Insect science

Insect Science (2017) 00, 1–10, DOI 10.1111/1744-7917.12476

ORIGINAL ARTICLE

Effects of *Aphidius gifuensis* on the feeding behavior and potato virus Y transmission ability of *Myzus persicae*

Ying-Qin He[1], Yong-Qiang Zhang[1], Juan-Ni Chen[1], Wen-Long Chen[2], Xian-Yi Zeng[1], Hai-Tao Chen[3] and Wei Ding[1]

[1]*College of Plant Protection, Southwest University, Chongqing, China;* [2]*Guizhou Key Laboratory of Plant Disease and Pest Management for Mountain Agriculture, Institute of Entomology, Guizhou University, Guiyang, China and* [3]*Chongqing Institute of Tobacco Science, Chongqing, China*

Abstract The stylet penetration behavior of aphids when feeding on plants is associated with virus acquisition and inoculation. *Aphidius gifuensis* (Ashmead) is a primary endoparasitoid of *Myzus persicae* (Sulzer) which is the most efficient vector of plant viruses. Information about the effects of parasitoid on aphid and virus transmission can provide an essential foundation for designing effective biological control strategies. This study aimed to investigate the effects of *A. gifuensis* on the feeding behavior and potato virus Y (PVY) transmission ability of *M. persicae*. The results showed that after *M. persicae* was parasitized by *A. gifuensis*, the duration of the first probe significantly decreased. Additionally, *A. gifuensis* exerted remarkable effects on aphid feeding in phloem ingestion. The contribution of the E1 waveform to the phloem phase was significantly higher in all parasitized aphids than in the control group. Although the time of infestation increased for parasitized aphids, the total duration of phloem sap ingestion decreased. Interestingly, the percentage of time *M. persicae* spent in the xylem and phloem phases only changed significantly on day 5. The percent transmission of PVY by the aphids parasitized on day 5 was lower than that in the control, but no significant differences were detected. The significance of this work is the demonstration that *A. gifuensis* can impede the feeding behavior of *M. persicae*, which sheds light on the biological basis of *A. gifuensis* as a natural enemy, but unfortunately does not provide an immediate solution for disrupting the transmission of PVY.

Key words Braconidae; electrical penetration graphs; feeding behavior; *Myzus persicae*; parasitoids; virus transmission

Introduction

Relationships between agents in multitrophic interactions are complicated. As the primary insect vector of plant viruses, the behavior of aphid is closely related to the spread of viruses (Dader *et al.*, 2012). Parasitic wasps have long been used in biological control to mitigate the damage caused by aphids in the agricultural field. Recent studies have shown that the presence of parasitoids can alter aphid behavior, which may indirectly affect virus transmission (Smyrnioudis *et al.*, 2001; Hodge & Powell, 2008). Hodge and Powell (2008) showed the dispersal behavior of *Acyrthosiphon pisum* parasitized by *Aphidius ervi* can affect and increase the disease spread. However, in *Macrosiphum euphorbiae* parasitized by *A. ervi*, no significant differences were detected in virus transmission in parasitized and unparasitized aphids (Calvo & Fereres, 2011). It can be seen that the specific combinations between parasitoid, aphid and virus may cause the differences among their interactions.

Correspondence: Wei Ding, College of Plant Protection, Southwest University, Chongqing 400715, China. Tel.: +86 13883219136; fax: +86 23 68250218; email: dingw@swu.edu.cn

The green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), is a generalist insect herbivore that can use a wide range of cultivated crops. It can develop high population densities on many economically important crops, such as fruit, cruciferous leaf vegetables and tobacco (Blackman & Eastop, 2000; Davis *et al.*, 2007). *Myzus persicae* damages crops directly by feeding on the vascular bundles of plants and indirectly through the transmission of numerous viral diseases (Loebenstein *et al.*, 2001). In the last decade, *M. persicae* has caused serious damage to tobacco in China. Potato virus Y (PVY), the agent of an important viral disease of tobacco, is a member of the Potyviridae family of viruses. The most efficient vector of PVY is *M. persicae*, which transmits the virus in a non-persistent manner during brief probes of the plant epidermis (Ragsdale *et al.*, 2001; Hussain *et al.*, 2016). *M. persicae* causes more damage as a PVY vector than it does when directly feeding on crops. Munyaneza (2015) suggested that the management of insect-transmitted plant pathogens is critical to minimize disease transmission and spread in crops. Thus, *M. persicae* should be managed to control the occurrence of viral diseases.

Insecticides are commonly used as control measures to protect crops from *M. persicae*. As a result of frequent chemical control, *M. persicae* has evolved numerous strategies to resist commonly used insecticides (e.g., imidacloprid) (Elbert *et al.*, 2008). Concurrently, environmental concerns have increased interest in alternative pest control methods. Therefore, other effective and environmentally friendly methods to control *M. persicae* should be identified. *Aphidius gifuensis* Ashmead (Hymenoptera: Braconidae) is a parasitic natural enemy that attacks agricultural pest aphids, including *M. persicae*, *Schizaphis graminum* (Rondani) and *Aulacorthum solani* (Kaltenbach) (Hemiptera: Aphididae) (Yan *et al.*, 2005; Ohta & Honda, 2010). *Aphidius gifuensis* exhibits a number of desirable parasitic attributes, including high host searching ability, high fecundity, a wide host range, and adaptation to a wide range of temperatures (Jones *et al.*, 2003; Ohta & Ohtaishi, 2004). Consequently, *A. gifuensis* is a predominant endoparasitoid attacking *M. persicae* in commercial crops (Yamamoto, 1997; Ohta & Ohtaishi, 2004).

