Government of Saskatchewan
Ministry of Agriculture

ADF Project Final Report Format

1. **Investigating the role of plant hosts in the outbreaks of the aster leafhopper vectored Aster Yellows (AY)**
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4. **Abstract/ Summary**: *An outline on overall project objectives, methods, key findings and conclusions for use in publications and in the Ministry database (**Maximum of 500 words** or one* page).

Aster leafhoppers (*Macrosteles quadrilineatus* Forbes) (Hemiptera: Cicadellidae) are the main vector of Aster Yellows Phytoplasma (AYp) (*Candidatus* Phytoplasma asteris) in the Canadian Prairies. AYp causes Aster Yellows (AY) disease in over 300 plant species, but is especially problematic in canola. These insects are migratory and almost every spring, arrive in Saskatchewan. However, we need to know what they do when they arrive because typically, their arrival date precedes suitable crops in cultivated fields. We need to increase our knowledge about their food and reproductive host preferences on non-crop plants and compare plant preferences to discover the likely plants that sustain them prior to moving into crops. Also, the disease dynamics of AY in Saskatchewan is poorly understood. For example, the source of the AYp infecting leafhoppers each year is unknown, but AYp could originate from plants in the source areas of the leafhopper migration, which would be the case if the aster leafhoppers migrated with a high percentage of individuals infected. The alternative hypothesis is that the aster leafhoppers migrate into Saskatchewan and acquire the AYp infection from local plants. A combination of these hypotheses is also possible. However, little is known about the host range of Aster leafhoppers or their host-choice selection behavior in this geographical region that might drive leafhopper movement among plants. Several crop and noncrop species commonly found on the Canadian Prairies were evaluated as food and reproductive hosts for Aster leafhoppers through no-choice bioassays. To study possible effects of pathogen infection on host choice, AY-uninfected and AY-infected insects were used. Cereals and some noncrop plants like fleabane were suitable reproductive hosts for Aster leafhoppers, with higher numbers of offspring observed in treatments using both AY-uninfected and AY-infected insects, suggesting an egg-laying preference on these plant species. Development was similar across the different plant species, except for canola and sowthistle, where growth indexes were lower. Sex-ratios of Aster leafhopper adults did not differ among the plant species or with respect to AY infection. Potential fecundity differed across plant species and was affected by the AY infection status of the insect. These findings have implications for AY epidemiology and suggest that while cereals can be suitable host plants for Aster leafhopper oviposition and development, some noncrop species could act as alternate hosts for leafhoppers that migrate into the Canadian Prairies before emergence of cereal and canola crops. Host choice selection behaviour accounting for both feeding and reproductive choices by Aster leafhoppers was evaluated through two-choice bioassays, using domesticated and wild plants species commonly found in the Canadian Prairies. Leaf tissues from these plants were collected and stained to quantify the number of stylet sheaths and eggs using AY-infected and uninfected leafhoppers. When two domesticated or wild plant species were presented together, similar numbers of uninfected Aster leafhoppers were observed on both plant species in most combinations. In domesticated vs wild plant bioassays, AY-uninfected Aster leafhoppers

preferred to settle on the domesticated species. There was little to no association between settling preferences and stylet sheath and egg counts. These findings provide a better understanding of AY epidemiology and suggest that after domesticated species germination, leafhoppers could move from nearby wild plants into the preferred cereals to settle on them, influencing the risk of AYp infection in some of these species. Through field surveys for AY symptomatic and asymptomatic biennial and perennial non-crop plants we searched for and found reservoir plants that are the likely source of AYp infections acquired by migrating leafhoppers. We also captured and tested early-season migrant leafhoppers as they arrived in Saskatchewan to determine how many of them were infected with AYp when they migrated. Surprisingly, the migrant generation had very low levels of infection, while several common plant species were harbouring AYp infections in the early spring and summer (alfalfa) and are likely acting as reservoirs for AYp in Western Canada.

5. **Extension Messages:** *key outcomes and their importance for producers/processors and the relevant industry sector (**3-5 bullet points** <u>in lay language</u>).*
    - When given a choice between a crop (including canola and three cereals) and a non-crop (weedy plants common in field margins), aster leafhoppers preferred crop plants over weedy plants and this preference likely drives their movement into crops from field margins.
    - AYp infection status of aster leafhoppers only influenced one of the studied preferences, indicating that infection with the phytoplasma likely does not have a strong effect on the behaviour or reproductive potential of the vector insect on the tested plants. The only preference shown was for AY-uninfected leafhoppers to preferentially settle on noncrop hosts, which may indicate a preference for plants where AYp infection can be acquired.
    - Canola and sowthistle were poor reproductive hosts for aster leafhoppers, yet sowthistle was one of the plants identified as an AYp reservoir. Cereal plants and fleabane were very suitable for aster leafhopper oviposition and nymphal development. This suggests that prior to crop emergence, a plant species such as fleabane could act as a suitable reproductive and food choice for aster leafhoppers. However, in the presence of an additional plant species, fleabane and other weedy species are less preferred (for settling, probing, and ovipositing), indicating that little reproduction is happening on the dicotyledonous weeds in the field margins.
    - Each year, the percentage of migrant leafhoppers arriving infected with AYp was very low, but several perennial and biennial plants harboured AYp infections in the spring and summer and are likely acting as reservoir plants for AYp infection of aster leafhoppers (alfalfa, sowthistle, stinkweed, dandelion).
    - AYp infection studies of several plant species were conducted (*Arabidopsis thaliana*, barley, canola, dandelion, wheat, and sowthistle) and descriptions of resulting symptoms and pictures of non-symptomatic and AY-symptomatic plants are included for educational purposes.
    - Several of the potential reservoir plants (such as dandelion and sowthistle) were tested in no-choice and two-choice assays. The presence of stylet sheaths and the settling of leafhoppers on these plant species strongly suggest that dandelion and sowthistle are part of the AY disease cycle.

6. **Introduction:** *Brief project background and rationale (**Maximum of 1500 words or 1.5-3 pages**).*

Phytoplasmas are prokaryotic microorganisms associated with plant diseases in field crops, orchards, vegetables, and ornamental crops (Marcone et al. 1997; Weintraub & Beanland 2006; Marcone 2010). Within the plant, these microorganisms are mostly restricted to the phloem tissues, from where they can be acquired by leafhoppers, planthoppers, and psyllids during feeding (Weintraub & Beanland 2006; Weintraub & Wilson 2010; Olivier et al. 2009; Weintraub et al. 2019). Following phytoplasma infection of a plant, these microorganisms produce a series of compounds known as “effectors”, which target different molecules in the host and affect the development of different plant structures such as floral buds and pods (Sugio et al. 2011, MacLean et al. 2011, Sugio et al. 2014, Bertaccini et al. 2019). Symptoms of phytoplasma infection include abnormal presence of green pigmentation (virescence), development of leafy structures (phyllody) that cause malformation of floral structures, purpling or yellowing of plant tissue, and yet these symptoms can differ between cultivars and plant species. In barley, for example, phytoplasmas can lead to changes in the pigmentation of leaves (yellow, red, and/or purple) and changes to the size of grain heads, and these symptoms are similar to those produced by Barley Yellow Dwarf Virus (Alberta Agriculture and Forestry 2014). In canola, phytoplasma-infected plants may be either asymptomatic or show excessive branching and stunting (Olivier et al. 2010; Olivier et al. 2014). Symptom expression and disease development can be affected by temperature, as observed in previous studies (Chung et al. 2015, Bahar et al. 2018), yet limited studies of this kind have been conducted for phytoplasma diseases.

Aster Yellows (AY) disease is caused by at least fifteen distinct subgroups of phytoplasmas (Lee et al. 2004). Three of these subgroups can be transmitted by the aster (six-spotted) leafhopper *Macrosteles quadrilineatus* (Forbes), the main vector of AY in the Canadian Prairies (Olivier et al. 2009). While AY can greatly affect field crops like canola during outbreak years (2001, 2007, and 2012), disease incidence in canola is low in most years (<0.1%) (Alberta Agriculture and Forestry and Saskatchewan Ministry of Agriculture yearly surveys). *M. quadrilineatus*, is a migratory species that arrives on wind currents originating in the Southern United States (Nichiporik 1965; Olivier et al. 2009). Similarly to other migratory insect species, Aster leafhoppers are polyphagous and feed on multiple families of plants (Olivier et al. 2009; Weintraub & Beanland 2006), with a very wide range of plant hosts from different taxonomic groups as part of their diet. However, several aspects about their biology in the Canadian Prairies remain largely unknown, including their host range and host selection behavior in this region, and whether these can be affected by infection with phytoplasmas. Furthermore, it is unclear if plant species on which leafhoppers prefer to reproduce and develop on can be infected with AY and become AY reservoirs.

