Effects of Phytoplasma Infection on Aster Leafhopper Settling Behavior, Oviposition, and Development

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INTRODUCTION

- Aster Yellows Phytoplasma (AYp) is a mollicute that infects a variety of hosts. Research into the pathosystem involving AYp, insect vectors, and the host plant (**Figure 1**) is needed to understand to what degree infected plants might influence insect behaviour and disease dynamics. In Canada, AYp is mainly transmitted by aster leafhoppers (*Macrosteles quadrilineatus* Forbes) (Hemiptera: Cicadellidae).
- When a plant senses a pathogen, defense pathways are activated, resulting in the production of secondary metabolites which can influence the plants' physiological state. In some instances, the presence of these metabolites can affect the feeding behaviour of herbivorous insect species.
- Previous work has shown that vector preference can change significantly depending on the infection status of the insect vector. This is referred to as the "Conditional Vector Preference", in which uninfected vectors can be expected to prefer infected plants and infected vectors can be expected to prefer an uninfected plant (Roosien et al., 2013).
- The purpose of this research was to examine if plant infection with phytoplasmas can alter aster leafhoppers' settling behavior, oviposition, and development. This is important for understanding this pathosystem and particularly its effects on Canola which is especially susceptible to damage from AYp.

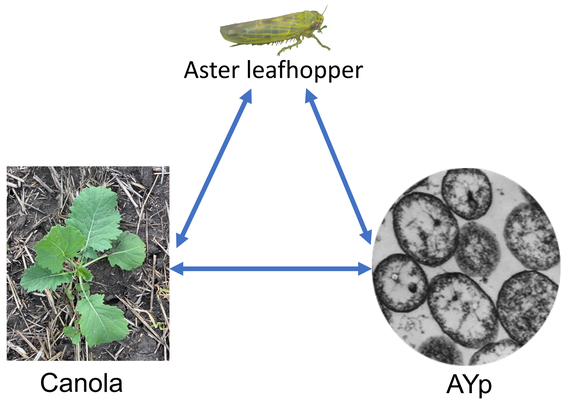

Figure 1: Pathosystem diagram of AYp, canola, and the aster leafhopper

CONCLUSIONS AND FUTURE DIRECTIONS

- While AY-uninfected aster leafhoppers did not exhibit a settling preference when simultaneously offered AY-uninfected and AY-infected canola plants, AY-infected leafhoppers preferred to settle on AY-infected canola plants during the early stages of the infection (**Figure 3**).
- Aster leafhopper oviposition and survivorship were greatly reduced on *B. napus* when compared to *A. thaliana*, even when *B. napus* was infected with AYp (**Table 1**).
- Although AY-infected leafhoppers exhibit a settling preference towards AY-infected plants, this preference is subtle and not likely to lead to increased spread of the phytoplasma.
- Both no-choice and two-choice will be repeated with Cucumber Mosaic Virus and Turnip Mosaic Virus to determine if plant infection with a virus can have similar trends on leafhopper behavior and development.

RESULTS

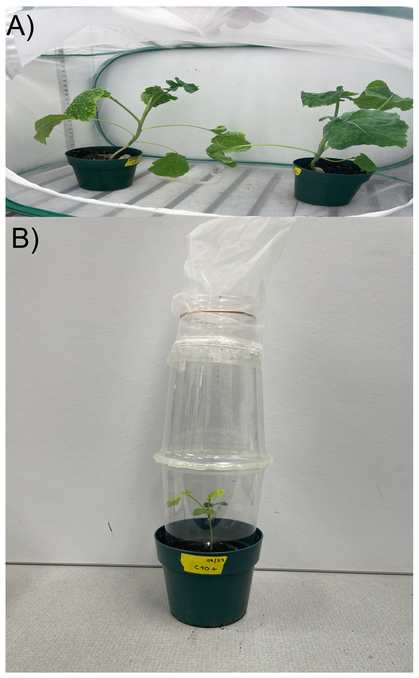

Figure 2: Example of A) two-choice and B) no-choice bioassays. A) Groups of 10 aster leafhoppers were released in the middle of the cage. Each cage contained one AY-uninfected canola and one AY-infected canola plant. B) Three pairs of aster leafhoppers were caged onto plants and allowed to reproduce for one week. Nymphal development was tracked and stage-specific life tables were generated.

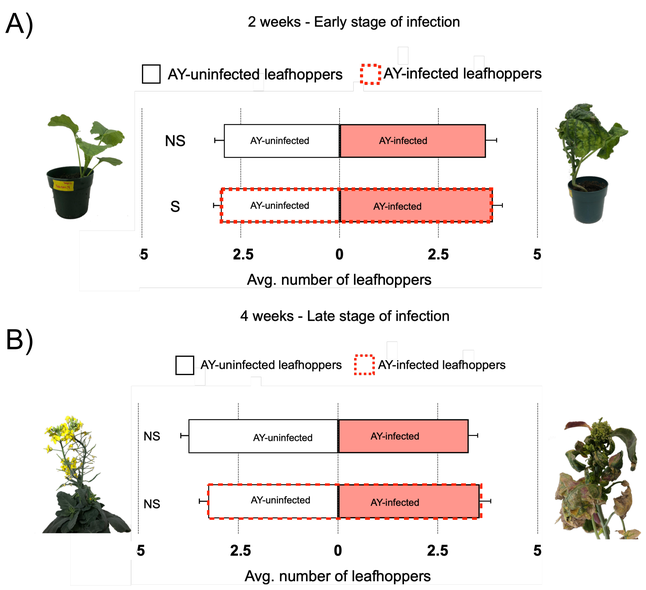

Figure 3: Settling behaviour of AY-uninfected and AY-infected aster leafhoppers in two-choice bioassays at A) two weeks and B) four weeks post-infection. Bars represent the average number of leafhoppers on each plant for each plant combination (Mean ± SEM). Results were evaluated using a PERMANOVA analysis and a significance level (α value) of 0.05 was used. "S" represents a plant combination in which a settling preference was observed, while "NS" indicates that aster leafhoppers distributed similarly on both test plants provided.

