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Effects of Phytoplasma Infection on Aster Leafhopper (*Macrostes quadrilineatus*) Settling Behavior and Development on *Brassica napus*

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ABSTRACT

Aster yellows phytoplasma (AYp) is a mollicute that infects numerous crops, including canola (*Brassica napus* L.), in which it is pathogenic, and is transmitted by the aster leafhopper (*Macrostes quadrilineatus* Forbes). Understanding how AYp infection alters vector behavior and development is critical for predicting disease dynamics in agricultural systems. We used two-choice and no-choice bioassays to assess settling preferences and developmental performance of AYp-infected and uninfected leafhoppers on AYp-infected and uninfected *B. napus*. *Arabidopsis thaliana* L. was included as a reference host for developmental comparisons. At 2 weeks post-infection, leafhoppers showed a significant preference for AYp-infected plants; by 4 weeks, this preference disappeared. Uninfected leafhoppers produced significantly more nymphs on AYp-uninfected plants than on AYp-infected plants, although survival on *B. napus* was poor, and no individuals completed development. Phytoplasma titers in plants increased over time and corresponded with symptom severity. These results show that while AYp infection briefly influences vector settling behavior, *B. napus* is a marginal host that does not support full development, providing insight into vector–pathogen interactions and disease epidemiology in canola systems.

1 | Introduction

Brassica napus L. (Brassicaceae) is a crucial agricultural crop for the Canadian agriculture sector. Between 2016 and 2019, *B. napus* production resulted in an average of C\$29.9 billion in economic impact annually while supporting 207 000 direct and indirect jobs (LMC International 2020). However, *B. napus* is highly susceptible to the bacterial pathogen *Candidatus* Phytoplasma asteris (AYp), the causal agent of Aster Yellows disease. This disease can be detrimental to infected plants, and common symptoms of infection include stunting, excessive greening of floral tissues, leaf chlorosis, phyllody and bladder-like siliques (Olivier

et al. 2006; Romero et al. 2023). These malformations of organs can result in reduced quantity and quality of the overall yield (Olivier et al. 2010). AYp has the most extensive and diverse host range of the known phytoplasmas, infecting over 350 plant species (Lee et al. 2000; Hollingsworth et al. 2008; Frost et al. 2013; Romero et al. 2023).

This plant pathogen is primarily spread by sap-sucking hemipterans, mainly of the family Cicadellidae (Weintraub and Beanland 2006; Wilson 2007). Nineteen known vector species in North America transmit AYp (Olivier et al. 2009; Romero et al. 2020), but the aster leafhopper (*Macrostes quadrilineatus*

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Forbes, Hemiptera: Cicadellidae) is the most common and has been determined to be the causal vector of several outbreaks of Aster Yellows disease in *B. napus* production (Weintraub and Jones 2010). Although aster leafhoppers are widely recognized as a vector of AYp and are frequently captured in canola fields across the Canadian Prairies, several studies to date have demonstrated that *B. napus* is a suboptimal host for their development, with most individuals failing to progress beyond early instars (Olivier et al. 2017; Romero et al. 2020). This paradox—where a vector commonly visits, feeds, and transmits AYp in a crop on which it performs poorly—presents an important and understudied ecological question. Canola remains a cornerstone of Canadian agriculture and is highly susceptible to Aster Yellows disease, with outbreaks causing significant yield losses in affected years. Understanding the early behavioral interactions between leafhoppers and canola, even in the absence of full vector development, is therefore critical for elucidating epidemiological pathways and informing integrated management strategies.

The interaction between pathogens, vectors, and host plants creates a dynamic triad with significant implications for disease spread and severity. Previous work has demonstrated that the presence of a pathogen in host plants can alter plant physiology, resulting in increased vector offspring and survivorship (Feres et al. 1989; Mayer et al. 2002; Sugio et al. 2011). Infection by AYp can alter the physiology and behavior of its primary vector, the aster leafhopper, extending its lifespan and enhancing reproductive output, which may amplify its role in the epidemiology of aster yellows disease (Beanland et al. 2000). Host plants infected with a pathogen can induce vector behavioral changes, such as altered host selection, further enhancing disease spread (Ingwell et al. 2012; Roosien et al. 2013). For instance, infected vectors may preferentially settle on or feed from healthy plants, optimizing the pathogen's dissemination across a plant population (Ingwell et al. 2012; Rajabaskar et al. 2014). Although the mechanisms behind these behavioral changes are not well understood, infected plants often alter their volatile organic compound profiles to emit odors that attract vectors, as seen with *Candidatus Phytoplasma mali*, which induces the release of β -caryophyllene in infected apple plants to attract its vector *Cacopsylla picta* Förster (Hemiptera: Psyllidae) (Mayer et al. 2008b). These altered cues can increase the number of transmitting vectors aggregating in infected fields, compounding the spread of the disease-causing pathogen. From an epidemiological perspective, these behavioral and physiological shifts can reshape disease dynamics by creating feedback loops that amplify vector populations and pathogen prevalence. For example, interactions between the English grain aphid, *Macrosiphum avenae* Fab. (Hemiptera: Aphididae), and three wheat cultivars infected with two isolates of barley yellow dwarf virus (BYDV) demonstrated that aphids feeding on BYDV-infected plants exhibited shorter developmental times, greater fecundity, and higher intrinsic rates of natural increase compared to those feeding on healthy plants, supporting the idea that the presence of the pathogen enhances host plant suitability for aphids, leading to increased reproduction, faster population growth, and more efficient virus spread (Feres et al. 1989). Further, *Dalbulus maidis* DeLong & Wolcott (Hemiptera: Cicadellidae) leafhoppers exhibited improved survival when feeding on maize infected with maize stunting pathogens, such as corn