Unlike other plant pathogens such as fungi, control of AY and other phytoplasma diseases is restricted to insecticide application targeting vectors and proper plant sanitation (Alberta Agriculture and Forestry 2014). There are currently no chemicals that can specifically target these microorganisms in crops and no commercial canola varieties with resistance to AY, yet differences in susceptibility to this disease have been reported for *Brassica rapa* and *B. napus* (Alberta Agriculture and Forestry 2014). As previously mentioned, symptom expression in some plant species infected with phytoplasmas can be affected by environmental factors or can be mistaken for abiotic stress or a viral disease, leading to a less accurate detection of diseases caused by phytoplasmas during the early stages of infection. Several polymerase chain reaction (PCR) methodologies have been developed for early detection and quantification of phytoplasma in infected plants, and a direct comparison of the analytical sensitivity and specificity of these techniques has been accomplished in the sister-project to this one (Pusz-Bochenska et al. 2020) but the relationship between AY titer and symptom expression in various plant species still needs to be examined.

In most springs and early summers, Aster leafhoppers migrate into the Canadian Prairies, with their trajectory and arrival determined by wind currents (Nichiporik 1965; Hoy et al. 1992; Olivier et al. 2009). The constant changes of weather parameters such as air temperature, atmospheric pressure, and precipitation greatly influence the nature of the migratory movement of Aster leafhoppers, adding some difficulties to the prediction of the time and geographical location of the arrival of these insects (Frost et al. 2013a). Previous work conducted by Frost et al. (2013b) examined the relationship between Aster leafhopper abundance and infectivity and time and location of sampling, observing that geographical location did not contribute greatly to the variation in insect abundance and infectivity. The majority of the variation in Aster leafhopper abundance and infectivity could be associated with temporal factors and complex interactions among the explanatory variables under study, suggesting that weather parameters and wind patterns occurring on a smaller geographical scale could have introduced additional individuals on a weekly basis and contributed to the great variation in abundance that was observed over time. Moreover, the geographical and temporal variables under study accounted for less than 50% of the variation in Aster leafhopper infectivity suggesting that collected insects could have been infected with more than one phytoplasma substrain, affecting insect transmission of this pathogen and therefore leafhopper infectivity, and that symptom expression in plants could have been misidentified, leading to an inaccurate detection of this disease in the field (Frost et al. 2013b). Furthermore, climate models have predicted an increased frequency and speed of winds in the Prairies (Cheng et al. 2014; Vavrus et al. 2017), which could potentially introduce high numbers of infective Aster leafhoppers into the region and increase the risk of AY infection in multiple susceptible crops.

Aster leafhopper (*Macrosteles quadrilineatus* Forbes) (Hemiptera: Cicadellidae) is the main vector of Aster Yellows Phytoplasma(AYp) (*Candidatus* Phytoplasma asteris) in the Canadian Prairies, AYp is the causal agent of Aster Yellows (AY) disease in over 300 plant species including cereals and oilseeds. These insects are migratory and almost every spring, migrate into Saskatchewan. However, we lack a good understanding about what these insects do when they arrive in Saskatchewan. We know that they infect canola with Aster Yellows, which usually causes complete yield loss in a plant and we know that they reproduce in Saskatchewan cereal crops and likely several other non-cereal crops as well. We don't know much about their food and reproductive host preferences on non-crop plants or what plant species they prefer over others. Additionally, the disease dynamics of Aster Yellows in Saskatchewan is poorly understood. For example, the source of the AYp iin each year is unknown but could originate from plants in the source areas of the leafhopper migration, which would be the case if the aster leafhoppers migrated with a high percentage of infectivity. The alternative hypothesis is that the aster leafhoppers migrate into Saskatchewan and acquire the AYp infection from local plants. A combination of these hypotheses is also possible. However, little is known about the host range of Aster leafhoppers or their host-choice selection behavior in this geographical region that might drive leafhopper movement among plants. By examining Aster leafhoppers´ host choice selection behavior, oviposition, and development on various plant species occurring in the Canadian Prairies, including perennial weeds, and by determining whether some of these plant species can be AY hosts and establish what the relationship between AYp titer and symptom expression is, this project will contribute to a better understanding of AY dynamics in the region.

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7. Objectives and the progress towards meeting each objective.

Objectives **(Please list the original objectives and/or revised objectives if Ministry-approved revisions have been made to original objectives. A justification is needed for any deviation from original objectives).**	**Status** **(e.g. completed/not completed)**
a) Establish the food and reproductive preferences of Aster leafhoppers in selected AY susceptible and AY tolerant wheat, canola and common weeds	**Completed**
b) Determine and quantify the ability of Aster leafhoppers to vector Aster Yellows Phytoplasma among selected common crop plants and weeds	**Completed**
c) Determine the ability of selected common weeds to serve as Aster yellow phytoplasma reservoirs	**Completed**
d) Survey of common weeds for the presence of leafhoppers and/or Aster Yellows phytoplasma	**Completed**

8. Methodology:

Laboratory bioassays

The following plants species (varieties) were used in experiments with Aster Yellows phytoplasma (AY)-infected and AY-uninfected leafhoppers: bread wheat (AAC Brandon), oat (CS Candem), barley (CDC Copeland), canola (AC Excel), spiny annual sow thistle (*Sonchus asper* Linnaeus), dandelion (*Taraxacum officinale* Weber), fleabane (*Erigeron annuus* Linnaeus), marigold (*Tagetes* sp.), and *Arabidopsis thaliana* (Columbia Ecotype). Plants were maintained in growth chambers within the University of Saskatchewan´s Agriculture Building under an 18-hour photoperiod, with temperatures at 21ºC during the day and 17ºC during the night. Pots were watered every three days, with the addition of a water-soluble fertilizer to meet minimum nutrient requirements. Leafhoppers (*Macrosteles quadrilineatus* Forbes) were originally sourced from colonies maintained at AAFC Saskatoon, and reared on Madagascar periwinkle as AY reservoir plants and barley as food and reproductive hosts. 30-day-old plants were used in all cases.

Two types of bioassays were conducted (no-choice and two-choice bioassays). For the **no-choice assays (Obj. a)**, three pairs of either AY-infected or AY-uninfected leafhoppers were caged onto a plant and allowed to reproduce for one week, after which adults were removed and nymphs were classified by instar and counted until all individuals became adults. The cage was constructed from two plastic cups and an organza bag. The bottoms of the plastic cups were removed and the cups were glued to one another. These cages were placed around an individual plant, making sure that the structure was inserted into the soil and did not move. An organza bag was then secured around the open top end of the glued cups with a rubber band to allow air flow (Figure 11 in *Appendices*). Growth indexes, defined as the proportion of individuals that survive to adulthood, were calculated for each plant species, as described by Romero et al. (2020). After completing the no-choice assays, the number of nymph and adult insects on each plant were counted and the sex of each adult was determined. Females were then dissected to determine how many eggs they contained. The ratio of males to females were calculated for each experimental unit. No-choice bioassays were replicated ten times. Following the no-choice assay, females were collected and dissected to determine the number of eggs in their ovarioles. Each female was placed in the center of a dissecting pad, covered with one droplet of Ringer's solution, and the content of its abdomen exposed using a dissecting needle. Following these steps, the number of eggs were counted (Figure 12 in *Appendices*). For this experiment, the sample size depended on the number of females obtained, but no more than 100 females per plant were dissected.

For the **two-choice bioassays (Obj. a)**, ten pairs of Aster leafhopper adults were released inside a large rectangular mesh cage containing two test plants at opposite ends of the cage. Following an acclimation period of 24 hrs, the location of all insects (on plant 1, plant 2 or off the plant) was monitored daily for a total of 96 hrs. (Figure 13 in *Administrative and Other Aspects*). *A. thaliana* was excluded from these bioassays and ten replicates were conducted for each plant combination. A subset of plant species was selected to conduct two-choice bioassays with AY-infected Aster leafhoppers (barley, canola, wheat, and dandelion), following similar procedures to those previously described. In addition to this, two-choice bioassays were repeated with AY-uninfected Aster leafhoppers reared entirely on fleabane, to test for possible effects of the rearing host on host-choice by leafhoppers.

Following completion of the two-choice bioassays **(Obj. a)**, plants were retained for further analysis. Leaves were removed and stained to count the number of stylet sheaths and eggs (Figure 14 in *Appendices*). Stylet sheaths are structures produced by piercing-sucking insects during feeding, which surround their mouthparts and provide mechanical stability and lubrication (Morgvjan et al. 2013, Will and Vilcinskas 2015). These stylet sheaths can be used as a proxy for feeding activity. For the staining process, leaves were treated as per the methods of Backus et al. (1988). These assays were replicated ten times.

Disease development (Obj. b) and c)) was evaluated on different plant species (*A. thaliana*, barley, canola, dandelion, sowthistle, and wheat) by caging ten adult Aster leafhoppers (AY-infected) for 1 week as above. After the exposure period, insects were killed with insecticide. Plants were then maintained in growth chambers at 24ºC until AY symptoms were expressed. During this period, leaf samples were periodically taken at regular intervals (2, 4, and 5 weeks following the infection period) to test for AY titer and infection using qPCR based methods. These assays were replicated ten times.

