Densovirus infection facilitates plant virus transmission by an aphid

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Supporting Information 2-original data used in this study: S1 to S24.

Summary

- The interactions among plant viruses, insect vectors, and host plants have been well studied; however, the roles of insect viruses in this system have largely been neglected.
- We investigated the effects of MpnDV infection on aphid and PVY transmission using bioassays, RNA Interference (RNAi), and GC-MS methods and green peach aphid (*Myzus persicae*), potato virus Y (PVY), and densovirus (*Myzus persicae nicotianae* densovirus, MpnDV) as model systems.
- MpnDV increased the activities of its host, promoting population dispersal and leading to significant proliferation in tobacco plants by significantly enhancing the titer of the sesquiterpene (E)-β-farnesene (EβF) via up-regulation of expression levels of the *MpFPPS1* gene. The proliferation and dispersal of MpnDV-positive individuals were faster than that of MpnDV-negative individuals in PVY-infected tobacco plants, which promoted the transmission of PVY.
- These results combined showed that an insect virus may facilitate the transmission of a plant virus by enhancing the locomotor activity and population proliferation of insect vectors. These findings provide novel opportunities for controlling insect vectors and plant viruses, which can be used in the development of novel management strategies.

Keywords: *Myzus persicae*, *Myzus persicae nicotianae densovirus*, locomotor activity, (E)-β-farnesene, *Potato virus Y*

Introduction

Hundreds of insect viruses have been isolated and identified over the last 100 years, and most belong to 16 established families (Ibarra and Del Rincón-Castro, 2009; Possee and King, 2014). Recently, the development of sequencing technologies (e.g., next-generation sequencing) has facilitated the exploration of viral species in both invertebrates and vertebrates. Although thousands of novel viruses have been identified, their taxonomies is often remains unclear (Shi et al., 2016; Shi et al., 2018; Kafer et al., 2019; Wu et al., 2020). The interactions between viruses and their insect hosts have been well studied in the last few decades, and insect viruses are now known to form diverse interactions with their hosts: harmful, in which they damage their hosts by killing them or reducing their fertility, and can be considered as potential biopesticides (Clem and Passarelli 2013; Haase et al., 2015; Palmer et al., 2018); beneficial, in which the host benefits from the virus by increasing their fitness (Jagdale and Joshi 2019; Wan et al., 2023); and both beneficial and harmful, in which the virus is either beneficial or harmful to their hosts depending on the context, for example, the virus decreases the fertility of the hosts but enhances their resistance to pathogens (Xu et al., 2020). Understanding the relationship between viruses and their insect hosts can facilitate the development of environmentally friendly management tools and strategies for controlling pests, such as baculoviruses for caterpillar pests (Haase et al., 2015; Lacey et al., 2015; Jagdale and Joshi 2019).

Besides being infected by insect viruses, piercing-sucking insects from Hemiptera and Thysanoptera are also vectors of most known plant viruses (approximately 1200 species) that threaten crop production with economic importance globally (Whitfield et al., 2015; Islam et al., 2020; Wang and Blanc 2021). Plant viruses commonly attract insect vectors by changing the volatile organic compounds of plants, or enhance the fitness of insect vectors by regulating the quality of plants to indirectly enhance their transmission efficiency (Musser et al., 2003; Fereres and Moreno 2009; Guo et al., 2010; Li et al., 2019; Liu et al., 2019). In addition, plant viruses can modify the behavior and performance of insect vectors to directly enhance their transmission efficiency (Medina-Ortega et al., 2009; Rajabaskar et al., 2013; Carmo-Sousa et al.,

2016). Regardless of the mechanism used by plant viruses, beneficial or both beneficial and harmful relationships between plant viruses and their insect vectors are expected because plant viruses need to modify the behaviors and increase the population dispersal of their vectors to enhance their transmission.

Interactions between microorganisms within common insect hosts have been extensively studied. For example, *Wolbachia* bacteria decrease the titer of dengue virus in their mosquito vectors (Hoffmann et al., 2011; Pan et al., 2012; Rances et al., 2012), *Wolbachia* enhances the titers of some pathogenic viruses in their insect hosts (Graham et al., 2012; Amuzu, et al. 2018), and symbiotic viruses enhance insect host resistance to baculoviruses (Xu et al., 2014; Xu et al., 2020). These interactions have been used to develop novel strategies for controlling insect-borne diseases and insect pests. However, the role of insect viruses in plant–virus–insect vector systems has been largely neglected.

Green peach aphids (*Myzus persicae*) are one of the most economically important aphid crop pests worldwide. They are highly polyphagous and feed on more than 400 plant species from 40 different families (Emden et al., 1969; Blackman and Eastop 2000). Besides directly feeding on plants, *M. persicae* can indirectly cause losses of economically important plants by efficiently transmitting over 100 plant viruses from approximately 30 different families, including persistent viruses (e.g., Potato leaf roll virus, PLRV) and non-persistent viruses (e.g., Potato virus Y, PVY) (Kennedy et al., 1962; Ng and Perry 2004; Gadhave et al., 2020). Previously, we identified a densovirus infecting *M. persicae* (*Myzus persicae nicotianae* densovirus, MpnDV) (Tang et al., 2016) and demonstrated that PVY infection could be helpful for the performance and dispersal of *M. persicae* (Liu et al., 2019; Chen et al., 2020). The MpnDV–PVY system is an ideal model for testing the interactions between insect and plant viruses within an aphid–virus–plant system. In this study, we showed, for the first time, that an insect virus facilitates the transmission of a plant virus by enhancing the locomotor activity and population proliferation of its common insect vector. This has important implications for devising novel sustainable management strategies against insect-transmitted plant viruses.

Materials and methods

Aphid culture and feeding device preparation

The MpnDV-infected *M. persicae* were collected in Xichang, Sichuan province, China (27.50ºN, 102.21ºE), in 2012, while the MpnDV-free tobacco aphids were collected in Qingdao, Shandong province, China (36.44ºN, 120.58ºE), in 2015. Both were maintained on tobacco plants (*Nicotiana tabacum* 'K326') in cages in climate chambers, at 25 ± 1 ℃ with 65 ± 1% relative humidity and 16:8 light:dark photoperiod. A feeding device was prepared as previously described to determine the life-history parameters of individual aphids (van Munster et al., 2003). Briefly, this included a polypropylene Petri dish (diameter: 6 or 10 cm) containing a fitted tobacco leaf disc on a layer of 1% water agar. For each experiment, the neonatal nymphs were produced by adult aphids within 6 hours.

MpnDV detection and preparation

Total DNA from individual aphids was extracted using a TIANamp Virus DNA Kit (Tiangen, Beijing, China), and total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), as described previously (Tang et al., 2016). First-strand cDNA was synthesized using oligo(dT), TransScript One-Step gDNA Removal, and cDNA Synthesis SuperMix (TransGen Biotech, Beijing, China) for viral detection. Specific primers amplifying a 445 bp fragment were designed according to the MpnDV genomic sequence (GenBank accession no: KT239104) to determine the presence of MpnDV. The actin and cytochrome oxidase I (*COI*) gene of *M. persicae* were used to determine the quality of the cDNA and DNA templates with specific primers Mpactin-F/Mpactin-R and MpCOI-F/MpCOI-R designed based on the sequences (GenBank accession no: MF083568 and KY522912), respectively. All of the primers used in this study are shown in Table S1 (Supporting Information 1). The PCR reaction was performed as follows: 30 seconds at 94 ℃, 30 seconds at 55 ℃, and 45 seconds at 72 ℃ for 40 cycles.

MpnDV was isolated from positive individuals as described previously (Xu et al., 2020). Briefly, 200 newborn MpnDV-infected aphids were raised on ten feeding devices. When these aphids were 2-day-old adults, 200 wingless aphids were ground

in liquid nitrogen, and some were used for DNA extraction and virus detection. The remaining sample was used to extract the MpnDV-filtered liquid. Approximately 400 mg of tissue was transferred to 1 mL PBS buffer (0.01 M, pH 7.4). The homogenate was centrifuged at 6500 × g for 15 min at 4 ℃, and the supernatant immediately filtered with Sartorius Minisart 0.2 μm PES (Invitrogen, Grand Island, USA). As a control, MpnDV-free filtered liquid was extracted from 200 MpnDV-free individuals as a mock inoculation. All the samples were stored at −80 ℃.

