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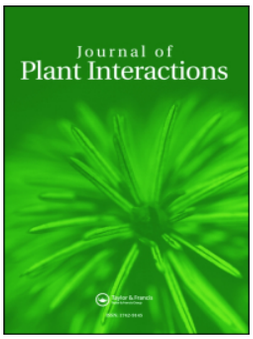

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An aphid-transmitted polerovirus is mutualistic with its insect vector by accelerating population growth in both winged and wingless individuals

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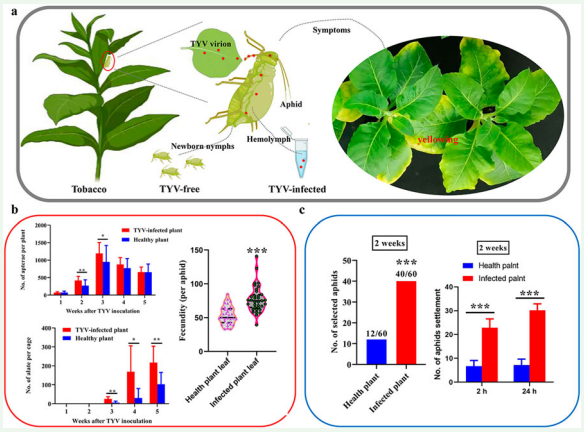
ABSTRACT

The occurrence and increased dispersion of plant viruses and insect vectors are serious global threat to the production of agricultural crops. Facing novel pathogenic plant viruses, the ability to accurately identify plant virus species, and understand the interaction between plant viruses, host plants and their insect vectors would provide an important basis for formulating effective plant virus control measures. In this study, we explored the transmission mechanism, pathogenic symptoms, host range and the interactions between virus and aphid vectors of a novel polerovirus from Nicotiana tabacum, named Tobacco yellow virus (TYV). The results indicate that TYV can be transmitted by Myzus persicae in a persistent manner, and cause yellowing and shrinking of tobacco leaves. TYV can successfully infect a total of 9 plant species belonging to 3 families. The effect of TYV-infected tobacco plants on M. persicae behavior and life characteristics was found to be stage-dependent. TYV can directly and indirectly manipulate the performance and life history traits of M. persicae vectors to promote their own transmission. These results provide a certain theoretical basis for the possibility of control strategies of the virus, and the in-depth exploration of the interaction among plant virus, vector aphid and host plants.



Mutulistic relationship between TYV and aphid vector by attracting aphids feeding and promoting aphids population growth of both winged and wingless individuals. (a) TYV transmission mechanism in aphid and pathogenic symptoms of tobacco. (b) TYV-infected tobacco plants promoted aphids population growth of both winged and wingless individuals. (c) TYV-infected tobacco plants attracted both winged and wingless aphids feeding.

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Tobacco yellow virus; tobacco; *Myzus persicae*; selection behavior; life characteristics

Introduction

Plant viruses can reduce crop yield and quality leading to devastating crop losses. About three-quarters of known plant viruses depend on insect vectors for their transmission between host plants (Ghosh et al. 2021; Shi et al. 2021). A number of studies have been performed to reveal the interactions between plant viruses and insect vectors (e.g. Mauck 2016; Eigenbrode et al. 2018). In general, viruses with different transmission modes have different influences on their insect vectors. A total of four modes of transmission have

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been reported to date: (a) *noncirculative-nonpersistent*, which are viruses acquired or inoculated by probing or feeding insects, with the insects attracted by virus-infected plants, resulting in more probing, lower reproduction and greater alate production; (b) *noncirculative-semipersistent*, which contrasts with (a), whereby insects acquiring or inoculating viruses need relatively long times; (c) *circulative-persistent-nonpropagative*, where virus acquisition, retention and inoculation by their vectors normally take many hours or days, and infectious insect vectors prefer noninfected plants, and perform more phloem feeding, often resulting in greater reproduction and alate production; (d) *circulative-persistent-propagative*, compared with (c), these viruses can replicate within their insect vectors and usually capable of vertical transmission to offspring (Eigenbrode et al. 2018; Wang and Blanc 2021; Jayasinghe et al. 2022; Ray and Casteel 2022).

The majority of poleroviruses are transmitted by various aphid species. However, recent studies have identified whiteflies can also transmit some viruses exhibiting high genome identity with other aphid-borne poleroviruses (Ghosh et al. 2019; Costa et al. 2020). Transmission of poleroviruses occurs in a persistent *circulative non-propagative* manner (Hogenhout et al. 2008; Boissinot et al. 2020). The genus *Polerovirus* belongs to the family Solemoviridae consisting of plant viruses that possess polycistronic, positive-sense, single-strand RNA genomes (Walker et al. 2021). The genome RNA of poleroviruses, ranging from 5.6 to 6.2 kb, consists of 5'- and 3'- untranslated regions (UTR) and 7 ORFs, four of which (ORF3a, ORF4, ORF6, and ORF7) are unique to poleroviruses (Sõmera et al. 2015; Walker et al. 2021). Poleroviruses can infect many major crops, including *Solanaceae*, *Gramineae*, *Malvaceae*, *Cucurbitaceae*, *Amaranthaceae*, among others (Delfosse et al. 2021; LaTourrette et al. 2021; Sun et al. 2021). The infection of poleroviruses is restricted to plant phloem cells. The typical symptoms of the virus-infected plants are leaf yellowing and curling, dwarfing of plants, and brittleness of foliage (Boissinot et al. 2020). Additionally, studies have reported the emergence of new poleroviruses, due to the frequent genome RNA recombination among poleroviruses themselves, as well as recombination between poleroviruses and other viruses (Knierim et al. 2010; Pagán and Holmes 2010; Dombrovsky et al. 2013; Costa et al. 2020).