For **field surveys (Obj. d)**, sampling occurred during the spring season of each project year and the extended season of 2021. The number of sites varied across sampling years: In 2018, **plant surveys** occurred at the research farm sites in fields and field margins and any plants that suspected of having aster yellows symptoms were identified and tested. In 2019 and spring 2020, the focus was on alfalfa as reservoirs and in this case, leaf, shoot, root and flowers were tested for the presence of AYp. Spring surveys of 2020 and 2021 had the purpose of identifying potential weedy reservoir plants for the AYp. This survey sampled asymptomatic plants as well as any that appeared "yellowed". Sampling during spring 2020 was hampered by the Covid-19 restrictions on travel within AAFC, limiting the sites to the AAFC Saskatoon Farms (n=10 plants in a sample collected with 3 plants per sample tested). In late summer 2020, alfalfa samples were collected from 14 sites (10 plants/site) and noncrop plants exhibiting symptoms were surveyed, when the AAFC weed survey was allowed to continue. After consultation of the literature, five to six perennial plant species previously described as AYp hosts (perennial or biennial) and commonly found in field margins in Saskatchewan were selected for sampling as part of the plant surveys. Other plants that were symptomatic and found in research fields or on field margins were additionally tested when found. Plants were surveyed for symptoms of phytoplasma infection in the spring and random samples of any symptomatic and ten asymptomatic plants were taken (10 plants from each of the five or six species per site). Not all plant species were present at each site. Plants were visually inspected for the presence of leafhoppers before sampling and sweep sampling targeted areas where other plants of these species were growing. Margins of leaves of the portion of plants were visually surveyed for the presence of aster leafhopper eggs using a hand lens. For the **insect samples**, 38 cm diameter sweep nets were used to collect insect samples from the field margins (ditches) next to canola and wheat fields. Nets were swept in 180 degree arc 50-times through the prevailing canopy of the field margins which was typically dominated by brome grass, in a single transect that ran parallel to the field edge. Presence of leafhoppers was determined by examining the insect samples collected under a microscope after freezing the sample bag at -20 ºC. Any aster leafhoppers that were caught were then screened for the presence of AYp with PCR. Most samples were tested with 16S ribosomal primers using nested PCR (samples from 2018 and 2019). However, different methods including the use of the *cpn60* gene, qPCR and LAMP methods were also applied to some samples in order to determine which one is most efficient for early detection of AY. The LAMP method using the *cpn60* gene target was used once it was validated and detected the presence of Aster Yellows phytoplasma at an order of magnitude lower than the "gold standard" 16S nested PCR and took less time to complete (Pusz-Bochenska et al. 2020). 16S PCR was also used in most cases to validate positive LAMP results, but also to sequence the 16S region to ensure that it was the phytoplasma that caused Aster Yellows and to determine which strain of AYp was present in plant samples. More detailed methods for each project year are presented below in the results, including details on surveys for plant reservoirs of AYp.

9. Results and discussion:

No-choice bioassays: Growth indexes (GIs)

Growth indexes (GIs), are defined as the proportion of individuals that survive on a plant and become adults. Based on the no-choice bioassays, GIs were calculated for the various plant species and leafhopper infection status (AY-uninfected or AY-infected) combinations. Results were analyzed with a Generalized Linear Mixed-Effects Model (GLMM), with a negative binomial distribution to account for overdispersion and with 'plant species', 'insect condition', and their interaction as fixed effects (**Figure 1**). No significant effect of insect condition was found (X^2= 1.15, df = 1, P = 0.99), but there were differences among the plant species (X^2= 19.88, df = 8, P = 0.01). As for the interaction term, no significant effect was found (X^2= 1.67, df = 8, P = 0.99). Post-hoc comparisons indicated that: a) GIs are similar between uninfected and AY-infected leafhoppers; b) canola and sowthistle are unsuitable reproductive hosts compared to barley, having lower GIs; and c) development of Aster leafhopper nymphs reared on *Arabidopsis*, barley, dandelion, fleabane, marigold, oat, and wheat was similar.

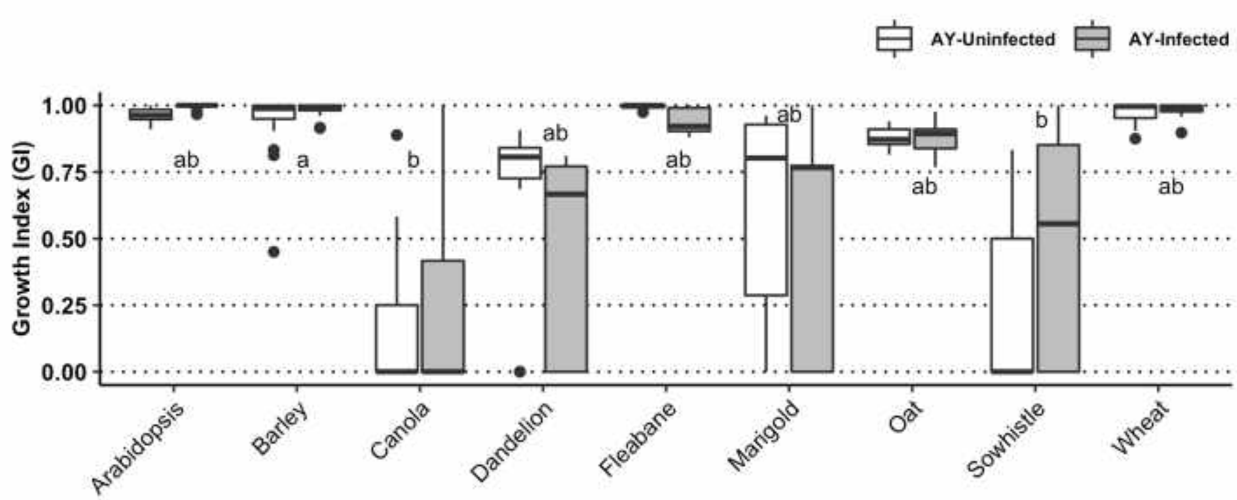

Figure 1: Quantification of insect growth on different plant species. Growth indexes (GIs), defined as the proportion of individuals that survive to adult, are calculated for each plant species, as described by Prager et al. (2014). Mean and standard errors (SEM) are presented. White bars represent results with AY-uninfected-parental generations, while grey bars represent results with AY-infected ones. Different letters indicate statistically significant differences in the GI observed in each treatment (GLMM followed by Tukey's test with adjustment for multiple comparisons, with an α-value of 0.05).

No-choice bioassays: number of individuals, female-to-male proportion, and individual female egg load

In addition to calculating the GIs, the number of offspring (**Figure 2A**), which was further separated into the number of nymphs (**Figure 2B**) and adults (**Figure 2C**); the female-to-male ratio (**Figure 3**); and the individual female egg load (the number of eggs within a female's ovaries) (**Figure 4**) were also examined. Similarly to GIs, these variables were analyzed with a GLMM, with a negative binomial distribution to account for overdispersion and with 'plant species', 'insect condition' (AY+ or AY- leafhoppers), and their interaction as fixed effects.

When examining the number of offspring (**Figure 2A**), we observed a significant effect of the insect condition (X^2= 4.49, df = 1, P = 0.03), a significant effect of the plant species (X^2= 332.97, df = 8, P < 0.001), and a significant two-way interaction (X^2= 26.38, df = 8, P < 0.001). These results suggest that different plant species are not equally preferred for oviposition and that in some cases, oviposition behavior may also be affected by whether an insect is infected with AY or not. In *A. thaliana*, for example, the difference in the number of offspring between the AY-uninfected and the AY-infected treatments suggests that AY-uninfected Aster leafhoppers laid more eggs than AY-infected ones (**Figure 2A**). However, in other plant species such as barley, dandelion, fleabane, oat, and wheat, similar numbers of offspring were observed when comparing AY-uninfected and AY-infected treatments (**Figure 2A**). Overall, more offspring were observed on barley, fleabane, oat, and wheat, suggesting that these plant species are more suitable hosts for leafhopper oviposition. Unlike the aforementioned plant species, canola and sowthistle were less preferred for oviposition, as almost no offspring were observed on these plants. These findings support previous ones (**Figure 1**) and strongly suggest that canola and sowthistle are unsuitable hosts for leafhopper oviposition and nymph development.

Offspring numbers were grouped as nymphs (**Figure 2B**) and adults (**Figure 2C**) and analyzed separately. When examining the number of nymphs, we observed a significant effect of the insect condition (X^2=6.29, df = 1, P = 0.01), a significant effect of the plant species (X^2=96.24, df = 8, P < 0.001), and a significant two-way interaction (X^2=33.12, df = 8, P < 0.001). In *A. thaliana*, more nymphs were observed in the AY-uninfected treatment when compared to the AY-infected one (**Figure 2B**). In other plant species, similar numbers of nymphs were found when comparing between AY-uninfected and AY-infected treatments. Overall, nymph counts in barley, dandelion, fleabane, marigold, oat, and wheat were similar and higher than those in canola and sowthistle (**Figure 2B**). We found no significant effect of the insect condition on the number of adults (AY-uninfected or AY-infected; X^2= 2.19, df = 1, P = 0.14), but there was a significant effect of the plant species (X^2= 332.81, df = 8, P < 0.001) and a significant two-way interaction between these factors (X^2= 17.47, df = 8, P = 0.02). Barley, oat, fleabane, and wheat had the most adults regardless of infection status, while canola had the fewest adults (**Figure 2C**).