stunt spiroplasma (*Spiroplasma kunkelii*) and maize bushy stunt phytoplasma, compared to healthy maize, highlighting the indirect fitness benefits these pathogens can confer on their insect vectors (Ebbert and Nault 2001).

Acknowledging the complex epidemiology of AYp, its year-to-year variability in incidence, and its intricate interactions with host plants and insect vectors, this study aimed to investigate how the presence of AYp influences vector host selection and population dynamics. To address this, we developed a study system involving AYp-infected and uninfected aster leafhoppers, AYp-infected and uninfected *B. napus*, and *Arabidopsis thaliana* L. plants. Using two-choice bioassays, we examined the settling behavior of aster leafhoppers across all combinations of infected and uninfected vectors and host plants. These assays were conducted at two time points—2 and 4 weeks post-infection—to assess whether increasing phytoplasma presence alters vector behavior. Additionally, no-choice bioassays were employed to evaluate the effects of phytoplasma infection on insect development. Because studies have suggested that aster leafhoppers do not complete development on canola, our study focuses on early-stage interactions, rather than full life cycle performance. To confirm and quantify the level of phytoplasma infection, phytoplasma concentration was determined using quantitative PCR (qPCR). This comprehensive approach allowed for a detailed analysis of the pathogen's role in shaping vector-plant interactions and its potential impact on disease dynamics. We hypothesized that (1) AYp infection would alter aster leafhopper settling preferences over time, and (2) the infection status of the host plant and vector would interact to influence reproductive success and early developmental outcomes.

2 | Materials and Methods

2.1 | Plant Species and Growing Conditions

The following Brassicaceae species were used in this study: *Brassica napus* var. AC Excel and *Arabidopsis thaliana* (WT Col-0 ecotype). Seeds of *B. napus* were sown in individual pots containing a standard propagation mix. Three seeds were sown per pot to ensure germination, with seedlings thinned to one plant after emergence. Plants were watered every 3 days and fertilized at each watering with a 20-20-20 water-soluble fertilizer (20% N, 20% P₂O₅, 20% K₂O). *A. thaliana* seeds were sown densely in propagation mix, and after germination, five to seven seedlings were transplanted into individual pots for bioassays. Both species were maintained under identical controlled growth chamber conditions (18-h photoperiod, 21°C day/17°C night) at the University of Saskatchewan's controlled growth facility. To synchronize growth stages for bioassays, *A. thaliana* was seeded 14 days before *B. napus*, due to its longer germination period. All dimensions and model specifications for pots, cages, and other rearing materials are provided in Table S1.

These experiments were conducted on *B. napus* plants under two conditions: AYp-infected and AYp-uninfected. AYp is fastidious, and therefore, it is not possible to mechanically infect plants. To acquire AYp-infected *B. napus* plants, AYp-uninfected plants underwent insect-mediated inoculation using inoculative aster leafhoppers. For the inoculations, AYp-infected aster

leafhoppers were kept in cup cages containing individual pots, as described by Romero et al. (2020, 2023). Briefly, this involved placing plastic cups around the plant, with an organza bag secured on top of the cage to ensure the insects could not escape while allowing airflow (Figure S1). Three pairs of male and female leafhoppers from an AYp-infected colony were introduced into the cup cage, where they could access the AYp-uninfected plant. The insects remained on the plant for 10 days at a consistent 24°C to facilitate the infection of the *B. napus* plants. After the infection period, the AYp-infected leafhoppers were removed, and the plants were maintained at a steady 24°C for an additional 14 days to allow the phytoplasma to replicate within the infected plants.

2.2 | Aster Leafhoppers

Aster leafhoppers were obtained from colonies maintained at the University of Saskatchewan for over 4 years (Romero et al. 2020), which originated from field collections in 2007. They were housed in 30 cm³ insect-rearing cages with mesh walls to ensure proper ventilation. Colonies were fed on barley (*Hordeum vulgare* L.; var. CDC Copeland), an optimal host for aster leafhoppers, following the methods outlined by Romero et al. (2020). Plants were replaced biweekly to promote plant health and facilitate the oviposition of new eggs. The colonies were kept in growth chambers under an 18-h photoperiod, with temperatures of 21°C during the day and 17°C at night. Relative humidity was not artificially modified during this study and remained around 45% ± 10%. Adult male and female leafhoppers, identified by examining external genitalia as described by Romero et al. (2020), were used in this study.

