Virus infection of a weed increases vector attraction to and vector fitness on the weed

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Weeds are important in the ecology of field crops, and when crops are harvested, weeds often become the main hosts for plant viruses and their insect vectors. Few studies, however, have examined the relationships between plant viruses, vectors, and weeds. Here, we investigated how infection of the weed Datura stramonium L. by tomato yellow leaf curl virus (TYLCV) affects the host preference and performance of the TYLCV vector, Bemisia tabaci (Gennadius) Q. The results of a choice experiment indicated that B. tabaci Q preferentially settled and oviposited on TYLCV-infected plants rather than on healthy plants. In addition, B. tabaci Q performed better on TYLCV-infected plants than on healthy plants. These results demonstrate that TYLCV is indirectly mutualistic to B. tabaci Q. The mutually beneficial interaction between TYLCV and B. tabaci Q may help explain the concurrent outbreaks of TYLCV and B. tabaci Q in China.

Plant–pathogen–vector systems are characterized by complex direct and indirect interactions1,2. The effects of the virus-infected plant on the vectors can be deleterious3, neutral4, or beneficial1,5 depending on the combination of virus, vector, and plant. Although many studies have been conducted on crop–pathogen–vector systems, research regarding the interactions between viruses, vectors, and weed is lacking. Weeds, however, are important components of crop ecosystems. When crops are harvested, for example, weeds often become the main host of plant viruses and their insect vectors.

Datura stramonium L. (Solanaceae) is a summer weed that originated in the Americas and has a worldwide distribution6. D. stramonium can produce 100 seed capsules per plant, and each capsule contains 200–300 seeds7. Seeds are dormant at maturity8 and may remain viable for 39 years in the soil9. With its rapid growth and high requirements for water, light, and nutrients, D. stramonium is highly competitive10–12. D. stramonium is also a host for many plant viruses, including tomato yellow leaf curl virus (TYLCV)13.

TYLCV is a single-stranded DNA (ssDNA) plant virus in the genus Begomovirus, family Geminiviridae. It causes an economically important disease of the Solanaceae in many tropical and subtropical regions worldwide14,15. In China, TYLCV has been documented in 11 provinces16. This exotic virus was first detected in symptomatic tomato plants in March 2006 in Shanghai, China17. Since then, it has spread north to Heilongjiang, Liaoning, Neimenggu, Hebei, Beijing, Shandong, Shanxi, Jiangsu, Zhejiang, and Hubei provinces, where it has caused extensive damage to tomato crops18. TYLCV is transmitted by the whitefly Bemisia tabaci (Homoptera: Aleyrodidae) in a circulative and persistent manner13,18,19. B. tabaci is transmitted by the whitely Bemisia tabaci (Homoptera: Aleyrodidae) in a circulative and persistent manner13,18,19.

Bemisia tabaci (Gennadius) is an important crop pest worldwide. It damages crops by direct feeding and by transmitting plant viruses20. The most severe damage results from its transmission of begomoviruses. To date, more than 200 species of begomoviruses are known to be exclusively transmitted by B. tabaci21,22. B. tabaci has been regarded as a species complex consisting of many biotypes that are morphologically indistinguishable but that differ in host range, virus transmission, insecticide resistance, or the symbionts that they harbor23,24,25,26. The two most invasive and destructive biotypes, B. tabaci biotype B (hereafter referred to as B) and biotype Q (hereafter referred to as Q), are regarded as the Middle East–Minor Asia 1 and the Mediterranean genetic group, respectively27.

In China, B. tabaci was first recorded in the late 1940s28. The crop damage and economic losses caused by this pest, however, did not become serious until the introduction of B. tabaci B in the mid-1990s29. Since then, B has spread rapidly across the entire country and has caused serious losses to many crops30. As a new invasive whitefly,
Q was first found in Yunnan Province of China in 2003\textsuperscript{30}. During the past several years, Q has gradually displaced earlier well-established B populations in field and protected crop systems and has become the dominant \textit{B. tabaci} in most parts of China\textsuperscript{31,32}.