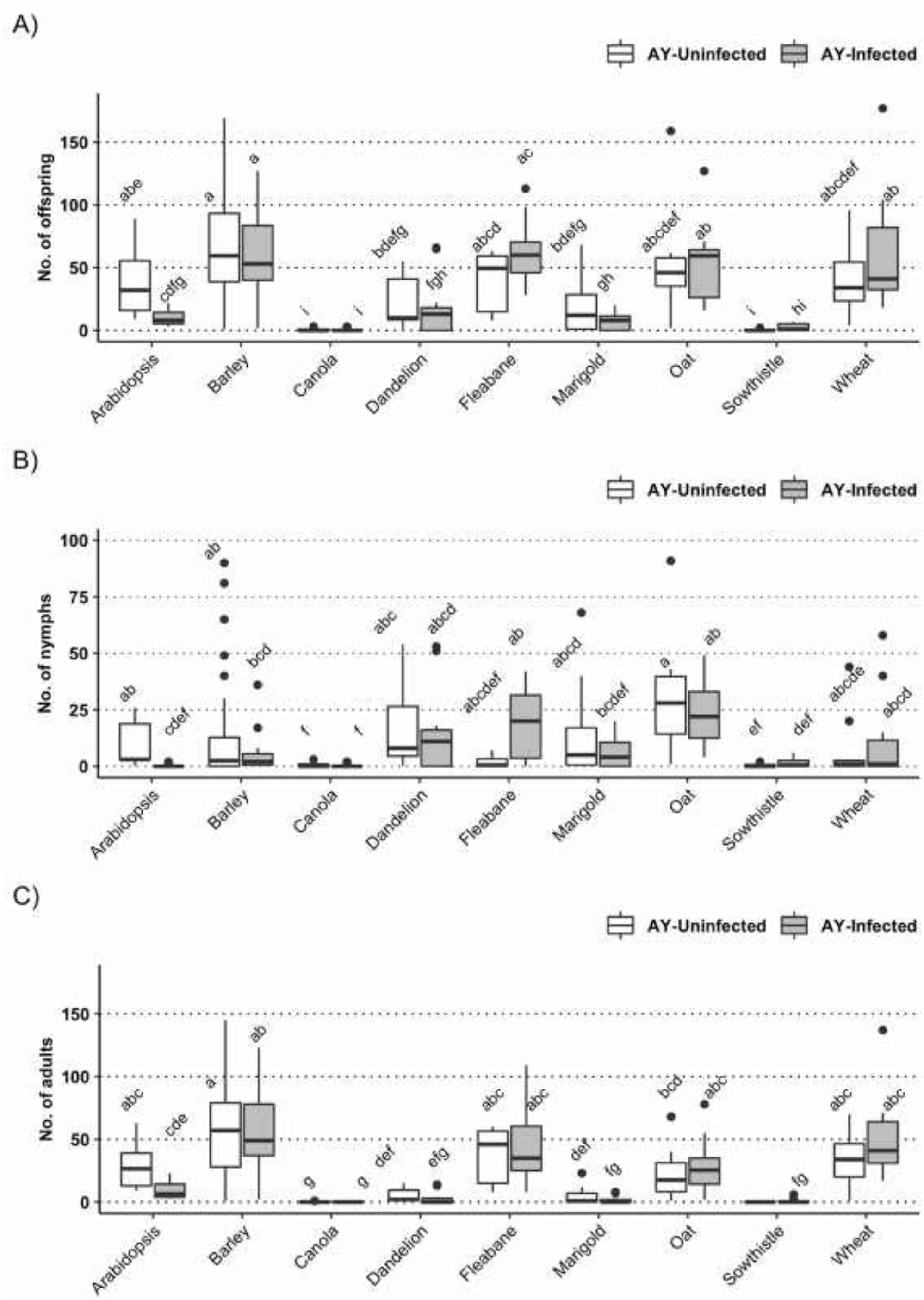

Figure 2: The median, 25th and 75th quartiles for the number of aster leafhopper offspring (A) for each plant species and aster yellows (AY) infection status (AY-uninfected or AY-infected) combination. For additional analyses, offspring numbers were separated into nymphs (B) and adults (C). White boxes represent bioassays in which AY-uninfected leafhopper pairs were used, while gray boxes represent bioassays with AY-infected leafhopper pairs. Different letters indicate statistically significant differences in the number of nymphs or adults observed in each treatment (GLMM followed by Tukey's test with adjustment for multiple comparisons, with an α-value of 0.05).

Following the completion of the no-choice bioassays, the sex of all adults was determined and the ratio of females to males was calculated (**Figure 3**). Canola treatments were excluded from the statistical analysis as a total of only three offspring became adults across all replicates. We found no significant effects of insect condition (AY-uninfected or AY-infected; X^2= 0.01, df = 1, P = 0.91), plant species (X^2= 0.99, df = 7, P = 0.99), or the two-way interaction between these factors (X^2= 0.84, df = 6, P = 0.99), indicating that sex ratios were similar among all treatments.

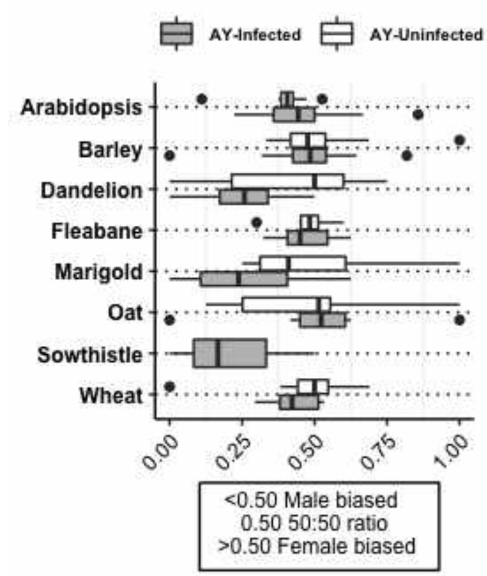

Figure 3: The median, 25th and 75th quartiles for the offspring sex-ratio for each plant species and aster yellows (AY) infection status (AY-uninfected or AY-infected) combination. White boxes represent bioassays in which AY-uninfected leafhopper pairs were used, while gray boxes represent bioassays with AY-infected leafhopper pairs. Canola treatments were excluded from the statistical analysis as only three adults were observed in all replicates.

Female adults were dissected and the number of eggs contained in their ovaries was determined (**Figure 4**). Again, canola treatments were excluded from the analyses. Sowthistle bioassays were also excluded because adults were only in three replicates. The pairing of marigolds and AY-infected leafhopper pairs was excluded from the analysis because no adults were observed in this group. In those treatments examined, we found a significant effect of the insect condition (AY-uninfected or AY-infected; X^2= 39.85, df = 1, P < 0.001; Fig. 4), as well as of the plant species (X^2= 111.47, df = 7, P < 0.001). Additionally, there was a significant two-way interaction between these factors (X^2= 42.37, df = 6, P < 0.001). In *A. thaliana*, a higher egg load was observed in females from the AY-infected treatment when compared to the AY-uninfected one (**Figure 4**). A similar observation can be made about females from barley bioassays, as females from the AY-infected treatment had a higher number of eggs than those from the AY-uninfected treatment (**Figure 4**). In fleabane, oat, and wheat, however, a similar egg load was observed when comparing between AY-uninfected and AY-infected treatments (**Figure 4**).

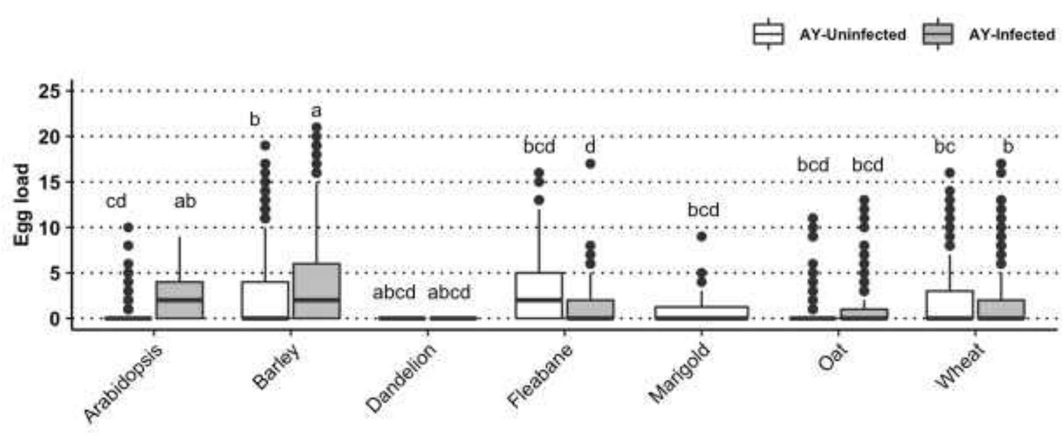

Figure 4: The median, 25th and 75th quartiles for the individual egg load of offspring female adults for each plant species and aster yellows (AY) infection status (AY-uninfected or AY-infected) combination. White boxes represent bioassays in which AY-uninfected leafhopper pairs were used, whereas gray boxes represent bioassays with AY-infected leafhopper pairs. Canola bioassays were removed as only male adults were observed. Sowthistle bioassays were removed because adults were observed in only three replicates and few of them were females. Marigolds and AY-infected leafhopper pairs were removed from the analysis because no adults were observed on these plants. Different letters indicate statistically significant differences in the individual female egg load observed in each treatment (GLMM followed by Tukey's test with adjustment for multiple comparisons, with an α-value of 0.05).