AYp-infected aster leafhopper colonies were maintained as outlined above, with some modifications. The AYp-infected colony was kept at 24°C to ensure adequate phytoplasma replication and survival. Furthermore, AYp-infected periwinkle (*Catharanthus roseus* L.) was added to the insect rearing cage alongside barley to enhance pathogen acquisition due to its ability to endure long-term AYp infection without plant senescence. AYp-infected colonies were housed in separate growth chambers to prevent cross-contamination of the phytoplasma, and the infection status of AYp in both plants and insect colonies was confirmed using qPCR, as detailed below.

2.3 | Two-Choice Bioassays

Two-choice bioassays were conducted following the procedures described by Romero et al. (2022) in cages, which contained two 28-day-old *B. napus* plants: one AYp-uninfected and one AYp-infected (Figure S2). The cages featured a clear UV-transparent vinyl window positioned upward, allowing for unobstructed observations and light entry. The remaining five sides were constructed of mesh to promote airflow to the plants. Plants were randomly assigned to one of the two sides of the cage, ensuring a maximum distance between them without touching the cage sides. At the beginning of the bioassay, five pairs of aster leafhoppers, consisting of one male and one female each, were released into the center of the choice cage and allowed to acclimate for 36 h. After this period, the leafhoppers' positions were recorded

daily for 96 h between 13:00 and 15:00 every 24 h. The positions were defined as (1) on the uninfected plant, (2) on the infected plant, or (3) off the plants entirely. This procedure was replicated 10 times, using AYp-infected leafhoppers and AYp-uninfected leafhoppers separately. Experiments were conducted on two different occasions—2 and 4 weeks post-infection—to assess potential differences related to the phytoplasma concentration within plant tissues and the symptoms exhibited by the plants. In one set of experiments, the aster leafhoppers (AYp-uninfected and AYp-infected) were presented with an AYp-uninfected *B. napus* plant and an AYp-infected *B. napus* (2 weeks post-infection, corresponding to an early phytoplasma infection). In another set of experiments, insects were presented with an AYp-uninfected *B. napus* plant and an AYp-infected *B. napus* plant (4 weeks post-infection, reflecting the later stages of phytoplasma infection). This allowed us to evaluate the effects of different infection levels on vector behavior, as AYp concentration increased over time in both *A. thaliana* and *B. napus*, and symptoms vary between the early and later stages of the infection (Romero et al. 2023). Following the bioassays, plant and insect samples were collected and stored at −80°C for later analysis.

2.4 | No-Choice Bioassays

For no-choice bioassays, the procedures were similar to those described by Romero et al. (2020). The bioassays were conducted using the same cup cages that were used to infect plants with AYp in the two-choice bioassays (Figure S1). Experiments utilized 10 replicates (a caged plant as a replicate) for both AYp-infected and AYp-uninfected treatments, employing 28-day-old *B. napus* plants. Additionally, 10 replicates were performed with 28-day-old *A. thaliana*, which served as a reference plant. No-choice bioassays involved both AYp-infected and AYp-uninfected leafhoppers. To conduct bioassays, three male/female pairs of aster leafhoppers were placed in each cup cage to facilitate reproduction (mating and oviposition) for 1 week. After this period, the leafhopper pairs were removed from the caged plants, and the plants were monitored until the first instar nymphs were observed. Regular observations were made to track nymphal development. During these observations, the number and instar of each nymph were recorded. Reproductive success was measured by the number of first instar nymphs that appeared on each replicate. Observations were conducted every 72 h until 90% of the nymphs on the reference plant (AYp-uninfected *A. thaliana*, AYp-uninfected leafhoppers) reached the adult stage, which occurred 34–36 days into the observations. A stage-specific life history table (Table 1) was generated using the recorded data, following a methodology akin to that of Price et al. (2011).

2.5 | DNA Extraction and Quantitative PCR (qPCR)

DNA extractions for qPCR were conducted on pools of two individual insects per extraction. The extraction was performed using a Qiagen DNeasy Blood and Tissue Kit (QIAGEN GmbH, Hilden, Germany), adhering to the manufacturer's protocols. After the extractions, DNA concentration was measured using a NanoDrop One Microvolume UV-Vis Spectrophotometer (ThermoFisher Scientific, Lenexa, KS, USA). The DNA concentration obtained

TABLE 1 | A stage-specific life table of AYp-uninfected and AYp-infected aster leafhoppers was reared on different hosts, including the model system *A. thaliana*, and *B. napus*. For this set of experiments, *A. thaliana* was used as a reference.

