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RAPID COMMUNICATION

Effect of *Cucumber mosaic virus* infection on aphid colony development

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Abstract

Knowing the tritrophic interactions between plant-virus-insect is important in developing sustainable pest management practices. *Myzus persicae* is a well-known plant viral vector which can transmit over 40 plant viruses. We studied the impact of *Cucumber mosaic virus* (CMV) infection in *Nicotiana tabacum* on the colony development of *M. persicae* to understand how plant virus infection can affect vector growth and reproduction. Aphid growth, reproduction and fecundity were significantly affected by the virus infection. The mean relative growth rate of *M. persicae* on healthy plants was 0.29 $mg^{-1} \cdot mg^{-1} \cdot day^{-1}$ and was significantly higher than that of CMV-infected plants (0.23 $mg^{-1} \cdot mg^{-1} \cdot day^{-1}$). In contrast, the percentage of survival was significantly higher on CMV-infected plants. The estimated survival percentages of aphids at 20 days after introduction to CMV-infected and healthy plants were 55.8 and 25.8%, respectively. Therefore, the total population of aphids on CMV-infected plants was significantly higher on the 25th day after the introduction of aphids. The total population of aphids on the CMV-infected plants was 1,225 compared to that of healthy plants which was 713. Similarly, mean fecundity over a 30 day observation period was 61.25 and 35.65 for aphids grown on CMV-infected and healthy plants, respectively. Jasmonic acid (JA) upstream gene *OPR3* and downstream gene *COI1* was measured to quantify the changes in JA expression in the plants under the virus infection. Both genes tested were significantly downregulated in CMV-infected plants. From our results, it was evident that the JA related insect resistance was reduced in CMV-infected plants and hence aphid colony development was increased.

Keywords: *Cucumber mosaic virus*, growth, jasmonic acid, *Myzus persicae,* reproduction

The tritrophic interactions between plant-virus-insect have recently been of great interest, since it has become evident that such interactions play major roles in a given ecosystem and plant protection (Vinoth and Shivaprasad 2020). Revealing how the plant virus, the host plant, and insect vectors interact has tremendous implications in sustainable pest management (Dietzgen *et al.* 2016; Shi *et al.* 2021). In the current study, we investigated the effects on *Myzus persicae* reared on *Nicotiana tabacum* with and without *Cucumber mosaic virus* (CMV) infection in an attempt to determine the tritrophic interactions between the host plant, virus and insect vector.

CMV, the type species of the genus Cucumovirus, has an extensive host range and can be vectored by around 80 aphid species in a non-persistent manner (Jacquemond 2012). *Myzus persicae* is an efficient vector of CMV and is frequently used in transmission assays (Escriu *et al.* 2000; Ziebell *et al.* 2011). However, it is unknown how the *M. persicae* population dynamics contribute to the epidemiology of the CMV (Donnelly *et al.* 2019).

Plant-virus-insect tritrophic interactions are greatly influenced by changes occurring on phytohormones pathways such as of salicylic acid (SA) and jasmonic acid (JA). We investigated how CMV infection affects the aphid population growth on *N. tabacum* by quantifying the reproduction capacity, growth rate and survival. Our results will be helpful for exploiting sustainable pest management strategies in crops and thereby effectively manage plant viral diseases, through interpreting ecological interactions between plants and aphids.

Nicotiana tabacum plants and *M. persicae* were grown at 24°C with 16 h of artificial light. Stock colonies of aphids were reared on healthy *Brassica rapa* plants. Tobacco plants were inoculated with purified virions of CMV (CMV strain O) at the 4-leaf stage and the virus infection was confirmed by ELISA. One-day-old aphids were collected by isolating new-born aphids within 24 h after birth. Under our laboratory rearing conditions, we did not observe sexual reproduction in *M. persicae*. Therefore, all experiments were conducted using viviparous and apterous female *M. persicae* only.