Two-choice bioassays

The number of leafhoppers on each plant was evaluated using the permutational multivariate analyses of variance technique (PERMANOVA). Insects recorded as being "off the plant" were excluded from the analysis given that their values were low and consistent across all treatments. Results are presented in Tables 1 (AY-uninfected insects) and 2 (AY-infected insects). Figures 5A (AY-uninfected insects) and 6A (AY-infected insects) contain a visual representation of the results´ statistical significance.

Plants from the two-choice bioassays were retained for further evaluation. Leaf samples were stained with McBride´s solution and the number of stylet sheaths and eggs on each plant were counted. These were analyzed with a paired t-test for each combination of the plant species being offered during the two-choice bioassay and the insects´ condition (AY-uninfected or AY-infected). When residuals were not normally distributed, Wilcoxon tests were used. Results are presented in Tables 1 (AY-uninfected insects) and 2 (AY-infected insects). Figures 5B-C (AY-uninfected insects) and 6B-6C (AY-infected insects) contain a visual representation of the results´ statistical significance.

For each plant species in each combination, the relation between the number of probing events and eggs was examined using Spearman's correlation (**Table 3**). For the subset of plant combinations for which both AY-uninfected and AY-infected insects were examined, differences in the total number of stylet sheaths between bioassays with AY-uninfected and AY-infected were analyzed with a Mann-Whitney test for each plant combination (**Figure 7**). The total number of stylet sheaths corresponds to the sum of stylet sheath counts from each plant species present at each time in the two-choice arena. Results from bioassays with AY-uninfected leafhoppers reared on fleabane have been included in **Table 4**, in the *Appendices* section.

When examining settling behavior of AY-uninfected Aster leafhoppers (**Figure 5A** and **Table 1**), we observed differences depending on the plant combination provided during the bioassays. In most crop-crop combinations, similar numbers of leafhoppers were observed on both plant species provided. In two combinations in which canola was presented together with oat or wheat, however, a preference for settling on the cereals over canola was observed (**Figure 5A** and **Table 1**). These same patterns of preference and non-preference were also observed when examining the number of stylet sheaths (**Figure 5B** and **Table 1**), as plant species that had been preferred for settling were found to contain a higher number of stylet sheath structures. This suggests that for these plant combinations, settling and probing behavior are associated with one another. In crop-noncrop combinations, AY-uninfected Aster leafhoppers exhibited a preference for settling on the crops over the noncrops

(**Figure 5A** and **Table 1**). It is interesting to note that while canola had been characterized as an unsuitable host for leafhopper oviposition and development (no-choice bioassays, **Figures 1 and 2**), it was preferred for settling over several noncrop species. Unlike crop-crop combinations, settling and probing behaviors when a crop and a noncrop were presented together were not strongly associated with one another. In some cases, plant species were preferred for settling, but similar numbers of stylet sheath structures were observed on both plant species in that combination (**Figures 5A** and **5B**, and **Table 1**). When two noncrops were presented together, both preference and non-preference for settling on these plant species were observed (**Figure 5A** and **Table 1**). Similar to crop-noncrop combinations, settling and probing behaviors did not seem to be strongly correlated to one another. Overall, these findings present a complex scenario in which plant species preferred for settling (**Figure 5A** and **Table 1**) might not always be the most suitable ones for other biological aspects such as oviposition and nymphal development (**Figures 1 and 2**) and suggests that plant acceptance and use will be highly dependent on the context in which that plant species is encountered.

As for AY-uninfected Aster leafhoppers´ oviposition behavior, both preference and non-preference were found in crop-noncrop combinations (**Figure 5C** and **Table 1**). Interestingly, fewer eggs were found on canola when it was presented together with wheat or oat (**Figure 5C** and **Table 1**). However, this was not the case in barley-canola bioassays, as similar numbers of eggs were observed on both plant species (**Figure 5C** and **Table 1**). In most crop-noncrop bioassays, a higher number of eggs was laid on the crops over the noncrops. When canola was presented together with a noncrop, a preference for ovipositing on this plant species was observed in one plant combination (canola-dandelion). In other combinations, canola seems to represent a host of similar suitability to noncrops such as fleabane, marigold, and sowthistle, as similar numbers of eggs were found on both plant species (**Figure 5C** and **Table 1**). Altogether, these results suggest that plant species preferred for settling and/or probing might not necessarily be equally suitable for ovipositing and that plant acceptance and use will be highly dependent on the context in which that plant species is encountered.

When examining the effect of the rearing host (**Table 4** in *Appendices*), AY-uninfected Aster leafhoppers reared entirely on fleabane exhibited a similar settling behavior to leafhoppers reared on barley (**Figure 5A** and **Table 1**). When Aster leafhoppers were presented with barley and fleabane, differences in the number of stylet sheaths and eggs were observed between insects that had been reared on barley (**Figures 5B** and **5C**, and **Table 1**) and those reared on fleabane (**Table 4**). When oat and fleabane were presented together, a higher number of stylet sheaths were present in oat in both cases. However, in barley-fleabane bioassays with leafhoppers reared on barley, stylet sheath counts were similar between both plant species (**Figure 5B**), whereas in bioassays with insects reared on fleabane, a higher number of stylet sheaths was observed in barley when compared to fleabane (**Table 4**). Similar observations can be made when examining the number of eggs laid in two-choice bioassays, as insects from both rearing hosts exhibited a similar oviposition behavior in oat-fleabane combinations (**Figure 5C** and **Table 4**) yet behaved differently when barley and fleabane were presented together (**Figure 5C** and **Table 4**). While this would suggest that the rearing host plant can introduce additional variability in the bioassays, we note that different fleabane plant generations were used over the course of these bioassays. The first generation was field collected during the summer of 2018, while later generations were obtained by continuous inbreeding under laboratory conditions. It is therefore possible that the high inbreeding in these later generations could have affected plant traits associated with attractiveness and palatability to Aster leafhoppers, leading to differences in the number of produced stylet sheaths and possibly explaining these patterns.

A) **Settling behavior**

	Ba	Ca	O	Wh	Da	Fb	Ma	Th
Ba		0.437	0.087	0.058	**0.001**	**0.001**	**0.002**	**0.002**
Ca	=		0.001	**0.004**	**0.011**	**0.001**	**0.013**	**0.001**
O	=	←		0.310	**0.001**	**0.001**	0.132	**0.006**
Wh	=	←	=		**0.001**	**0.001**	0.001	**0.001**
Da	↑	↑	↑	↑		**0.002**	0.984	0.706
Fb	↑	↑	↑	↑	↑		0.079	**0.001**
Ma	↑	↑	=	↑	=	=		**0.001**
Th	↑	↑	↑	↑	=	←	←	

B) **Stylet sheaths**

	Ba	Ca	O	Wh	Da	Fb	Ma	Th
Ba		0.428	0.437	0.110	**0.002**	0.125	**0.009**	0.598
Ca	=		0.001	**0.004**	**0.037**	**0.001**	0.813	**0.030**
O	=	←		0.359	**0.012**	**0.001**	**0.003**	0.153
Wh	-	←	-		**0.006**	**0.002**	**0.003**	0.153
Da	↑	←	↑	↑		**0.018**	**0.004**	0.767
Fb	=	↑	↑	↑	↑		**0.037**	0.229
Ma	↑	=	↑	↑	↑	↑		**0.005**
Th	-	←	←	-	-	-	←	

C) **Oviposition**

	Ba	Ca	O	Wh	Da	Fb	Ma	Th
Ba		0.543	**0.048**	0.830	**0.002**	0.269	**0.009**	**0.016**
Ca	=		**0.018**	**0.003**	**0.002**	0.294	0.309	0.150
O	←	←		0.999	**0.005**	**0.002**	**0.037**	0.474
Wh	=	←	=		**0.006**	**0.006**	**0.001**	**0.048**
Da	↑	↑	↑	↑		0.726	**0.004**	0.999
Fb	=	=	↑	↑	=		**0.011**	0.527
Ma	↑	-	↑	↑	←	←		0.359
Th	↑	=	=	↑	=	=	=	

Figure 5: Results from two-choice bioassays using AY-uninfected Aster leafhoppers reared on barley. The following abbreviations have been used: “Ba” = barley, “Ca” = canola, “O” = oat, “Wh” = wheat, “Da” =dandelion, “Fb” = fleabane, “Ma” = marigold, and “Th” = sowthistle. For all panels (A-C), P-values are presented above the diagonal (black cells), while symbols indicating whether a preference (arrows) or no preference was observed (“=”) are provided below the diagonal. For plant combinations in which a preference was observed, the arrow points to the plant species that was preferred. A significance level (α-value) of 0.05 was used. Crop-crop combinations are indicated by a white background, crop-noncrop combinations by a light grey background, and noncrop-noncrop bioassays by a dark grey background. A) Settling behavior results were evaluated using a PERMANOVA analysis. Details about the percentage and number of insects on each plant can be found in Table 1. B) Probing events were used as proxy for feeding activity and results were evaluated using a paired t-test for each combination. Details about the number of stylet sheaths on each plant can be found in Table 1. C) Oviposition event results were evaluated using a paired t-test for each combination. Details about the number of eggs on each plant can be found in Table 1.