Age interval, x	Reference	Number of individuals alive at beginning of x, lx			
		AYp-inf. <i>B. napus</i>		AYp-uninf. <i>B. napus</i>	
		AYp-inf. ALH	AYp-uninf. ALH	AYp-inf. ALH	AYp-uninf. ALH
Instar I	358	28	30	5	55
Instar II	358	27	26	—	37
Instar III	342	—	—	—	—
Instar IV	337	—	—	—	—
Instar V	322	—	—	—	—
Adult	305	—	—	—	—

Age interval, x	Reference	Number of individuals dying during x, dx			
		AYp—inf. <i>B. napus</i>		AYp—uninf. <i>B. napus</i>	
		AYp—inf. ALH	AYp—uninf. ALH	AYp—inf. ALH	AYp—uninf. ALH
Instar I	0	1	4	5	18
Instar II	16	27	26	—	37
Instar III	5	—	—	—	—
Instar IV	15	—	—	—	—
Instar V	17	—	—	—	—
Adult	—	—	—	—	—

Age interval, x	Reference	dx as percentage of lx, 100qx			
		AYp—inf. <i>B. napus</i>		AYp—uninf. <i>B. napus</i>	
		AYp—inf. ALH	AYp—uninf. ALH	AYp—inf. ALH	AYp—uninf. ALH
Instar I	0.00	3.57	13.33	100.00	32.73
Instar II	4.47	100.00	100.00	—	100.00
Instar III	1.46	—	—	—	—
Instar IV	4.45	—	—	—	—
Instar V	5.28	—	—	—	—
Adult	0.00	—	—	—	—

Note: Stages included five nymphal instars and adults. The number of individuals alive at the beginning, the number dying at each stage, and the survivorship are provided. The number of Instar I was assumed to be the number of eggs, and all eggs successfully hatched.

from insect samples ranged from 40 to 487 ng/ μ L before dilution with DEPC-treated water. When necessary, samples were diluted with nuclease-free water (Invitrogen, Carlsbad, CA, USA) until the DNA concentration reached ≤ 50 ng/ μ L to mitigate the concentration of potential PCR inhibitors.

To confirm AYp infection or non-infection, plants from two-choice bioassays were tested via qPCR for both the 2-week and 4-week post-infection bioassays. Extractions were performed on 2.54 cm² plant tissue samples harvested immediately after completing two-choice bioassays. The tissue samples were collected from the leaf margins of the oldest leaves and stored in a -80°C freezer until the DNA extraction. AYp-uninfected *B. napus* and AYp-infected *B. napus* extractions were done separately to minimize the risk of cross-contamination. The extractions were conducted

using a Qiagen DNeasy Plant Mini Kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's protocol with one modification. The final DNA was eluted with 32 μ L of UltraPure DNase/RNase-Free Distilled Water (Invitrogen, Carlsbad, CA, USA) instead of the provided buffer solution to decrease potential inhibitory effects associated with the elution buffer. DNA concentration was measured after extraction using a NanoDrop One, and DNA concentration in all samples ranged from 7 to 30 ng/ μ L.

AYp concentration in both plants and insects was determined using probe-based qPCR targeting the gene that encodes the chaperonin protein (chaperonin-60, cpn60), as outlined in the procedures described by Pusz-Bochenska et al. (2022) and Romero et al. (2023). This was conducted on a QuantStudio 3 Real-Time PCR machine (ThermoFisher Scientific, Lenexa, KS,

USA) with 20 μ L reactions comprising 10 μ L of SsoAdvanced Universal Probes Supermix (Bio-Rad, California, USA), 0.6 μ L of forward primer (10 μ M), 0.6 μ L of reverse primer (10 μ M), 0.4 μ L of probe (10 μ M), 2 μ L of template DNA (7–20 ng/ μ L), and 6.4 μ L of nuclease-free water. The phytoplasma cpn60 sequence was amplified using primers H279p (5'-GAT III GCA GGI GAT GGA ACM ACI AC-3') and H280p (5'-TGR TTI TCI CCA AAA CCA GGI GCA TT-3'), along with the probe (FAM: ATC CTT CAA CAA CTT CTA ATT CTG-BHQ1). A set of standards was employed to create a calibration curve with known copies of cpn60, ranging from 10^7 to 10^1 copies of cpn60 per 2 μ L of solution. The thermal cycling profile consisted of 95°C for 180s, followed by 40 cycles of 95°C for 30s, 59°C for 30s, and 72°C for 30s, culminating in a final melt curve of 95°C for 15s, 60°C for 60s, and 95°C for 10s. Concentrations were quantified using QuantStudio Design and Analysis Software v. 2.6.0 (ThermoFisher Scientific, Waltham, MA, USA).