Generally, when attacking their hosts, parasitoids lay their eggs on or in the body of an insect host, which is then used as food for the developing nymph. The host is ultimately killed. Before the actual death, survivors exposed to natural enemies may exhibit behavioral and/or physiological alterations. Mitsunaga *et al.* (2016) reported that the lifetime fecundity of *M. persicae* parasitized by *A. gifuensis* is 6.40 nymphs, which is lower than that of control (61.43 nymphs). Moreover, the growth of the wings of *M. persicae* is suppressed when it is parasitized (Liu & Hughes, 1984). Extensive information regarding the biological and ecological characteristics of the interactions between *A. gifuensis* and *M. persicae* is available, but few studies have examined the effects of these interactions on the feeding behavior of the aphids and their subsequent virus transmission efficiency. The feeding behavior of sap-sucking insects can be monitored electronically using electrical penetration graphs (EPGs) (Tjallingii, 1978, 1985, 1988). EPG signals reflect the stylet tip positions within plant tissues (Tjallingii & Hogen-Esch, 1993) and thus provide detailed information on the feeding activities of many sap-sucking insects, such as aphids, thrips, whiteflies, psyllids and mealybugs (Huang *et al.*, 2012; He *et al.*, 2015). It is known that most plant viruses are transmitted by insect vectors and are dependent on the feeding behavior and dispersal of their vectors for their spread among plants (Ng & Falk, 2006; Hogenhout *et al.*, 2008; Fereres & Moreno, 2009; Sandanayaka *et al.*, 2013). Therefore, the stylet penetration behavior of insects when feeding on plants is associated with virus acquisition and transmission. The common sense of a biological control agent in a pest/vector model system will generally be assumed as a way to control the target vector in order to reduce vector-borne disease in the field. Consequently, natural enemy-vector-virus interactions should be investigated to evaluate the continuing spread of insect-transmitted viruses.

In the present work, we studied the feeding behavior of parasitized *M. persicae* to investigate the direct effects of *A. gifuensis* on the feeding activities of aphids. In addition, potted plant experiments were performed to assess the potential indirect effects of *A. gifuensis* on the PVY transmission efficiency of *M. persicae*. We employed EPGs to monitor the behavioral variables of parameters of *M. persicae* feeding on tobacco plants. The information obtained in this paper may contribute to unravel the behavioral mechanisms of insect vectors underlying natural enemies.

Materials and methods

Insects

A strain of *A. gifuensis* was established from *M. persicae* mummies on tobacco leaves in our laboratory at the College of Plant Protection, Southwest University, Chongqing, China. The *M. persicae* specimens were originally collected from a tobacco farm in Chongqing (29°59′ N, 106°54′ E) in 2014 and were subsequently placed on

tobacco plants in a separate climate-controlled room. Stock cultures of *A. gifuensis* and *M. persicae* were maintained in separate insect-proof screened cages (400 × 400 × 400 mm) in the laboratory at 25 ± 1°C, 60% ± 10% RH, and a 16 : 8 L : D photoperiod. All insects were kept under laboratory conditions for more than 20 generations before the experiments.

Parasitism

Adult *A. gifuensis* females prefer the second- or third-instar nymph of aphids (Huang, 1982). Therefore, the second-instar nymphs of *M. persicae* were selected as the parasitic object in our experiment. Before the parasitism experiment, pairs of *A. gifuensis* were individually placed in glass vials to mate for 30 min. A successfully mated female *A. gifuensis* was then selected for the test. More than 2500 second-instar red *M. persicae* nymphs were placed on tobacco seedlings grown in 100 mm diameter plastic pots. Subsequently, the pots were individually placed in netted plastic cages (400 × 400 × 400 mm). When the aphids had settled on the leaves, the mated female *A. gifuensis* individuals were transferred into the cages for oviposition at a ratio of 50 aphids to one *A. gifuensis*. The parasitoids were removed after 24 h, and the aphids were maintained under the same conditions as described above. After set times (3, 4 and 5 d), parasitized aphids were identified under a binocular microscope. Unparasitized individuals were used as a control group under the same conditions.

PVY source plants

The tobacco veinal necrotic strain of PVY (PVY^N) was kindly provided by Shandong Agricultural University, China. We used the tobacco variety Yunyan87 at the four true-leaf stage. Tobacco plants infected with PVY were produced by mechanical inoculation of healthy seedlings with PVY^N using the method of Shrestha *et al.* (2014). Healthy plants were mock-inoculated to mimic the effects of mechanical inoculation. These virus sources were maintained inside climate chambers at 25 ± 1°C, 60% ± 10% RH and a 16 : 8 L : D photoperiod. After 12 days, tobacco plants that showed symptoms were used as the PVY inoculum for the transmission experiments.

EPG recording

The EPG waveforms were recorded using a DC-EPG Giga-4 instrument (EPG systems, Wageningen University, the Netherlands) with 10^9 Ohm input resistance on healthy tobacco plants in the four true-leaf stage. Before recording, apterous aphids were removed from healthy plants after parasitized by *A. gifuensis* on days 3, 4 and 5. Unparasitized aphids were used as the control group. Subsequently, the aphids were individually connected via their dorsum to a gold wire (15 μm diameter; 20–30 mm length) using a droplet of water-based silver glue and were connected to the input probe of the EPG. The other electrode was placed in the soil of each potted plant. The aphids were starved for 1 h between the time of wiring and the beginning of EPG monitoring and were then placed on the abaxial side of the youngest fully expanded leaf of the tobacco seedlings. The host plants, insects and EPG probes were maintained inside a Faraday cage to avoid electrical noises. EPGs were continuously recorded for 6 h with a fresh insect and a new host plant for each replicate. We recorded 15 aphids for each treatment. All experiments were performed at 25 ± 1°C and 60% ± 10% RH. The acquired data were recorded by Stylet+ for Windows software (EPG Systems, Wageningen University, the Netherlands), and all behavioral variables were processed using the EPG-Excel Data Workbook developed by Sarria *et al.* (2009).