Table 1: Results from two-choice bioassays using AY-uninfected Aster leafhoppers reared on barley. The following abbreviations have been used: “Ba” = barley, “Ca” = canola, “O” = oat, “Wh” = wheat, “Da” =dandelion, “Fb” = fleabane, “Ma” = marigold, and “Th” = sowthistle. For each plant combination, the first plant is referred to as “Plant 1”, while the second plant is “Plant 2”. The average percentages and number of leafhoppers on each plant, the p-values from PERMANOVA analyses, the number of probing events and eggs on each plant, and the p-values from paired-t tests have been provided. “(W)” indicates that residuals were not normally distributed and a Wilcoxon test was used instead. In these cases, refer to Table 5 (in *Appendices*) for median and interquartile range values. A significance level (α-value) of 0.05 was used.

Plant combination Plant 1 – Plant 2	Plant 1 Avg. % of leafhoppers	Plant 2 Avg. % of leafhoppers	Plant 1 Avg. no. of insects	Plant 2 Avg. no. of insects	PERMANOVA p-value (Figure 1)
Ba – Ca	53.2 ± 3.0	46.8 ± 3.0	4.5 ± 0.3	4.3 ± 0.3	0.478
Ba – O	53.0 ± 2.6	47.0 ± 2.6	5.9 ± 0.5	5.2 ± 0.4	0.087
Ba – Wh	60.6 ± 3.3	39.4 ± 3.3	4.7 ± 0.4	2.7 ± 0.3	0.058
Ba – Da	83.0 ± 2.2	17.0 ± 2.2	7.9 ± 0.3	1.8 ± 0.3	0.001
Ba – Fb	75.9 ± 3.4	24.1 ± 3.4	7.7 ± 0.5	2.7 ± 0.4	0.001
Ba – Ma	88.4 ± 2.6	11.6 ± 2.65	6.5 ± 0.3	0.9 ± 0.2	0.002
Ba - Th	62.2 ± 3.6	37.8 ± 3.6	6.2 ± 0.4	3.8 ± 0.4	0.002
Ca – O	24.9 ± 2.6	75.1 ± 2.6	2.7 ± 0.3	8.6 ± 0.3	0.001
Ca – Wh	34.6 ± 3.4	65.4 ± 3.4	3.5 ± 0.4	6.8 ± 0.5	0.004
Ca – Da	62.5 ± 3.2	37.5 ± 3.2	4.7 ± 0.3	2.9 ± 0.3	0.011
Ca – Fb	90.8 ± 1.7	9.2 ± 1.7	5.9 ± 0.3	0.7 ± 0.1	0.001
Ca – Ma	63.1 ± 4.2	36.9 ± 4.2	2.9 ± 0.3	1.5 ± 0.2	0.013
Ca – Th	75.1 ± 3.3	24.9 ± 3.3	5.7 ± 0.3	2.0 ± 0.3	0.001
O – Wh	54.0 ± 3.8	46.0 ± 3.8	4.3 ± 0.4	3.6 ± 0.4	0.310
O – Da	75.4 ± 3.6	24.6 ± 3.6	5.8 ± 0.4	2.1 ± 0.3	0.001
O – Fb	75.1 ± 3.2	24.9 ± 3.2	6.2 ± 0.3	2.3 ± 0.3	0.001
O – Ma	47.9 ± 3.1	52.1 ± 3.1	3.5 ± 0.2	2.2 ± 0.3	0.132
O – Th	63.4 ± 3.8	36.6 ± 3.8	7.1 ± 0.6	3.5 ± 0.3	0.006
Wh – Da	74.3 ± 3.2	25.7 ± 3.2	6.7 ± 0.4	2.3 ± 0.3	0.001
Wh – Fb	75.6 ± 2-9	24.4 ± 2.9	8.1 ± 0.4	2.2 ± 0.4	0.001
Wh – Ma	67.4 ± 2.8	32.6 ± 2.8	5.5 ± 0.4	2.6 ± 0.2	0.001
Wh – Th	64.4 ± 2.8	35.6 ± 2.8	7.1 ± 0.4	3.9 ± 0.3	0.001
Da – Fb	63.7 ± 4.2	36.3 ± 4.2	5.55 ± 0.5	2.6 ± 0.3	0.002
Da – Ma	46.6 ± 3.7	53.4 ± 3.7	3.5 ± 0.4	3.7 ± 0.3	0.984
Da – Th	46.9 ± 3.5	53.1 ± 3.5	3.9 ± 0.4	4.3 ± 0.4	0.706
Fb – Ma	37.4 ± 3.1	62.6 ± 3.1	2.8 ± 0.3	4.6 ± 0.3	0.070
Fb – Th	34.3 ± 4.2	65.7 ± 4.2	1.9 ± 0.2	4.3 ± 0.4	0.001
Ma - Th	26.6 ± 3.1	73.4 ± 3.1	2.4 ± 0.3	6.2 ± 0.3	0.001

Table 1 (Continuation): Results from two-choice bioassays using AY-uninfected Aster leafhoppers reared on barley. The following abbreviations have been used: "Ba" = barley, "Ca" = canola, "O" = oat, "Wh" = wheat, "Da" =dandelion, "Fb" = fleabane, "Ma" = marigold, and "Th" = sowthistle. For each plant combination, the first plant is referred to as "Plant 1", while the second plant is "Plant 2". The average percentages and number of leafhoppers on each plant, the p-values from PERMANOVA analyses, the number of probing events and eggs on each plant, and the p-values from paired-t tests have been provided. "(W)" indicates that residuals were not normally distributed and a Wilcoxon test was used instead. In these cases, refer to Table 5 (in *Appendices*) for median and interquartile range values. A significance level (α-value) of 0.05 was used.

Plant combination Plant 1 – Plant 2	Plant 1 No. of probing events	Plant 2 No. of probing events	Paired t-test p-value (Fig. 2)	Plant 1 No. of eggs	Plant 2 No. of eggs	Paired t-test p-value (Fig. 3)
Ba – Ca	85.0 ± 26.1	36.0 ± 31.6	0.437	20.0 ± 8.4	10.0 ± 8.1	(W) 0.543
Ba – O	84.6 ± 25.1	142.8 ± 52.9	0.428	13.6 ± 4.3	41.6 ± 8.2	0.048
Ba – Wh	124.6 ± 60.1	22.3 ± 11.7	(W) 0.110	18.4 ± 7.8	15.0 ± 7.3	(W) 0.830
Ba – Da	104.0 ± 23.2	8.8 ± 4.8	(W) 0.002	29.2 ± 7.9	0.2 ± 0.2	(W) 0.002
Ba – Fb	116.8 ± 45.5	42.6 ± 3.9	(W) 0.125	6.4 ± 4.2	0.8 ± 0.6	(W) 0.269
Ba – Ma	65.5 ± 23.2	3.3 ± 1.7	(W) 0.009	23.0 ± 5.8	0.9 ± 0.5	(W) 0.009
Ba - Th	190.7 ± 62.9	140.8 ± 43.7	0.598	18.0 ± 3.1	1.7 ± 0.9	(W) 0.016
Ca – O	13.6 ± 5.8	407.6 ± 63.4	(W) 0.001	1.9 ± 0.7	16.1 ± 2.1	(W) 0.003
Ca – Wh	35.9 ± 10.5	384.9 ± 88.8	(W) 0.004	4.1 ± 1.3	14.9 ± 3.5	0.019
Ca – Da	101.4 ± 24.2	221.3 ± 35.9	(W) 0.037	26.8 ± 7.7	4.1 ± 1.4	(W) 0.002
Ca – Fb	28.8 ± 11.8	67.5 ± 17.1	(W) 0.001	6.6 ± 2.6	3.4 ± 1.7	(W) 0.294
Ca – Ma	52.9 ± 22.2	40.6 ± 9.4	(W) 0.813	6.3 ± 2.2	4.3 ± 1.8	(W) 0.309
Ca – Th	3.5 ± 2.3	24.0 ± 6.1	(W) 0.030	1.6 ± 0.6	0.6 ± 0.2	(W) 0.150
O – Wh	43.0 ± 17.4	81.4 ± 33.1	(W) 0.359	22.6 ± 7.7	18.9 ± 3.8	(W) 0.999
O – Da	423.4 ± 98.1	174.2 ± 57.1	(W) 0.014	13.3 ± 4.2	0.5 ± 0.3	(W) 0.006
O – Fb	642.3 ± 106.5	140.9 ± 29.1	0.001	29.6 ± 7.6	3.9 ± 1.7	(W) 0.037
O – Ma	255.4 ± 70.9	60.4 ± 7.8	0.021	40.8 ± 11.3	16.3 ± 3.7	(W) 0.037
O – Th	187.8 ± 72.2	466.0 ± 115.6	0.047	9.4 ± 4.4	5.6 ± 2.1	0.474
Wh – Da	274.5 ± 61.1	66.9 ± 22.2	(W) 0.006	35.1 ± 7.0	0.1 ± 0.1	(W) 0.006
Wh – Fb	188.2 ± 59.0	28.7 ± 14.0	(W) 0.002	39.0 ± 4.9	2.4 ± 0.9	(W) 0.006
Wh – Ma	277.9 ± 66.8	37.3 ± 12.4	0.003	25.9 ± 4.1	5.3 ± 1.8	0.001
Wh – Th	87.4 ± 39.6	260.0 ± 87.1	0.048	14.2 ± 5.1	1.0 ± 0.8	(W) 0.004
Da – Fb	241.5 ± 36.6	98.4 ± 30.4	(W) 0.018	3.2 ± 1.1	4.0 ± 1.3	(W) 0.726
Da – Ma	224.5 ± 34.5	65.6 ± 17.9	(W) 0.004	6.0 ± 2.3	18.6 ± 4.1	0.004
Da – Th	301.2 ± 47.9	323.6 ± 35.6	0.767	3.5 ± 1.5	2.6 ± 0.9	(W) 0.999
Fb – Ma	129.2 ± 30.2	46.1 ± 10.3	0.037	1.6 ± 0.8	9.1 ± 1.5	(W) 0.011
Fb – Th	81.4 ± 35.6	149.3 ± 27.4	0.229	3.7 ± 1.8	2.0 ± 0.9	(W) 0.527
Ma - Th	14.7 ± 12.2	252.7 ± 42.1	(W) 0.005	1.0 ± 0.5	2.3 ± 1.1	(W) 0.359