Monoclonal line construction

The monoclonal line was established from a single MpnDV-free parthenogenetic female. MpnDV-positive colonies were constructed by feeding them with the filtered liquid with MpnDV five times, as described previously (Xu et al., 2014; van Munster et al., 2003). Briefly, 200 μL MpnDV filtered liquid was daubed on the surface of a tobacco leaf using cotton swabs. After 1 hour, 50 neonatal nymphs from the monoclonal line were placed on the leaf for 24 hours. They were then transferred to feeding devices (one individual per feeding device) until they produced offspring. Subsequently, the individuals were collected separately for DNA extraction and viral detection. The offspring of MpnDV-positive individuals were transferred to MpnDV-positive colonies. Several MpnDV-negative offspring from the same generation were transferred into MpnDV-negative colonies. The offspring of 100 MpnDV-positive aphids and 100 MpnDV-negative aphids were used to construct the MpnDV-positive and MpnDV-negative colonies, which were kept on six tobacco plants in one cage, respectively (Supporting Information 1-Fig. S1). Aphids were equally sampled from six host plants with MpnDV-positive and MpnDV-negative colonies for each experiment.

RNA-seq was performed using an Illumina NovaSeq 6000 instrument (Majorbio, Shanghai, China) to exclude the possibility that additional viruses were present in the MpnDV-positive and -negative colonies. Total RNA was extracted from 200 MpnDV-free and MpnDV-infected adults, respectively. Ribosomal RNA was depleted from the total RNA, then these samples were fragmented and used to synthesize double-stranded cDNA templates. After size selection and quantification, libraries

were sequenced using the Illumina NovaSeq 6000 platform to approximately 6 gigabase in depth. After sequencing and quality control, the clean reads were separately aligned to the reference genome (*Myzus persicae* clone G006, GenBank: GCA_001856785.1) in orientation mode. Sequences mismatched to the host genome were assembled using Trinity (v2.8.5) software (Grabherr et al., 2011). All of the assembled unigenes were searched against the NCBI protein nonredundant (NR), SwissProt and clusters of eukaryotic Orthologous Groups (KOG) databases using DIAMOND (v0.9.24) (e-value < 1E-5) (Buchfink et al., 2015); the Kyoto Encyclopedia of Genes and Genomes (KEGG) database using KAAS (r140224) (default) (Moriya et al., 2007); the Gene Ontology (GO) database using Blast2GO (v2.9.0) (default) (Conesa et al., 2005); the Interpro database using InterProScan5 (V5.11-51.0) (default) (Quevillon et al., 2005); and the Pfam database using HMMER (v3.2.1) (default) (Eddy, 2011). The RNA-Seq data were submitted to the NCBI Sequence Read Archive database.

MpnDV transmission mode

Horizontal transmission of MpnDV was determined using previously described methods (Li et al., 2022). Briefly, 10 adult MpnDV-infected aphids were removed from the feeding device after feeding for 72 hours, and 10 newborn nymphs of the MpnDV-free strain were fed on this device for 48 hours. Thereafter, the nymphs were independently transferred to a new feeding device. A total of 54 individual adults were collected for DNA extraction and viral detection. Nine MpnDV-positive adult aphids were placed separately on a feeding device for vertical transmission. Neonatal nymphs produced over 3 days were immediately transferred to a new feeding device before they started feeding on the leaves. A total of 105 adult individuals were collected for DNA extraction and viral detection.

Fluorescence *In Situ* Hybridization of MpnDV

In situ hybridization was performed to observe the cellular tropism of MpnDV in aphid embryos, as reported previously (Li et al., 2022). The adult MpnDV-infected strains were dissected in cold 70% ethanol under a stereoscopic microscope to obtain aphid embryos. Then more than 50 intact aphid embryos were fixed in Carnoy's

199 solution (chloroform-ethanolacetic acid [6:3:1]) for 10 hours, decolorized overnight in
200 an alcoholic 6% H_2O_2 solution, and pre-hybridized three times in hybridization buffer
201 (20 mM Tris-HCl [pH 8.0], 0.9 M NaCl, 0.01% sodium dodecyl sulfate, 30%
202 formamide) for 6 hours each time. Embryos were then incubated overnight in a
203 hybridization buffer containing 100 pmol/mL of each fluorescent probe and 0.5 mg/ml
204 49,69-diamino-2-phenylindole (DAPI). Finally, the embryos were washed in a buffer
205 (0.3 M NaCl, 0.03 M sodium citrate, 0.01% sodium dodecyl sulfate) and observed
206 under a laser confocal microscope (LSM 510 META, Carl Zeiss). In the hybridization,
207 a reported fluorescent probe was used to target the *M. persicae* primary symbiont
208 *Buchnera aphidicola* 16S rRNA (Li et al., 2011), and a newly designed fluorescent
209 probe targeting MpnDV NS1 mRNA, MpnDV-Alexa Fluor 488 (5′-Alexa Fluor
210 488-TCGTCGTCTACATAGTTGGA-3′) were employed. DAPI was used to
211 counterstain the nuclei of aphid cells. No probe or RNase digestion control
212 experiments were performed to confirm the specificity of the detection.

213 **Quantification of the MpnDV in *M. persicae***

214 MpnDV copy numbers were quantified using TaqMan real-time quantitative PCR
215 (qPCR) using a standard curve, as described previously (Xu et al., 2014). Briefly, a
216 445 bp fragment of MpnDV was amplified with specific primers, cloned into the
217 pEASY-T Cloning Vector (TransGen Biotech), and sequenced. Specific qPCR primers
218 and probe were designed based on these fragments. To construct a standard curve,
219 qPCR was performed on a 7500 Fast Real-Time PCR system (Applied Biosystems,
220 Foster City, CA, USA) using serial plasmid dilutions. The reaction system was as
221 follows: 2 x reverse transcriptase (RT)-PCR buffer (10 μL), forward and reverse
222 primers (0.4 μL each, 20 pmol/μL), TaqMan probe (0.8 μL, 40 pmol/μL), 50 x
223 RT-PCR enzyme mix (0.2 μL), DNA template (2 μL), and double-distilled (dd) H_2O
224 (6.2 μL). The thermal cycling conditions were 95 ℃ for 30 seconds, 40 cycles of
225 95 ℃ for 5 seconds, and 60 ℃ for 34 seconds. Individuals collected from different
226 stages (1^{st} to 4^{th} instar nymphs and 1-day- to 4-day-old adults) were used for MpnDV
227 quantification, with 10 individuals for each group and six replicates for each stage. All
228 of the primers used in this study are shown in Table S1 (Supporting Information 1).

Determination of life-history parameters of *M. persicae*

In total, 400 neonates from MpnDV-infected or MpnDV-free cultures were placed in the feeding device to test the effect of MpnDV infection on the life-history parameters of *M. persicae*, including nymph duration, adult longevity, fecundity, nymph mortality, and aphid weight. Individual status was checked daily at 8:00 am. The weights of fourth-instar nymphs, 1-day- and 2-day-old adults were recorded, with 20 individuals per group and six replicates for each treatment. The tobacco leaves were changed every 5 days.

Determination of the population distribution and dynamics of *M. persicae*

The population distribution and dynamics of MpnDV-positive and -negative aphids on tobacco plants were performed in insect-proof cages (110 × 60 × 40 cm) at a constant temperature of 25 ± 1 ℃ with 65 ± 1% relative humidity and 16:8 light:dark photoperiod. Ten newborn nymphs were transferred to the first true leaf (bottom) of the plant (six-leaf-stage plants, four plant replicates), and the number of aphids feeding on each leaf was counted daily for 3 weeks.