At a given temperature and when confined to a clip cage, the mean relative growth rate (MRGR) of an aphid is mainly determined by the quality of the host plant (Dixon 1987). MRGR of aphids was evaluated using 35, 1-day-old aphids reared individually in clip cages on healthy and CMV-infected *N. tabacum* plants separately. One-day-old aphids were selected as explained above. First, weight measurements were taken on the 3rd day, when the aphids were 3-days-old. These aphids were individually weighed (W_1) using a microbalance and returned to the clip cage. On the 8th day the weight (W_2) of the same aphid (8-days-old) was measured again. The combined data from 35 clip cages is presented in Figure 1A. The mean relative growth rate was calculated using the formula $MRGR = (\log_e W_2 - \log_e W_1)/t$, where: t – time in days (5 days). The *MRGR*s of *M. persicae* were significantly affected by CMV infection indicating that the CMV-infected plants were a poor source of nutrition compared to healthy plants.

The significant weight reduction of aphids feeding on CMV-infected plants could adversely affect the survival of the aphids. Therefore, we then investigated the effect of CMV infection on the survival of the aphid. Aphid survival on healthy and CMV-infected *N. tabacum* plants was evaluated by using a total of 80 1-day-old aphids per treatment. Ten, 1-day-old aphids were placed in one clip cage and two clip cages were fixed on each plant on the 3rd and 4th fully expanded healthy and systematically infected leaves. Each clip cage had an inside dimension of 2.54 cm. The aphid survival was observed daily for 20 days. The experiment was carried out thrice at 24°C with 16 h of artificial light. The combined data from all replicates are presented (Fig. 1B). Kaplan-Meier estimate and the 95% confidence interval at 1 day, 5 days, 10 days, 15 days, and 20 days are shown in Table 1. After 1 day, the mean survival percentage of aphids on healthy plants was 89.2% (83.8–94.9%), which was significantly lower than in CMV-infected plants where it was 97.5% (94.7%, 100%). By 20 days, the estimated survival percentages of aphids on CMV-infected and healthy plants were 55.8% (47.6%, 65.5%) and 25.8% (19.1%, 35.0%), respectively. Figure 1B indicates that the aphid survival percentage was always higher on CMV-infected plants regardless of the time in days. Furthermore, a log-rank non-parametric test was used to compare the entire survival curve. The test provided strong evidence that CMV-infected treatment gave better survival results than healthy treatment with a p-value of <0.0001 at a 5% significance level. Despite the lower *MRGR* of aphids fed CMV-infected plants, the number of survived aphids remained higher.

Because aphids had enhanced survivability on CMV-infected plants, yet poor *MRGR*, we investigated the total population of aphids grown on healthy and

Table 1. Kaplan-Meier estimate and 95% confidence interval for survival probabilities of healthy and CMV-infected treatment

Treatment	Time point [days]	Kaplan-Meier estimate	Confidence interval for survival probabilities
Healthy	1	0.892	(0.838, 0.949)
	5	0.725	(0.649, 0.809)
	10	0.517	(0.435, 0.614)
	15	0.375	(0.298, 0.472)
	20	0.258	(0.191, 0.350)
CMV-infected	1	0.975	(0.947, 1.000)
	5	0.908	(0.858, 0.961)
	10	0.733	(0.658, 0.817)
	15	0.642	(0.561, 0.733)
	20	0.558	(0.476, 0.655)

CMV-infected *N. tabacum* plants. The total populations were measured by placing 30 1-day-old aphids on healthy and CMV-infected *N. tabacum* plants and counting all aphids 25 days after the introduction of aphids to test plants. Mean data of four replicates is presented in Figure 1C. The total populations of aphids on the CMV-infected plants were significantly higher than that of healthy plants (*Chi*-square, *p*-value <0.001). This may be a result of the higher survival rate observed in aphids on CMV-infected plants.