PVY transmission experiments

This experiment aimed to detect the effect of *A. gifuensis* on the transmission of PVY from and to tobacco plants by parasitized *M. persicae*. We used PVY and the same aphid/plant combination previously described. Aphids parasitized on day 5 were selected as the test object, and unparasitized individuals were used as the control. In this study, we selected the tobacco variety Yunyan87 as the test plants, and plants inoculated with PVY were used as the virus source. All virus source plants and test plants were kept in wooden cages covered with aphid-proof netting in different compartments of a room at 25 ± 1°C and a photoperiod of 16 : 8 L : D. Plants with four expanded leaves were used in the transmission assays.

A total of 1200 *M. persicae* individuals were placed in glass vials for a 1 h starvation period. Batches of aphids were transferred to the upper side of a leaf on a virus source plant for a 10 min acquisition access period and subsequently moved onto the test plants (10 aphids per plant) for a 10 min inoculation access period. These aphids were removed from the test plants and killed by imidacloprid after the inoculation period. In each treatment, 20 tobacco test plants were used, and the assay was replicated three times. After the transmission assays, the test

plants were retained in the previously described cages for approximately 4 weeks until the visual inspection of symptoms.

Statistical analyses

The EPG waveforms were categorized as previously described (Tjallingii, 1994; Zeng *et al.*, 2016). Seven distinct waveforms were identified in this study: np, C, potential drop (pd), F, G, E1 and E2. The EPG signals were recorded and exported using the software PROBE 3.4 (Wageningen University, The Netherland) in Windows XP. In total, 17 non-phloem phase parameters and 11 phloem phase parameters were calculated and compared among the four treatments. Some EPG variables did not follow a normal distribution, and thus, normality and homogeneity of variance were assessed prior to analysis. The duration and percentage of each EPG variable were transformed using $\log_{10}(n + 1)$ and arcsine, respectively, to satisfy the assumption of normality. The feeding behavior data were statistically analyzed using one-way analysis of variance and the least significant difference test at a significance level of 0.05 (P-value = 0.05). Fisher's exact test was used to evaluate the significance in the difference in proportions of individuals performing each type of activity. The transmission rate of PVY was compared by an independent-samples t-test. All statistical analyses were done with IBM SPSS Statistics 17.0 (IBM Corp., Armonk, NY).

Results

Probing and feeding behavior of parasitized aphids

In the present study, as shown in Figure 1, four behavioral phases were identified during aphid probing. Each phase contained one or more waveforms: (i) non-probing (waveform np, which indicates the absence of stylet contact with the plant tissue); (ii) the pathway phase (including waveforms C, F and pd, which represent an intercellular stylet pathway, stylet penetration difficulty mechanics, and brief intracellular stylet punctures, respectively); (iii) the xylem phase (waveform G); and (iv) the phloem phase (including waveforms E1 and E2, which reflect saliva into the sieve element and passive phloem sap ingestion, respectively).

Parasitized aphids had different behavior than control aphids on several EPG variables related to pathway phase, xylem phase and phloem phase (Table 1). There were differences on the behavior depending on days of parasitization. Three and four days parasitized aphids increased the number of probes and short probes (C < 3 min) than that of control and aphids of 5 d parasitized, although these differences were statistically significant only on day 4 (Table 1, variables 2 and 3). No differences were observed in total duration of np, number of probes to the first E1, duration of F, number of G, time to first probe from the start of EPG, duration from the first C to the first pd, number of pd, mean duration of pd, and total duration of C waveforms among the groups (Table 1, variables 1, 5, 7 and 8, and Table S1, variables 1–5). Independently, on the day after the aphids had been parasitized, they had shorter duration of first probe than control aphids (Table 1, variable 4). Aphids on day 5 had increases in the number of F and duration of G (Table 1, variables 6 and 9).

A. gifuensis significantly affected the feeding activities of *M. persicae* in feeding on phloem phase compared with the control. Aphids parasitized on day 5 had decreases on the individual percent with E1 and E2 waveforms and on the duration of the first E (Table 1, variables 13 and 14). The time from first probe to first E2 did not differ among the four treatments (Table 1, variable 16). During the E1 period, the number of E1 occurrence and total duration of E1 waveforms were lower in unparasitized aphids than in parasitized aphids on days 3 and 4 (Table 1, variables 10 and 11). In addition, the contribution of time (percentage) of E1 to the phloem phase was also significantly lower in the control group than in the parasitized aphids (Table 1, variable 12). When *M. persicae* began to ingest phloem, the EPG exhibited a typical E2 waveform. The number of E2 waves was only reduced in aphids parasitized on day 5, whereas aphids parasitized on days 3 and 4 were increased compared with unparasitized ones (Table 1, variable 15). Additionally, the number of sustained E2 (>10 min) and total duration of E2 waveforms were reduced significantly in aphids parasitized on day 5 (Table 1, variables 17 and 18).

Compared with the control group, the percentage of time in the xylem phase significantly increased in aphids parasitized on1 day 5 (Fig. 2, % in G). Although the time of infestation increased for parasitized aphids, the percentage of time spent salivating in the phloem phase decreased (Fig. 2, % in E1). Additionally, the percentage of time spent ingesting in the phloem phase decreased gradually from 36.40% in the control aphids to 8.05% in the aphids parasitized on day 5 (Fig. 2, % in E2). There were no significant differences in percentage of time in the pathway phase among the four treatments (Fig. 2, % in C and F).