Previously, we reported a novel single-stranded RNA virus (Tobacco yellow virus, TYV) from *Myzus persicae* reared on tobacco leaves by RNA-seq analyses, which belongs to the genus *Polerovirus* (Liu et al. 2020). As expected, our preliminary results showed that TYV could only be transmitted by aphids, and the infected plants exhibited leaf-yellowing symptoms (Liu et al. 2020). In this study, we examined TYV transmission-mode, host-range, symptoms, and impact on the infected plants, in addition to interactions between the virus and aphid vector. Our results indicate that plant viruses can manipulate the performance and life history traits of aphid vectors to promote their own transmission, which provides a new vision for understanding the interaction among insect vectors, viruses, and host plants.

Materials and methods

Aphid colonies and virus isolates

The tobacco aphids, *Myzus persicae*, were collected in Qingdao, Shandong province, China (36.44°N, 120.58°E) in 2015, and the monoclonal line established from a single parthenogenetic female was maintained on tobacco plants (*Nicotiana tabacum* 'K326') in cages in climate chambers, at 25°C, 65% relative humidity, and 14 h photoperiod. The TYV-infected plants were collected in the tobacco field, and the virus was detected using specific primers by RT–PCR as previously described (Liu et al. 2020). The virus was transmitted to a new tobacco plant by aphids, and maintained in the same climate chambers.

Virus detection and quantification

The aphids and tobacco leaves were collected and ground up under liquid nitrogen separately to extract total RNA. cDNA templates were prepared and TYV detection was performed using specific primers (TYV-F and TYV-R) by RT–PCR and Sanger sequencing, as described previously (Xu et al. 2017; Liu et al. 2020). The quantification of TYV from both tobacco plants and aphids was determined by TaqMan real-time quantitative PCR with a standard curve method, as described previously (Liu et al. 2019). The primer and probe sequences and cycling parameters are shown in Supporting Information 1, Table S1.

Virus transmission mode

Mechanical friction method: tobacco plants at the six-true leaf stage were inoculated by mechanical friction, as described previously (Liu et al. 2019). Briefly, TYV-infected tobacco tissue was ground on ice and the filtering medium was smeared (30 times each leaf) on the surface of third- and fourth-leaf using cotton swabs. A total of 21 days post-virus inoculation, the fifth leaf was collected for virus detection. The experiment was performed in five replicates and the mock-inoculated using healthy tobacco tissue was used as the control.

Aphid transmission method: ten apterous adult aphids were starved for 2 h. These were then transferred to TYV-positive leaves for 12-h feeding duration. Then these aphids were transferred to healthy seedlings for a 3-day feeding duration. As a control, ten *M. persicae* from non-infected plants were also transferred to healthy seedlings for a 3-day feeding to obtain the mock-inoculated plants. After 3 weeks, leaves were collected and analyzed by RT–PCR for TYV.

Hemolymph isolation and virus detection

The method used for isolation of aphid hemolymph was performed according to Liu et al. (2006). Briefly, a thin layer of melted wax was placed onto a piece of parafilm. The adult aphid was then placed on the melted wax layer until the body of the aphid was completely covered. The parafilm was then removed from the hot plate to allow the wax to harden. A hind leg was removed from this aphid, and the hemolymph from the wound was flushed three times using 20 ul RNase-free water. The mixed liquid was collected for total RNA extraction.

Host range study

According to the host range of *M. persicae* and poleroviruses (Blackman and Eastop 2000; LaTourrette et al. 2021), a total of 10 cultivated crop species belonging to 3 families were used in this study, including *Nicotiana tabacum*, *Solanum melongena*, *Solanum tuberosum*, *Solanum lycopersicum*, *Capsicum annuum*, *Brassica rapa*, *Brassica oleracea*, *Brassica napus*, *Cucumis sativus*,

and *Cucurbita moschata*. Ten viruliferous *M. persicae* reared on TYV-infected tobacco plants were transferred to the healthy seedlings for a 3-day inoculation period. After 3 weeks, plant leaves were collected and analyzed by RT–PCR for TYV.

Influence on host plants

The 4-true leaf tobacco plants were inoculated by viruliferous aphids, and mock-inoculated plants using non-viruliferous aphids as the control. The symptoms and development states of inoculated and mock-inoculated plants were recorded every three days.

Host selection by aphids

The apterous aphid selection behavior was observed using a Y-shaped olfactory apparatus, as described previously (Liu et al. 2019). Briefly, the test instrument was placed in a dark box followed by two gas paths linking with each arm. The TYV-infected and healthy plants were placed in the source bottles of each gas path, respectively. The airflow velocity was 200 ml/min/arm. In order to have a non-biased steady gaseous environment, the air extractor was allowed to run for 2 h before aphid testing. Then a single aphid (starved for 2 h) was placed in the Y-shaped base arm. When the aphid passed the halfway point of each arm, this was recorded as a selective reaction, and when no choice behavior in 5 min this was recorded as 'no choice.' The plants inoculated post 7, 14, and 28 d were selected and measured, including control plants. Each of the paired plants (TYV-infected vs. healthy plants) was tested for 20 apterous adult aphids. Three replicates (20 apterous aphids per replicate) of each group were analyzed, and a total of 60 aphids were tested in each group, with each aphid tested only once.