With the above information we further wanted to evaluate the effect of CMV infection on the fecundity of the aphids. We randomly selected and placed 10 1-day-old female aphids individually on clip cages fixed on *N. tabacum* plants to measure the progeny development. Two clip cages were fixed on each plant on the 3rd and 4th fully expanded healthy leaves and on systematically infected leaves. The number of progenies developed was recorded and removed each day until the termination of progeny development or death of the mother aphid. The combined data from 20 clip cages is presented (Figs. 1D, E). Interestingly, the mean number of progenies developed per aphid on CMV-infected plants was nearly two times higher than that of healthy plants. When we compared the total number of fecundities between CMV-infected and the healthy treatment, the IQR (third quartile-first quartile) of CMV-infected treatment was 12.5 (66.0–53.5) and 7.5 (33.5–26.0) for the healthy treatment. This indicates that CMV-infected treatment tends to give higher fecundity. The mean numbers of progenies developed per aphid were 61.25 and 35.65 for aphids grown on CMV-infected and healthy plants, respectively. Two samples of independent t-tests showed that the difference was significant ($p < 0.0001$) at the level of 5% (Fig. 1D). The mean number of progenies developed each day is shown in Figure 1E and it is clear that the aphids on CMV-infected plants reached reproductive maturity earlier than the aphids on healthy *N. tabacum*. Interestingly, *M. persicae* grown on Jalapeño peppers infected with *Pepper cryptic virus* 1 or *Arabidopsis* infected with CMV showed lower fecundity rates (Westwood *et al.* 2013; Safari *et al.* 2019). Therefore, the tritrophic interactions between plant-virus-insect might be specific to the virus type and host plant.

Taken together, these results show that the aphid colony development was significantly enhanced by the infection of CMV. Thus, the CMV somehow increased the aphid population despite the poor growth rate observed. Recent research has shown that the plant virus infection can modify the plant resistance to insects by interfering with jasmonic acid (JA) signalling pathway