PVY transmission by aphids

The percent transmission of PVY by the aphids parasitized on day 5 was 22.67%, which was lower than that in

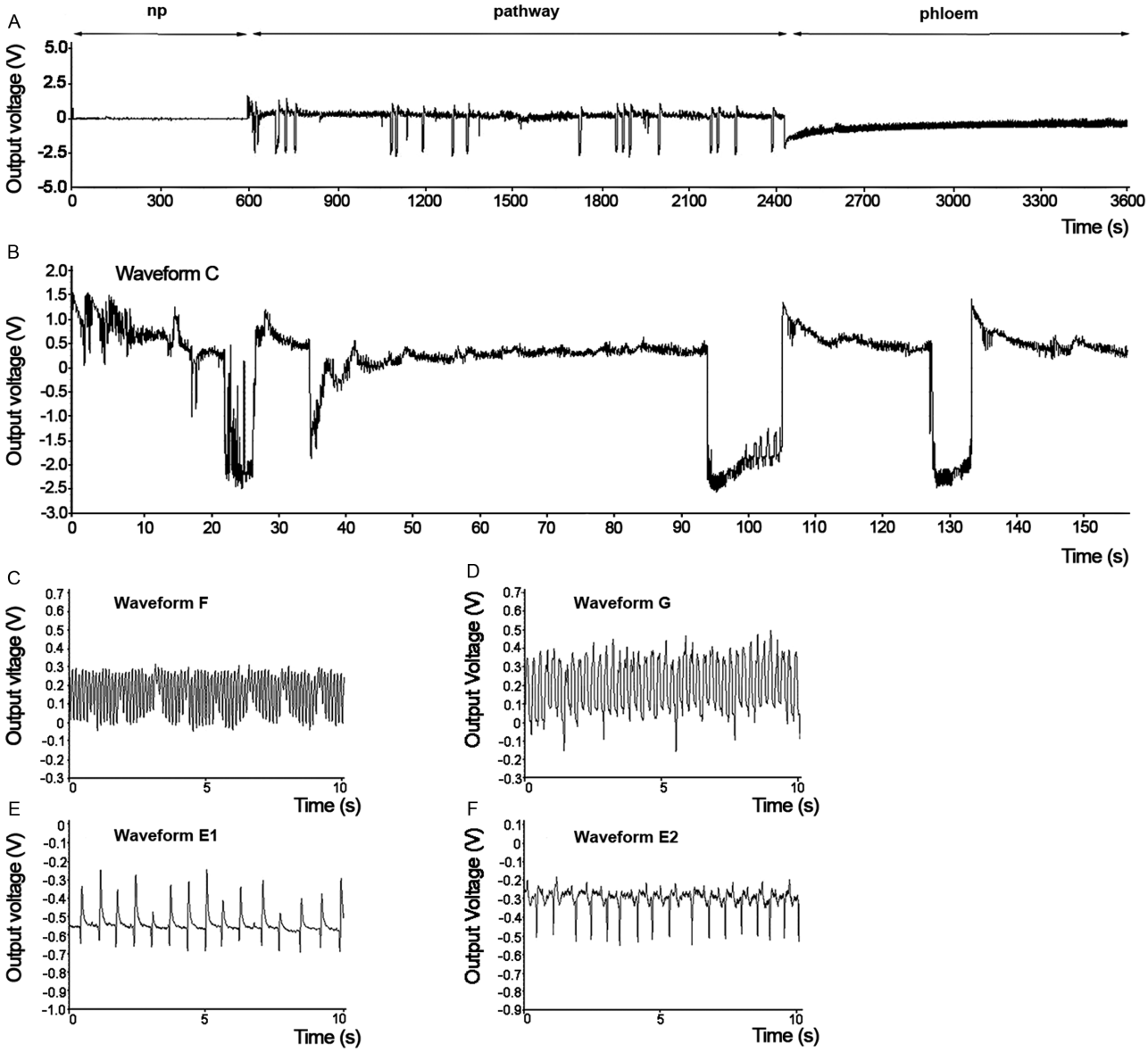

Fig. 1 (A) Typical electrical penetration graph (EPG) waveforms of *Myzus persicae* when feeding during 1 h on tobacco and (B–F) characteristic waveforms in detail. C, pathway phase; F, derailed stylet mechanics; G, xylem ingestion; E1, phloem salivation; and E2, phloem ingestion.

the control group (28.33%), but no significant difference was detected ($P > 0.5$).

Discussion

Aphidius gifuensis is a primary endoparasitoid against *M. persicae* in agricultural fields. Thus, the biological and ecological characteristics of *A. gifuensis* in relation to its use as a biological control agent against *M. persicae* have been studied previously by other authors (Ohta *et al.*, 2001; Takayuki *et al.*, 2016). These studies have mainly concentrated on the fertility, longevity, host range and temperature dependence of this species (Fukui & Takada, 1988; Ohta & Ohtaishi, 2004). Such information is useful for the rational utilization of *A. gifuensis* in controlling the population dynamics of aphids. Because it is an efficient vector of many viral diseases, further information is required on the specific characteristics of *M. persicae* parasitized by *A. gifuensis*, particularly in regard to its

Table 1 Electrical penetration graph variables of unparasitized and parasitized *Myzus persicae* probing tobacco plants.