In addition, alate aphid selection behavior was performed in insect-proof cages (110*60*40 cm). Treatment and control groups were prepared the same as the apterous aphids. TYV-infected and healthy plants were placed separately at both ends of the cage, 60 cm apart. The alate aphids (starved for 2 h) were released at the halfway point between the treatment and control plants. The number of alate aphids on each tobacco plant was counted after the released 2 and 24 h. Six replicates (40 aphids per replicate) of each group were analyzed.

Determination of life characteristics of aphids

In order to compare the population dynamics of apterous and alate aphids feeding on TYV-infected and mock-inoculated tobacco plants, 10 newborn nymphs were transferred to one plant (24-plant replicates), and the number of apterous aphids and alate aphids (removed during each inspection) were counted every 7 days, for a total of five times.

The life table parameters of single aphids were determined in a feeding device. The tobacco leaf disc from TYV-infected (inoculated post 14 and 28 d) and mock-inoculated plants were used to prepare the feeding device, as described previously (van Van Munster et al. 2003). A single newborn nymph was placed in the feeding device, and the status of development and reproduction was checked daily until the death of the aphid (50 aphid replicates per group). The tobacco leaves were changed every five days. Developmental period duration, reproductive period, fecundity, and life-longevity of each aphid were recorded.

Statistics

Statistical analyses were conducted using Graphpad Prism 8 and SPSS 17.0. TYV virus replication in tobacco plants was analyzed using one-way analysis of variance followed by the least-significant-difference test. Independent samples *t* test was used to determine the significance of leaf size, aphid population, life history characteristics, and selective behavior of alate aphids. The selective behavior of apterous aphids was analyzed using χ^2 test.

Results

Virus transmission mode and host range of TYV

The results showed that aphids feeding on TYV-infected plants successfully harbor viruses after a 12 h acquisition period (Figure S1). TYV could be transmitted to new plants by the aphids, but not by mechanical friction (Table S2, Figure S2). In order to further test the transmission routes of TYV inside the aphid vector, the hemolymph and the offspring of adults were collected for virus detection. The results demonstrated that TYV virions can enter the hemolymph of the aphid and complete the circulative transmission. However, the newborn nymphs from viruliferous adults were negative for TYV (Table S2, Figure S3).

Ten commonly cultivated crop types were selected for host-range tests. The research revealed that TYV successfully infected tobacco, eggplant, potato, tomato, pumpkin, rape, cucumber, cabbage, and Chinese cabbage (Table S3, Figure S4). Only pepper was not infected.

Dynamics of TYV copy numbers in tobacco plants

A standard curve was generated using the gradient dilution templates of a recombinant plasmid, using absolute quantification (Figure S5). A total of 13 stages of tobacco leaves were collected for virus quantification. The results determined that TYV copy number varied significantly in different stages ($F = 21.64$, $df = 26$, $P < 0.0001$) (Figure 1(a)). The TYV copy number increased significantly within the first 11 days; then from 11 to 40 days after inoculation, the TYV copy number was stable; then the TYV copy number gradually decreased after 40 days inoculation. We selected tobacco plants 27 days after inoculation to examine the spatial distribution of the virus. Seven leaves were collected for virus quantification from each plant. The results indicated that the TYV copy number was different depending on leaf position ($F = 2.949$, $df = 14$, $P = 0.045$) (Figure 1(b)). The TYV copy number of 4^{th}- and 5^{th}-leaf were significantly higher than that of 6^{th}- and 7^{th}-leaf ($P < 0.05$). However, there was no significant difference among 1^{st}- to 5^{th}-leaf ($P > 0.05$).

Symptoms of tobacco plants infected with TYV

Visually there were no significant symptoms of infected plants within 1–2 weeks post-TYV inoculation (Figure 2 (a)). After 3 weeks of infection, the old leaves (nether leaves) of TYV-infected tobacco gradually displayed symptoms of yellowing and slight curling (Figure 2(b)). After 5–6 weeks post-inoculation, compared with mock-inoculated plants, the whole tobacco plant presented symptoms of yellowing and dwarfing (Figure 2(c, d)). Meanwhile, by comparing the leaf size of infected and healthy tobacco plants, we