2.6 | Statistical Analysis

Statistical analyses were conducted using R version 4.3.1 (R Core Team 2025). For the two-choice bioassays, the number of leafhoppers on each plant was assessed using permutational multivariate analyses of variance (PERMANOVA; Oksanen et al. 2015). In this model, the plant infection status served as the explanatory variable, while the count of leafhoppers on each plant acted as the response variable. Leafhoppers noted as “off the plants” were excluded from the analysis as they were not the focus of this study, and their counts were low and consistent across all treatments. The package “lme4” (Bates et al. 2015) was utilized to analyze the reproductive success of aster leafhoppers in no-choice bioassays, employing a generalized linear mixed-effects model (GLMM) with a negative binomial distribution to accommodate overdispersion and the prevalence of zeros. The number of leafhoppers in each replicate was designated as the response variable, with “Leafhopper Infection Status,” “Plant Infection Status,” and their interactions included as fixed effects. The model also incorporated a random effect term for each replicate. Post hoc tests were conducted using the package “emmeans” (Lenth 2024) to obtain estimated marginal means from the model. Tukey’s HSD test and Bonferroni’s correction were applied for pairwise comparisons between multiple groups that exhibited significant interactions and to derive their associated *p*-values. AYp concentrations were assessed using a generalized linear model (GLM) with a negative binomial distribution to account for over-dispersed data. In this model, the number of copies of cpn60 served as the response variable, with “Time,” “Plant Infection Status,” and their two-way interaction specified as fixed effects. A Wald chi-square test was performed to ascertain whether there was a significant association between “Time” and the concentration of phytoplasma DNA present in the plant tissue.

3 | Results

3.1 | Two-Choice Bioassays: Leafhopper Settling Behavior

Leafhopper settling behavior showed a clear temporal shift. At 2 weeks post-infection, AYp-infected leafhoppers preferred

AYp-infected *B. napus* (3.88 ± 0.25 , mean $\pm$ SEM) over AYp-uninfected plants (3.00 ± 0.20 ; $F=13.2$; $df=1, 18$; $p<0.001$). By 4 weeks, this preference was no longer evident: neither AYp-uninfected ($F=1.8$; $df=1, 18$; $p=0.17$) nor AYp-infected leafhoppers ($F=0.6$; $df=1, 18$; $p=0.49$) showed significant settling differences between plant types. Mean settling counts were comparable for both infection statuses (e.g., AYp-uninfected leafhoppers: 3.75 ± 0.31 on uninfected plants vs. 3.28 ± 0.23 on infected plants). These results demonstrate that any early preference for infected plants disappears as infection progresses (Figure 1).

3.2 | No-Choice Bioassays: Reproductive Success and Development of Leafhoppers

Reproductive success differed significantly between plant infection treatments. AYp-uninfected *B. napus* supported higher offspring production than AYp-infected plants ($X^2=23.16$, $df=1$, $p<0.001$). In contrast, the infection status of the parent leafhoppers alone had no significant effect ($X^2=0.55$, $df=1$, $p=0.46$). However, a significant interaction between plant and vector infection status was found ($X^2=33.68$, $df=1$, $p<0.001$): on uninfected plants, AYp-uninfected leafhoppers produced a higher number of nymphs (3.28 ± 0.31) than AYp-infected leafhoppers (0.79 ± 0.22), whereas on infected plants, offspring production was similar across vector infection statuses (1.66 ± 0.11 vs. 1.79 ± 0.12 ; Figure 2).

Overall, AYp-uninfected leafhoppers had more offspring (5.50 ± 0.32) than AYp-infected leafhoppers (3.39 ± 0.21). The plant species significantly affected the number of aster leafhopper offspring observed ($X^2=39.22$, $df=1$, $p<0.001$). Aster leafhopper reproductive success was significantly reduced in all *B. napus* replicates (2.09 ± 0.11) compared to the number of offspring observed in *A. thaliana* replicates (5.74 ± 0.28). *A. thaliana* replicates with AYp-uninfected leafhoppers had a greater

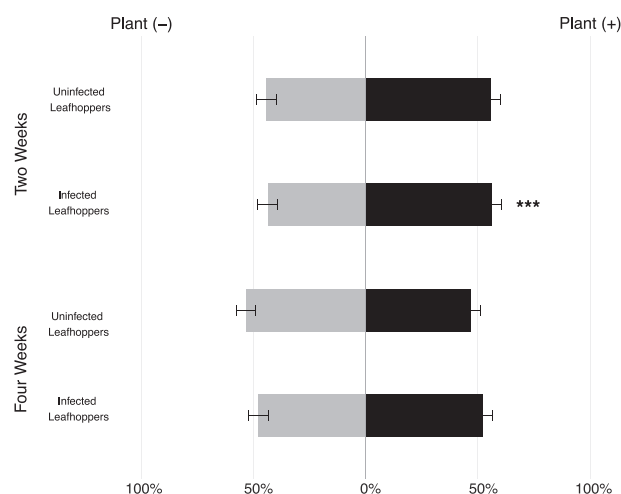


FIGURE 1 | Leafhoppers prefer AYp-infected plants early (2 weeks post-infection) but show no preference later (4 weeks). The figure depicts the percentage of insects choosing the AYp-uninfected (light grey) or AYp-infected (dark grey) plants. The *p*-values from permutational multivariate analyses of variance (PERMANOVA), which compare insect preference to an equal distribution, are indicated by asterisks *** ($p<0.001$).