Related to	No.	Variables	Control	Parasitized			*F*	df	*P*
				Day 3	Day 4	Day 5			
Probing, pathway, and cell puncture	1	Total duration of np (min)	57.54 ± 10.67 a	79.72 ± 19.22 a	62.56 ± 15.39 a	96.50 ± 19.60 a	0.97	3,56	0.420
	2	Count probes	8.73 ± 1.90 b	13.80 ± 2.82 ab	16.80 ± 3.69 a	8.53 ± 1.40 b	2.40	3,56	<0.05
	3	Number of short probes (C < 3 min)	2.73 ± 1.13 b	7.80 ± 2.26 ab	9.33 ± 2.79 a	3.67 ± 1.10 b	2.67	3,56	<0.05
	4	Duration of first probe (min)	51.25 ± 12.59 a	18.73 ± 10.40 b	20.67 ± 8.56 b	19.00 ± 7.15 b	3.58	3,56	<0.05
	5	Number of probes to the first E1	6.27 ± 1.88 a	7.93 ± 1.68 a	7.08 ± 1.72 a	5.13 ± 1.22 a	0.41	3,44	0.747
Derailed stylet mechanics	6	Number of F	0.93 ± 0.30 b	0.99 ± 0.35 b	1.80 ± 0.58 ab	3.33 ± 0.97 a	2.88	3,56	<0.05
	7	Duration of F (min)	45.87 ± 13.09 a	78.55 ± 20.86 a	62.17 ± 14.69 a	86.04 ± 13.80 a	0.85	3,56	0.473
Xylem ingestion	8	Number of G	0.67 ± 0.33 a	0.60 ± 0.25 a	0.60 ± 0.32 a	0.61 ± 0.29 a	0.01	3,56	0.998
	9	Duration of G (min)	3.25 ± 1.52 b	6.19 ± 2.92 ab	5.77 ± 2.65 ab	14.56 ± 6.10 a	0.39	3,56	0.760
Phloem salivation and ingestion	10	Number of E1	0.92 ± 0.28 b	2.33 ± 0.47 a	2.60 ± 0.77 a	0.80 ± 0.24 b	3.78	3,56	<0.05
	11	Total duration of E1	3.50 ± 1.79 b	31.12 ± 11.77 a	14.94 ± 6.17 ab	4.92 ± 4.41 b	3.69	3,56	<0.05
	12	Contribution of E1 to phloem phase (%)	3.84 ± 1.72 b	49.44 ± 9.53 a	31.63 ± 9.05 a	44.25 ± 13.53 a	3.69	3,44	<0.05
	13	Individual percent with E1 and E2 (%)	93 (14) a	80 (12) ab	73 (11) ab	40 (6) b	–	–	<0.05
	14	Duration of first E	80.12 ± 24.05 a	34.77 ± 15.56 ab	35.11 ± 22.22 ab	10.93 ± 7.19 b	3.74	3,56	<0.05
	15	Number of E2	1.07 ± 0.23 ab	1.27 ± 0.34 a	1.67 ± 0.43 a	0.40 ± 0.13 b	3.15	3,56	<0.05
	16	Time from first probe to first E2 (min)	129.42 ± 30.73 a	141.99 ± 31.52 a	118.14 ± 28.86 a	144.58 ± 39.43 a	0.21	3,37	0.886
	17	Number of sustained E2 (>10 minutes)	0.87 ± 0.17 a	0.73 ± 0.18 a	0.73 ± 0.21 a	0.20 ± 0.11 b	3.33	3,56	<0.05
	18	Total duration of E2 (min)	114.64 ± 23.80 a	61.22 ± 15.13 ab	79.53 ± 27.79 ab	21.00 ± 11.97 b	5.14	3,37	<0.05

Data are means ± SE. Means followed by different small letters indicate significant differences ($\alpha = 0.05$).

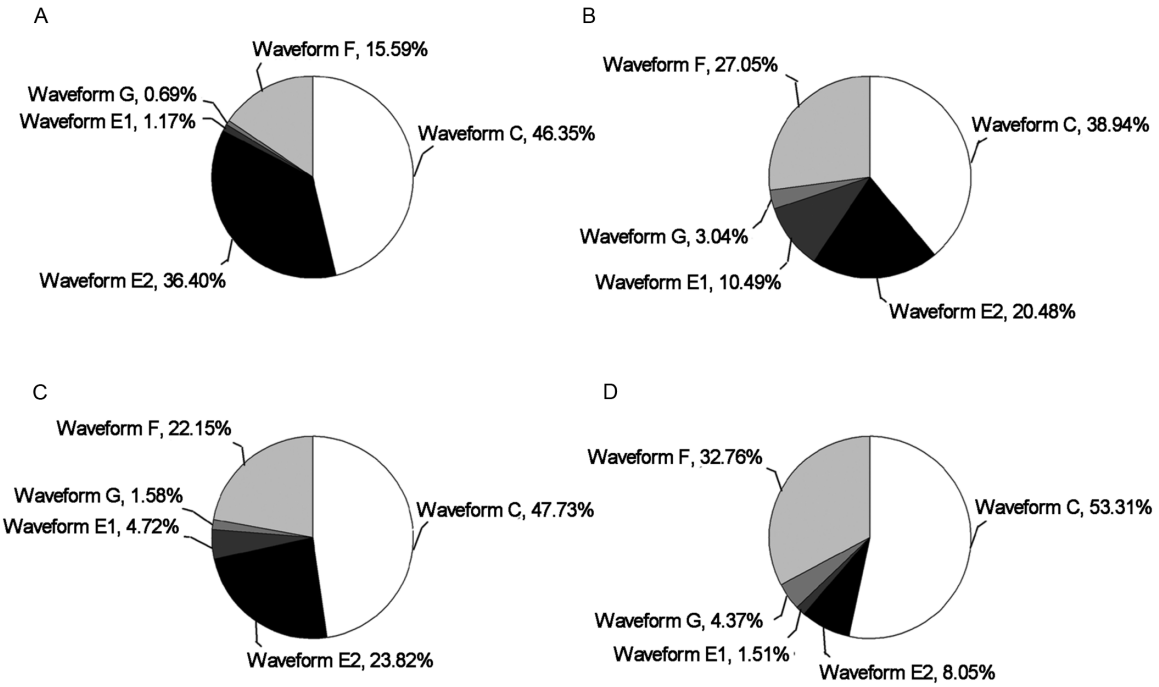

Fig. 2 Mean (± SE; $n = 15$) percentage of the total time spent ingesting in the pathway, xylem and phloem phases by unparasitized and parasitized *Myzus persicae* aphids on tobacco. A: unparasitized aphids; B: aphids parasitized on day 3; C: aphids parasitized on day 4; D: aphids parasitized on day 5.

feeding behavior and virus transmission efficiency. In the present study, we analyzed the effects that *A. gifuensis* on *M. persicae* feeding behavior. Moreover, the experiment was coupled with research on PVY transmission after parasitization.