Determination of walking movement of *M. persicae*

The movement paths of the aphids were tracked and measured using an insect locomotion compensator (TrackSphere LC-300, Syntech, Netherlands). During the experimental period, individuals were maintained at 25 ± 1 ℃. The sphere was cleaned with 75% ethanol before each trial. Before recording individual aphids (starved for 2 hours), they were allowed to acclimatize to the sphere for 2 minutes, after which each aphid was recorded for 10 minutes. Eighty adult aphids were measured, and MpnDV-free aphids were used as controls with the same treatment.

Determination of the population distribution and dispersal of *M. persicae*

Dispersal tests were carried out at a constant temperature of 25 ± 1 ℃ with 65 ± 1% relative humidity and 16:8 light:dark photoperiod. Each pair of donor and recipient tobacco plants was placed approximately 95 cm apart in trays (110 × 40 × 5 cm) within an insect-proof chamber. Ten newborn nymphs were transferred to the first leaf (bottom) of the donor tobacco plants (six-leaf-stage), and the population quantities of the donor and recipient tobacco plants were determined daily for 3 weeks.

Quantitative titers of EβF in *M. persicae* using GC-MS

The samples were prepared as previously described (Cheng and Li, 2019). Briefly, the MpnDV infected or MpnDV free wingless adults (1-day-old) were collected and weighed in a 1.5 mL centrifuge tube (n = 20) and then comprehensively ground under liquid nitrogen, followed by immediately added to 200 μL hexane. One μL decane (diluted by 1000 times) was used as the internal standard. as the internal standard. After centrifugation at 10000 × g for 5 min, the supernatant was transferred to a 2.0 mL chromatography vial. The GC-MS procedure was performed using an Agilent Technologies 7010B GC/MS Triple Quadrupole (Santa Clara, CA, USA). The splitflow of carrier gas helium was 2.25 mL/min with a 4 μL injection volume; mass spectra were obtained in the EI mode at 70 eV. EβF quantity was estimated based on the peak area ratio of EβF to decane. Each sample was analyzed in triplicate.

Detection of *MpFPPS* gene expression using qPCR

The relative transcript level of *MpFPPS* was detected using qPCR on a 7500 Fast Real-time PCR System (Applied Biosystems) using *Actin* and *GAPDH* as reference genes. Three replicates (100 adult aphids per replicate) of each treatment were analyzed, and total RNA and cDNA were prepared as described above. The reaction system was 20 μL in volume, comprising 10 μL of TB Green Premix Ex Taq (Tli RNaseH Plus) (2X), 0.4 μL of ROX Reference Dye II, 0.4 μL of upstream and downstream primers, 6.8 μL of sterile water, and 2 μL of template. The reaction conditions for fluorescence quantification were: 95°C for 30 seconds and 40 cycles of 95°C for 5 seconds and 60°C for 30 seconds. All of the primers used in this study are shown in Table S1 (Supporting Information 1).

***Myzus persicae farnesyl diphosphate synthase 1* (*MpFPPS1*) gene silencing using plant-mediated RNAi**

Specific primers were employed to prepare templates to express dsRNA that showed the best knockdown effect against *MpFPPS1* in a previous study (Cheng and Li, 2019). A GFP fragment was amplified as a dsRNA negative control as described previously (Cheng and Li, 2019). The EcoRI/KpnI and BamHI/XbaI restriction sites were added to each primer pair, respectively. The PCR products were cloned into the

pKANNIBAL vector after double digestion and ligation, and a recombinant vector containing two target fragments (forming a hairpin structure) was obtained. Then the pKANNIBAL-dsFPPS1 fragment was cloned into the pCAMBIA1300 vector using the SacI and SbfI restriction enzymes. The recombinant plasmid, pCAMBIA-RNAi-FPPS1, was verified using PCR and sequencing. Subsequently, the vector was transfected into *Agrobacterium tumefaciens* strain LBA4404 using electroporation. *Nicotiana tabacum* (ecotype K326) plants were transformed using the leaf disc co-cultivation method, as previously described (Gallois and Marinho, 1995). Finally, transgenic kanamycin-resistant plants were identified. Transgenic plants were further analyzed using RT-PCR. These transgenic plants were then transferred to pots of soil for continued growth, and plants with six true leaves were used for aphid feeding.

Determination of the effect of MpnDV and PVY on wing dimorphism of *M. persicae*

The tests were carried out at a constant temperature of 20 ± 1 ℃ with 65 ± 1% relative humidity and 16:8 light:dark photoperiod. The PVY-infected tobacco plants (1 day after PVY inoculation, six-leaf-stage) and PVY-free tobacco plants were placed in an insect-proof chamber, respectively. Twenty newborn nymphs were placed on the third leaf of PVY-infected plants using a leaf cage (diameter, 6 cm). At the adult stage, the aphids were removed after producing 100 offspring in each leaf cage. After 10 days, the proportion of winged aphids was recorded in these 100 offspring. PVY-free tobacco plants were used as control, with each treatment consisting of six replicates. Additionally, the population dynamics of winged aphids on tobacco plants were recorded under identical conditions. Twenty newborn nymphs were transferred to the first true leaf (bottom) of the plant (six-leaf-stage plants, six plant replicates), and the number of aphids feeding on each leaf was counted daily for 4 weeks.

Determination of PVY uptake and retention by *M. persicae*

One-day-old adult aphids were transferred to PVY-infected tobacco plants (10 days after PVY inoculation) and allowed to feed for 10 minutes, after which they were transferred to clean Petri dishes. After 0 , 8 , 16 , 24 and 30 hours, 10 aphids were

collected into a 1.5 mL tube for RNA extraction. Total RNA and cDNA were extracted as described above. PVY copy numbers were quantified using TaqMan real-time qPCR as described previously (Liu et al., 2019).

Determination of the population dispersal and PVY transmission of *M. persicae*

For wingless aphids, the tests were carried out at a constant temperature of 25 ± 1 ℃ with 65 ± 1% relative humidity and 16:8 light:dark photoperiod. One donor plant (1 day after PVY inoculation, six-leaf-stage) and 10 recipient plants were placed approximately 85 cm apart in a tray (110 × 40 × 5 cm) within an insect-proof chamber. Twenty newborn nymphs were transferred to the first leaf (bottom) of the donor tobacco plants, and the population quantities of the donor and recipient tobacco plants were determined daily. When aphids spread from donor plants to recipient plants, the recipient plants were changed every 2 days. The aphids on the recipient plants were counted and removed immediately. After 10 days, the PVY transmission rate to recipient plants was determined using RT-PCR.

For winged aphids, the tests were carried out at a constant temperature of 20 ± 1 ℃ with 65 ± 1% relative humidity and 16:8 light:dark photoperiod. One donor plant (1 day after PVY inoculation, six-leaf-stage) and 10 recipient plants were placed approximately 85 cm apart in a tray (110 × 40 × 5 cm) within an insect-proof chamber. A layer of water was placed at the bottom of the tray to prevent the wingless aphids from dispersing. Twenty newborn nymphs were transferred to the first leaf (bottom) of the donor tobacco plants, and the population quantities of the donor and recipient tobacco plants were determined daily. When winged aphids were spread from the donor plant to the recipient plants, the recipient plants were changed every 2 days. The aphids on the recipient plants were counted and removed immediately. After 10 days, the PVY transmission rate to recipient plants was determined using RT-PCR. Tobacco plants were inoculated with PVY-GFP (PVY^N, GenBank accession: X97895) (Tian and Valkonen, 2015) by mechanical friction, and 20 newborn nymphs were transferred to the plants as described above to show the replicates of PVY transmitted by winged aphids. After 18 days, the recipient plants were provided for 2 days, as described above. The recipient plants were observed using a handheld UV lamp after

6 days.

Statistical analyses

Statistical analyses were conducted using Graphpad Prism 8.3.0 and SPSS 17.0. All the data demonstrated approximate normality and homogeneity of variance before the parametric testing. MpnDV replication in *M. persicae* was analyzed using ANOVA with Tukey's LSD difference test. Student's *t*-test (or Welch's *t*-test) was used to determine the significance of the life-history characteristics, aphid population size, and walking movements of *M. persicae,* as well as other pairwise comparisons.