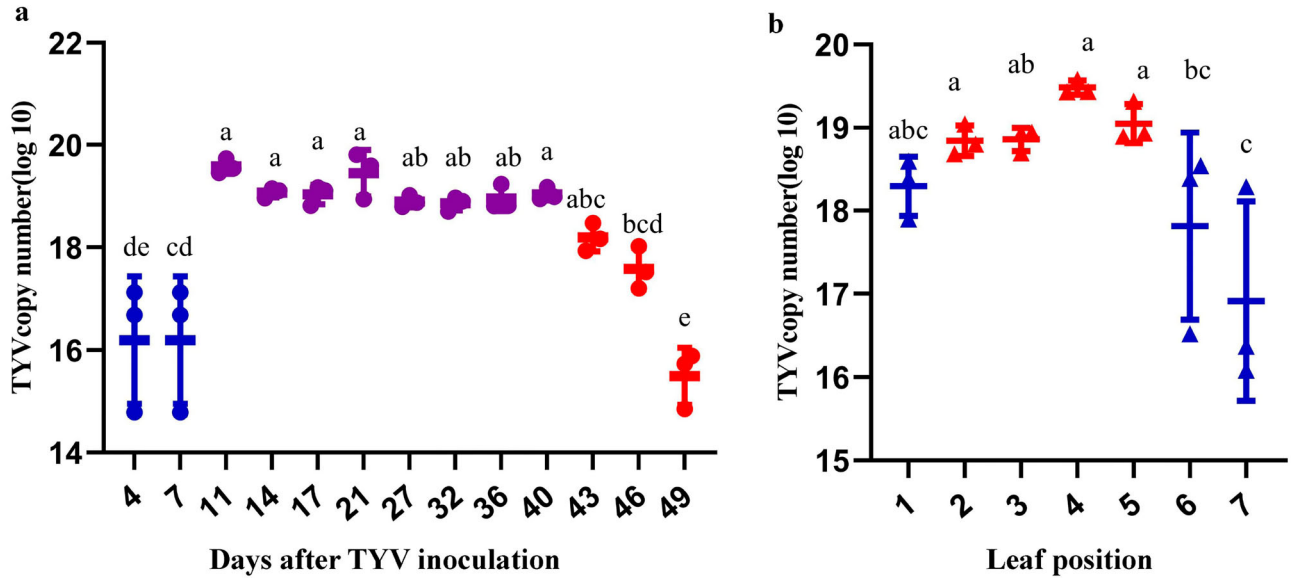

Figure 1. Virus quantification by TaqMan real-time PCR. a: TYV copy number of tobacco plants at different times after inoculation ($n = 3$, $F = 21.64$, $df = 26$, $P < 0.0001$). b: Spatial distribution of viral copy number in different leaf positions after inoculated for 27 days ($n = 3$, $F = 2.949$, $df = 14$, $P = 0.045$). The first leaf is the youngest leaf. Mean ± SD, different letters showed significant differences at $P < 0.05$ level (one-way ANOVA).

confirmed that, after 6 weeks of inoculation, both the length and width of 1st~5th leaf, as well as the length of the 6th leaf were markedly smaller ($P < 0.05$) (Figure 2(e)). Additionally, we also tested the effect of TYV on potato plants. As expected, the TYV-infected potato plant presented obvious symptoms of yellowing, curling, and dwarfing (Figure S6).

Aphid population dynamics feeding on TYV-infected tobacco plants

The long-term effects of TYV on the aphid vector were determined through comparatively examining aphid population dynamics on virus-infected versus non-infected plants. The results indicated that there were significant differences in the population quantity both of apterous and alate aphids (Figure 3). Interestingly, the regulatory effect of TYV-infected tobacco plants on apterous population was stage-dependent. There was no significant difference in the number of apterous aphid in the three virus infection period: TYV infection 1 week ($t_1 = 0.195$, $P_1 = 0.847$), TYV infection 4 weeks ($t_4 = 1.529$, $P_4 = 0.133$), TYV infection 5 weeks ($t_5 = 0.064$, $P_5 = 0.950$) (Figure 3(a)). However, the number of apterous aphids in infected tobacco plants was significantly higher than that in healthy tobacco plants at 2 and 3 weeks ($t_2 = 3.408$, $P_2 = 0.001$; $t_3 = 2.054$, $P_3 = 0.046$) (Figure 3(a)). As a result, the population of aphids on infected and non-infected tobacco plants increased within 1–3 weeks, reaching the peak population at 3 weeks; and then gradually decreased after 3 weeks. By comparing the population of winged aphid produced, the number of winged aphids on infected tobacco plants was significantly higher than that of healthy tobacco plants, at 3, 4, and 5 weeks after TYV infection ($t_3 = 4.109$, $P_3 = 0.001$; $t_4 = 2.695$, $P_4 = 0.017$; $t_5 = 3.022$, $P_5 = 0.009$) (Figure 3(b)).

M. persicae life history characteristics

The results of the single aphid life table showed that the effects of TYV-infected tobacco leaves on nymph duration, reproductive duration, fecundity, and longevity of *M. persicae* were different depending upon the plant-infection stage (Figure 3(c, d)). Aphids feeding on leaves 2 weeks post TYV-infection, nymph duration was significantly shortened ($t = 5.320$, $P < 0.001$); fecundity was significantly increased ($t = 5.774$, $P < 0.001$); and reproductive duration was significantly extended ($t = 4.708$, $P < 0.001$). However, there was no significant effect on aphid longevity ($t = 1.420$, $P = 0.160$) (Figure 3(c)), compared with the control group. On tobacco leaves 4 weeks post TYV-infection, there was no significant effect on aphid development duration ($t = 0.985$, $P = 0.329$), reproductive duration ($t = 1.582$, $P = 0.119$), and longevity ($t = 1.655$, $P = 0.103$); but there was for fecundity ($t = 2.423$, $P = 0.018$) (Figure 3(d)).

