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Dissecting the relationships between the insect vector *Empoasca paraparvipenis* with *Camptotheca acuminata* witches' broom phytoplasma

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Abstract

Phytoplasmas are phloem-limited bacteria that are primarily transmitted by hemipteran insects and are emerging threats to *Camptotheca acuminata* Decne plants due to their associations with a witches' broom disease. Despite numerous studies, there has been no report on insect transmission of phytoplasma among *C. acuminata*. Here, transmission characteristics of the leafhopper, *Empoasca paraparvipenis* Zhang and Liu, 2008 and the phytoplasma in plant leaves through PCR quantification are described. The interaction between *C. acuminata*-phytoplasma and insect vectors was examined by analysing the impact on the life characteristics and progeny population in a temperature-dependent manner. Phytoplasma-infected *C. acuminata* plant exhibited symptoms including shorter internodes, weak and clustered branches, shrunken and yellowed leaves, and red leaf margins. The acquisition and transmission time of bacterial-infected third-instar nymphs of insect vectors were 10 (11.11%) and 30 min (33.33%), respectively. A single insect vector can infect a plant after 72 h of feeding, and the incidence rate of disease increases with the number of insects following 11–100% from single to 20 insects. The development time of the infected insect vectors (1–3 instars) was significantly shorter than that of the healthy insects, and the development duration of instar individuals was longer. In progeny populations, the higher the phytoplasma concentration (88–0% for 1–5 instars nymph, female and male adults), the shorter the development time and the longer the adult lifetime (both male and female). These findings provided research evidence of phytoplasma transmission by insect vectors; however, further investigation of the mechanisms for prevention and management of phytoplasma diseases is needed.

Introduction

Phytoplasmas, previously known as mycoplasma-like organisms, are a large group of plant-pathogenic, without cell wall, non-helical bacteria associated with diseases that affect vegetables, cash crops, fruit trees, weeds and ornamental plants, generally associated with stunting, proliferation, virescence and yellowing (Dermastia, 2019). Phytoplasmas are localised in host plant phloem sieve tube cells (Marcone, 2009, 2014). Most of them colonise hemipteran insects such as Cicadellidae, Delphacidae and Psyllidae which feed on phloem sap (Hemmati *et al.*, 2021; Wang *et al.*, 2021a). According to research on the diseases spread by the insect vector *Empoasca paraparvipenis* Zhang and Liu, 2008, the leafhopper not only spreads tomato giant bud disease and tobacco yellow dwarf disease but also other diseases such as alfalfa witches' broom and potato purple top disease (Bertaccini *et al.*, 2019; Trivellone *et al.*, 2022).

Insect transmission is a complex process in a phytoplasma–insect–plant relationship. The process of phytoplasma transmission by insect vectors typically includes three main stages: feeding, incubation, and inoculation (Mou *et al.*, 2022). During feeding, the insect vector ingests phytoplasma via the phloem sap of the diseased plant, that phytoplasma is absorbed into the midgut and invades the insect's intestinal cells, adjacent muscle cells, and haemolymph. This period to acquire the phytoplasma is referred to as the acquisition access period (Bosco and Tedeschi, 2013; Ranebennur *et al.*, 2022). Depending on the insect species, feeding period can range from a few minutes to days to ensure that enough phytoplasma is obtained (Palermo *et al.*, 2001; Marzachì *et al.*, 2004).

Furthermore, many insect carriers that feed on phloem can acquire phytoplasma but cannot transmit it. As a result, identifying the transmission ability of insects, rather than only their ability to acquire and carry phytoplasma is critical in determining phytoplasma insect vectors

(Kumari *et al.*, 2019). Previously, it was found that phytoplasma vector *Asymmetrasca decedens* was the most efficient vector responsible for carrying '*Candidatus* Phytoplasma phoenicium' (Abou-Jawdah *et al.*, 2014). In addition, nine potential alternative insect vectors were found to transmit '*C. Phytoplasma solani*' to grapevines in Italy (Quaglino *et al.*, 2019). Based on this, we assume that there is not one vector species involved in carrying the pathogen from the infected to the healthy host, but different vectors could be involved in carrying the pathogen. Therefore, the detection of phytoplasma in the insect body alone cannot prove that the insect is a vector; only the transmission test provides conclusive evidence (Killiny and Almeida, 2014).

Camptotheca acuminata is a tall plant that grows in China and is used as an ornamental, source of timber, and in the medicine (Zhang *et al.*, 2002; Xing, 2004). The number of wild *C. acuminata* plants is seriously affected by environmental changes, diseases, and insect pests. *Camptotheca acuminata* witches' broom associated with the presence of phytoplasma is a systemic disease resulting in stunted growth, shortened internodes, and smaller leaves in plants (Wei *et al.*, 2006; Xu *et al.*, 2009; Wang, 2018). This severely impedes the development of the *C. acuminata* plant industry and the pharmaceutical industry. The biology, ecology and management strategies of *C. acuminata* leafhoppers were studied in our previous work (Wang *et al.*, 2021c). Still, the transmission characteristics of phytoplasma diseases and insect vectors have not been examined yet. Therefore, the current study focuses on the transmission characteristics of leafhopper insects in the *C. acuminata* community to investigate the interaction between *C. acuminata*- phytoplasma and insect vectors (*E. paraparvipenis*).