Results

Distribution, transmission of MpnDV and its effects on life-history parameters and population dynamics of *M. persicae*

We successfully constructed MpnDV-negative and -positive aphid strains using PCR with specific primers (Supporting Information 1-Fig. S2). Using the RNA-seq method, we proved that there was only one virus (MpnDV) in the aphids used (SRA accession: SRR28495334 and SRR28495335; dataset for MpnDV-positive aphids: 8.2 GB, for MpnDV-negative aphids: 7.8 GB). Using *Buchnera aphidicola* as a reference, the cellular infection pattern of MpnDV was observed in aphid embryos (Fig. 1A, Supporting Information 1-Fig. S3). The results showed that *B. aphidicola* was only observed in primary bacteriocytes. In contrast, MpnDV infected diverse aphid cells, including those around primary bacteriocytes. Control experiments (without probe or RNase digestion) confirmed the specificity of the observed signals (data not shown).

A standard curve was generated using gradient dilution templates of the recombinant plasmids to quantify MpnDV (Supporting Information, 1-Fig. S4; 2-S1). The results indicated that MpnDV copy numbers varied significantly at different stages, with copy numbers increasing significantly in nymphs over the three days and there were no significant differences in older individual infection levels (Fig. 1B; Supporting Information 2-S2). MpnDV could be horizontally transmitted among individuals of *M. persicae* by feeding on virus-contaminated tobacco leaves, with an infection rate of 57.4% (31/54), and the vertical transmission rate was about 91.4% (96/105) (Supporting Information 1-Table S2).

There were no significant effects of MpnDV infection on the life-history parameters of aphids fed on devices with a single individual (Fig. 1C, 1D; Supporting Information, 1-Fig. S5A-5C, 2-S3). Interestingly, the number of MpnDV-positive aphids was significantly higher than the negative ones in tobacco plants on days 14 and 21 after aphid inoculation (Fig. 1E; Supporting Information 2-S4). Additionally, the distribution of MpnDV-positive and -negative aphids on each leaf of the tobacco plants was also recorded. The results indicated that the numbers of MpnDV-positive aphids were significantly higher than the negative ones on mature leaves (4^{th} to 6^{th} leaves) and young leaves (7^{th} to 9^{th} leaves) 14 and 21 days after aphid inoculation (Fig. 1F; Supporting Information 2-S4). Consistently, the proportion of MpnDV-positive individuals in mature and young leaves was significantly higher than that of MpnDV-negative individuals (Fig. 1F).

To determine the effect of aphid distribution on population dynamics, 20 newborn nymphs were evenly inoculated on four leaves (1^{st} to 4^{th} leaves of six-leaf-stage plants), and 20 newborn nymphs were inoculated on one leaf (1^{st} leaf of six-leaf-stage plants) as a control. The results indicated that the number of aphids was significantly higher than that in the control group 21 days after aphid inoculation (Fig. 1G; Supporting Information 2-S5). Additionally, the fecundity of aphids feeding on young leaves was significantly higher than on the old leaves (1^{st} to 3^{rd} leaves) on the same tobacco plant (Fig. 1H; Supporting Information 2-S6). These results combined showed that MpnDV infestation improves the population proliferation of *M. persicae* by promoting the dispersal of its aphid host on tobacco leaves.

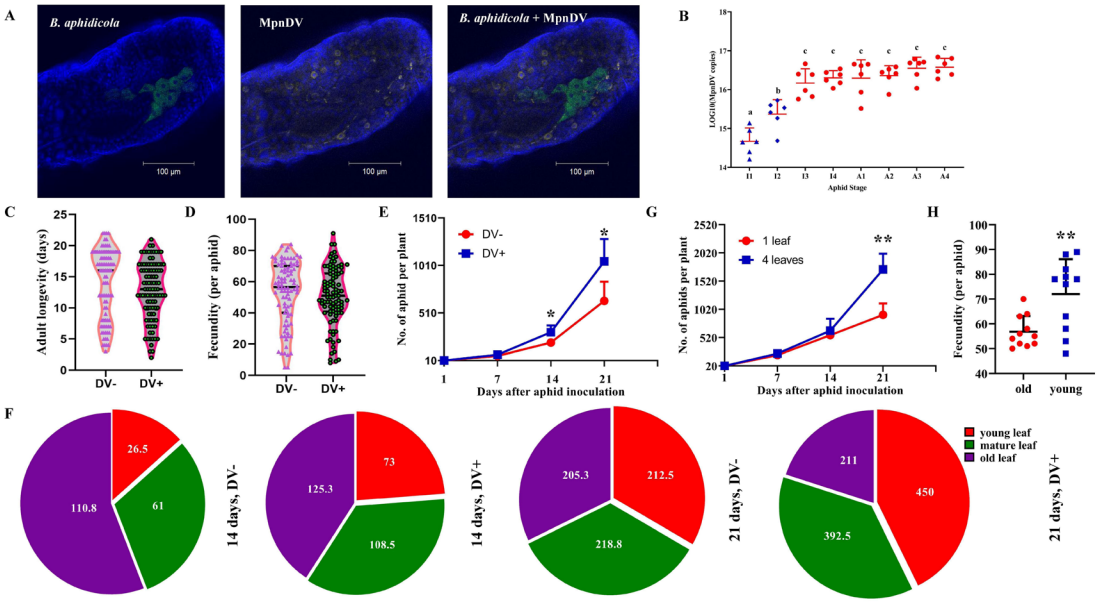


Fig. 1 The effect of MpnDV on *Myzus persicae* life-history characteristics. A:whole-mount *in situ* hybridization of MpnDV-infected aphid embryos. Nuclei of aphid cells (blue), *Buchnera aphidicola* (green) and MpnDV (yellow). B: population dynamics of MpnDV in different stages of *M. persicae* (F = 25.34, df = 7, 40, P < 0.0001, one-way ANOVA, different letters are significantly different at the 0.05 level; I1–I4: 1st instar nymph to 4th instar nymph, A1–A4: 1-day-old adult to 4-day-old adult). C: adult longevity (n: DV- = 92, DV+ = 98, t = 1.734, df = 188, P = 0.0846). D: fecundity (n: DV- = 92, DV+ = 98, t = 0.8748, df = 188, P = 0.3828). E: aphid population dynamics in tobacco plants (n = 4, on 14 days, t = 2.821, df = 6, P = 0.03; on 21 days, t = 2.689, df = 6, P = 0.036). F: Proportion and number of aphids on tobacco plant (n = 4, on 14 days: for mature leaves, t = 3.335, df = 6, P = 0.016; for young leaves, t = 3.08, df = 6, P = 0.022; on 21 days: for mature leaves, t = 2.518, df = 6, P = 0.045; for young leaves, t = 2.72, df = 6, P = 0.035). Leaves were numbered from the bottom to the top of the plant, starting with the first true leaf. Old leaves (1st to 3rd leaves), mature leaves (4th to 6th leaves), young leaves (7th to 9th leaves). G: aphid population dynamics in tobacco plant after even inoculation (n = 4, on 21 days, t = 4.676, df = 6, P = 0.0034). H: fecundity of aphids feeding on young and old leaves (n = 11, t = 3.25, df = 13.89, P = 0.006). Mean with SD, Independent samples t-test (C-G), Welch's t-test (H), "*" represents p < 0.05, "**" represents p < 0.01.

MpnDV enhanced the locomotor activity and population dispersal of *M. persicae*

To determine the effect of MpnDV on aphid behavior, the locomotor activity of *M. persicae* individuals was recorded. The results showed that the average speed and travel length of MpnDV-positive individuals were significantly higher than those of MpnDV-negative individuals (Fig. 2 A, 2B; Supporting Information 2-S7, S8).