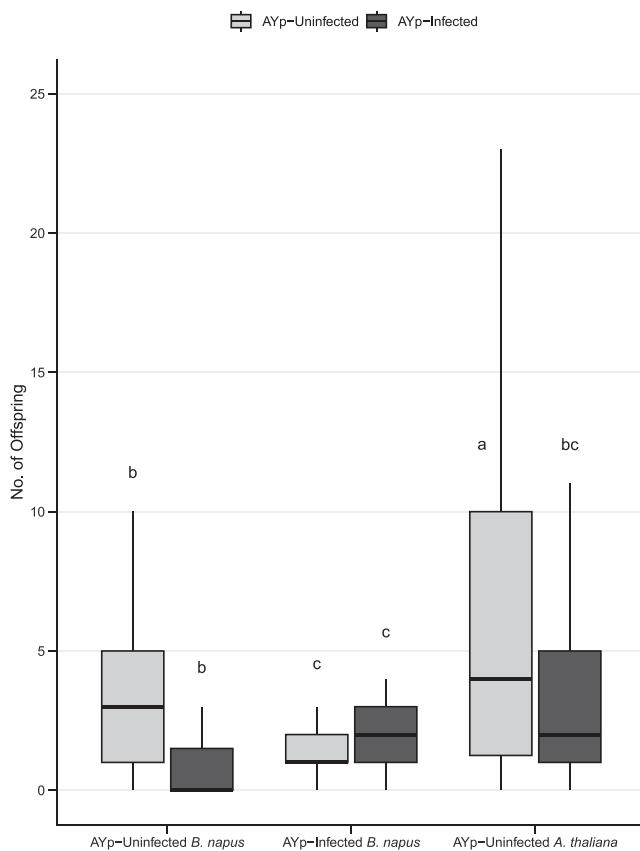


FIGURE 2 | Reproductive success of aster leafhoppers is highest on AYp-uninfected plants, with overall offspring production reduced on *B. napus* compared to *A. thaliana*. The boxplot figure illustrates the number of offspring observed for both plant species and aster yellows phytoplasma (AYp) infection status. Boxes are drawn between the 25th and 75th percentiles, with the median marked with a horizontal black line. Whiskers indicate the largest and smallest values within 1.5 times the interquartile range from the ends of the boxes. Light-grey boxes indicate no-choice bioassays where AYp-uninfected aster leafhoppers were used, and dark-grey boxes indicate where AYp-infected aster leafhoppers were used. Different letters indicate statistically significant differences in the number of offspring observed for each treatment combination (GLMM followed by Tukey's test with Bonferroni's correction for multiple comparisons, using an α -value of 0.05).

TABLE 2 | Number of *cpn60* Copies per ng of DNA (mean $\pm$ SEM) after qPCR in AYp-uninfected and AYp-infected *B. napus* plants.

	Plant infection condition	
	AYp-uninfected	AYp-infected
Time		
2 weeks	0.059 $\pm$ 0.025	449.709 $\pm$ 258.524
4 weeks	0.208 $\pm$ 0.078	3595.408 $\pm$ 1383.364

number of offspring (7.49 ± 0.23) than those with AYp-infected leafhoppers (4.03 ± 0.13).

Developmental progression also differed between host plants. On *A. thaliana*, nymphs successfully developed to adulthood

within 39–41 days. On *B. napus*, however, no nymphs advanced beyond the second instar, regardless of plant infection status (Table 1). Notably, AYp-infected *B. napus* showed reduced first-instar mortality (8.6%) compared to uninfected plants (18.1%), and AYp-infected leafhoppers on infected plants had the lowest mortality between first and second instars (3.6%) among treatments. Despite these differences, all nymphs on *B. napus* died by 21–25 days.

3.3 | Validation of AYp-Infection Status and Plant Concentration

qPCR confirmed infection treatments and documented concentration progression. Phytoplasma concentration significantly differed between infected and uninfected treatments ($F = 3980.15$, $df = 1, 76$, $p < 0.001$) and increased significantly between 2 and 4 weeks post-infection ($F = 96.78$, $df = 1, 76$, $p < 0.001$). The interaction between “Time” and “Plant Infection Status” was not found to be significant ($F = 0.51$, $df = 1, 76$, $p = 0.47$). AYp-infected *B. napus* had significantly greater *cpn60* gene copy numbers at 4 weeks post-infection compared to 2 weeks post-infection (Table 2). These concentration changes coincided with distinct visual symptoms: AYp-uninfected plants remained healthy, while AYp-infected plants displayed pronounced stunting, virescence of floral tissues, phyllody, and an absence of reproductive structures, with symptom severity increasing by 4 weeks post-infection (Figure 3).

All aster leafhoppers used in the bioassays were also tested by qPCR to confirm their infection status. Although quantitative concentrations were obtained from these samples, they are not reported here because the timing of pathogen acquisition in individual insects was unknown, and concentrations could not be standardized across individuals.