Materials and methods

Insects, plants, and pathogen

The adults and nymphs of the leafhopper (*E. paraparvipenis*) were collected using a net from infected leaves in the *C. acuminata* community in Wenshan (23°21′47.02″ N 104°15′1.69″ E), Yunnan Province, China and placed in an insect feeding cage. The leafhopper-rearing *C. acuminata* plants were obtained from Huatao Gardening Farm, followed by their transplant into pot. Healthy *C. acuminata* plants were screened for phytoplasma infection with polymerase chain reaction (PCR) and placed in an insect cage to be cultured in the greenhouse for the pathogen transmission test. The phytoplasma was detected through a *Taq*Man probe-based quantitative PCR (qPCR) method that specifically identifies the member of phytoplasma group 16SrXXXII as performed in our previous study (Wang *et al.*, 2021b).

DNA extraction and PCR amplification

OMEGA plant DNA extraction kit (D5511-02) was used to extract DNA from the leaves of *C. acuminata* as per manufacturer instructions (Omega Bio-tek, Inc., Norcross, GA, USA). Total DNA was extracted from leafhoppers using an insect DNA extraction kit according to manufacturer instructions. The 16S rRNA gene of phytoplasma was amplified by PCR using the DNA of the infected *C. acuminata* plants and the leafhoppers, concerning the universal primer of phytoplasma designed by Lee (Lee, 1993; Schneider *et al.*, 1993). PCR amplification was carried out with Phanta Max Super-Fidelity DNA polymerase and 2 Phanta buffer (Nanjing, China). Electrophoresis was carried on 1% agarose gel to detect PCR products. The PCR product was purified with the Cycle-Pure Kit for PCR Product Purification (Omega Bio-tek, Inc.).

Quantitative determination of phytoplasma in leafhoppers and plants by a fluorescent dye method

A real-time fluorescent dye method was used to screen the universal primers 16SrXXXIIF (ACGCTGTTCTGTTATAGA) and 16SrXXXIIR (GTTCCTCCATATATTTACACA), which were synthesised by Beijing Qingke Biotechnology Co., Ltd. qPCR SYBR Green Master Mix (Nanjing Novozan Biotechnology Co., Ltd.) and Bio-Rad (CFX96, USA). qPCR was used with 16S rRNA gene recombinant plasmid of *C. acuminata* phytoplasma as the internal reference gene; in addition to the host plant, the pathogen-acquired leafhopper DNA was used as the template. A qPCR reaction was carried out with three technical replications according to the SYBR green reagent and qPCR instrument instructions, with a melting curve of 60–95°C.

Identification of insect transmission ability

At 28°C, 20 highly infected insect nymphs (collected from diseased hosts) were transferred in each group to healthy *C. acuminata* plants for 5, 10, 30 min, 1, 3, 6, 24, and 48 h. Each treatment ($n = 3$) was repeated three times with three plants in each replicate, and cages of plants were covered with a net. After 10 days of feeding with infected insects, the plants were collected, and DNA was extracted separately to detect the bacterial load in the plants.

Determination of the transmission effect of different numbers of insects

At 28°C, the numbers of infected nymphs 1, 3, 5, 10, 15, and 20 were allowed to feed on healthy *C. acuminata* plants. Each treatment ($n = 3$) was carried out three times with three plants in each replicate, and the insects were removed after 72 h. The DNA of insects and plants was then extracted, and the rate of diseased insects and plants was detected by amplification through PCR.

Determination of the transmission effect of different development stages of insects

At 28°C, the infected insects (collected from diseased host) were allowed to feed on the healthy *C. acuminata* plants according to their age: instar (1, 2, 3, 4, 5, adult male, adult female). Each treatment treated three seedlings of the *C. acuminata* plant, with three replications. Treatments were placed in the insect cage, and the insects were removed after 3 days. Then the total DNA of insects and plants was extracted, the susceptibility was detected by PCR amplification, and the disease occurrence rate was calculated.

Effects of Camptotheca acuminata phytoplasma strains on the life parameters of its insect vector

Healthy and infected first-instar insects were separately caged in groups of 30 on non-infected plants and maintained at temperatures of 16, 20, 25, 28, 30, and 31.5°C. Insect development duration of all stages was observed until death. After the emergence of insects, the female and male leafhoppers were identified with the naked eye, and the life spans were compared under the conditions of being infected and healthy. After the death of

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the leafhopper, qPCR was performed to confirm the infected insect vector.

Effects of Camptotheca acuminata *phytoplasma strains on the progeny populations of its insect vectors*

At 28°C, fifth instars were reared to adult in groups of 80 on healthy and infected plants. Female and male adults were collected after 2 weeks and paired for mating as groups of equal 30 males and 30 females held separately in the same cage. After 3 days, each treatment group was split into two subgroups each consisting of 15 males and 15 females caged on healthy *C. acuuminata* seedlings ($n = 3 \times 3$). The offspring of these adults were reared to adults. During the period, the progeny populations were recorded by observing their life span (male 9 days; female 17 days).