In addition, the effect of MpnDV on aphid population dispersal from donor plants to neighboring plants was recorded. The results showed that the number of MpnDV-positive aphids was significantly higher than the number of MpnDV-negative aphids on both the donor and recipient tobacco plants 21 days after aphid inoculation (Fig. 2 C, D; Supporting Information 2-S9). As a result, the number of MpnDV-positive aphids was significantly higher than that of MpnDV-negative aphids in all insect-proof cages (Fig. 2 E; Supporting Information 2-S9). Together, these results indicated that MpnDV infection increases population proliferation by promoting the locomotor activities and population dispersal of its host, thus possibly improving the transmission of plant viruses by insect vectors.

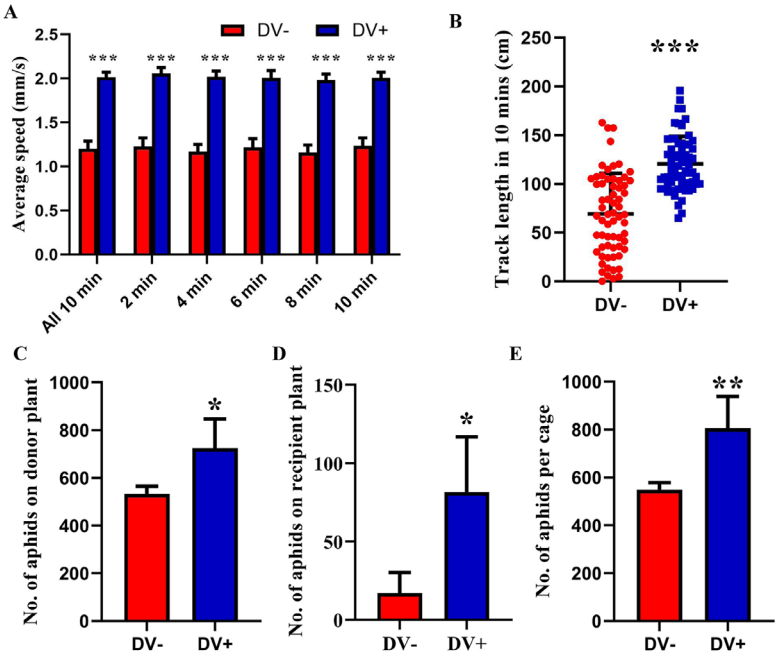


Fig. 2 MpnDV enhanced the locomotor activity and population dispersal of *Myzus persicae*. A: Average aphid speed over 10 minutes and every 2 minutes (n: DV- = 62, DV+ = 63, df_{all} = 108.7, t_{all} = 7.798, P_{all} < 0.0001; df_2 = 108.2, t_2 = 6.822, P_2 < 0.0001; df_4 = 123, t_4 = 7.99, P_4 < 0.0001; df_6 = 123, t_6 = 6.16, P_6 < 0.0001; df_8 = 123,

$t_8 = 7.43$, $P_8 < 0.0001$; $df_{10} = 111.6$, $t_{10} = 6.861$, $P_{10} < 0.0001$). B: Track lengths of individuals in 10 minutes (n: DV- = 65, DV+ = 63, $t = 8.234$, $df = 112.8$, $P < 0.0001$). C: Number of aphids on the donor plant ($n = 4$, $t = 3.064$, $df = 6$, $P = 0.022$). D: Number of aphids on recipient plant ($n = 4$, $t = 3.419$, $df = 6$, $P = 0.014$). E: Number of aphids in whole cage ($n = 4$, $t = 3.824$, $df = 3.297$, $P = 0.027$). Mean with SD, Independent samples *t*-test (A, C, D), Welch's *t*-test (A, B, E), "*" represents $p < 0.05$, "**" represents $p < 0.01$, "***" represents $p < 0.001$.

MpnDV infection enhanced the EβF titer in *M. persicae*

The (E)-β-farnesene (EβF) titer in the aphid was determined using GC-MS. The results indicated that the EβF titer in MpnDV-positive individuals was significantly higher than that in the negative ones (Fig. 3A, B; Supporting Information 2-S10). The *farnesyl diphosphate synthase* (FPPS) gene plays key roles in the synthesis of EβF. Interestingly, the expression level of *MpFPPS1* of MpnDV-positive individuals was significantly higher than the negative ones (Fig. 3C; Supporting Information 2-S11), suggesting that MpnDV enhanced the EβF titer in virus-positive aphids by increasing the expression levels of *MpFPPS1*.

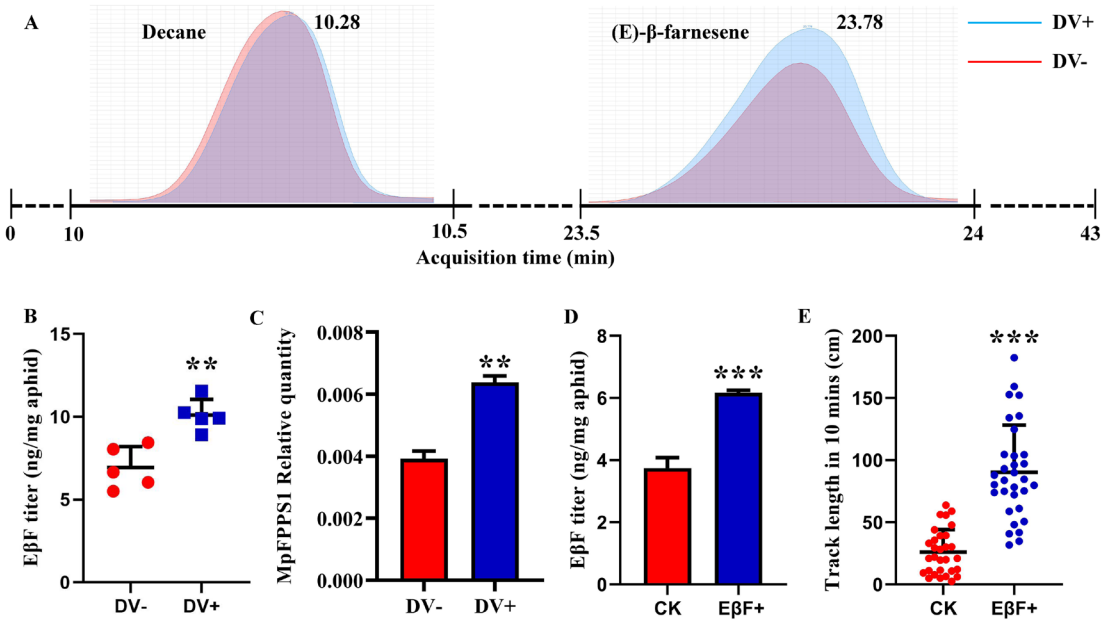


Fig. 3 MpnDV infection enhanced the (E)-β-farnesene (EβF) titer in *Myzus persicae*. A: Gas phase mass spectrogram of n-decane and EβF of MpnDV-positive

463 and -negative aphids. B: EβF titer in MpnDV-positive and -negative aphids (n = 5, t =
464 4.473, df = 8, P = 0.0021). C: Relative expression of *Myzus persicae* farnesyl
465 diphosphate synthase 1 (*MpFPPS1*) in aphids (n = 3, t = 7.421, df = 4, P = 0.0018). D:
466 The concentration of EβF in aphid after supplementation by oral administration (n = 3,
467 t = 11.98, df = 4, P = 0.0003). E: Track length of individuals after supplementation
468 with EβF (n: CK = 30, feeding EβF = 32, t = 8.565, df = 44.95, P < 0.0001). CK:
469 aphids feeding on artificial diet, EβF+: aphids feeding on artificial diet with EβF
470 compound. Mean with SD, Independent samples t-test (B, C, D), Welch's t-test (E),
471 "**" represents p < 0.01, "***" represents p < 0.001.

