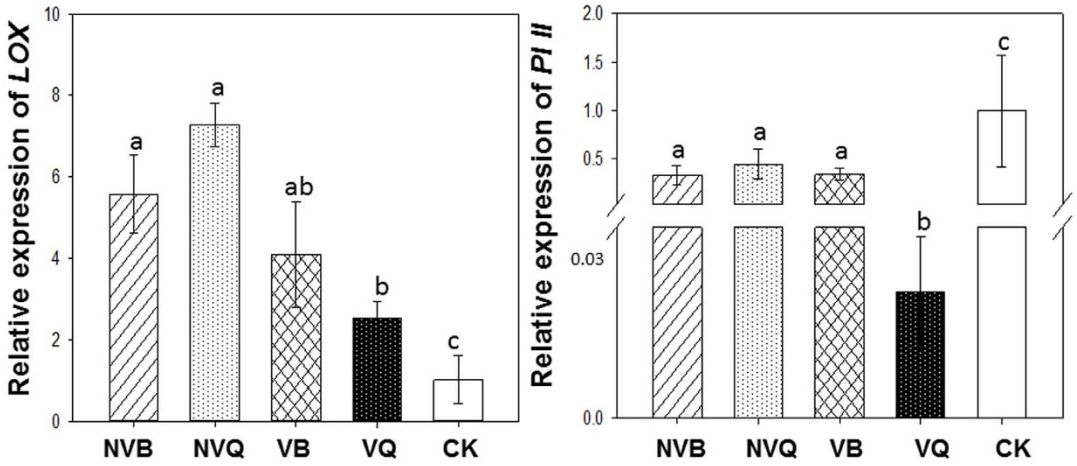

Figure 6 | Relative expression of *LOX* and *PI II* genes in tomato leaves without *Bemisia tabaci* infestation or after infestation (for 1 day) with viruliferous or nonviruliferous *Bemisia tabaci* B and Q. The virus was TYLCV. Values are normalized to *ACT* and *UBI*. NVB: non-viruliferous B; VB: viruliferous B; NVQ: nonviruliferous Q; VQ: viruliferous Q; CK: noninfested leaves. Values are means ± SE. Different letters indicate significant differences ($P < 0.05$).

According to this previous study and the current study, feeding by viruliferous Q greatly reduced JA content but did not increase SA content relative to feeding by nonviruliferous Q. Overall, viruliferous Q seems to have the ability to suppress plant defenses.

An increase in PI activity contributes to the plant defense response against insects and pathogens, and JA is known to mediate the induction of PIs[43]. The previous study showed that PI activity in leaves infested by viruliferous Q was lower than the activity in leaves infested by nonviruliferous Q[8]. Most importantly, our study demonstrated that the PI activity in leaves infested by viruliferous Q was lower than in leaves infested by viruliferous B and was much lower than in noninfested leaves. There was also a positive correlation between JA content and PI activity, i.e., both JA content and PI activity were significantly reduced by viruliferous Q. This result provides additional evidence that viruliferous Q can suppress JA-related plant defenses.

The JA-responsive upstream gene *LOX* and downstream gene *PI II* are two important genes in JA signaling pathway[24,25,43–46]. Our results showed that *LOX* was induced and *PI II* was reduced, which are consistent with previous study indicating that the upstream gene *LOX2* was induced, whereas the downstream gene *VSP1* was reduced after *B. tabaci* nymph infestion[45]. For example, *LOX2* transcript was significantly induced by *B. tabaci* nymph feeding at 14 d but not at 7 d after infestation[45]. In our study, the gene expression of *LOX* was induced at 1 d after infestation, so we speculate that there may be a different process of gene response in tomato and *Arabidopsis*. Besides, the JA-related defense response induced by adults may be different from nymphs because nymphs have a long-term interaction with plants whereas adults may induce a transient response. Furthermore, our data indicated that JA pathway was differently manipulated by nonviruliferous and viruliferous *B. tabaci* B and Q. The gene expression was always reduced to a lower level by infestion of viruliferous Q than by infestion of nonviruliferous Q, nonviruliferous B, and viruliferous B, regardless of *LOX* or *PI II*.