Aphid response to TYV-infected and non-infected plants

Apterous adult aphid did not show any distinct preference to either TYV-infected or non-infected tobacco plants within 1 week of plant infection ($\chi^2_1 = 0.348$, $P_1 = 0.555$). However, significantly more apterous aphids selected TYV-infected tobacco plants 2 weeks after inoculation ($\chi^2_2 = 15.077$, $P_2 < 0.001$) and 4 weeks after inoculation ($\chi^2_4 = 5.898$, $P_4 = 0.015$) (Figure 4(a)). In addition, dual-choice settling bioassays were performed to determine the alate aphid settling preference of TYV-infected versus non-infected tobacco plants. The results showed that there were no significant differences in aphid preference for TYV-infected vs. healthy plants 1 week after inoculation ($t_2 = 0.227$, $P_2 = 0.825$; $t_{24} = 1.048$, $P_{24} = 0.319$) (Figure 4(b)). At 2 weeks post-inoculation, more aphids markedly selected TYV-infected tobacco plants ($t_2 = 8.937$, $P_2 < 0.001$; $t_{24} = 15.314$, $P_{24} < 0.001$) (Figure 4(b)). Interestingly, more aphids markedly selected TYV-infected tobacco plants 4 weeks after inoculation (at the 2 h recording time) ($t_2 = 3.919$, $P_2 = 0.003$) (Figure 4(b)), but there were no significant differences in aphid preference between treatment and control groups at the 24 h recording time ($t_{24} = 1.827$, $P_{24} = 0.098$) (Figure 4(b)).

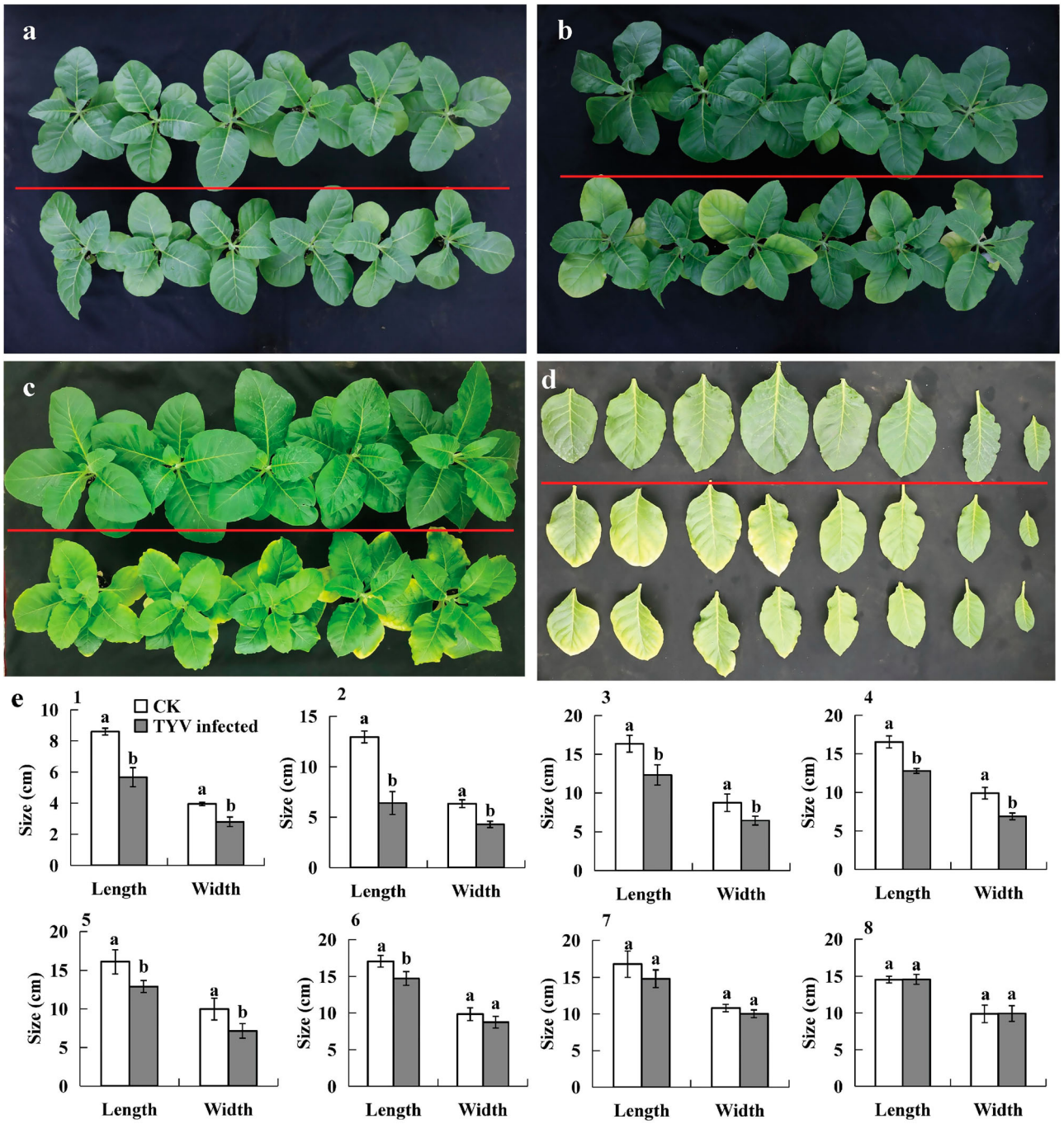

Figure 2. Symptoms of tobacco plants after TYV infection. a: 2 weeks after TYV infection; b: 3 weeks after TYV infection; c: 5 weeks after TYV infection; d: 6 weeks after TYV infection; above the red line: healthy plants, below the red line: TYV-infected plants. e: Effect of TYV infection on leaf size (length and width) after 6 weeks inoculation, 1–8: 1st~8th leaf position, CK: healthy plants, Mean ± SD, different letters showed statistical significance ($P < 0.05$) ($n = 4$, Independent samples t test).