Statistical analysis

The phytoplasma content in fluorescence qPCR was calculated using the $2^{-\triangle\triangle CT}$ method (Schmittgen and Livak, 2008). The data were statistically analysed using analyses of variance in IBM SPSS Statistics 20.0 software; the means were subjected to Duncan's multiple range test at $P \leq 0.05$ and multiple comparisons in the Data Processing System (DPS) software (Hangzhou Ruifeng Information Technology Co., LTD, Zhejiang, China) were used to analyse the amount of phytoplasma expression and life spans. The amplification diagram and fusion curve of phytoplasma were both from real-time fluorescence qPCR.

Results

Symptoms of indoor and outdoor phytoplasma-infected Camptotheca acuminata *plant*

To gain insight into the detailed symptoms, continuous observation of *C. acuminata* in the field and greenhouse (Wenshan, Yunnan Province, China) revealed that phytoplasma-infected plants had apparent symptoms all year till plant death (fig. 1). The symptoms were short internodes, branches weak and clustered, leaves shrunk and yellowed and leaf margins somewhat red (fig. 1A, B1) in the infected plant (fig. 1B2) as compared to the field-healthy *C. acuminata* (fig. 1B1). The infected plants (fig. 1C) symptoms were identical to those of the field plants, with exceptions for the clumps in comparison with the healthy indoor *C. acuminata* plants (fig. 1D2). After phytoplasma invaded the leaves, lesions on the leaves extended from the leaf tip or edge to the leaf vein.

Pathogen-transmission by Empoasca paraparvipenis

Bacterial-infected third-instar nymphs of insect vectors were fed on healthy *C. acuminata* plants during different time intervals for 5, 10, 30 min, 1, 3, 6, 24, and 48 h. After 10 days, the occurrence rate of *C. acuminata* at different inoculation times was 0.00, 11.11, 33.33, 33.33, 33.33, 44.44, 77.77, and 66.66%, respectively (table 1). Except for 5, 10, and 30 min after inoculation, phytoplasma detection revealed amplification bands in the leaves of *C. acuminata* (fig. S1A). The melting curve for *C. acuminata*

Figure 1. *In vitro* and *in vivo* symptoms of *C. acuminata* (AB *in vivo* symptoms; CD *in vitro* symptoms; B1 and D1 infected *C. acuminata*; B2 and D2 healthy *C. acuminata*).

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Table 1. Pathogen transmission ability of *Empoasca paraparvipenis* during different time intervals

Transmission time	qPCR detection of internal reference gene average Ct	qPCR detection of plant phytoplasma average Ct	Relative expression level of phytoplasma in leaves	qPCR detected positive/healthy plants	Pathogen transmission ability (%)
5 min	19.1	36.04	0.0017 ± 0.0001d	0/9	0.00
10 min	18.9	35.43	0.0031 ± 0.0007d	1/9	11.11
30 min	18.77	30.05	0.0976 ± 0.01d	3/9	33.33
1 h	18.97	26.9	0.67 ± 0.01d	3/9	33.33
3 h	19.16	27.64	1.00 ± 0.13d	3/9	33.33
6 h	19.03	25.18	3.36 ± 0.23c	4/9	44.44
24 h	19.05	23.55	10.68 ± 0.87b	7/9	77.77
48 h	19.22	23.26	14.54 ± 0.49a	6/9	66.66

Note: Each treatment was repeated three times and the means were subjected to Duncan's multiple range test at $P \leq 0.05$. Different letters showed significant differences.

leaves was a single peak, and the three replicates at 5 and 10 min in the amplification curve were negative amplification. In contrast, the other six treatments were positive amplification from 30 min to onwards (figs S1BC and S2AB).

The incidence rate of *C. acuminata* was noted as 33.33% 10 days later for 30 min, and the Ct value of phytoplasma amplification in leaves was 30.05, indicating positive amplification. The rate of *C. acuminata* after 24 h was 77.77%, and the Ct value of phytoplasma amplification in leaves was 23.55. The leaves phytoplasma concentration and incidence rate increased as pathogen transmission time increased (fig. 2). The third-instar nymphs had the shortest transmission time of 30 min. The higher the phytoplasma content in the body, the shorter the time to show symptoms and the higher the incidence rate.

Transmission effects of different insect vector numbers

The highly infected insect vector third-instar nymphs of leafhoppers were allowed to feed for 72 h on healthy *C. acuminata* plants in groups of 1, 3, 5, 10, 15, and 20. After 10 days, the pathogen detection concentration of plants was 11.11, 11.11, 44.44, 33.33, 77.77, and 100%, respectively. Plants and leafhoppers were tested for phytoplasma, which revealed that the leafhoppers DNA was all amplified *in vivo* with a single band size (fig. 3A), whereas phytoplasma amplification bands with a fragment size of about 1200 bp were found in plant leaves (fig. 3B). The phytoplasma content in leafhoppers and plants was detected using qPCR, and the results showed that the melting curves and internal reference genes were single peaks, indicating that the amplification products were single with good specificity, and the samples were positive (15 < Ct < 35) (fig. S3AB).