The concurrence of the spread of TYLCV with the invasion of Q rather than B suggests a more mutualistic relationship between TYLCV and Q than B. We have recently demonstrated that Q is a better vector of TYLCV than B\textsuperscript{16} in that Q acquires significantly more viral DNA than the B and attains the maximum viral load in a substantially shorter time. Although TYLCV is transmitted horizontally by both whiteflies, transmission frequencies are higher with Q than B\textsuperscript{44}. We hypothesize that weed hosts are important in the epidemiology of TYLCV. To test this hypothesis, we investigated whether \textit{B. tabaci} Q prefers to settle and oviposit on TYLCV-infected rather than on healthy \textit{D. stramonium} plants. We also compared the fitness of \textit{B. tabaci} Q when feeding on TYLCV-infected vs. healthy \textit{D. stramonium}.

**Results**

**Symptoms and viral load in TYLCV-infected and healthy \textit{D. stramonium}**. Compared to the healthy \textit{D. stramonium} plants (Fig. 1A), TYLCV-infected plants exhibited yellow leaf curling and stunting (Fig. 1B). The viral load was significantly higher in the TYLCV-infected plants than in the healthy plants ($F_{1, 22} = 2545.161, df = 22, P < 0.0001$, Fig. 2).

**Whitefly settling and oviposition preference**. More Q adults settled on TYLCV-infected plants than on healthy plants ($F_{1, 16} = 7591.451, P < 0.0001$) (Fig. 3). Oviposition was greater on TYLCV-infected plants than on healthy plants ($F_{1, 22} = 83.223, P < 0.001$) (Fig. 4).

**The fitness of \textit{Bemisia tabaci} Q on TYLCV-infected and healthy \textit{D. stramonium}**. Although virus infection of \textit{D. stramonium} plants did not affect whitefly egg to adult development time ($F_{1, 22} = 0.744, P = 0.398$) (Fig. 5A), it did affect other whitefly fitness parameters. TYLCV infection of \textit{D. stramonium} plants increased female body length ($F_{1, 98} = 10.587, P = 0.002$) (Fig. 5B), male body length ($F_{1, 98} = 7.005, P = 0.009$) (Fig. 5C), survival (egg to adult) ($F_{1, 22} = 7.476, P = 0.012$) (Fig. 5D), longevity ($F_{1, 58} = 32.715, P < 0.001$) (Fig. 5E), and fecundity ($F_{1, 58} = 39.435, P < 0.001$) (Fig. 5F).

**Discussion**

Virus–vector relationships can range from parasitic to mutualistic, depending on whether the effect of a virus on its vector is deleterious, neutral, or beneficial. Given the concurrent outbreak and spread of TYLCV with the invasion of Q rather than B, we tested the hypothesis that the relationship between TYLCV and Q in China is mutualistic.

This hypothesis was supported by the results of the current study. First, TYLCV infection of a host plant (\textit{D. stramonium}) indirectly improved the overall performance (in terms of survival, fecundity, longevity, and female and male body size) of Q (Fig. 5). Second, \textit{B. tabaci} Q preferred to settle and oviposit on TYLCV-infected plants rather than on healthy plants (Fig. 3 and Fig. 4). Additional evidence of a mutualistic relationship between \textit{B. tabaci} Q and TYLCV was provided by Pan et al. (2012)\textsuperscript{16}, who reported that \textit{B. tabaci} Q transmits TYLCV with high efficiency.

These findings are consistent with previous reports that pathogens and parasites can induce changes in their hosts that influence host–vector interactions\textsuperscript{33,34}. Through these studies, we know that vectors of persistently transmitted viruses tend to be attracted to infected host plants and that persistently transmitted viruses tend to improve host plant quality for the vectors and to promote long-term feeding of the vector on the host plant\textsuperscript{44}. The status of the vector (viruliferous or not) can also influence its behavior in a way that benefits the virus. In a recent study, we conducted two experiments testing the effect of TYLCV (a persistently transmitted virus) infection of the host (tomato) on vector (\textit{B. tabaci} B and Q) feeding behavior using an electrical penetration graph (EPG). \textit{B. tabaci} B and Q both appeared...
to find TYLCV-infected plants more attractive than healthy plants, probing them more quickly and exhibiting a greater number of feeding bouts; TYLCV infection of host plant did not, however, alter the total time spent feeding. Interestingly, viruliferous whiteflies fed more readily than nonviruliferous whiteflies and spent more time salivating into sieve tube elements. Because vector salivation is essential for viral transmission, this virus-mediated alteration of behavior should directly benefit TYLCV fitness.