472 To determine the effect of EβF titer on aphid locomotor activity, the titers of EβF in
473 the aphid were increased by feeding on artificial diet with EβF compound and
474 decreased by feeding on transgenic plants with dsRNA targeting the *MpFPPS1* gene.
475 The EβF titer of aphids *in vivo* was significantly increased by feeding on the
476 compound (Fig. 3D; Supporting Information 2-S12). Consistently, the locomotor
477 activities of aphids feeding on EβF were higher than that of non-treated individuals
478 (Fig. 3E; Supporting Information 2-S13). Transgenic plants with dsRNA were
479 obtained, and the targeting of the *MpFPPS1* gene was analyzed by RT-PCR (Fig.
480 4A-4C, Supporting Information 1-Fig. S6). After feeding the transgenic plants for 5
481 days, the relative expression level of *MpFPPS1* was significantly reduced by about
482 20% (Fig. 4D; Supporting Information 2-S14). Consistently, the EβF titer of these
483 aphids was significantly decreased, and the locomotor activity of aphids was
484 significantly inhibited (Fig. 4E, 4F; Supporting Information 2-S15). These results
485 indicated that MpnDV infestation promoted the locomotor activity of *M. persicae* by
486 increasing the titer of EβF via up-regulation of expression levels of the *MpFPPS1*
487 gene.

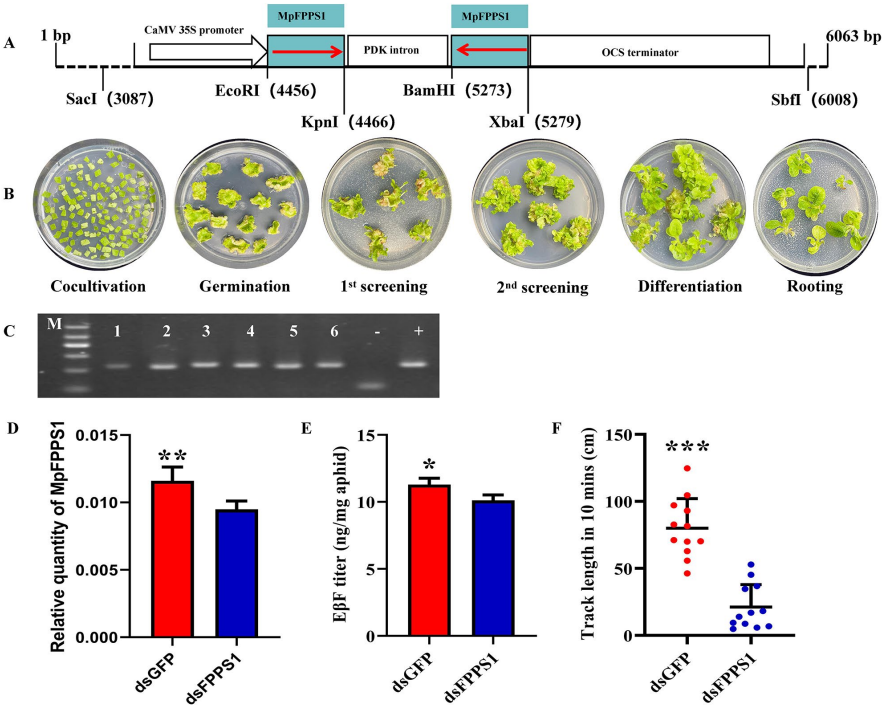


Fig. 4 *Myzus persicae* farnesyl diphosphate synthase *1* (*MpFPPS1*) gene silencing by plant-mediated RNAi. A: Map of the recombinant vector of *pKANNIBAL* containing the two target fragments for the expression of ds*MpFPPS1* (EcoRI, KpnI, BamHI, XbaI, SacI and SbfI restriction sites are indicated). B: Tobacco plant transformation using the leaf disc co-cultivation method. C: The detection of *MpFPPS1* in transgenic plants by RT-PCR (M: marker, Lanes 1-6: transgenic plants, "–" = negative control, "+" = positive control). D: Relative expression of *MpFPPS1* in aphid fed on transgenic tobacco plants (n = 6, t = 4.333, df = 10, P = 0.0015). E: (E)-β-farnesene (EβF) titer in aphid after fed on transgenic tobacco plants (n = 3, t = 3.757, df = 4, P = 0.0198). F: Track length of individuals fed on transgenic tobacco plants (n = 12, t = 7.353, df = 22, P < 0.0001). Mean with SD, Independent samples t-test, "*" represents p < 0.05, "**" represents p < 0.01, "***" represents p < 0.001.

MpnDV infection accelerated PVY transmission by *M. persicae*

The effect of MpnDV infection on the transmission of PVY by aphids was also determined. The results showed that MpnDV had no significant effect on the acquisition quantity, retention time, or transmission efficiency of PVY by aphids (Supporting Information, 1-Fig. S7A, 7B; 2-S16). There was no significant difference

between the copy number of PVY in plants inoculated with MpnDV-infected aphids and MpnDV-free aphids (Supporting Information, 1-Fig. S7C; 2-S17). Meanwhile, PVY had no significant effects on the horizontal or vertical transmission efficiency of MpnDV or on the copy number of MpnDV in aphids (Supporting Information, 1-Fig. S7D-7F; 2-S18).

Winged aphids are indispensable for long-distance population dispersal and viral transmission. The effects of MpnDV on wing dimorphism in aphids were determined at the same population density. The results indicated no significant difference in the proportion of winged aphids between MpnDV-positive and -negative aphids (Fig. 5A; Supporting Information 2-S19). However, PVY significantly increased the proportion of winged aphids among both MpnDV-positive and -negative aphids (Fig. 5A; Supporting Information 2-S19). In addition, the proportion and number of winged aphids on the tobacco plants were recorded. Although the numbers of wingless and winged of MpnDV-positive aphids were significantly higher than those of the negative aphids in tobacco plants 21 and 28 days after aphid inoculation, the proportion of winged MpnDV-positive aphids did not differ from that of the negative aphids (Fig. 5B; Supporting Information, 1-Fig. S8A-8C, 2-S20).

The effects of MpnDV on the dispersal of winged aphid population and PVY transmission were also determined. The results showed that the number of MpnDV-positive winged aphids was significantly higher than that of the MpnDV-negative ones in recipient tobacco plants (Fig. 5C; Supporting Information 2-S21). Meanwhile, MpnDV increased the PVY transmission rate of winged aphids in recipient tobacco plants (Fig. 5D; Supporting Information 2-S22). Additionally, MpnDV also promoted the dispersal of wingless aphids and improved the rate of PVY transmission of wingless aphids in recipient tobacco plants (Supporting Information, 1-Fig. S8D, 8E; 2-S23, S24). The PVY infection status of recipient tobacco plants was observed using a handheld UV lamp. The fluorescence intensity of PVY-GFP in the recipient tobacco plants after 6 days inoculated with MpnDV-positive aphids was higher than that in the negative aphids (Fig. 5E, F, G). These results showed that MpnDV promotes the diffusion of the aphid population, which increases the

transmission of PVY. The dispersal ability of MpnDV-infected aphids over short and long distances was significantly improved in PVY-infected tobacco plants.