4 | Discussion

In this study, we used two-choice and no-choice bioassays to assess whether AYp infection in either plants or the leafhopper vector influenced the vector's performance or preference. Our results showed a preference for infected plants at 2 weeks. However, by 4 weeks, this preference disappeared. This transition coincided with a marked increase in AYp concentration and symptom severity in infected plants, as confirmed by qPCR measurements, indicating that infection progressed substantially between these two time points. The no-choice bioassays revealed that *B. napus* is not a suitable host for aster leafhoppers under any condition, as they were unable to complete their development on this host. Nevertheless, leafhoppers exhibited different oviposition patterns depending on infection status. It is important to note that this limitation applies specifically to the no-choice experiments assessing reproductive performance and development, as aster leafhoppers did not survive beyond the second instar on *B. napus*. Consequently, our findings regarding performance and survival are limited to early instar stages, while behavioral interpretations are based on adult responses. These findings highlight a key aspect of the pathosystem: while *B. napus* is clearly not a suitable developmental host, aster leafhoppers are still encountered in canola fields and known to



FIGURE 3 | Visual symptoms of aster yellows phytoplasma (AYp) infection. The figure compares AYp-infected *Brassica napus* at 2 weeks post-infection (left) with an AYp-uninfected control (right). Infected plants exhibited early symptoms including mild stunting and virescence, which progressed to phyllody and the absence of reproductive structures by 4 weeks post-infection (not shown). AYp-uninfected plants remained phenotypically normal throughout the experiment.

transmit aster yellows phytoplasma in this crop. The poor performance observed here underscores that the presence of vectors in a field does not necessarily equate to successful reproduction, but it can still contribute to pathogen spread through transient feeding and short-term survival.

Numerous factors influence insect host plant selection (Todd et al. 1990; Bell et al. 1995; Howse et al. 1998; Singh et al. 2019), and pathogens can modify plant traits in ways that affect these choices (Hogenhout et al. 2008; Mauck et al. 2010, 2016). In some cases, these pathogen-induced alterations are hypothesized to facilitate pathogen transmission by manipulating vector behavior (Mayer et al. 2008a, 2008b; Rajabaskar et al. 2014). In our trials, AYp-infected aster leafhoppers did not preferentially settle on AYp-uninfected *B. napus*, as would be expected if the pathogen actively manipulated its vector to enhance transmission. Instead, at 2 weeks post-infection, AYp-infected leafhoppers preferred AYp-infected *B. napus*, which contradicts the manipulation hypothesis as selecting an already infected host does not facilitate further pathogen spread. However, this preference disappeared 4 weeks post-infection, with leafhoppers showing no clear preference for either option provided. In this study, the increase in AYp concentration between 2 and 4 weeks suggests that infected plants were undergoing significant physiological changes during the period when vector preference shifted (Table 2). Liu et al. (2017) found that plant defences can counteract pathogen manipulation, which may explain the delayed loss of preference in *B. napus* at 4 weeks but highlights dynamic temporal changes in vector behavior, possibly influenced by pathogen interactions with plant or insect physiology (Werner et al. 2009; Eigenbrode et al. 2018). Given that vector manipulation syndromes may require extended evolutionary timescales to develop, the *B. napus*—*M. quadrilineatus*—*Ca. P. asteris* pathosystem, being relatively recent in evolutionary terms, may not have had sufficient time for such adaptations to

arise. Alternatively, the mechanisms necessary for behavioral manipulation may be absent in this system, or manipulation may confer no selective advantage (Gandon 2018).

While pathogen-driven alterations of vector behavior have been well documented in some pathosystems (Werner et al. 2009; Martini et al. 2015; Mauck et al. 2016), it remains unclear whether these cases represent widespread phenomena or isolated occurrences. A notable example is provided by Mauck et al. (2010), who found that aphid vectors were attracted to cucumber mosaic virus (CMV)-infected host plants despite their reduced quality. This attraction was hypothesized to result from deceptive host plant signalling induced by CMV infection, potentially enhancing host-location cues to draw insect vectors. Given the complex interactions between phytoplasmas and their host plants, it remains challenging to determine whether the observed differences in vector settling behavior stem from specific physiological changes or broader plant-level modifications. Phytoplasma infections can induce physiological and biochemical changes in host plants, such as altered amino acid distribution in leaves and phloem sap (Gai et al. 2014), disrupted photosynthesis leading to abnormal carbohydrate accumulation (Xue et al. 2018; Negro et al. 2020), and the emission of volatile organic compounds that directly influence vector behavior (Oluwafemi et al. 2011; Mann et al. 2012). These pathogen-induced modifications are potentially one of the mechanisms by which host plant attractiveness is affected for aster leafhoppers.

4.1 | Effects of Phytoplasma Infection on Life History

In this study, survivorship was significantly reduced on *B. napus* compared to the reference host *A. thaliana*, regardless of the plant or insect vector's infection status. AYp-uninfected

aster leafhoppers confined to *B. napus* did not survive past the second instar, while AYp-infected leafhoppers on AYp-uninfected *B. napus* died during the first instar. Aster leafhoppers exhibited significantly reduced reproductive success on *B. napus* compared to the reference plant *A. thaliana*. This agrees with previous work that suggests *B. napus* is a poor reproductive host for aster leafhoppers, while *A. thaliana* is a suitable host (Romero et al. 2020, 2022). Aster leafhoppers rely on suitable host plants for survival and development, but *B. napus* presents challenges due to its chemical and mechanical defences. High glucosinolate levels in *B. napus* are hydrolyzed into toxic compounds upon tissue damage, deterring phloem feeders (Halkier and Gershenzon 2006; Travers-Martin and Müller 2007; Robert et al. 2019). Furthermore, the tetraploid nature of *B. napus* may alter cell wall structure, making stylet penetration more difficult (Corneillie et al. 2019). Similarly, leaf toughness has been negatively correlated with host preference in the leafhopper *Dalbulus maidis* (Bellota et al. 2013), indicating that comparable mechanical barriers in *B. napus* could further contribute to reduced feeding efficiency for aster leafhoppers. These combined defences likely contribute to aster leafhoppers' poor survival and reproductive success on *B. napus*.

