Plant Pathogen effects on Hemipteran Settling Behavior

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INTRODUCTION

- When a plant senses a pathogen, secondary metabolites are produced, affecting the plants' physiological state. Moreover, the presence of these metabolites can affect the feeding behaviour of herbivorous insect species.
- Aster Yellows Phytoplasma (AYp) is a mollicute that infects a very wide 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).
- 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 affect aster leafhoppers' settling behavior, oviposition, and development.

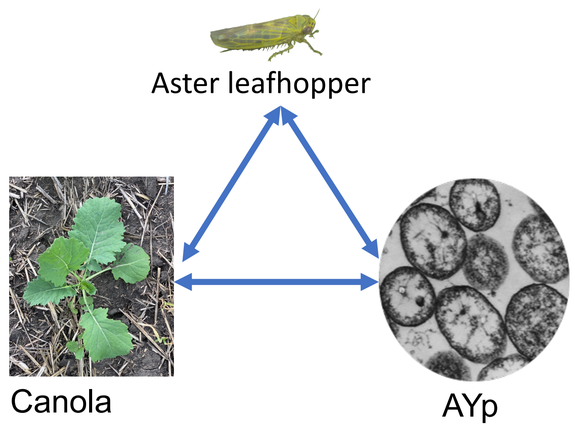

Figure 1: Pathosystem diagram of AYp, canola, and the aster leafhopper

CONCLUSIONS AND FUTURE DIRECTIONS

- AYp-infected leafhoppers preferred to settle on AYp-infected plants (Figure 2A).
- AYp-infected leafhoppers oviposition was highly reduced when compared to AYp-uninfected insects on identical plants (Figure 3B).
- Leafhopper developmental was similar in both *A. thaliana* and AYp-uninfected *B. napus* (Figure 3A-C).
- Survivorship of leafhoppers on uninfected canola did not exceed 23 days, and nymphs were not able to develop further than second instar (Figure 3A-B).
- No-choice using AYp-infected plants are currently on-going.
- 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