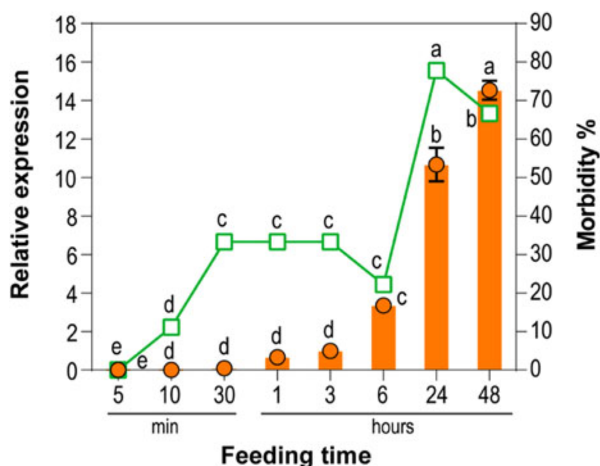

Figure 2. Pathogen-transmission ability of insect vector. Relative expression of phytoplasma in *C. acuminata* leaves in different pathogen transmission time and ability of *E. paraparvipenis.* Each treatment was repeated three times, and the means were subjected to Duncan's multiple range test at $P \leq 0.05$. Different letters showed significant differences over time.

One leafhopper fed on healthy *C. acuminata* for 72 h, and 10 days later, the plant incidence rate was 11.11%, with a Ct value of 29.45 for phytoplasma amplification in the leaves. After 72 h of feeding on healthy *C. acuminata*, the plant incidence rate reached 100%, with a Ct value of 22.41 for phytoplasma amplification in leaves (table S2). The incidence rate of *C. acuminata* increased with insect population density, showing that the denser the population, the higher the transmission efficiency (fig. 4AB). Regression analysis indicated that a positive relationship was found between a number of vectors per plant and relative expression in the plant or the proportion of plants that become infected, as shown in the figure.

Transmission effects of different stages of insect vector

Leafhopper at different ages (1–5 instar nymphs, female and male adults) were fed on healthy *C. acuminata* plants for 3 days, and the incidence rate of *C. acuminata* was checked after 10 days, which was 88.88, 66.67, 100.00, 77.77, 55.55, 11.11, and 0%, respectively. Phytoplasma detection showed that there were amplified bands in leafhoppers at each instar. The band size was single (fig. 5A), while the leaves of *C. acuminata* fed by all stages had about 1200 bp amplification bands except for males (fig. 5B). The detection of phytoplasma in the leafhoppers and leaves of *C. acuminata* showed positive amplification except for the none of the amplification of the leaves fed by male adults (15 < Ct < 35) (fig. S4AB).

The amplified Ct values of phytoplasma in different stages (1–5 instar nymphs, female adults and male adults) of leafhopper were 23.46, 22.06, 21.68, 22.12, 23.87, 24.95, and 27.38, respectively. The phytoplasma amplified Ct values in the leaves of *C. acuminata* were 24.20, 23.43, 22.21, 22.26, 23.38, 23.95, and 34.36,

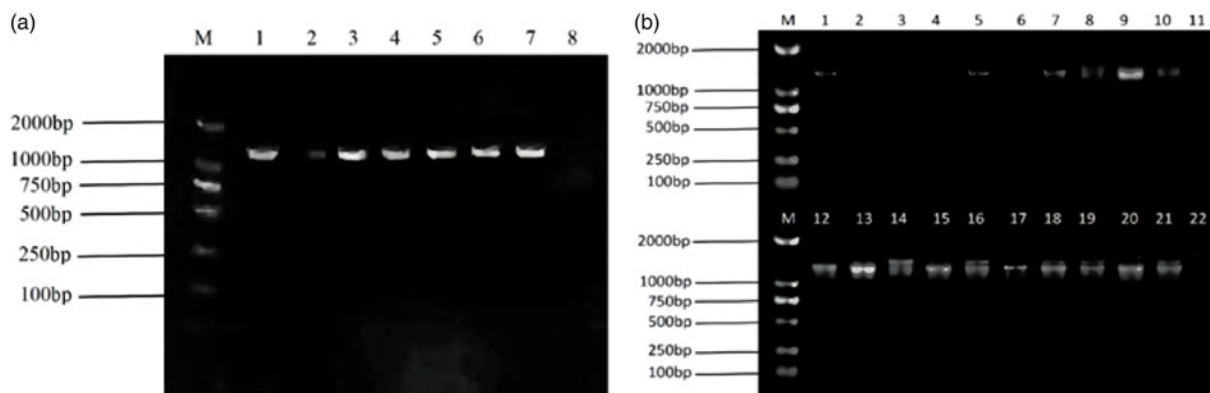

Figure 3. Gel electrophoresis of phytoplasma: (A) in different numbers of *E. paraparvipenis* (M: marker; 1: positive control; 2: 1 insect; 3: 3 insect; 4: 5 insect; 5: 10 insect; 6: 15 insect; 7: 20 insect; 8: negative control.); (B) in *C. acuminata* leaves at different amount inoculation (M: marker; 1, 12: positive control; 2–4: 1 insect; 5–7: 3 insect; 8–10: 5 insect; 11–13: 10 insect; 16–18: 15 insect; 19–21: 20 insect; 11, 22: negative control).

respectively (table 2). It shows that the green leafhopper can acquire phytoplasma and transfer it from the first instar nymph to the male adult. The amount of phytoplasma expression in leafhoppers and plants and the pathogen transmission ability of leafhoppers at different stages were as follows: 3rd age > 4th age > 2nd age > 1st age > 5th age > female adult > male adult. The shortest (feeding by the third-instar nymph) and longest (feeding by the female adult) times for showing symptoms in *C. acuminata* were 3 and 6 days, respectively, indicating that the strength of the transmission ability determines the concentration of phytoplasma, which then determines the process of plant infection from phytoplasma to symptoms.