As shown in Table 1, the parasitized aphids exhibited a significantly shorter duration of the first probe compared with the control group (Table 1, variable 4), thus indicating that *M. persicae*, once parasitized by *A. gifuensis*, became less efficient from the beginning of the pathway phase. In addition, the number of F events, total duration and the percentage of time spent having stylet penetration difficulties by *M. persicae* after parasitization were higher than those of the control (Table 1, variables 6 and 7, and Fig. 2, % in F). These results indicated that aphids parasitized by *A. gifuensis* experienced more obstacles in the intercellular stylet pathway to reach the phloem. Waveform G is associated with the active ingestion of fluid from xylem vessels in aphids (Spiller *et al.*, 1990). Pompon *et al.* (2010) found that xylem ingestion may help insects resist dehydration. In our study, the total duration and percentage of time spent drinking from the xylem produced by parasitized aphids on day 5 were longer and higher, respectively, than those produced by the control group (Table 1, variable 9, and Fig. 2, % in G). This result demonstrates that parasitized aphids on day 5 may need to ingest a larger amount of xylem sap to maintain their water balance.

Successful phloem feeding requires overcoming a number of phloem-related plant properties and reactions (Tjallingii, 2006). Proteins in aphid saliva play an important role in feeding activities, countering sieve plate occlusion, and for some species, resistance factors in plants (Will & van Bel, 2006; Hogenhout *et al.*, 2009). The disruption of the symbiosis of *M. persicae* with *Buchnera aphidicola* negatively affects the feeding behavior producing changes on it physiology, which leads to impeding host plant acceptance (Machado-Assefh & Alvarez, 2016). In the present study, the contribution of E1 to the phloem phase (%) in parasitized aphids changed significantly (Table 1, variable 12). Aphids rely on the phloem sap ingested from plants as their only source of nutrients. On day 5 in the parasitized aphids, the duration of the first E and total duration of the E2 waves were significantly reduced (Table 1, variables 14 and 18). The changes found on the aphid behavior regarding salivation (E1 waveform) and ingestion (E2 waveform) on phloem sieve elements after being parasitized by *A. gifuensis* could be related with changes occurring in the aphid leading to a constraint on host plant acceptance. Additionally, compared with the

control, the total duration of E2 in aphids parasitized on days 3 and 4 was also reduced, but the difference was not statistically significant. This phenomenon may be related to the growth and development of *A. gifuensis*. During the early process of development, nymphs of *A. gifuensis* need nutrition supplied by the aphids, which causes the aphids to ingest more phloem sap from tobacco plants and may explain why the phloem ingestion time of aphids parasitized on days 3 and 4 was not obviously shortened. Similarly, Couchman and King (1979) also found that the food uptake of *Brevicoryne brassicae* parasitized by *Diaeretiella rapae* do not differ compared with unparasitized aphids when the parasitoids developed in aphids at the embryonic and second-instar nymph stages. However, feeding decreased remarkably when the third-instar stage is reached.

The transmission experiments showed that the transmission of PVY by *M. persicae* parasitized on day 5 was not significantly reduced compared with the control. In a previous study, two of the pd (intracellular stylet punctures) subphases, that is, II-1 and II-3, which are associated with salivation and ingestion events, were experimentally demonstrated to be related to the inoculation and acquisition of non-persistent viruses, respectively (Martin *et al.*, 1997; Powell, 2005). In addition, the transmission of Cucumber mosaic virus by *Aphis gossypii* requires the occurrence of the pd waveform, and the virus transmission efficiency is positively correlated with the number of pd (Zhang *et al.*, 2001; Wang *et al.*, 2003). In our experiments, no significant differences were observed in the number of pd and mean duration of pd between the control and parasitized aphids (Table S1, variables 3 and 4). This result indicates that *A. gifuensis* does not immediately disrupt the transmission of PVY by affecting pd parameters in the parasitized aphids.

Persistent aphid-transmitted viruses are acquired from plants by aphids performing passive phloem ingestion (E2 waveform) and are inoculated into plants by salivation into the phloem sieve elements (E1 waveform) (Zeng *et al.*, 2016). In the present study, reductions in the proportion of individuals that produced E1 and E2 waveforms, on the duration of the first E waveform, on the total duration of the E2 waveform, and the percentage of time spent ingesting in the phloem phase were obtained when aphids parasitized on day 5 fed on the plants (Table 1, variables 13, 14 and 18, and Fig. 2, % in E2). Although *A. gifuensis* reduced the feeding activities of *M. persicae* on phloem phase, this alteration would not be expected to reduce the transmission of persistently transmitted viruses. Calvo and Fereres (2011) suggested that *Aphidius ervi* did not affect the Turnip yellows virus transmission rate of *Macrosiphum euphorbiae* underlaboratory conditions. Additionally, parasitoid performance is negatively affected by the presence of a circulative virus within the vector's body. Compared with the control, some phloem-feeding variables of *M. persicae* that were parasitized on days 3 and 4 were reduced, but no significant differences were detected. Thus, we infer that the transmission efficiency also showed no obvious change. However, the wings of *M. persicae* did not develop normally after being parasitized by *A. gifuensis.* This information clearly shows that despite the limited effect on the spread of the virus caused by the way *A. gifuensis* reduces *M. persicae* feeding behavior, parasitism can still decrease the transmission of diseases by reducing aphid movement and the spread of these vectors within and between fields (Chen *et al.*, 2016).

In conclusion, this study revealed the effects of *A. gifuensis* on the feeding behavior and PVY transmission ability of *M. persicae* on tobacco plants. The results showed that *A. gifuensis* exerted adverse effects on aphid feeding, but the transmission of viruses was not significantly reduced. Therefore, considering that the effects of parasitism by *A. gifuensis* on aphid virus transmission via feeding behavior are limited, we suggest that this method for the prevention of this viral disease should be combined with other control methods. Furthermore, the assessment of the influence of *A. gifuensis* on the ability of aphids to transmit viruses in the laboratory is insufficient, and these conclusions should be verified under field conditions. The use of natural enemies to control pests is likely to increase in the future. Therefore, understanding the effects of *A. gifuensis* on *M. persicae* feeding activities and its virus transmission ability could add to the knowledge of insect-virus interactions under the effects of natural enemies. In addition, this new knowledge has the potential to help develop new strategies for the biological control of aphids and viral diseases.

Acknowledgments

This study was funded by the Research Project Technology of *Aphidius gifuensis* against *Myzus persicae* (NY20140101070001).