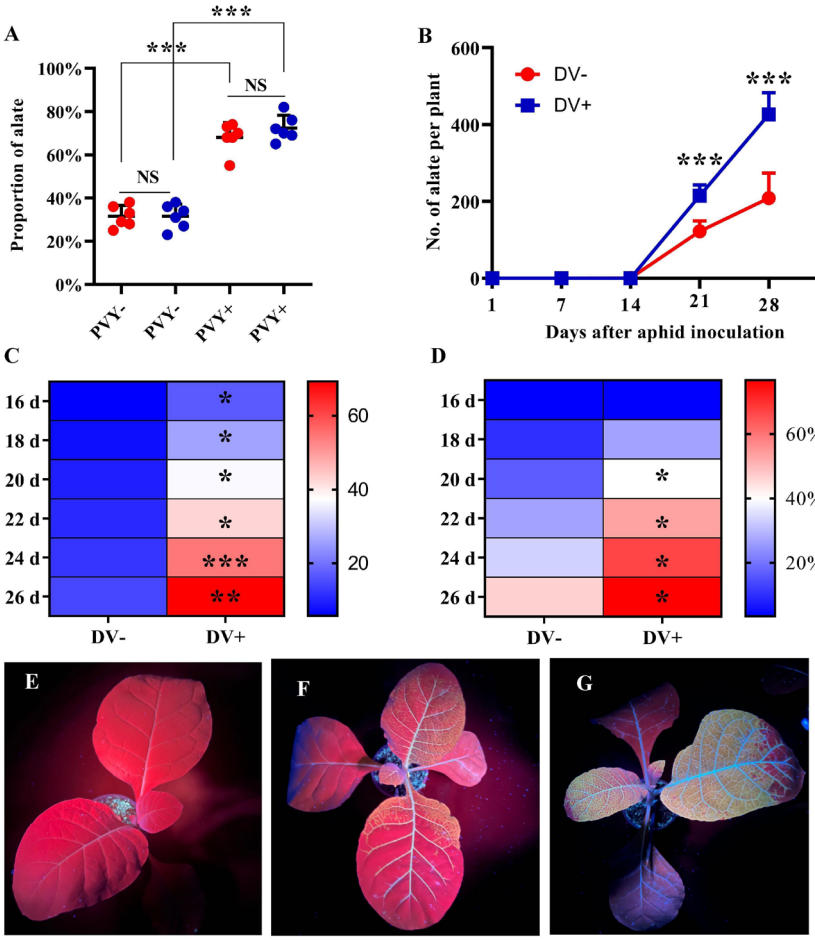


Fig. 5 MpnDV infection accelerated PVY transmission by *Myzus persicae*. A: The proportion of winged aphids was significantly increased by feeding on PVY-infected tobacco plants (n = 6, PVY-/DV- *VS* PVY-/DV+: t = 0.011, df = 10, P = 0.992; PVY+/DV- *VS* PVY+/DV+: t = 1.185, df = 10, P = 0.263; PVY-/DV- *VS* PVY+/DV-: t = 10.24, df = 10, P < 0.0001; PVY-/DV+ *VS* PVY+/DV+: t = 11.25, df = 10, P < 0.0001; red dot: MpnDV-free aphids, blue dot: MpnDV-infected aphids, PVY-: PVY-free plants, PVY+: PVY-infected plants). B: MpnDV infection significantly increased the number of winged aphids on tobacco plants (n = 6, on 21 days, t = 5.659, df = 10, P = 0.0002; on 28 days, t = 6.155, df = 10, P < 0.0001). C: MpnDV infection significantly enhances the dispersal of winged aphids. There were significantly more MpnDV-positive winged aphids moving from donor plants to recipient plants than from negative plants at different times (DV-: number of MpnDV-negative aphids,

552 DV+: number of MpnDV-positive aphids; n = 3, on 16 days: t = 3.982, df = 4, P =
553 0.016; on 18 days: t = 8.062, df = 4, P = 0.0013; on 20 days: t = 8.796, df = 4, P =
554 0.0009; on 22 days: t = 3.884, df = 4, P = 0.018; on 24 days: t = 5.633, df = 4, P =
555 0.005; on 26 days: t = 9.485, df = 4, P = 0.0007). D: MpnDV infection significantly
556 enhances PVY transmission by aphids. The PVY infection rate of recipient tobacco
557 plants was monitored by RT-PCR (DV-: PVY infection rate transmitted by
558 MpnDV-negative winged aphids, DV+: PVY infection rate transmitted by
559 MpnDV-positive winged aphids; n = 3, on 16 days: t = 0, df = 4, P > 0.999; on 18
560 days: t = 1.53, df = 4, P = 0.201; on 20 days: t = 3.515, df = 4, P = 0.025; on 22 days: t
561 = 2.848, df = 4, P = 0.047; on 24 days: t = 3.452, df = 4, P = 0.026; on 26 days: t =
562 3.525, df = 4, P = 0.024). E-G: PVY infection detected using GFP under a UV lamp
563 (E: PVY-free plant, F: PVY-GFP-infected plant inoculated with MpnDV-negative
564 aphids, G: PVY-GFP-infected plant inoculated with MpnDV-positive aphids). Mean
565 with SD, Independent samples t-test, "*" represents $p < 0.05$, "**" represents $p < 0.01$,
566 "***" represents $p < 0.001$.
567
568 **Discussion**
569 Interactions between insects and their vectored viruses, including insect and plant
570 viruses, have been extensively studied and used to develop new strategies for crop
571 protection (Ye et al., 2018; Xu et al., 2014, 2020; Wan et al., 2023). In this study,
572 using MpnDV–aphid–PVY–tobacco as a model system, we firstly demonstrated that
573 the insect virus (MpnDV) could promote population proliferation and dispersal of
574 aphids by enhancing their titer of EβF, leading to an enhanced transmission of a plant
575 virus (PVY). Additionally, PVY increased the frequency of winged aphids, facilitating
576 the long-distance dispersal of the aphid population. Subsequently, transmission of
577 both MpnDV and PVY was promoted, suggesting a potentially mutualistic interaction
578 between an insect virus and a plant virus in their common host.
579 Insect viruses can be efficiently transmitted both vertically and horizontally (Chen
580 et al., 2006; Mondotte et al., 2018). Vertical transmission of viruses can be subdivided
581 into two mechanisms: transovum transmission (virus on the surface of eggs) and

582 transovarial transmission (virus within eggs) (Yang et al., 2019; Xu et al., 2014). In
583 this study, we found that MpnDV can be transmitted both transovarially vertically and
584 orally horizontally. However, because of the ovoviviparous reproductive mode of
585 aphids, it cannot be completely excluded that MpnDV was horizontally transmitted to
586 the newly hatched nymphs in the body of the MpnDV-positive mother aphid, which
587 could also be considered vertical transmission, but not transovarial. Therefore, we
588 used the term “vertical transmission” rather than “transovarial transmission” in this
589 study. The negative effects of *Myzus persicae* densovirus on *M. persicae* have been
590 shown previously (Van Munster et al., 2003). However, our results, based on the
591 life-history parameters of single individuals, indicated that there were no significant
592 effects of MpnDV on its host, which might be due to the different virus strains used in
593 these two studies. Interestingly, MpnDV infection significantly promoted population
594 proliferation by increasing aphid dispersal in tobacco plants. The distribution of
595 individuals on plants can affect population dynamics, and our results suggested that
596 the population proliferation of *M. persicae* on tobacco plants was significantly faster
597 in populations that were evenly distributed on the host plant than when they were
598 aggregated. Additionally, there were more MpnDV-positive than MpnDV-negative
599 individuals on the young leaves of tobacco plants, suggesting that MpnDV infection
600 might promote the dispersal of *M. persicae*. It was previously reported that *Dysaphis*
601 *plantaginea* densovirus (DplDNV) infection promotes aphid dispersal (Ryabov et al.,
602 2009). Indeed, the MpnDV-positive individuals moved faster than the
603 MpnDV-negative ones, as measured using the insect locomotion compensator,
604 suggesting that MpnDV infection promoted population dispersal of its host and a
605 potentially mutualistic relationship between MpnDV and its host. However, this
606 approach was based on laboratory studies of aphids in environments without predators.
607 It cannot be completely ruled out that there could be negative effects resulting from
608 aphids moving around more and faster in a natural setting.