The effects of viruliferous *B. tabaci* Q can be explained in several related ways. First, TYLCV infection may reduce levels of carbohydrates and amino acids in leaves and in the phloem in ways that reduce plant quality for viruliferous B but not for viruliferous Q. Second, the feeding behaviors of *B. tabaci* B and Q may differ. Our recent study showed that when nonviruliferous whiteflies fed on healthy and TYLCV-infected tomato plants, *B. tabaci* Q engaged in more phloem salivation and phloem sap ingestion than *B. tabaci* B[12]. Third, virus infection could change the primary and secondary compounds produced by the host plant, which in turn could differentially affect the performance of *B. tabaci* B and Q. Further experiments are needed to investigate the biochemical and physiological mechanisms underlying the effects of TYLCV on *B. tabaci* B and Q.

In conclusion, we found that the interactions between *B. tabaci* B and Q were differentially altered by TYLCV and the JA-related plant defense response was reduced more by *B. tabaci* Q carrying TYLCV than by B carrying TYLCV. These different responses to TYLCV-carrying B and Q are likely to favor the spread of Q and TYLCV in China. More research is needed to clarify the molecular mechanisms by which viruliferous *B. tabaci* Q suppresses plant defense.

Methods

Host plants. Tomato plants (*Lycopersicon esculentum*, cv. Zhongza 9) were grown in pots containing a mixture of vermiculite and organic fertilizer at 25 ± 1°C, 60 ± 100% RH, and L14: D10 in a greenhouse. TYLCV-infected plants were produced by *Agrobacterium tumefaciens*-mediated inoculation at the 3–4 true-leaf stage with a cloned TYLCV genome (GenBank accession ID: AM282874) that was originally isolated from Shanghai, China[46]. Viral infection of test plants was confirmed by characteristic leaf curl symptoms and by molecular analysis[10].

Establishment of viruliferous and nonviruliferous B and Q colonies. B populations were originally collected from infested cabbage (*Brassica oleracea*. cv. Jingfeng 1) in Beijing, China in 2004[10], and Q populations were originally collected from infested poinsettia (*Euphorbia pulcherrima* Wild. ex Klotz.) in Beijing, China in 2009[10]. We obtained viruliferous B and Q colonies by placing four TYLCV-infected tomato plants in each of two cages (60 × 60 × 60 cm). We then transferred 300 nonviruliferous B and Q adults to each of the two cages, one biotype per cage. We simultaneously established nonviruliferous B and Q colonies by transferring 300 nonviruliferous B and Q adults to cages with virus-free tomato plants, one biotype per cage. All colonies were maintained for more than six generations in greenhouses. The purity of these populations was monitored by sampling 20 adults per generation using the CAPS (cleavage amplified polymorphic sequence) molecular diagnostic technique and the molecular marker mitochondrial cytochrome oxidase I gene (*mtCOI*)[47].

Fecundity and longevity of viruliferous and nonviruliferous B and Q on plants previously exposed to viruliferous and nonviruliferous Q and B. Eighty tomato plants with eight true leaves were selected. Five leaves on each plant were placed in clip cages and each clip cage had 60 newly emerged nonviruliferous or viruliferous B or Q. In other words, each tomato plant was inoculated by 300 viruliferous or nonviruliferous whiteflies for seven days. Control plants with the same size were placed in five clip cages per plant without whiteflies. There were each of 10 plants with nonviruliferous B, nonviruliferous Q, viruliferous B, viruliferous Q respectively, and 40 noninfested control plants. After seven days, all whiteflies adults, instars and eggs were removed gently with a brush from the clip cages, and the control plants were also treated with a brush, and then one adult female (two days since eclosion) was placed in one of five clip cages on each plant. The adult females were either B or Q. For example, one viruliferous Q was added to one of five leaves on each of 10 plants previously exposed to viruliferous B and to one of five leaves on 10 non-infested control plants. Similarly, one viruliferous B was added to one of five leaves on each of 10 plants that had been previously exposed to viruliferous Q and to 10 non-infested

Table 1 | Primer sequences used for qPCR analysis

Gene	GeneBank accession no.	Primer sequence
LOX	NM_001247330	F: 5′-ACTCATCAGCACCGACATCG-3′ R: 5′-ACTCTCCAGAAAGAACTCCTGC-3′
PI II	K03291	F: 5′-CCTATTCAAGATGTCCCCGTTC-3′ R: 5′-GGGCAATCCAGAAGATGG-3′
ACT	BT013707	F: 5′-AGGCAGGATTTGCTGGTGATGATGCT-3′ R: 5′-ATACGCATCCTTCTGTCCCATTCCGA-3′
UBI	X58253	F: 5′-TCGTAAGGAGTGCCCTAATGCTGA-3′ R: 5′-CAATCGCCTCCAGCCTTGTTGTAA-3′

control plants. After one day, the whitefly on one leave was replaced with another leave of the five leaves. The same was done with nonviruliferous B and Q. The new eggs deposited on the leaves during the first 5 days were counted. The mean longevity of *B. tabaci* was calculated after all whiteflies had died.