Effects of Camptotheca acuminata *phytoplasma on the life parameters of its insect vectors*

There were significant differences in the development duration of adults and nymphs between the infected and the healthy leafhoppers at 20, 25, 28, 30, and 31.5°C ($P < 0.05$). At different temperatures, the development duration of the infected nymphs was significantly shorter than that of the healthy ones, while the development duration of the infected adults was significantly longer than that of the healthy ones (fig. 6A). Above 25°C, the development duration of the first, second and third instars with pathogen was significantly shorter than healthy ones, while the fourth and fifth instars, female and male adults, and pre-oviposition period with pathogen were significantly longer than those of the healthy leafhoppers (fig 6BC).

Effects on progeny populations of vectors

The statistical data revealed that the progeny populations of the highly pathogenic leafhoppers and the healthy leafhoppers had significantly different life parameters after surviving on the highly pathogenic *C. acuminata* and the healthy *C. acuminata* at 28°C ($P < 0.05$). The leafhoppers on the healthy *C. acuminata* plants (B1) have the longest growth time, whereas the susceptible *C. acuminata* (A2) has the shortest (table S1).

Discussion

The potential significance of many insect vectors identified as a new carrier of pathogens must be considered for their growing abundance in a different host and their developmental cycle. Many phytophagous insects, such as the brown planthopper, black-tailed leafhopper, and white-backed planthopper, are monophagous or oligophagous and can only feed on one or a few plant genera gradually as the number of leafhoppers increases, and it was directly proportional to the leafhopper population. Due to more frequent feeding activities, the higher concentration

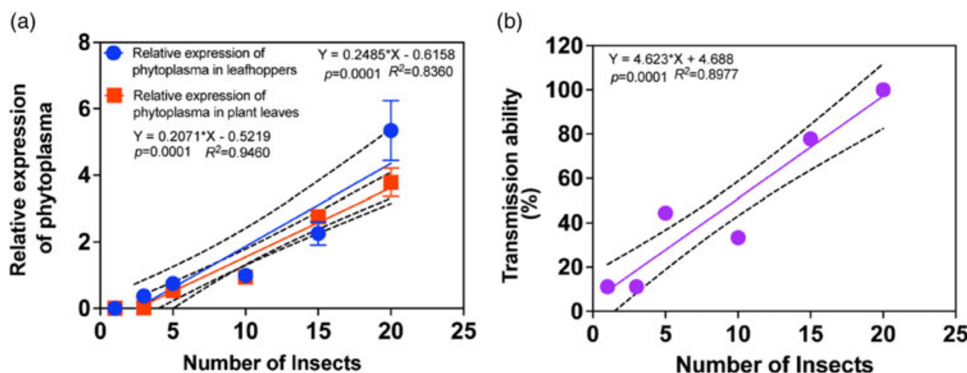

Figure 4. Regression analysis indicating transmission effects of different insect vector numbers. (A) Relative expression of phytoplasma in *E. paraparvipenis* and *C. acuminata* at different insect inoculation. (B) Pathogen transmission ability of *E. paraparvipenis* in different insect numbers. Each treatment was repeated three times.

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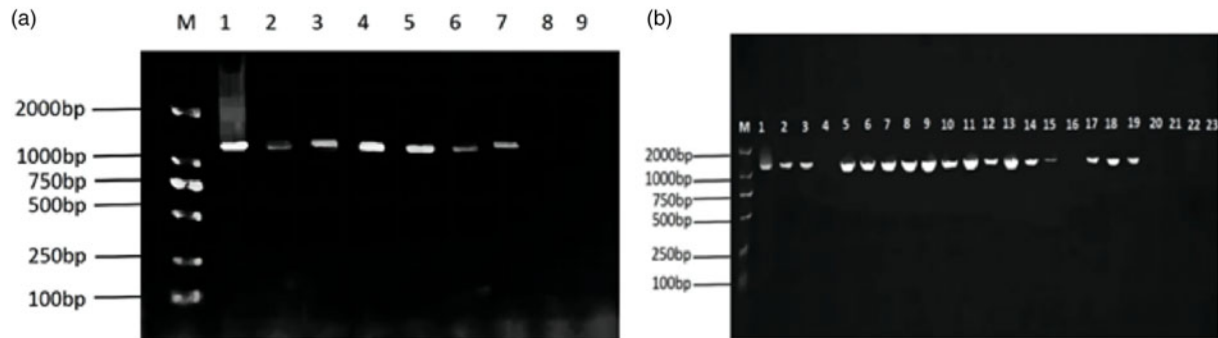

Figure 5. Gel electrophoresis of phytoplasma: (A) in *E. paraparvipenis* at different ages (M: marker; 1: positive control; 2: 1st instar; 3: 2nd instar; 4: 3rd instar; 5: 4th instar; 6: 5th instar; 7: female adults; 8: male adults; 9: negative control); (B) in leaves of *C. acuminata* at different ages vaccination (M: marker; 1: positive control; 2–4: 1st instar nymphs; 5–7: 2nd instar nymphs; 8–10: 3rd instar nymphs; 11–13: 4th instar nymphs; 14–16: 5th instar nymphs; 17–19: female adults; 20–22: male adults; 23: negative control).