609 Pheromones are related to the movement of insects, e.g., EβF, a key component of
610 aphid alarm pheromone, promotes the dispersal of aphids (de Vos et al., 2010; Lin et
611 al., 2016; Wang et al., 2022). Interestingly, MpnDV infection increased the titer of

612 EβF significantly. *MpFPPS* genes were shown to be related to the titer of EβF in the
613 green peach aphid (Cheng and Li, 2019). MpnDV infection up-regulated the
614 expression level of the *MpFPPS1* gene significantly, suggesting MpnDV infection
615 enhanced the titer of EβF in *M. persicae* by up-regulation of the gene. To determine
616 whether the titer of EβF was related to the movement of *M. persicae*, we increased the
617 titer of EβF by feeding aphids on the compound and decreased the titer of EβF with
618 transgenic plants expressing dsRNA targeting the *MpFPPS1* gene. Consistently,
619 increasing the titer of EβF could enhance the movement of *M. persicae* significantly
620 and decreasing the titer of EβF could inhibit the movement of *M. persicae*
621 significantly, suggesting that MpnDV infection promoted the movement of *M.*
622 *persicae* by increasing the titer of EβF. As described above, MpnDV infection
623 enhances population proliferation by promoting aphid movement. However, aphids
624 could also become habituated to the presence of excess EβF, which made them less
625 able to perceive alarm pheromone warnings from conspecifics and escape from
626 predators. This might be disadvantageous for aphids infected with MpnDV in natural
627 settings. Previously, the EβF received with odorant receptors (ORs) and
628 odorant-binding proteins (OBPs) was shown to deter aphids *in vitro* (Mostafavi *et al*.,
629 1996; wang et al., 2015; Zhang et al., 2017; wang et al., 2022). However, the
630 relationship between the concentration of EβF *in vivo* and behavior of the aphid was
631 still unknown. We showed that the concentration of EβF *in vivo* could increase the
632 locomotor activity of aphids; however, the mechanism needs further investigation.
633 Endosymbionts play critical roles in plant virus transmission by insects, including
634 viral acquisition, retention, circulation, and inoculation in insect bodies, as well as
635 vertical viral transmission from parents to offspring (Kliot et al., 2019; He et al., 2021;
636 Ma et al., 2021). Although previous studies have revealed that insect viruses can
637 interact with insect-borne plant viruses in their common insect hosts (Pinheiro et al.
638 2019; Wan et al. 2023), the role of insect viruses in insect vectors during the
639 transmission of plant viruses is unclear. MpnDV promoted the population dispersal of
640 *M. persicae*, suggesting that the insect virus might help the transmission of plant
641 viruses vectored by the green peach aphid. Indeed, our results indicated that MpnDV

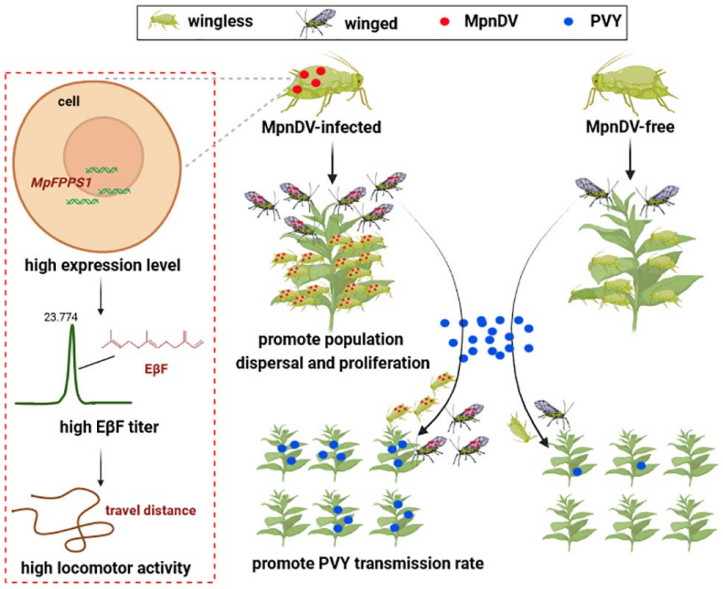
infection increased the population growth and dispersal of aphids, which promoted the transmission of PVY in tobacco.

The transmission modes of plant viruses by insect vectors are divided into four categories: non-circulative non-persistent, non-circulative semi-persistent, circulative non-propagative, and circulative propagative (Whitfield et al., 2015; Dietzgen et al., 2016). Plant viruses transmitted in a circulative persistent manner showed more complicated interactions with symbionts in their common insect hosts; for example, rice gall dwarf virus (RGDV) and *Recilia dorsalis* filamentous virus (RdFV) cooperatively hijacked the sperm-specific proteins of leafhopper for transmission of the two viruses (Wan et al. 2023), potato leafroll virus (PLRV) changed the copy numbers of the densovirus by small RNA in the green peach aphid; however, PVY with non-circulative non-persistent transmission mode did not change the titers of MpDNV (Pinheiro et al. 2019). Consistent with this, we found that PVY infection had no significant effect on the horizontal and vertical transmission efficiency of MpnDV or on the copy number of MpnDV in aphids. However, PVY infection significantly increased the proportion of winged aphids, possibly improving the population dispersal of *M. persicae.* MpnDV infestation had no significant effect on the acquisition quantity, retention time, or transmission efficiency of PVY by the aphids. However, MpnDV increased the transmission of PVY by promoting the diffusion of the aphid population. Although there was no direct interaction between MpnDV and PVY in the aphid body, MpnDV interacted with PVY, promoting long-distance migration of the aphid population by increasing the number and proportional rate of winged aphids, suggesting an indirect positive relationship between MpnDV and PVY in their common aphid hosts.

Aphid wing morphs are mainly responsible for long-distance dispersal (Hayes et al., 2019; Zhang et al., 2019). DplDNV can decrease aphid fecundity, but promote population dispersal by increasing the number of winged individuals (Ryabov et al., 2019). The perception of alarm pheromone (EβF) alone was not sufficient to trigger wing production in aphids; however, the EβF exposure was proved to enhance the proportion of winged aphids, possibly by increasing walking behavior to result in a

672 “pseudo-crowding” effect (Kunert *et al*., 2005). MpnDV infection significantly
673 enhanced the titer of EβF; however, it did not change the proportional rate of winged
674 individuals, which might be due to that there was relatively sufficient space on whole
675 tobacco plants. However, there were more winged aphids that promoted population
676 proliferation in the MpnDV-positive aphids than in the negative ones, suggesting that
677 MpnDV infection increased the long-distance dispersal of the green peach aphid.
678 Plant virus infection usually induces more winged aphids in infected plants by
679 changing the nutritional quality of host plants (Blua and Perring, 1992; Casteel et al.,
680 2014). Our previous study showed that PVY infestation decreased the nutritional
681 quality of tobacco plants, leading to the earlier emergence of alates in tobacco plants
682 (Chen et al., 2020). Interestingly, we here showed that PVY significantly increased
683 the proportion of winged individuals. Taken together, MpnDV and PVY may
684 cooperatively promote the long-distance population dispersal of their common hosts,
685 which would also facilitate the transmission of the two viruses, suggesting a
686 potentially mutualistic relationship between insect virus and insect-borne plant virus.
687 These results identified novel challenges for plant protection and provided
688 opportunities for developing novel management strategies to control insect vectors
689 and plant viruses by decreasing the transmission of insect viruses.

690 In summary, our study highlights the interactions among insect, insect virus and
691 insect-borne plant virus. For the first time, we demonstrate that the insect virus
692 (MpnDV) could promote population proliferation and dispersal of aphids by
693 enhancing their titer of EβF, which promoted the transmission of a plant virus (PVY)
694 (Fig. 6). Additionally, PVY increased the proportion of winged individuals.
695 Consequently, we show that MpnDV infection facilitates the transmission of PVY by
696 enhancing the activity and number of their aphid vectors. These findings identify
697 novel challenges in controlling insect vectors and plant viruses that can be used to
698 develop novel management strategies.



699

700 **Fig. 6 Proposed model of MpnDV-modulated locomotor activity of insect hosts to**
701 **enhance plant virus transmission.** MpnDV infection enhanced the titer of EβF via
702 up-regulation of the expression of *MpFPPS1* gene, which increased the activities of
703 its host and promoted population dispersal and proliferation in tobacco plants. Thus,
704 MpnDV may promote PVY transmission by enhancing the movement and population
705 dispersal of their common host.

706

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710 Science and Technology Innovation Program (ASTIP-TRIC04, KLTPMIMT2022-01).

711 **Competing Interests**

712 The authors declare that they have no conflict of interest.

713 **Author contributions**

714 XP, RG, LY and DY designed research; DY, LT and HY performed research; XP, RG,
715 LY, DY, LT, HY, SC and WX analysed data; DY, XP, LT, RG, WiK and LY wrote the
716 paper.

717 **Data availability**

718 The data that supports the findings of this study are available in the supplementary
719 material of this article.

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