Disclosure

The authors declare that they have no conflicts of interest.

References

Blackman, R.L. and Eastop, V.F. (2000) Aphids on the wolrd's crop. *An Identification and Information Guide*, pp. 80–120. John Wiley & Sons, Chichester.

Calvo, D. and Fereres, A. (2011) The performance of an aphid parasitoid is negatively affected by the presence of a circulative plant virus. *BioControl*, 56, 747–757.

Chen, J., Yang, Y., Wu, C.Y., Long, L.W., Zou, G.J. and Pan, H.P. (2016) Control effect of using *Aphidius gifuensis* to control *Myzus persicae* and aphids-transmitted virus. *Journal of Anhui Agricultural Sciences*, 44, 188–190.

Couchman, J.R. and King, P.E. (1979) Effect of the parasitoid *Diaeretiella rapae* on the feeding rate of its host *Brevacoryne brassicae*. *Entomologia Experimentalis et Applicata*, 25, 9–15.

Dader, B., Moreno, A., Vinuela, E. and Fereres, A. (2012) Spatio-temporal dynamics of viruses are differentially affected by parasitoids depending on the mode of transmission. *Viruses*, 4, 3069–3089.

Davis, J.A., Radcliffe, E.B. and Ragsdale, D.W. (2007) Resistance to green peach aphid, *Myzus persicae* (Sulzer), and potato aphid, *Macrosiphum euphorbiae* (Thomas), in potato cultivars. *American Journal of Potato Research*, 84, 259–269.

Elbert, A., Haas, M., Springer, B., Thielert, W. and Nauen, R. (2008) Applied aspects of neonicotinoid uses in crop protection. *Pest Management Science*, 64, 1099–1105.

Fereres, A. and Moreno, A. (2009) Behavioural aspects influencing plant virus transmission by homopteran insects. *Virus Research*, 141, 158–168.

Fukui, M. and Takada, H. (1988) Fecundity, oviposition period and longevity of *Diaeretiella rapae* (M'Intosh) and *Aphidius gifuensis* Ashmead (Hymenoptera: Aphidiidae), two parasitoids of *Myzus persicae* (Sulzer) (Homoptera: Aphididae). *Japanese Journal of Applied Entomology and Zoology*, 32, 331–333.

He, W.B., Li, J. and Liu, S.S. (2015) Differential profiles of direct and indirect modification of vector feeding behaviour by a plant virus, *Scientific Reports*, 5, 76–82.

Hogenhout, S.A., Ammar, E.D., Whitfield, A.E. and Redinbaugh, M.G. (2008) Insect vector interactions with persistently transmitted viruses. *Annual Review of Phytopathology*, 46, 327–359.

Hogenhout, S.A., van der Hoorn, R.A.L., Terauchi, R. and Kamoun, S. (2009) Emerging concepts in effector biology of plant associated organisms. *Molecular Plant–Microbe Interactions*, 22, 115–122.

Huang, F., Tjallingii, W.F., Zhang, P.J., Zhang, J.M., Lu, Y.B. and Lin, J.T. (2012) EPG waveform characteristics of solenopsis mealybug stylet penetration on cotton. *Entomolgia Exerimentalis et Applicata*, 143, 47–54.

Huang, S.G. (1982) Study on the parasitic performance of *Aphidius gifuensis* (Ashmead). *Guangxi Agricultural Sciences*, 7, 33–35.

Jones, D.B., Giles, K.L., Berberet, R.C., Royer, T.A., Elliot, N.C. and Payton, M.E. (2003) Functional responses of an introduced parasitoid and an indigenous parasitoid on greenbug at four temperatures. *Environmental Entomology*, 32, 425–432.

Liu, S.S. and Hughes, R.D. (1984) Effect of host age at parasitization by *Aphidius sonchi* on the development, survival, and reproduction of the sowthistle aphid, *Hyperomyzus lacurcae. Entomologia Experimentalis et Applicata*, 36, 239–246.

Loebenstein, G., Berger, P.H., Brunt, A.A. and Lawson, R.H. (2001) *Virus and Virus-like Diseases of Potatoes and Production of Seed-potatoes*. Springer, the Netherlands.

Hodge, S. and Powell, G. (2008) Complex interactions between a plant pathogen and insect parasitoid via the shared vector-host: consequences for host plant infection. *Oecologia*, 157, 387–397.

Hussain, A., Arif, M., Abbas, A., Hussain, B., Ali, M. and Jaffar, S. (2016) A review on aphid-borne virus (*Potato Virus Y*). *Journal of Entomology and Zoology Studies*, 4, 189–192.

Machado-assefh, C.R. and Alvarez, A.E. (2016) Probing behavior of aposymbiotic green peach aphid (*Myzus persicae* Sulzer) on susceptible *Solanum tuberosum* and resistant *Solanum stoloniferum* plants. *Insect Science*, https://doi.org/10.1111/1744-7917.12372.

Martin, B., Collar, J.L., Tjallingii, W.F. and Fereres, A. (1997) Intracellular ingestion and salivation by aphids may cause the acquisition and inoculation of non-persistently transmitted plant viruses. *Journal of General Virology*, 78, 2701–2705.

Mitsunaga, T., Nagasaka, K. and Moriya, S. (2016) Differences in the reproductive durations of *Myzus persicae* (Hemiptera: Aphididae) parasitized by three aphidiid parasitoids. *Applied Entomology and Zoology*, 51, 297–304.

Munyaneza, J.E. (2015) Zebra chip disease, *Candidatus* Liberibacter, and potato psyllid: A global threat to the potato industry. *American Journal of Potato Research*, 92, 230–235.

Ng, J.C. and Falk, B.W. (2006) Virus-vector interactions mediating nonpersistent and semipersistent transmission of plant viruses. *Annual Review of Phytopathology*, 44, 183–212.