Previous research has shown that leafhoppers infected with AYp lay more eggs than uninfected leafhoppers on known host plants like oat (*Avena sativa* L.) and romaine lettuce (*Lactuca sativa* L.) (Beanland et al. 2000). We expected to observe a similar pattern in *B. napus*. However, no such difference was detected. The similarity in nymph emergence (used here as a proxy for reproductive success) between AYp-infected and AYp-uninfected leafhoppers may be attributed to factors influencing overall colony fitness. One potential explanation is age-dependent variation, where older individuals nearing the end of their natural lifespan may have been inadvertently selected for the bioassays, reducing observed fecundity (Romero et al. 2025). Additionally, prolonged inbreeding within the colonies may have contributed to a decrease in reproductive capacity, as suggested by Romero et al. (2024). This decline in fitness may have masked potential differences in fecundity between infected and uninfected leafhoppers, leading to the observed similarities in egg-laying patterns.

Uninfected aster leafhoppers produced significantly more nymphs on AYp-uninfected plants than on AYp-infected plants, indicating that phytoplasma infection of the host plant reduced its suitability for reproduction. The preference-performance hypothesis, also known as the “mother knows best” principle, suggests that reproductive females select host plants that maximize offspring survival (Valladares and Lawton 1991; Johnson et al. 2006; Romero et al. 2022). This leads to variation in host plant selection, with females preferentially ovipositing on plants that enhance offspring development, accelerate growth, or reduce predation risk. As infected plants will likely die, it might be expected that aster leafhoppers will prefer to lay eggs on uninfected plants. In other pathosystems, pathogen-infected insect vectors have been found to have decreased fecundity (Bressan et al. 2005; Vézilier et al. 2012; Arismendi and Carrillo 2015), which is consistent with the findings of this study.

Obligate pathogens like AYp can alter host plant physiology to create more favorable feeding sites, such as prolonging

vegetative growth and inducing sterile flowers, which increase nutrient availability (Bai et al. 2006; Huang et al. 2021). While *B. napus* alone is a poor reproductive host for aster leafhoppers, AYp infection may partially offset its unsuitability. Beanland et al. (2000) found that aster leafhoppers lived longer on AYp-infected plants, likely due to pathogen-induced changes such as increased photosynthate accumulation or improved feeding sites. In our study, infected leafhoppers placed on infected plants produced nymph numbers and developmental outcomes comparable to those of uninfected leafhoppers on infected plants, suggesting that matching infection status between the vector and host plant may mitigate some of the limitations of this otherwise marginal host, consistent with the patterns described by Maramorosch (1958).

5 | Conclusions and Future Directions

While AYp successfully reproduces within *B. napus*, facilitating its spread and symptom severity, several critical aspects of this pathosystem remain unclear. The mechanisms underlying vector-mediated transmission in this system are poorly understood, and studying the effects of AYp-infected *B. napus* on aster leafhopper oviposition and development is particularly challenging due to the plant's unsuitability as a reproductive host. Despite this, aster leafhoppers still feed on *B. napus*, raising the question of why they utilize a host that cannot support their full development. Addressing these gaps requires further research into the ecological and biochemical interactions driving the *B. napus*—*M. quadrilineatus*—*Ca. P. asteris* pathosystem. Future studies should examine the factors influencing leafhopper engagement with *B. napus*, including the role of ploidy level and cell wall structure in reducing vector fitness and the potential for AYp to manipulate plant and vector physiology. Additionally, investigating how aster leafhoppers detect and respond to infected plants—whether through visual, olfactory, or gustatory cues—could provide insights into the potential for pathogen-induced behavioral manipulation. A deeper understanding of these complex interactions will clarify the ecological dynamics of this pathosystem and inform management strategies for vector-borne diseases in agricultural systems.

Author Contributions

Jeremy R. Irvine: conceptualization, investigation, writing – original draft, methodology, validation, writing – review and editing, visualization, formal analysis. **Berenice Romero:** supervision, conceptualization, investigation, funding acquisition, methodology, validation, writing – review and editing, formal analysis. **Sean M. Prager:** supervision, resources, conceptualization, funding acquisition, methodology, validation, writing – review and editing, data curation.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** eea70036-sup-0001-FigureS1.jpg. **Figure S2:** eea70036-sup-0002-FigureS2.jpg. **Table S1:** Specifications for pots, cages, and other materials used in bioassays.