Table 2. Pathogen transmission effects of different development ages of *Empoasca paraparvipenis*

Insect stages	qPCR detection of average Ct in leafhoppers	Relative expression of phytoplasma in leafhoppers	qPCR detection of plant phytoplasma average Ct	Relative expression level of phytoplasma in leaves	qPCR detected positive/healthy plants
1st instar	23.46	20.08 ± 1.12b	24.2	0.99 ± 0.01bc	8/9
2nd instar	22.06	49.37 ± 5.42a	23.43	1.73 ± 0.15b	6/9
3rd instar	21.68	53.91 ± 5.80a	22.21	4.47 ± 0.79a	9/9
4th instar	22.12	51.1 ± 6.05a	22.26	3.71 ± 0.13a	7/9
5th instar	23.87	16.51 ± 4.68bc	23.38	1.75 ± 0.30b	5/9
Adult female	24.95	5.56 ± 0.44cd	23.95	1.42 ± 0.14b	1/9
Adult male	27.38	1 ± 0.04d	34.36	0.0008 ± 0.0001c	0/9

Note: Each treatment was repeated three times and the means were subjected to Duncan's multiple range test at $P \leq 0.05$. Different letters showed significant differences.

of phytoplasma in plants and the higher transmission efficiency were recorded. The investigation of several leafhopper stages revealed that nymphs show higher transmission than adults. This could be because nymphs (1–4th instars) are more involved in feeding and moulting activities to grow and survive. The third-instar nymph has the best transmission ability because of its higher survival and frequent feeding activities. In contrast, the fourth-instar nymph exhibited higher survival, but its feeding activity was relatively weak in the latter stage due to the sex differentiation process. Female adults die a few days after oviposition, so there were fewer feeding activities; male adults, on the other hand, die after mating for a few days after emergence and do not eat, so their ability to spread pathogens was nearly minimal.

Plant pathogens are mostly transmitted by specific insect vectors, such as rice stripe mosaic virus (RSMV) is transmitted by leafhoppers (Sun *et al.*, 2017), and papaya ringspot virus is transmitted by peach, cotton, and bean aphids. The pathogen directly affects the biological characteristics of insects (Agasyeva *et al.*, 2024), such as survival rate, egg production, reproductive duration, feeding behaviour, and host selection behaviour. It has an indirect effect on the ecological adaptability of insect vectors to host plants (Yan, 2020). It was found that the leafhopper's growth, development, and reproduction were adversely affected after it became the vector of RSMV (Li *et al.*, 2020). Several studies showed that most viruses promote the egg production of insect vectors (Carr *et al.*, 2018), whereas the infection of tomato spotted wilt virus reduces egg production in thrips (Zhu *et al.*, 2011). The oviposition selection of insect vectors is also indirectly affected by plants, and insect vectors tend to lay eggs on healthy or infected plants (Wan *et al.*, 2020).

Interestingly, here we found that the leafhoppers *E. paraparvipenis* feed on an emerging vector host *C. acuminata* tree, which is not surprising due to growing climatic conditions, environmental factors, and pathogen–vector evolution to occupy diverse hosts as well as biology of the vector and the phytoplasma. The impact of the phytoplasma of *C. acuminata* witches' broom disease on the growth and development of the green leafhopper *E. paraparvipenis* at different temperatures were evaluated, as well as the life parameters of the infected and healthy leafhoppers using *C. acuminata* as its host plant. The results showed that with the increase in temperature, the development duration of both the infected and the healthy leafhoppers was significantly decreased, and the development duration of the first to third instars of the infected leafhoppers was shorter than that of healthy nymphs. However, the duration of the fourth and fifth instars, female, and male adults and pre-oviposition stage of the infected leafhoppers was significantly longer than that of the healthy leafhoppers.

An increasing number of emerging pathogens, new insect vectors for these pathogens and different vascular pathogens, including phytoplasma, result in devastating losses to agriculture. Due to rising climate change, there is a great evolution in the insect vector or pathogen, causing devastating losses to agriculture