Ohta, I. and Honda, K. (2010) Use of *Sitobion akebiae* (Hemiptera: Aphididae) as an alternative host aphid for a banker-plant system using an indigenous parasitoid, *Aphidius gifuensis* (Hymenoptera: Braconidae). *Applied Entomology and Zoology*, 45, 233–238.

Ohta, I. and Ohtaishi, M. (2004) Fertility, longevity and intrinsic rate of increase of *Aphidius gifuensis* Ashmead (Hymenoptera: Braconidae) on the green peach aphid, *Myzus persicae* (Sulzer) (Homoptera: Aphididae). *Applied Entomology and Zoology*, 39, 113–117.

Ohta, I., Miura, K. and Kobayashi, M. (2001) Life history parameters during immature stage of *Aphidius gifuensis* Ashmead (Hymenoptera: Braconidae) on green peach aphid, *Myzus persicae* (Sulzer) (Homoptera: Aphididae). *Applied Entomology and Zoology*, 36, 103–109.

Pompon, J., Quiring, D., Giordanengo, P. and Pelletier, Y. (2010) Role of xylem consumption on osmoregulation in

Macrosiphum euphorbiae (Thomas). *Journal of Insect Physiology*, 56, 610–615.

Powell, G. (2005) Intracellular salivation is the aphid activity associated with inoculation of non-persistently transmitted viruses. *Journal of General Virology*, 86, 469–472.

Ragsdale, D., Radcliffe, T., Difonzo, C. and Connelly, M.S. (2001) Action thresholds for an aphid vector of potato leaf roll virus. *American Phytological Society*, 99–110.

Sandanayaka, W.R.M., Blouin, A.G., Prado, E. and Cohen, D. (2013) Stylet penetration behaviour of *Pseudococcus longispinus* in relation to acquisition of grapevine leaf roll virus 3. *Arthropod-Plant Interactions*, 7, 137–146.

Sarria, E., Cid, M., Garzo, E. and Fereres, A. (2009) Excel Workbook for automatic parameter calculation of EPG data. *Computers and Electronics in Agriculture*, 67, 35–42.

Shrestha, D., Wenninger, E.J., Hutchinson, P.J., Whitworth, J.L., Mondal, S., Eigenbrode, S.D. and Bosque-Perez, N.A. (2014) Interactions among potato genotypes, growth stages, virus strains, and inoculation methods in the *potato virus Y* and green peach aphid pathosystem. *Environmental Entomology*, 43, 662–671.

Smyrnioudis, I.N., Harrington, R., Clark, S.J. and Katis, N. (2001) The effect of natural enemies on the spread of *barley yellow dwarf virus* (BYDV) by *Rhopalosiphum padi* (Hemiptera: Aphididae). *Bulletin of Entomological Research*, 91, 301–306.

Spiller, N.J., Koenders, L. and Tjallingii, W.F. (1990) Xylem ingestion by aphids-a strategy for maintaining water balance. *Entomologia Experimentalis et Applicata*, 55, 101–104.

Takayuki, M., Koukichi, N. and Seiichi, M. (2016) Differences in the reproductive durations of *Myzus persicae* (Hemiptera: Aphididae) parasitized by three aphidiid parasitoids. *Applied Entomology and Zoology*, 51, 297–304.

Tjallingii, W.F. (1978) Electronic recording of penetration behavior by aphids. *Entomologia Experimentalis et Applicata*, 24, 721–730.

Tjallingii, W.F. (1985) Electrical nature of recorded signals during stylet penetration by aphids. *Entomologia Experimentalis et Applicata*, 38, 177–186.

Tjallingii, W.F. (1988) Electrical recording of stylet penetration activities. *Aphids, Their Biology, Natural Enemies and Control* (eds. A.K. Minks & P. Harrewijn), pp. 95–108. Elsevier, Amsterdam, the Netherlands.

Tjallingii, W.F. and Hogen-Esch, T. (1993) Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. *Physiological Entomology*, 18, 189–200.

Tjallingii, W.F. (1994) Sieve element acceptance by aphids. *European Journal of Entomology*, 91, 47–52.

Tjallingii, W.F. (2006) Salivary secretions by aphids interacting with proteins of phloem wound responses. *Journal of Experimental Botany*, 57, 739–745.

Wang, B., Chen, J.Q., Zhang, P.F., Ma, L. and Wang, Y.M. (2003) Effects of post-acquisition fast on *cucumber mosaic virus* transmission by the cotton aphid, *Aphis gossypii. Acta Entomologica Sinica*, 46, 259–266.

Yamamoto, M. (1997) Studies on the hymenopterous parasitoids reared from eggplant in greenhouse in Shiga Prefecture Japan. *Special Bulletin of Shiga Agricultural Experimental Station*, 20, 1–70.

Yan, S.Y., Du, M.F., Yin, X.M., An, S.H., Wang, T.C. and He, D.X. (2005) Effect of parasitism by *Aphidius gifuensis* on the activity of protective and detoxification enzymes in *Schizaphis graminum*. *Journal of Henan Agricultural University*, 49, 488–493.

Zeng, X.Y., He, Y.Q., Wu, J.X., Tang, Y.M., Gu, J.T., Ding, W. and Zhang, Y.Q. (2016) Sublethal effects of cyantraniliprole and imidacloprid on feeding behavior and life table parameters of *Myzus persicae* (Hemiptera: Aphididae). *Journal of Economic Entomology*, 109, 1595–1602.

Zhang, P.F., Chen, J.Q., Zhang, X., Wang, B. and Jiang, Q.F. (2001) The feeding behavior and the acquisition of CMV by the cotton aphid *Aphis gossypii*. *Acta Entomologica Sinica*, 44, 395–401.

Will, T. and van Bel, A.J.E. (2006) Physical and chemical interactions between aphids and plants. *Journal of Experimental Botany*, 57, 729–737.

Manuscript received October 12, 2016
Final version received April 10, 2017
Accepted April 12, 2017

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. EPG variables of unparasitized and parasitized *Myzus persicae* probing tobacco plants.