To examine whether infection of Aster leafhoppers with AYp would affect their settling behavior, we repeated the two-choice bioassays using AY-infected leafhoppers and a subset of plant species (**Figure 6** and **Table 2**). In plant combinations such as barley-dandelion, canola-wheat, and wheat-dandelion, settling behavior was similar between uninfected and AY-infected Aster leafhoppers (**Figures 5A** and **6A**). In other cases, however, settling behavior differed between these insect groups. When barley and canola were presented together, for example, a similar number of AY-uninfected Aster leafhoppers were observed on both plant species (**Figure 5A** and **Table 1**). When AY-infected Aster leafhoppers were offered this plant combination, leafhoppers preferred to settle on barley (**Figure 6A** and **Table 2**). This was also the case for bioassays with barley and wheat, as AY-uninfected Aster leafhoppers exhibited no settling preference (Figure 5A and Table 1), while AY-infected insects preferred to settle on barley (**Figure 6A** and **Table 2**). In dandelion-canola bioassays, AY-uninfected leafhoppers preferred to settle on canola, while AY-infected insects preferred to settle on dandelion.

A) **Settling behavior**

	Ba	Ca	Wh	Da
Ba		**0.001**	**0.015**	**0.001**
Ca	↑		**0.001**	**0.001**
Wh	↑	←		**0.001**
Da	↑	←	↑	

B) **Stylet sheaths**

	Ba	Ca	Wh	Da
Ba		**0.002**	0.326	0.239
Ca	↑		**0.002**	**0.002**
Wh	=	←		**0.011**
Da	=	←	↑	

C) **Oviposition**

	Ba	Ca	Wh	Da
Ba		**0.013**	0.123	**0.001**
Ca	↑		**0.011**	0.577
Wh	=	←		**0.010**
Da	↑	=	↑	

Figure 6: Results from two-choice bioassays using AY-infected Aster leafhoppers reared on barley. The following abbreviations have been used: “Ba” = barley, “Ca” = canola, “Wh” = wheat, and “Da” =dandelion. For all panels (A-C), P-values are presented above the diagonal (black cells), while symbols indicating whether a preference (arrows) or no preference was observed (“=”) are provided below the diagonal. For plant combinations in which a preference was observed, the arrow points to the plant species that was preferred. A significance level (α-value) of 0.05 was used. Crop-crop combinations are indicated by a white background and crop-noncrop combinations by a light grey background. A) Settling behavior results were evaluated using a PERMANOVA analysis. Details about the percentage and number of insects on each plant can be found in Table 2. B) Probing events were used as proxy for feeding activity and results were evaluated using a paired t-test for each combination. Details about the number of stylet sheaths on each plant can be found in Table 2. C) Oviposition event results were evaluated using a paired t-test for each combination. Details about the number of eggs on each plant can be found in Table 2.

Table 2: Results from two-choice bioassays using AY-infected Aster leafhoppers reared on barley. The following abbreviations have been used: "Ba" = barley, "Ca" = canola, "Wh" = wheat, and "Da" =dandelion. For each plant combination, the first plant is referred to as "Plant 1", while the second plant is "Plant 2". The average percentages and number of leafhoppers on each plant, the p-values from PERMANOVA analyses, the number of probing events and eggs on each plant, and the p-values from paired-t tests have been provided. "(W)" indicates that residuals were not normally distributed and a Wilcoxon test was used instead. In these cases, refer to Table 5 (in *Appendices*) for median and interquartile range values. A significance level (α-value) of 0.05 was used.

Plant combination Plant 1 – Plant 2	Plant 1 Avg. % of leafhoppers	Plant 2 Avg. % of leafhoppers	Plant 1 Avg. no. of insects	Plant 2 Avg. no. of insects	PERMANOVA p-value (Figure 1)
Ba – Ca	68.6 ± 3.0	31.4 ± 3.0	5.5 ± 0.4	2.4 ± 0.2	0.001
Ba – Wh	60.3 ± 3.9	39.7 ± 3.9	5.2 ± 0.4	3.7 ± 0.5	0.015
Ba – Da	65.5 ± 3.7	34.5 ± 3.7	5.7 ± 0.4	3.4 ± 0.4	0.001
Ca – Wh	19.4 ± 2.5	80.6 ± 2.5	1.3 ± 0.2	5.4 ± 0.3	0.001
Ca – Da	21.9 ± 3.3	78.1 ± 3.3	1.4 ± 0.2	4.6 ± 0.2	0.001
Wh – Da	79.3 ± 3.7	20.7 ± 3.7	4.7 ± 0.2	1.2 ± 0.2	0.001

Table 2 (Continuation): Results from two-choice bioassays using AY-infected Aster leafhoppers reared on barley. The following abbreviations have been used: "Ba" = barley, "Ca" = canola, "Wh" = wheat, and "Da" =dandelion. For each plant combination, the first plant is referred to as "Plant 1", while the second plant is "Plant 2". The average percentages and number of leafhoppers on each plant, the p-values from PERMANOVA analyses, the number of probing events and eggs on each plant, and the p-values from paired-t tests have been provided. "(W)" indicates that residuals were not normally distributed and a Wilcoxon test was used instead. In these cases, refer to Table 5 (in *Administrative and Other Aspects*) for median and interquartile range values. A significance level (α-value) of 0.05 was used.

Plant combination Plant 1 – Plant 2	Plant 1 No. of probing events	Plant 2 No. of probing events	Paired t-test p-value (Fig. 2)	Plant 1 No. of eggs	Plant 2 No. of eggs	Paired t-test p-value (Fig. 3)
Ba – Ca	196.6 ± 48.3	9.5 ± 3.7	(W) 0.002	11.8 ± 2.3	1.9 ± 0.8	(W) 0.013
Ba – Wh	142.6 ± 61.3	105.0 ± 39.7	0.326	49.2 ± 18.3	13.2 ± 5.1	0.123
Ba – Da	155.6 ± 39.5	96.7 ± 29.7	0.239	17.6 ± 4.5	1.9 ± 0.7	(W) 0.001
Ca – Wh	16.5 ± 7.9	273.4 ± 36.5	(W) 0.002	2.3 ± 0.9	15.9 ± 3.1	(W) 0.011
Ca – Da	1.7 ± 0.9	154.4 ± 45.5	(W) 0.002	1.1 ± 0.7	0.9 ± 0.5	(W) 0.577
Wh – Da	256.7 ± 54.8	82.4 ± 12.2	0.011	7.9 ± 2.3	0.5 ± 0.4	(W) 0.010

When examining stylet sheath and egg counts from bioassays with AY-infected Aster leafhoppers, AY-uninfected insects exhibited similar probing and oviposition behavior to AY-infected leafhoppers in most plant combinations (**Figure 5B-C** and **Figure 6B-C**). Comparison of egg laying behavior between groups of AY-uninfected and AY-infected Aster leafhoppers revealed that insects exhibited similar patterns of preference/no preference in most plant combinations, including barley-wheat, barley-dandelion, canola-wheat, and dandelion-wheat combinations (**Figures 5C** and **6C**).

To provide additional information about the effect of insect infection with AYp on probing behavior, the total number of stylet sheaths was determined for plant combinations for which AY-uninfected and AY-infected leafhoppers had been examined. Differences between insect groups were determined for each plant combination separately. In most plant combinations, analyses revealed similar numbers in the total stylet sheath count between uninfected and AY-infected insect groups (**Figure 7**). When comparing the total number of stylet sheaths in bioassays in which barley and dandelion were presented, a higher number of total stylet sheaths was observed in the AY-infected Aster leafhopper treatment (p = 0.021, **Figure 7**). The opposite effect was observed in bioassays with canola and dandelion, in which samples from AY-infected Aster leafhoppers had a lower number of total stylet sheaths when compared to those from uninfected insects (P = 0.012, **Figure 7**).