Viral load with TAS-ELISA. After seven days inoculation by different whiteflies, the infested and non-infested tomato leaves were tested for TYLCV with a triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA). A kit supplied by Adgen Phytodiagnotics (Neogen Europe (Ayr), Ltd) was used. Leaf samples of 0.1 g were ground in 1 ml of extraction buffer. Each of the three treatments (viruliferous B-infested leaves; viruliferous Q-infested leaves, and non-infested leaves) was represented by 12 replicates. Absorbance was read with a spectrophotometer at the wavelength of 405 nm. The samples were considered positive for TYLCV when the mean optical density (OD) values at 405 nm were over three times that of the healthy controls.

JA content in leaves infested by viruliferous and nonviruliferous B and Q. Endogenous JA was quantified according to Flors et al. (2008)[48] and Huang et al. (2012)[49]. Tomato plants with eight true leaves were used. Six leaves with 50 adults (or no whiteflies) per leave on each plant were placed in clip cages. There were five treatments: nonviruliferous B, nonviruliferous Q, viruliferous B, viruliferous Q, or no whiteflies. After infestation times of 0.5 h, 1 h, 1 d, 3 d, 5 d, and 7 d, the clip cages and whiteflies within were removed, and the corresponding leaves were collected. The entire plant received the same treatment, and each treatment was represented by nine replicates. The leaves were frozen, and a 0.5-g sample was ground with 3 ml of 80% methanol and kept at −20°C for 12 h before an internal standard containing 6 μl of [9,10]-dihydro-JA (50 ng μl^{-1}) was added. The mixture was centrifuged at 7500 *g* for 10 min. The first supernatant was transferred and saved, and the precipitate was re-suspended in 2 ml of 100% methanol before the mixture was centrifuged again at 7500 *g* for 10 min. The first and second supernatants were combined and adjusted to pH 2.5–3.0 with 3 M HCl and then extracted with an equal volume of ethyl acetate. After the organic fraction was evaporated, the solid residue was re-suspended in 0.1 M acetic acid and loaded on a C18 column (Waters, Milford, MA, USA). The C18 column was then eluted, and the eluents were collected and evaporated. After esterification of the residue with excess diazomethane, the sample volume was adjusted to 50 μl with acetic acid and analyzed using GC/MS system (6890N/5973N; Agilent Technologies, Santa Clara, CA, USA) with a DB-5-MS column (30 m × 0.32 mm × 0.25 μm, J&W Scientific, Agilent Technologies). Endogenous JA and the internal standard were analyzed using full-scan mode.

PI activity in leaves infested by viruliferous and nonviruliferous B and Q. Tomato plants with 6–7 true leaves were used. Six leaves with 50 adults (or no whiteflies) per leave on each plant were placed in clip cages for one day. There were five treatments: nonviruliferous B, nonviruliferous Q, viruliferous B, viruliferous Q, or no whiteflies. The entire plant received the same treatment, and each treatment was represented by nine replicates. Leaf tissue of 600 mg was ground in liquid nitrogen, homogenized in 1 000 μl of extraction buffer (0.1 M Tris-HCl buffer, pH 8.2, and 20 mM $CaCl_2$; 1 : 3 w/v), and centrifuged at 17 200 *g* for 30 min at 4°C. A mixture containing 50 μl of the supernatant, 50 μl of trypsin (4.7×10^{-5} M), and 500 μl of extraction buffer was incubated at room temperature for 5 min. Controls include 500 μl of extraction buffer and 50 μl of trypsin. A 500 μl volume of the mixture was added to 500 μl of extraction buffer and 500 μl of Na-Benzoyl-D, L-arginine 4-nitroanilide hydrochloride (1.2 mM)[31]. Trypsin activity was detected at 410 nm with a spectrophotometer. The difference between the absorbance at 150 and 60 s was used to determine trypsin activity. Measurements were performed in triplicate for each sample, were converted to mg of trypsin inhibited per gram of protein[50], and were corrected for the dilution[51].