Discussion

The typical symptoms of infection with a *Polerovirus* (Solemoviridae) are yellowing, curling, and dwarfing (Zhang et al. 2014; Boissinot et al. 2020). In this study, we found that 3 weeks post-inoculation, TYV-infected tobacco plants gradually displayed visual symptoms of yellowing and slight curling (nether leaves), dwindling (upper leaves), and dwarfing (whole plant). These symptoms of TYV infection were typical to those of poleroviruses, which supported a scientific basis for classifying the virus to the genus *Polerovirus*. The obvious symptoms of yellowing, curling, and dwarfing were also observed in TYV-infected potato plants (Figure S6). In addition, this current research revealed that TYV can also successfully infect nine common plants, including tobacco, eggplant, potato, tomato, pumpkin, rape, cucumber, cabbage, and Chinese cabbage (Figure S4). The resistance of different cultivars of the same crop species to the same plant virus also showed diversity (Legnani et al. 1996; Chen et al. 2015; Gallois et al. 2018). Although TYV virion was not detected in the selected pepper cultivar, more research should be performed to determine whether pepper is a host for TYV. Undoubtedly, the disease prevalence and potential harm resulting from this generalist virus need further observation and examination.

Poleroviruses are mainly transmitted by aphids in a persistent manner, and can not be infected by mechanical friction (Garret et al. 1996; Bragard et al. 2013; Zhang et al. 2014). With the development of *situ* hybridization technology, the circulative process of persistent viruses in insect vectors has been further illustrated (Kliot et al. 2014,