In addition, virus infection may alter plant volatiles in ways that might be beneficial or detrimental to the herbivorous vectors of the virus. In a preliminary experiment, the percentage of 1–dodecanol was significantly lower in the volatiles of TYLCV-infected vs. healthy D. stramonium plants, and squalene was only detected in the TYLCV-infected plants (GC, unpublished data). Further experiments are needed to investigate how virus-induced changes in plant volatiles affect host selection by B. tabaci Q.

That Q whiteflies had greater survival, fecundity, body size, and longevity when feeding on TYLCV-infected D. stramonium than on healthy plants (Fig. 5) demonstrates the occurrence of an indirect mutualism between Q and TYLCV mediated by the host plant. Apparently, TYLCV infection alters the D. stramonium plants, and the resulting changes are beneficial to Q. Our results are consistent with the conclusion that vectors perform better on plants infected with persistently transmitted viruses. Infection by a virus could influence the preference and performance of its herbivorous vectors in several ways. First, virus infection often alters plant morphology,
and these changes could be beneficial or detrimental to herbivorous vectors. Second, virus infection can change the composition and percentage of free amino acids in the phloem or in other ways change the nutritional composition of the plant tissue and thereby change the fitness of herbivorous vectors. Finally, viruses can benefit a herbivorous vector by suppressing plant defenses against herbivores. Additional experiments are required to determine whether these mechanisms or other mechanisms explain why TYLCV infection generally improves the fitness of B. tabaci Q on D. stramonium.

Our previous research showed that TYLCV infection can improve the performance of its vector B. tabaci Q on tomato. Here, we have shown that TYLCV infection improves vector attraction to and fitness on its weed host D. stramonium. Overall, the results suggest that the indirect and direct interactions between TYLCV and B. tabaci Q
are mutually beneficial and may help explain the concurrent outbreaks and spread of TYLCV and *B. tabaci* in China.

**Methods**

**Bemisia tabaci** laboratory population. Since its collection in Beijing, China, in 2009, the *B. tabaci* Q population used in this study has been maintained on *Poinsettia* (*Euphorbia pulcherrima* Wild. ex. Klotz.) in isolated whitely-proof screen cages with natural lighting and controlled temperature (26±2°C) in a glasshouse. The purity of the population has been monitored by determining the cleavage amplified polymorphic sequence (CAPS) and mitochondrial cytochrome oxidase I genes (mtCOI) for 15 adults per generation.

Plant cultures and TYLCV inoculation. *Datura stramonium* L. was grown in a potting mix (a mixture of peat moss, vermiculite, organic fertilizer, and perlite) in isolated whitely-proof screen cages (60×40×80 cm; two plants per cage). About 300 adult whiteflies were collected between 07:00 and 09:00 am for each replicate in 24 cages with insects and plants drawn from the same pool, and the replicate cages were arranged in parallel with each side having six replicate cages (healthy vs. virus-infected plants) under natural lighting and ambient temperature (26±2°C) in a glasshouse. The length of each newly emerged adult was measured with a stereomicroscope (Leica, M205C). At the beginning, there were a total of 24 cages with insects and plants drawn from the same pool, and the replicate cages were arranged in parallel with each side having six replicate cages (healthy vs. virus-infected plants) under natural lighting and ambient temperature (26±2°C) in a glasshouse. 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.

Whitefly settling and oviposition preference. Experiments concerning whitely settling and oviposition preference were performed as described by Omondi et al. (2003). Briefly, individual plants of healthy and TYLCV-infected *D. stramonium* of approximately the same size were arranged in opposite corners of a screen cage (60×40×80 cm; two plants per cage). About 300 adult whiteflies were collected between 07:00 and 09:00 am for each replicate in 24 cages with insects and plants drawn from the same pool, and the replicate cages were arranged in parallel with each side having six replicate cages (healthy vs. virus-infected plants) under natural lighting and ambient temperature (26±2°C) in a glasshouse. 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.

Data analysis. Repeated-measures ANOVAs were used to compare the whitely settling preference on healthy and TYLCV-infected *D. stramonium* plants. One-way ANOVAs were used to compare whitely oviposition preference and life history parameters on the healthy and TYLCV-infected *D. stramonium* plants, and to compare the viral load in the healthy and TYLCV-infected *D. stramonium* plants. SPSS version 19.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. All proportional data were arcsine square root transformed before analyses.

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

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