Gene expression levels in leaves infested by viruliferous and nonviruliferous B and Q. Tomato leaves in clip cages were treated the same as leaves used for determination of PI activity. To determine how *B. tabaci* infestation affected the JA pathway and its related PI genes, the expression levels of the upstream *LOX* gene[25,44,45] and the downstream *PI II* gene were measured[52,53] of the JA signal pathway and used *actin* (*ACT*) and *ubiquitin 3* (*UBI*)[54] as reference genes (Table 1). Total RNA was extracted from 0.2 g of treated or control leaves, and 1.0 μg of RNA was used to synthesize the first-strand cDNA using the PrimeScript® RT reagent Kit (Takara Bio, Tokyo, Japan) with gDNA Eraser (Perfect Real Time, TaKara, Shiga, Japan). The 25.0 μl reaction system containing 10.5 μl of ddH_2O, 1.0 μl of cDNA, 12.5 μl of SYBR® Green PCR Master Mix (TIANGEN, Beijing, China), and 0.5 μl of each primer. Relative quantities of RNA were calculated using the comparative cycle threshold (Ct) ($2^{-\Delta\Delta Ct}$) method[55,56]. Three biological replicates and four technical replicates were analyzed.

Statistical analyses. Repeated-measures ANOVAs were used to compare the daily fecundity of nonviruliferous and viruliferous B or Q on plants that had been previously exposed to nonviruliferous and viruliferous Q or B and on noninfested control plants. T-test was used to compare the longevity of nonviruliferous and viruliferous B or Q on plants that had been previously exposed to nonviruliferous and viruliferous Q or B and on noninfested control plants, respectively. One-way ANOVA was used to compare the viral load in leaves infested by viruliferous B and Q. One-way ANOVA was also used to compare the JA content, PI activity, and relative gene expression of *LOX* and *PI II* in control leaves and leaves infested by nonviruliferous and viruliferous B and Q. SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