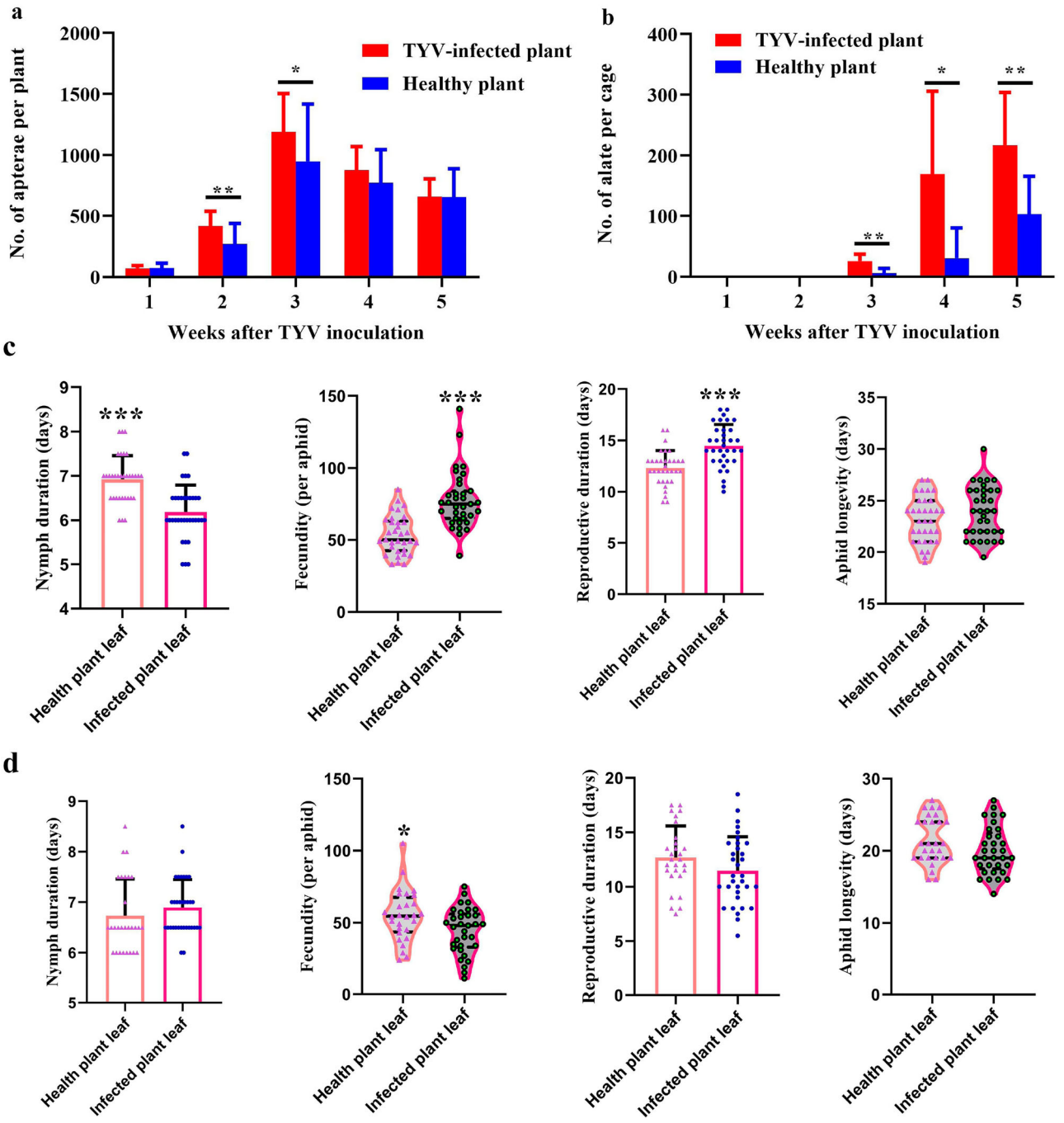

Figure 3. Effects of TYV-infected tobacco plants on *M. persicae* population dynamics and life history characteristics. a: Apterous aphid population dynamics; b: Alate aphid population dynamics; c: Effects of TYV-infected tobacco leaves for 2 weeks on *M. persicae* characteristics; d: Effects of TYV-infected tobacco leaves for 4 weeks on *M. persicae* characteristics; fecundity and aphid longevity were distribution plots, Mean ± SD, '*' stands for $p < 0.05$, '**' stands for $p < 0.01$, '***' stands for $p < 0.001$ (Independent samples *t* test).

2016). Persistent plant viruses move through the insect vector in a sequential path of stylet-midgut-haemolymph-salivary glands, and are finally transmitted back into the plant during insect feeding by breaking four barriers: the midgut infection barrier, the midgut escape barrier, the salivary gland infection barrier, and the salivary gland escape barrier, respectively (Hogenhout et al. 2008; Zhao et al. 2020). In this study, we confirmed that aphid hemolymph contained TYV virions (Figure S3). In addition, we found that TYV could be transmitted to new plants by aphids, but not by mechanical friction. Moreover, TYV could not be transmitted from parental generation to offspring (Table S2, Figure S2). Therefore, the results indicate that TYV could be transmitted by aphids in a persistent circulative manner.

In natural ecosystems, by virtue of the co-evolution among different species, plant viruses often regulate the behavior of insect vectors by changing the characteristics of host plants to facilitate virus prevalence (Mauck et al. 2012; Hammerbacher et al. 2019). It has been widely noted that the majority of poleroviruses significantly attract aphid vectors and improve aphid fitness by altering the physiological and metabolic state of the host plant (Eigenbrode et al. 2002; Jiménez-Martínez et al. 2004a, 2004b; Medina-Ortega et al. 2009; Werner et al. 2009; Stafford et al. 2011; Wu et al. 2014). As a novel species of poleroviruses, we explored the interactions of the TYV-aphid-host plant. The results showed that the influence of TYV-infected tobacco plants on aphids was determined by the infection period: prophase stage (infection 1-week