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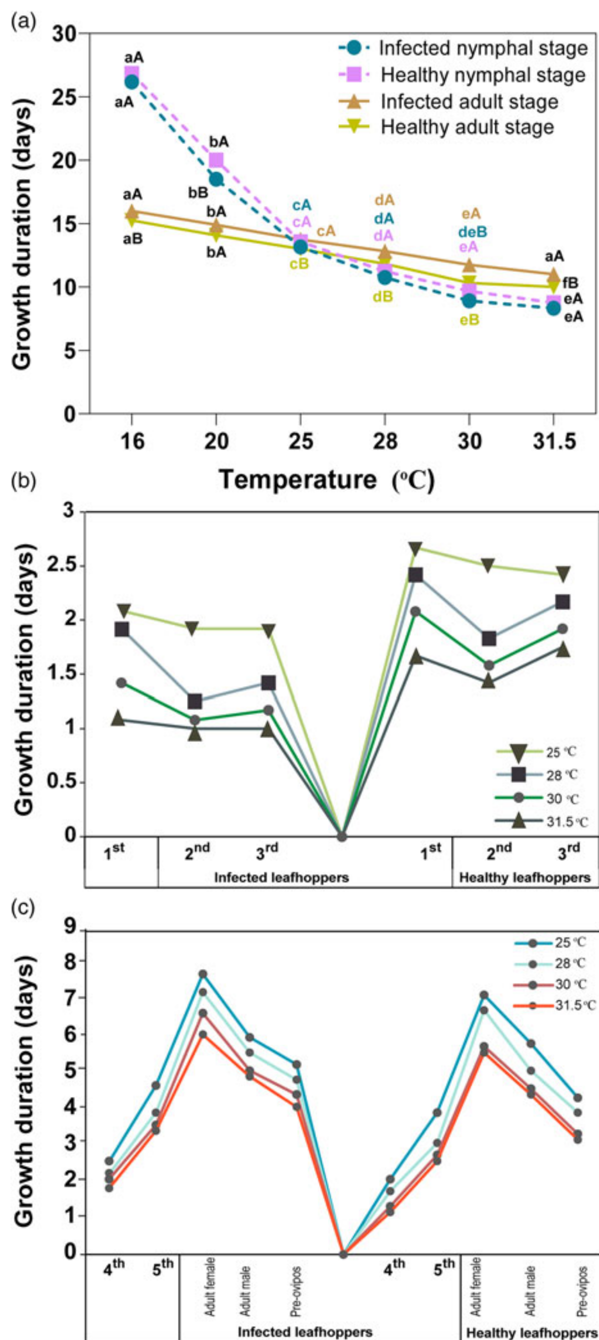

Figure 6. Development times of leafhoppers *E. paraparvipenis*. (A) Infected and healthy stages of insects. Different letters indicate the statistical difference among the column ($P \leq 0.05$). (B) 1–3 instar nymphs of the infected and healthy insects. (C) 4th instars, adults and pre-oviposition periods of the infected and healthy insects.

production. In the present study, different phytoplasma concentrations affect leafhopper life parameters differently. This revealed that *C. acuminata* phytoplasma is more conducive to green leafhopper survival and will increase the theirpopulation by decreasing the development time and increasing the number of female adults and eggs. Albeit, the molecular mechanisms for the new insect vector underlying this phenomenon are highly elusive and require in-depth investigation.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0007485324000646

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Competing interests. None.

References

Abou-Jawdah Y, Abdel Sater A, Jawhari M, Sobh H, Abdul-Nour H, Bianco P, Molino Lova M and Alma A (2014) *Asymmetrasca decedens* (Cicadellidae, Typhlocybinae), a natural vector of 'Candidatus Phytoplasma phoenicium'. *Annals of Applied Biology* **165**, 395–403.

Agasyeva IS, Ismailov VY, Petrishcheva M and Nastasiy AS (2024) Use of biological agents to control the number of *Halyomorpha halys* Stål. *Advancements in Life Sciences* **11**, 696–702.

Bertaccini A, Fiore N, Zamorano A, Tiwari AK and Rao GP (2019) Molecular and serological approaches in detection of phytoplasmas in plants and insects. *Phytoplasmas: Plant Pathogenic Bacteria-III: Genomics, Host Pathogen Interactions Diagnosis*, 105–136.

Bosco D and Tedeschi R (2013) Insect vector transmission assays. In Dickinson M and Hodgetts J. (eds), *Phytoplasma. Methods in Molecular Biology, vol 938*. Totowa, NJ: Humana Press, pp. 73–85.

Carr JP, Donnelly R, Tungadi T, Murphy AM, Jiang S, Bravo-Cazar A, Yoon J-Y, Cunniffe NJ, Glover BJ and Gilligan CA (2018) Viral manipulation of plant stress responses and host interactions with insects. *Advances in virus Research* **102**, 177–197.

Dermastia M (2019) Plant hormones in phytoplasma infected plants. *Frontiers in Plant Science* **10**, 477.

Hemmati C, Nikooei M, Al-Subhi AM and Al-Sadi AM (2021) History and current status of phytoplasma diseases in the Middle East. *Biology* **10**, 226.

Killiny N and Almeida RP (2014) Factors affecting the initial adhesion and retention of the plant pathogen *Xylella fastidiosa* in the foregut of an insect vector. *Applied and Environmental Microbiology* **80**, 420–426.

Kumari S, Nagendran K, Rai AB, Singh B, Rao GP and Bertaccini A (2019) Global status of phytoplasma diseases in vegetable crops. *Frontiers in Microbiology* **10**, 1349.

Lee IM (1993) Universal amplification and analysis of pathogen 16S rDNA for classification and identification of mycoplasma like organisms. *Phytopathology* **83**, 834–842.