1. Hohn, T. Plant virus transmission from the insect point of view. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 17905–17906 (2007).
2. Andret-Link, P. & Fuchs, M. Transmission specificity of plant viruses by vectors. *J. Plant Pathol.* **87**, 153–165 (2005).
3. Ingwell, L. L., Eigenbrode, S. D. & Bosque-Pérez, N. A. Plant viruses alter insect behavior to enhance their spread. *Sci. Rep.* **2**, 578 (2012).
4. Luan, J. B. *et al*. Suppression of terpenoid synthesis in plants by a virus promotes its mutualism with vectors. *Ecol. Lett.* **16**, 390–398 (2012).
5. Shi, X. B. *et al*. Plant virus differentially alters the plant's defense response to its closely related vectors. *PLoS One* **8**, e83520 (2013).
6. Pan, H. P. *et al*. Differential effects of an exotic plant virus on its two closely related vectors. *Sci. Rep.* **3**, 2230 (2013).
7. Brown, J. K., Frohlich, D. R. & Rosell, R. C. The sweetpotato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex? *Annu. Rev. Entomol.* **40**, 511–534 (1995).
8. Perring, T. M. The *Bemisia tabaci* species complex. *Crop Prot.* **20**, 725–737 (2001).
9. Jones, D. R. Plant viruses transmitted by whiteflies. *Eur. J. Plant Pathol.* **109**, 195–219 (2003).
10. Pan, H. P. *et al*. Rapid spread of *tomato yellow leaf curl virus* in China is aided differentially by two invasive whiteflies. *PLoS One* **7**, e34817 (2012).
11. Pan, H. P. *et al*. Factors affecting population dynamics of maternally transmitted endosymbionts in *Bemisia tabaci*. *PLoS One* **7**, e30760 (2012).
12. Liu, B. M. *et al*. Multiple forms of vector manipulation by a plant-infecting virus: *Bemisia tabaci* and *tomato yellow leaf curl virus*. *J. Virol.* **87**, 4929 (2013).
13. De Barro, P. J., Liu, S. S., Boykin, L. M. & Dinsdale, A. B. *Bemisia tabaci*: a statement of species status. *Annu. Rev. Entomol.* **56**, 1–19 (2011).
14. Czosnek, H. & Laterrot, H. A worldwide survey of *tomato yellow leaf curl viruses*. *Arch. Virol.* **142**, 1391–1406 (1997).
15. Moriones, E. & Navas-Castillo, J. *Tomato yellow leaf curl virus,* an emerging virus complex causing epidemics worldwide. *Virus Res.* **71**, 123–134 (2000).
16. Navas-Castillo, J., Fiallo-Olive, E. & Sanchez-Campos, S. Emerging virus diseases transmitted by whiteflies. *Annu. Rev. Phytopathol.* **49**, 219–248 (2011).
17. Pan, H. P. *et al*. Further spread of and domination by *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotype Q on field crops in China. *J. Econ. Entomol.* **104**, 978–985 (2011).
18. Chen, G. *et al*. Virus infection of a weed increases vector attraction to and vector fitness on the weed. *Sci. Rep.* **3**, 2253 (2013).
19. Stout, M. J., Thaler, J. S. & Thomma, B. P. H. J. Plant-mediated interactions between pathogenic microorganisms and herbivorous arthropods. *Annu. Rev. Entomol.* **51**, 663–689 (2006).
20. Thaler, J. S., Humphrey, P. T. & Whiteman, N. K. Evolution of jasmonate and salicylate signal crosstalk. *Trends Plant Sci.* **17**, 260–270 (2012).
21. Green, T. R. & Ryan, C. A. Wound-induced proteinase inhibitor in plant leaves: a possible defense mechanism against insects. *Science* **175**, 776–777 (1972).
22. Thaler, J. S., Karban, R., Ullman, D. E., Boege, K. & Bostock, R. M. Cross-talk between jasmonate and salicylate plant defense pathways: effects on several plant parasites. *Oecologia* **131**, 227–235 (2002).
23. Ryan, C. A. Protease inhibitors in plants: genes for improving defenses against insects and pathogens. *Annu. Rev. Phytopathol.* **28**, 425–449 (1990).
24. Zhang, H. Y., Xie, X. Z., Xu, Y. Z. & Wu, N. H. Isolation and functional assessment of a tomato proteinase inhibitor II gene. *Plant Physiol. Bioch.* **42**, 437–444 (2004).
25. Moran, P. J. & Thompson, G. A. Molecular responses to aphid feeding in *Arabidopsis* in relation to plant defense pathways. *Plant Physiol.* **125**, 1074–1085 (2001).
26. Tack, A. J. M. & Dicke, M. Plant pathogens structure arthropod communities across multiple spatial and temporal scales. *Funct. Ecol.* **27**, 633–645 (2013).
27. Zhang, T. *et al*. Begomovirus-whitefly mutualism is achieved through repression of plant defences by a virus pathogenicity factor. *Mol. Ecol.* **21**, 1294–1304 (2012).
28. Shi, X. B. *et al*. Three-way interactions between the tomato plant, *tomato yellow leaf curl virus*, and the whitefly *Bemisia tabaci* facilitate virus spread. *J. Econ. Entomol.* **107**, 000-000 (2014). (in press).