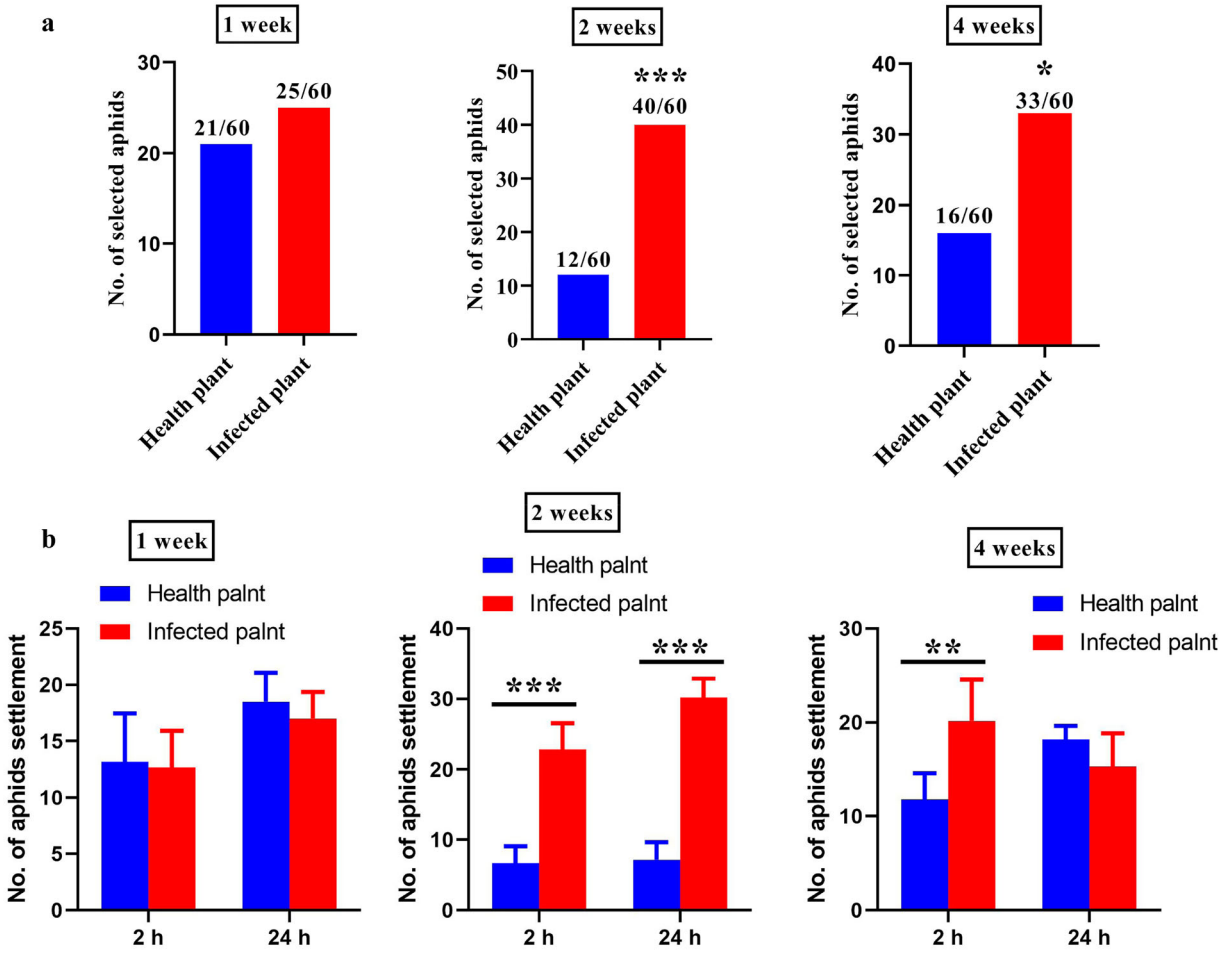

Figure 4. Selective behavior of *M. persicae* to TYV infected or healthy plant. a: Selective behavior of apterous aphids at different infection periods, the numbers above the bars stands for 'selective aphids/all test aphids,' '*' stands for $p < 0.05$, '***' stands for $p < 0.001$ (χ^2 test); b: Selective behavior of alate aphids at different infection periods and different times, Mean ± SD, '**' stands for $p < 0.01$, '***' stands for $p < 0.001$ ($n = 3$, Independent samples t test).

post-inoculation), the tobacco plants had no significant effect on host selection and fecundity of aphid vectors; metaphase stage (infection 2~3 weeks post-inoculation), both the wingless and winged aphids were attracted to the TYV-infected tobacco plants, meanwhile the development duration was significantly shortened, the fecundity was markedly increased, and the reproductive duration was extended in the aphids; anaphase stage (infection 4 weeks post-inoculation), the TYV-infected tobacco plants significantly attracted the aphids at the initial selection, but the winged aphids gradually migrated to healthy plants, whilst the fitness of wingless aphids was inhibited (Figures 3, 4).

Plant viruses often attract insects by altering plant secondary volatiles as well as causing leaf yellowing of host plants (Alvarez et al. 2007; Rajabaskar et al. 2014; Holland et al. 2019; Stukenberg and Poehling 2019). In this study, the selection behavior of apterous aphids to plant volatiles was measured by Y-shaped olfactory apparatus. In addition, the selection behavior of alate aphid between TYV-infected and non-infected tobacco plants was performed in insect-rearing cages, in the laboratory. The results showed that after 2 h, more aphids markedly selected TYV-infected tobacco plants (at 4 weeks after inoculation), but after 24 h, there were no significant differences in aphid preference between treatment and control groups. Meanwhile, after 4 weeks virus inoculation, leaf systematic yellowing of TYV-infected tobacco was observed. Thus, it was speculated that the change of tobacco leaf color promoted the selective response of alate aphid. We found that the influence of TYV-infected tobacco plants on the host selection behavior of aphids was determined by the infection period: tobacco plants in the metaphase stage of TYV infection attracted the feeding and colonization of aphid as well as promoted the population growth of aphids; however, in anaphase stage, tobacco plants attracted the aphid to probe, but then aphids tended to diffuse to healthy plants.

Different to the non-persistent virus transmission mechanism of 'attraction-probe-rapid spread,' persistent virus transmission often requires several minutes or even hours for the vector to acquire virus (Hogenhout et al. 2008). Hence, persistent viruses tend to manipulate the plant host in some way to attract insect vectors; for example, improve the nutritional quality of the host plants for insect vectors colonization, feeding, and virus acquisition (Eigenbrode et al. 2002; Jiménez-Martínez et al. 2004b). Moreover, it appears that by changing the nutritional quality of infected plants, plant viruses can improve the fecundity and longevity of aphid vectors, which subsequently leads to an increase in population density. As a result, the large population abundance produces a crowding effect, thus promoting the production of winged aphids in the offspring. The migration of viruliferous winged aphids is responsible for long-distance transmission and epidemic of plant virus (Müller et al. 2001; Casteel et al. 2014). In

this study, we found that the population of apterous and alate on TYV-infected tobacco plants (3~5 weeks post-inoculation) was significant higher than in healthy plants. In conclusion, we found that the novel plant virus named Tobacco yellow virus (TYV) can be transmitted by *M. persicae* in a persistent manner, and cause yellowing and shrinking of tobacco leaves. The regulatory effect of TYV-infected tobacco plants on *M. persicae* behavior and life characteristics appeared to be stage-dependent. The results provide a certain theoretical basis for the establishment of the virus, and the in-depth exploration of the interaction among plant virus, vector aphid, and host plants.

Author contributions

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Disclosure statement

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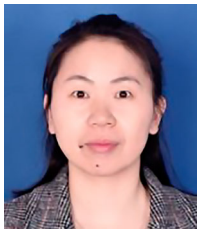

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