- AY-uninfected leafhoppers exhibited no preference for settling on an AY-uninfected or AY-infected canola plant during both observation periods (early and late stage of infection).
- AY-infected leafhoppers preferred to settle on the AY-infected canola during the early stage of plant infection but showed no preference for either plant in later stages of infection.

Table 1: Stage-specific life table of AY-uninfected and AY-infected aster leafhoppers 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. Stages included five nymphal instars as well as adult. The number of individuals alive at the beginning, the number of individuals dying at each stage, and the survivorship are provided. Number of Instar I was assumed to be the number of eggs and all eggs successfully hatched.

Age Interval, x	Number of individuals alive at beginning of x, l_x					Number of individuals dying during x, d_x					d_x as percentage of l_x, $100q_x$				
	Reference	AY-Positive canola AY-Negative Leafhoppers	AY-Positive canola AY-Positive Leafhoppers	AY-Negative canola AY-Negative Leafhoppers	AY-Negative canola AY-Positive Leafhoppers	Reference	AY-Positive canola AY-Negative Leafhoppers	AY-Positive canola AY-Positive Leafhoppers	AY-Negative canola AY-Negative Leafhoppers	AY-Negative canola AY-Positive Leafhoppers	Reference	AY-Positive canola AY-Negative Leafhoppers	AY-Positive canola AY-Positive Leafhoppers	AY-Negative canola AY-Negative Leafhoppers	AY-Negative canola AY-Positive Leafhoppers
Instar I	358	30	28	55	5	0	4	1	18	5	0.00	13.33	3.57	32.73	100.00
Instar II	358	26	27	37	-	16	26	27	37	-	4.47	100.00	100.00	100.00	-
Instar III	342	-	-	-	-	5	-	-	-	-	1.46	-	-	-	-
Instar IV	337	-	-	-	-	15	-	-	-	-	4.45	-	-	-	-
Instar V	322	-	-	-	-	17	-	-	-	-	5.28	-	-	-	-
Adult	305	-	-	-	-	-	-	-	-	-	0.00	-	-	-	-

- Aster leafhopper oviposition was lower in canola plants compared to *A. thaliana*. 1
- In AY-infected plants, oviposition was similar between AY-uninfected and AY-infected aster leafhoppers. 2
- In AY-uninfected plants, oviposition was higher when groups of AY-uninfected insects were used. The number of individuals was greatly reduced with the AY-infected leafhopper treatment. 3
- While aster leafhoppers were able to complete their development on *A. thaliana*, insects were not able to develop beyond Instars I and II on canola. 4

MATERIALS AND METHODS

AY-uninfected aster leafhoppers (*Macrosteles quadrilineatus* Forbes) were reared on barley (*Hordeum vulgare* var. CDC Copeland) and maintained under the following conditions: 21°C during the day (18 hrs) and 18°C during the night.
AY-infected aster leafhoppers were also reared on barley, but the insect colony was kept at a constant 24°C with periwinkle (*Catharanthus roseus*) plants infected with Aster Yellows phytoplasma (AYp) added to facilitate pathogen acquisition.

The following plant species were used: canola (*Brassica napus* var. AC Excel) and *Arabidopsis thaliana* (WT Col-0 Ecotype). While one canola plant was used per pot (10.16cmx8.89cm; 0.460L), there were five to seven *A. thaliana* plants per pot (10.16cmx8.89cm; 0.460L). Plants were watered every three days, with the addition of 20-20-20 water soluble fertilizer (20% N, 20% P_2O_5, 20% K_2O), and growing conditions were similar to those described for AY-uninfected aster leafhoppers. Canola plants were subjected to two treatments: AY-uninfected and AY-infected. To obtain AY-infected canola plants, individual plants were kept at constant 24°C and exposed to six infected aster leafhoppers (three females and three males) for 10 days (**Figure 2**). After this period, insects were removed, and plants were kept under the same conditions for 14 additional days.
AYp status of insects, plants, and colonies was confirmed via qPCR as described by Romero et al. (2022).

For the two-choice bioassay, procedures were similar to those described by Romero et al. (2022). Briefly, a group of five males and five females of AY-uninfected leafhoppers were added to the middle of a choice cage (**Figure 2A**) and allowed to acclimate for 24 hours. Each choice cage included an AY-infected canola plant as well as an AY-uninfected canola plant. Following the acclimation period, leafhopper position was recorded daily for 96 hours. Positions were defined as (1) on the uninfected plant, (2) on the infected plant, or (3) off the plants entirely. Settling behavior was evaluated at 2 weeks (early stage of AYp infection) and 4 weeks (later stages of AYp infection) following the onset of the infection. This was repeated using AY-infected aster leafhoppers and replicated 10 times in each case.

For the no-choice bioassays, procedures were similar to those described by Romero et al. (2020) and *A. thaliana* was used as a reference. Briefly, groups of AY-uninfected males and females (three and three, respectively) were caged onto a plant and allowed to reproduce for one week, after which insects were removed and plants were inspected for the presence of nymphs (**Figure 3**). Following this period, plants were monitored until first instar nymphs were observed and regular observations were conducted to track nymphal development. This was repeated using AY-infected aster leafhoppers and replicated 10 times in each case. Stage-specific life tables were generated following a similar methodology to of Price et al. (2011) (**Table 1**).

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