29. Sauge, M. H. *et al*. Genotypic variation in induced resistance and induced susceptibility in the peach–*Myzus persicae* aphid system. *Oikos* **113**, 305–313 (2006).
30. Poelman, E. H., Broekgaarden, C., Van Loon, J. J. A. & Dicke, M. Early season herbivore differentially affects plant defence responses to subsequently colonizing herbivores and their abundance in the field. *Mol. Ecol.* **17**, 3352–3365 (2008).
31. Sarmento, R. A. *et al.* A herbivorous mite down-regulates plant defence and produces web to exclude competitors. *PLoS One* **6**, e23757 (2011).
32. Sarmento, R. A. *et al.* A herbivore that manipulates plant defence. *Ecol. Lett.* **14**, 229–236 (2011).
33. Thompson, W. M. O. in *Introduction: whiteflies, geminiviruses and recent events. The whitefly, Bemisia tabaci (Homoptera: Aleyrodidae) interaction with geminivirus-infected host plants* (ed. Thompson, W. M. O.)1–13 (Springer Ltd., 2011).
34. Chu, D. *et al*. The introduction of the exotic Q biotype of *Bemisia tabaci* from the Mediterranean region into China on ornamental crops. *Fla. Entomol.* **89**, 168–174 (2006).
35. Zarate, S. I., Kempema, L. A. & Walling, L. L. Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. *Plant Physiol.* **143**, 866–875 (2007).
36. Lewsey, M. G. *et al.* Symptom induction and RNA silencing suppression by the *cucumber mosaic virus* 2b protein. *Plant Signal. Behav.* **5**, 705–708 (2010).
37. Koornneef, A. & Pieterse, C. M. Cross talk in defense signaling. *Plant Physiol.* **146**, 839–844 (2008).
38. Thaler, J. S., Humphrey, P. T. & Whiteman, N. K. Evolution of jasmonate and salicylate signal crosstalk. *Trends Plant Sci.* **17**, 260–270 (2012).
39. Koornneef, A. *et al*. Kinetics of salicylate-mediated suppression of jasmonate signaling reveal a role for redox modulation. *Plant Physiol.* **147**, 1358–1368 (2008).
40. Zhang, P. J. *et al*. Whiteflies interfere with indirect plant defense against spider mites in Lima bean. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 21202–21207 (2009).
41. Leon-Reyes, A. *et al*. Salicylate-mediated suppression of jasmonate-responsive gene expression in *Arabidopsis* is targeted downstream of the jasmonate biosynthesis pathway. *Planta* **232**, 1423–1432 (2010).
42. Mohase, L. & van der Westhuizen, A. J. Salicylic acid is involved in resistance responses in the Russian wheat aphid-wheat interaction. *J. Plant Physiol.* **159**, 585–590 (2002).
43. Turner, J. G., Ellis, C. & Devoto, A. The jasmonate signal pathway. *Plant Cell* **14**, S153–S164 (2002).
44. Sivasankar, S., Sheldrick, B. & Rothstein, S. J. Expression of allene oxide synthase determines defense gene activation in tomato. *Plant Physiol.* **122**, 1335–1342 (2000).
45. Zhang, P. J. *et al*. Feeding by whiteflies suppresses downstream jasmonic acid signaling by eliciting salicylic acid signaling. *J. Chem. Ecol.* **39**, 612–619 (2013).
46. Yan, L. H. *et al*. Role of tomato lipoxygenase D in wound-induced jasmonate biosynthesis and plant immunity to insect herbivores. *PLoS Genet.* **9**, e1003964 (2013).
47. Wu, J. B., Dai, F. M. & Zhou, X. P. First report of *tomato yellow leaf curl virus* in China. *Ann. Appl. Biol.* **155**, 439–448 (2006).
48. Chu, D., Wan, F. H., Zhang, Y. J. & Brown, J. K. Change in the biotype composition of *Bemisia tabaci* in shandong province of China from 2005 to 2008. *Environ. Entomol.* **39**, 1028–1036 (2010).
49. Flors, V. *et al*. Interplay between JA, SA and ABA signaling during basal and induced resistance against *Pseudomonas syringae* and *Alternaria brassicicola*. *Plant J.* **54**, 81–92 (2008).
50. Huang, L. C. *et al*. Lower incidence and severity of tomato virus in elevated CO_2 is accompanied by modulated plant induced defense in tomato. *Plant Biology* **14**, 905–913 (2012).
51. Kant, M. R., Ament, K., Sabelis, M. W., Haring, M. A. & Schuurink, R. C. Differential timing of spider mite-induced direct and indirect defenses in tomato plants. *Plant Physiol.* **135**, 483–495 (2004).
52. Kakade, M. L., Rackis, J. J., McGhee, J. E. & Puski, G. Determination of trypsin-inhibitor activity of soy products–collaborative analysis of an improved procedure. *Cereal. Chem.* **51**, 376–382 (1974).
53. Ei Oirdi, M. *et al*. *Botrytis cinerea* manipulates the antagonistic effects between immune pathways to promote disease development in tomato. *Plant Cell* **23**, 2405–2021 (2011).
54. Peña-Cortés, H., Fisahn, J. & Willmitzer, L. Signals involved in wound-induced proteinase inhibitor II gene expression in tomato and potato plants. *Proc. Natl. Acad. Sci. U. S. A.* **92**, 4106–4113 (1995).
55. Mascia, T., Santovito, E., Gallitelli, D. & Cillo, F. Evaluation of reference genes for quantitative reverse-transcription polymerase chain reaction normalization in infected tomato plants. *Mol. Plant Pathol.* **11**, 805–816 (2010).
56. Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. *Methods* **25**, 402–408 (2001).

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Author contributions

Y.J.Z., X.B.S., H.P.P. designed the experiment. X.B.S., H.Y.Z., Y.F., G.C. performed the experiment. X.G.J., W.X., Q.J.W., S.L.W., X.G.Z. contributed reagents/materials. X.B.S., H.P.P., Y.J.Z. wrote the paper.

Additional information

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