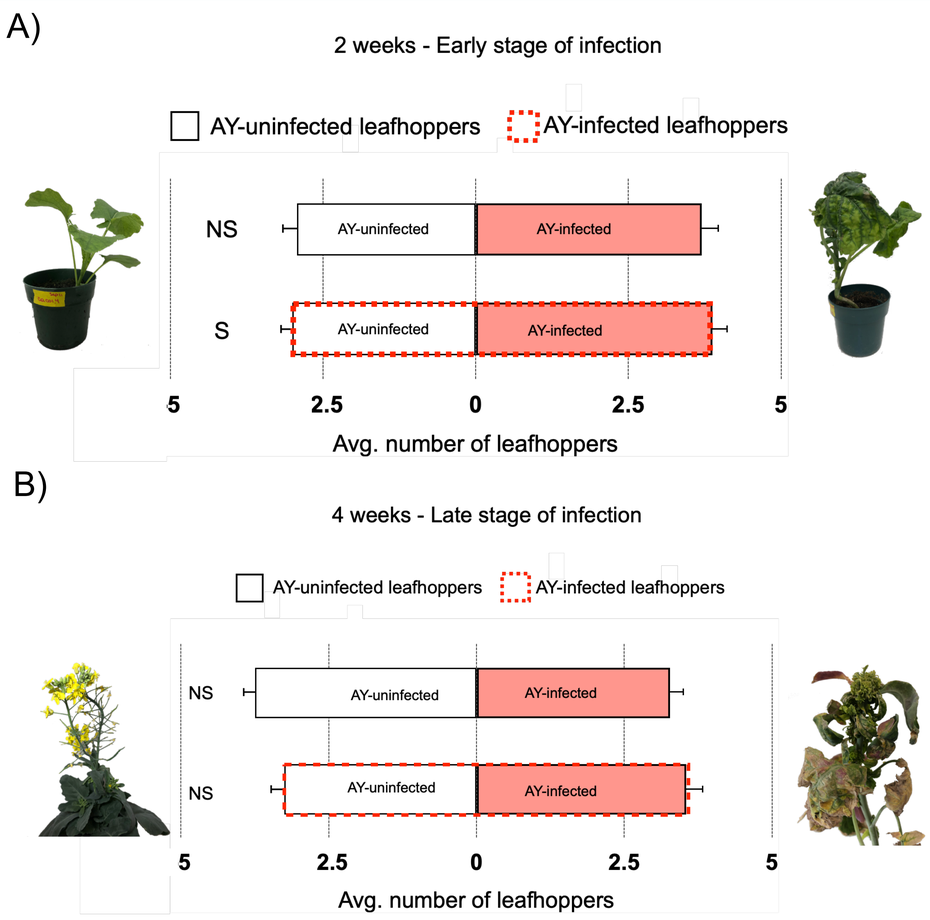

Figure 2: Settling behaviour of AY-uninfected and AY-infected aster leafhoppers in two-choice bioassays at A) two weeks and B) four weeks post-infection. Bars represent the average number of leafhoppers on each plant for each plant combination (Mean ± SEM). Results were evaluated using a PERMANOVA analysis 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.

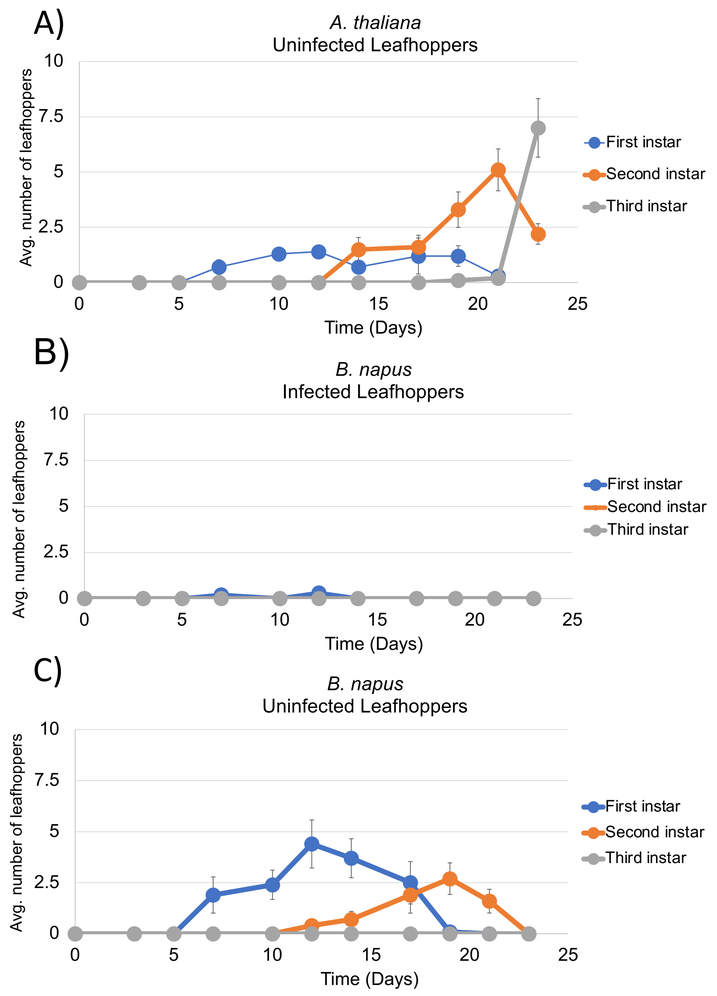

Figure 3: Preliminary results of no-choice bioassays tracking nymphal development for a period of 23 days in three different treatments:
A) Offspring from AY-uninfected leafhoppers reared on *A. thaliana* (reference), B) Offspring from AY-infected leafhoppers reared on *B. napus*, and C) Offspring from AY-uninfected leafhoppers reared on *B. napus.*

Figure 4: Cup cage used for infecting plants and no-choice bioassays.

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℃ during the day (18 hrs) and 18℃ during the night. AY-infected aster leafhoppers reared as previously described, with a few modifications. The insect colony was kept at constant 24℃ and periwinkle (*Catharanthus roseus*) plants infected with Aster Yellows phytoplasma (AYp) were 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℃ and exposed to six infected aster leafhoppers (three females and three males) for 10 days (Figure 4). After this period, insects were removed, and plants were kept under the same conditions for 14 additional days.

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 5) 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 4). 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.

Figure 5: Two-choice bioassay cage.

References

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