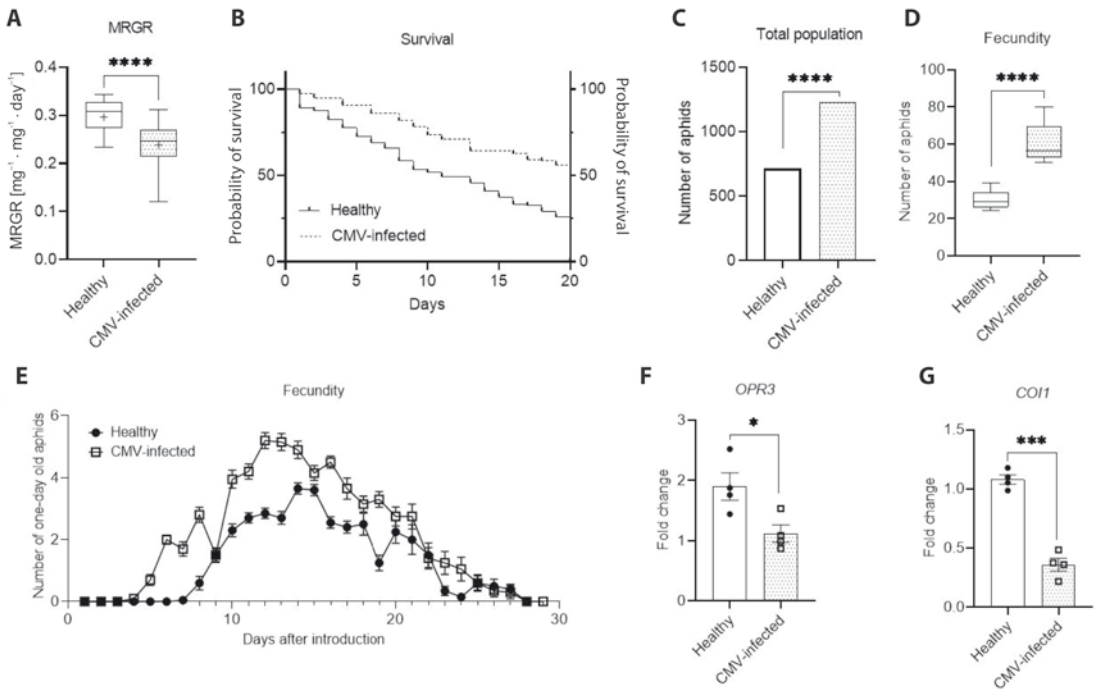

Fig. 1. Aphid performance on healthy and CMV-infected *Nicotiana tabacum* plants: A – the mean relative growth rate of *Myzus persicae* was nearly two times higher on the healthy plants ($p \leq 0.0001$, unpaired t-test); B – the survival percentage was significantly higher on CMV-infected plants (Table 1); C – the total population of aphids on healthy and CMV-infected plants (*Chi*-square, $p \leq 0.001$); D – mean fecundity over a 30 day observation period ($p \leq 0.0001$, unpaired t-test); E – mean daily number of progenies produced by *M. persicae* on healthy and CMV-infected plants; F – the relative expression of *OPR3* was significantly higher in healthy plants than in CMV-infected plants ($p = 0.0274$, $n = 4$, unpaired t-test); G – the relative expression of *COI1* was significantly higher in healthy plants than in CMV-infected plants ($p \leq 0.001$, $n = 4$, unpaired t-test) *$p \leq 0.05$; ***$p \leq 0.001$, ****$p \leq 0.0001$

(Shi *et al.* 2016; Gholi-Tolouie *et al.* 2018). It might be the effect of JA which enhanced the aphid survivability and consequently the colony development.

Therefore, in order to elucidate the possible role of JA in the aphid colony development, expression of JA upstream gene *OPR3* and downstream gene *COI1* was measured to quantify the changes in JA expression at a molecular level. The conventional phenol–chloroform method was used to extract total RNA from the plants followed by cDNA synthesis using Takara Perfect Real Time, PrimeScriptTM RT reagent Kit. q-PCR was performed using SYBR Green Master Mix (Applied Biosystems). For *OPR3* and *COI1* detection, primer pairs 5'-AGGCACTATGATTTCTC-3'/5'-GTTGATC CCATCTTTC-3' and 5'-CACTTGATAATGGTGT-3'/ 5'-AGGCCTTCATCGGATTCC-3' were used, respectively. The 18S rRNA gene was used as the reference gene (primer pair 5'-GCAAGACCGAAACT CAAGG-3'/5'-TGTTCATATGTCAAGGGCTGG-3'). The expression of both JA upstream and downstream genes was downregulated in CMV-infected plants (Fig. 1F, G). The phytohormone JA is known to be responsible for defense mechanisms in the host plant (Kloth *et al.* 2016; Nouri-Ganbalani *et al.* 2018; Van Dam *et al.* 2018). The down regulation of the above gene might have resulted from the previously observed increase in the aphid population in CMV-infected plants.

Our results showed that the CMV-infected plants decreased JA signalling related genes and is in line with the previous results of others (Shi *et al.* 2016, 2021). Proteinase inhibitor, TPI-2 plays an important role in tobacco cells and the protein products have shown insecticidal qualities (Zhang *et al.* 2004). The proteinase inhibitor activity might have been reduced in CMV-infected plants resulting in the host plant being more susceptible to aphids.

As a result, the aphid colony development was increased in CMV-infected plants possibly due to the reduction of JA related insect resistance. In our results we found that the final population was nearly two times in the CMV-infected plants. Increased reproduction and survival of aphids on these plants will help to build up aphid populations in nature and favor longer-term persistence of the vector within a given ecosystem (Westwood *et al.* 2013). Hence, aphids are benefited by CMV-mediated changes in host plants.

The interactions of aphids and plants have been clearly modified by the plant virus. Non-persistently transmitted viruses are efficiently transmitted when aphids quickly disperse from an infected plant. Therefore, the observed upsurge of an aphid population can delay the onward transmission of the virus as CMV is a non-persistently transmitted virus. However, it should be taken into consideration that, overcrowding can lead to the dispersal of viruliferous aphids and thereby the virus can spread to new hosts.

The majority of plant viruses depend on insect vectors for their onward transmission and aphids are by far the most common vector of plant viruses (Ng and Perry 2004). Aphid-vectored viruses are serious threats to many major agriculturally important crops. Changes in climate and development of insecticide resistance among aphids have further worsened the situation by widening the problem over many geographic areas (Westwood and Stevens 2010). An improved understanding of how plant-aphid interactions are modified by viruses could be used to formulate strategies to inhibit aphid vectors in the agricultural environment and thereby the infection of aphid transmitted viruses.

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