Li P, Jie Z and Yue Y (2020) Effects of rice stripe mosaic virus on the growth, reproduction and feeding behavior of the mediator electric leafhopper. *Acta Entomologica Sinica* **2**, 174–180.

Marcone C (2009) Movement of phytoplasmas and the development of disease in the plant. In *Phytoplasmas: Genomes, Plant Hosts and Vectors*. Wallingford: CABI International, pp. 114–131.

Marcone C (2014) Molecular biology and pathogenicity of phytoplasmas. *Annals of Applied Biology* **165**, 199–221.

Marzachì C, Milne RG and Bosco D (2004) Phytoplasma-plant-vector relationships. *Recent Research Developments in Plant Pathology* **3**, 211–241.

Mou D-F, Di Lella B, Halbert SE, Bextine B, Helmick EE and Bahder BW (2022) Acquisition and transmission of the lethal bronzing phytoplasma by *Haplaxius crudus* using infected palm spear leaves and artificial feeding media. *Phytopathology®* **112**, 2052–2061.

Palermo S, Arzone A and Bosco D (2001) Vector-pathogen-host plant relationships of chrysanthemum yellows (CY) phytoplasma and the vector leafhoppers *Macrosteles quadripunctulatus* and *Euscelidius variegatus*. *Entomologia experimentalis et applicata* **99**, 347–354.

Quaglino F, Sanna F, Moussa A, Faccincani M, Passera A, Casati P, Bianco PA and Mori N (2019) Identification and ecology of alternative insect vectors of 'Candidatus Phytoplasma solani' to grapevine. *Scientific Reports* **9**, 19522.

Ranebennur H, Rawat K, Rao A, Kumari P, Chalam VC, Meshram N and Rao GP (2022) Transmission efficiency of a 'Candidatus Phytoplasma australasia' (16SrII-D) related strain associated with sesame phyllody by dodder, grafting and leafhoppers. *European Journal of Plant Pathology* **164**, 193–208.

Schmittgen TD and Livak KJ (2008) Analyzing real-time PCR data by the comparative CT method. *Nature Protocols* **3**, 1101–1108.

Schneider B, Ahrens U, Kirkpatrick B and SeemuLler E (1993) Classification of plant-apthogenic mycoplasma-like organism using restriction-site

analysis of PCR-amplified 16S rDNA. *Microbiology-sgm* **139**, 519–527. https://doi.org/10.1099/00221287-139-3-519

Sun X, Dongsheng J and Ping Z (2017) Infection of rice stripe mosaic virus in the mediator electronic-leaf cicada. *Chinese Society of Plant Pathology* (Abstract), 285–287.

Trivellone V, Cao Y and Dietrich CH (2022) Comparison of traditional and next-generation approaches for uncovering phytoplasma diversity, with discovery of new groups, subgroups and potential vectors. *Biology* **11**, 977.

Wan Y, Hussain S, Merchant A, Xu B, Xie W, Wang S, Zhang Y, Zhou X and Wu Q (2020) Tomato spotted wilt orthotospovirus influences the reproduction of its insect vector, western flower thrips, *Frankliniella occidentalis*, to facilitate transmission. *Pest Management Science* **76**, 2406–2414.

Wang Z (2018) *Detection of* Camptotheca acuminata *mycoplasma and identification and verification of vector insect leafhopper*. Master thesis. Yunnan Agricultural University.

Wang C, Chien Y, Liao P, Chiu Y, Chen Y and Yang J (2021a) First report of 16SrII-V phytoplasma associated with green manure soybean (*Glycine max*) in Taiwan. *Plant Disease* **105**, 2012.

Wang Z, Yuan E, Su F, Wan Q and Cai H (2021b) Identification of Camptotheca acuminate witches-broom phytoplasma and TagMan-based fluorescence qPCR method for detection. *Acta Phytopathologica Sinica* **51**, 429–440.

Wang Z-H, Wang W-P, Yuan E-P, Su F, Mao Q-Y, Wan Q-L and Cai H (2021c) Identification of Camptotheca acuminate witches'-broom Phytoplasma and Taq man-based fluorescence qPCR method for detection. *Acta Phytopathologica* **51**, 429–440.

Wei W, Jiang L and Depo Y (2006) *Camptotheca acuminata* planting in limestone mountains and its comprehensive control of brown spot. *Practical Forestry Technology* **11**, 9–11.

Xing L (2004) Distribution status, medicinal value and development prospect of *Camptotheca acuminata*. *Journal of Shaanxi Normal University* **32**, 69–73.

Xu X, Gang X and Wanbin M (2009) Control test of *Camptotheca acuminata* canker. *Jiangsu Forestry Science and Technology* **36**, 44–54.

Yan F (2020) Plant pathogen-vector interaction: research progress and prospect. *Journal of Insects* **63**, 123–130.

Zhang S, Shunli C and Feiping Z (2002) Study on the life table of natural populations of *Camptotheca acuminata*. *Journal of Fujian Forestry University* **2**, 4–5.

Zhu XJ, Zhang ZJ and Lu YB (2011) Effects of inoculation of host plants with tomato spotted wilt virus on populations of the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). *Acta Entomologica Sinica* **54**, 425–431.

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