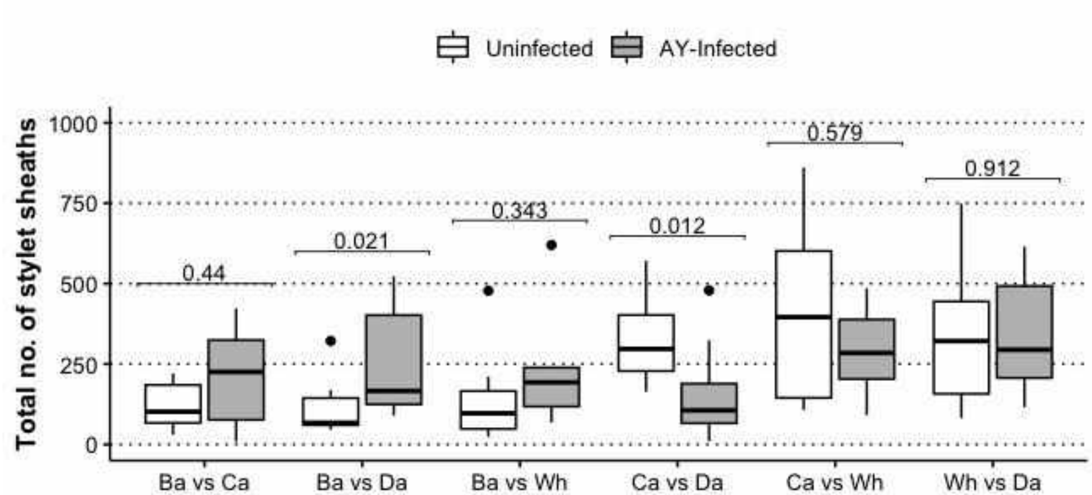

Figure 7: The median, 25th and 75th quartiles for the total number of stylet sheaths for the subset of plant combinations for which both AY-uninfected and AY-infected insects were examined. White boxes represent bioassays in which AY-uninfected leafhopper pairs were used, while gray boxes represent bioassays with AY-infected leafhopper pairs. The following abbreviations have been used: “Ba” = barley, “Ca” = canola, “Wh” = wheat, and “Da” =dandelion. The p-values from each Mann-Whitney test are provided right above the treatments being compared. A significance level (α-value) of 0.05 was used.

We analyzed the relationship between the stylet sheath and egg counts for each plant species in each plant using Spearman´s correlations (**Table 3**). In most cases, no correlation was observed between these variables (P > 0.05), yet for 12 plant species in some bioassays, a strong positive correlation between the number of stylet sheaths and the number of eggs was found (P < 0.05, **Table 3**).

Table 3: Relationship between stylet sheath and egg counts for each plant species in each two-choice bioassay. Spearman´s correlation coefficient and its significance are provided for each plant species in each plant combination under study. The following abbreviations have been used: “Ba” = barley, “Ca” = canola, “O” = oat, “Wh” = wheat, “Da” =dandelion, “Fb” = fleabane, “Ma” = marigold, and “Th” = sowthistle. For each pair of plant species, the first plant is referred to as “Plant 1”, while the second plant is “Plant 2”. A significance level (α-value) of 0.05 was used.

Rearing host	Insect infection status	Plant combination Plant 1 – Plant 2	Plant 1 Spearman´s correlation coefficient	Plant 1 Coefficient p-value	Plant 2 Spearman´s correlation coefficient	Plant 2 Coefficient p-value
Ba	AY-uninfected	Ba – Ca	0.40	0.52	1.00	<0.01
Ba	AY-uninfected	Ba – O	-0.30	0.68	0.30	0.68
Ba	AY-uninfected	Ba – Wh	0.74	0.06	-0.57	0.18
Ba	AY-uninfected	Ba – Da	0.25	0.47	0.23	0.51
Ba	AY-uninfected	Ba – Fb	1.00	0.02	0.33	0.58
Ba	AY-uninfected	Ba – Ma	0.59	0.07	0.21	0.55
Ba	AY-uninfected	Ba - Th	0.61	0.17	0.22	0.63
Ba	AY-uninfected	Ca – O	0.31	0.35	0.43	0.18
Ba	AY-uninfected	Ca – Wh	0.72	0.02	0.39	0.27
Ba	AY-uninfected	Ca – Da	0.20	0.58	0.71	0.02
Ba	AY-uninfected	Ca – Fb	0.75	0.01	0.50	0.14
Ba	AY-uninfected	Ca – Ma	0.65	0.04	0.76	<0.01
Ba	AY-uninfected	Ca – Th	0.10	0.77	-0.04	0.89
Ba	AY-uninfected	O – Wh	0.81	<0.01	0.43	0.21
Ba	AY-uninfected	O – Da	0.16	0.67	-0.04	0.90
Ba	AY-uninfected	O – Fb	0.32	0.37	0.41	0.24
Ba	AY-uninfected	O – Ma	0.84	<0.01	0.44	0.21
Ba	AY-uninfected	O – Th	0.52	0.13	-0.56	0.32
Ba	AY-uninfected	Wh – Da	0.72	0.02	0.06	0.87
Ba	AY-uninfected	Wh – Fb	0.53	0.11	0.57	0.08
Ba	AY-uninfected	Wh – Ma	0.34	0.33	0.80	<0.01
Ba	AY-uninfected	Wh – Th	0.10	0.95	0.22	0.72
Ba	AY-uninfected	Da – Fb	-0.05	0.87	0.45	0.19
Ba	AY-uninfected	Da – Ma	0.37	0.29	-0.01	0.97
Ba	AY-uninfected	Da – Th	0.15	0.67	0.24	0.50
Ba	AY-uninfected	Fb – Ma	0.24	0.50	0.45	0.19
Ba	AY-uninfected	Fb – Th	0.41	0.36	0.62	0.13
Ba	AY-uninfected	Ma - Th	0.46	0.17	0.04	0.92
Ba	AY-infected	Ba – Ca	0.51	0.13	0.12	0.72
Ba	AY-infected	Ba – Wh	0.50	0.45	0.15	0.80
Ba	AY-infected	Ba – Da	0.12	0.75	0.32	0.37
Ba	AY-infected	Ca – Wh	0.60	0.07	-0.01	0.99
Ba	AY-infected	Ca – Da	0.53	0.11	0.26	0.47
Ba	AY-infected	Wh – Da	0.84	<0.01	0.28	0.44
Fb	AY-uninfected	Ba – Fb	0.73	0.02	0.30	0.40
Fb	AY-uninfected	O - Fb	0.22	0.54	0.31	0.37

Disease development

Plant species for this set of experiments were selected based on previous results from the no-choice and two-choice bioassays: *A. thaliana* and cereals like barley and wheat represent suitable hosts for leafhopper oviposition and development, dandelion was characterized as an intermediate host for leafhopper oviposition and can be present in the field before crops emergence, and canola and sowthistle were observed to be poor hosts for leafhopper development. In particular with canola, despite being a poor host for leafhopper reproduction and development, it can be greatly affected by AY infection.

Due to the COVID-19 pandemic and associated restrictions, this part of the project experienced some delays. Disease development assays have been completed and part of the samples remain to be qPCR tested. Pictures of asymptomatic and symptomatic plants were included, as they might be helpful for outreach information for growers in the region (**Figures 8-13**).

Preliminary results from the first set of plants that were examined (*A. thaliana*, barley, canola, dandelion, and wheat) revealed differences in symptom expression following AY infection (**Figures 8-13**). In *A. thaliana*, some plants exhibited yellowing at 2 weeks and floral bud distortion at 4 weeks. Malformation of flower buds was more pronounced at 5 weeks post-infection period (**Figure 8**). In barley, however, plants showed almost no symptoms (**Figure 9**), except for some yellowing during the last sampling period (5 weeks post-infection period), which could be associated with other stress factors including drought, overwatering, nutrient availability, and/or suboptimal temperatures. Symptoms such as yellowing and malformation of flower buds could be observed in canola at 2 weeks post-infection period, while reddening and signs of phyllody were detected at 4 and 5 weeks (**Figure 10**). In dandelion, some plants exhibited reddening of leaf tips (**Figure 11**), starting at 2 weeks post-infection period, which has been also observed in phytoplasma-uninfected dandelion plants grown in chambers. Similarly to barley, almost no symptoms were observed in wheat plants (**Figure 12**). In sowthistle (**Figure 13**), most plants exhibited no symptoms, except for one plant, in which yellowing was observed in one leaf at 5 weeks post-infection period. For those plant species in which yellowing was observed, further testing is required to determine if this symptom is associated with a potential AY infection or with abiotic stress.

Figure 8: Examples of *A. thaliana* plants from the disease development assays at different sampling periods (2, 4, and 5 weeks following the last day of the infection period). Whole plant and close ups of some plants are provided.

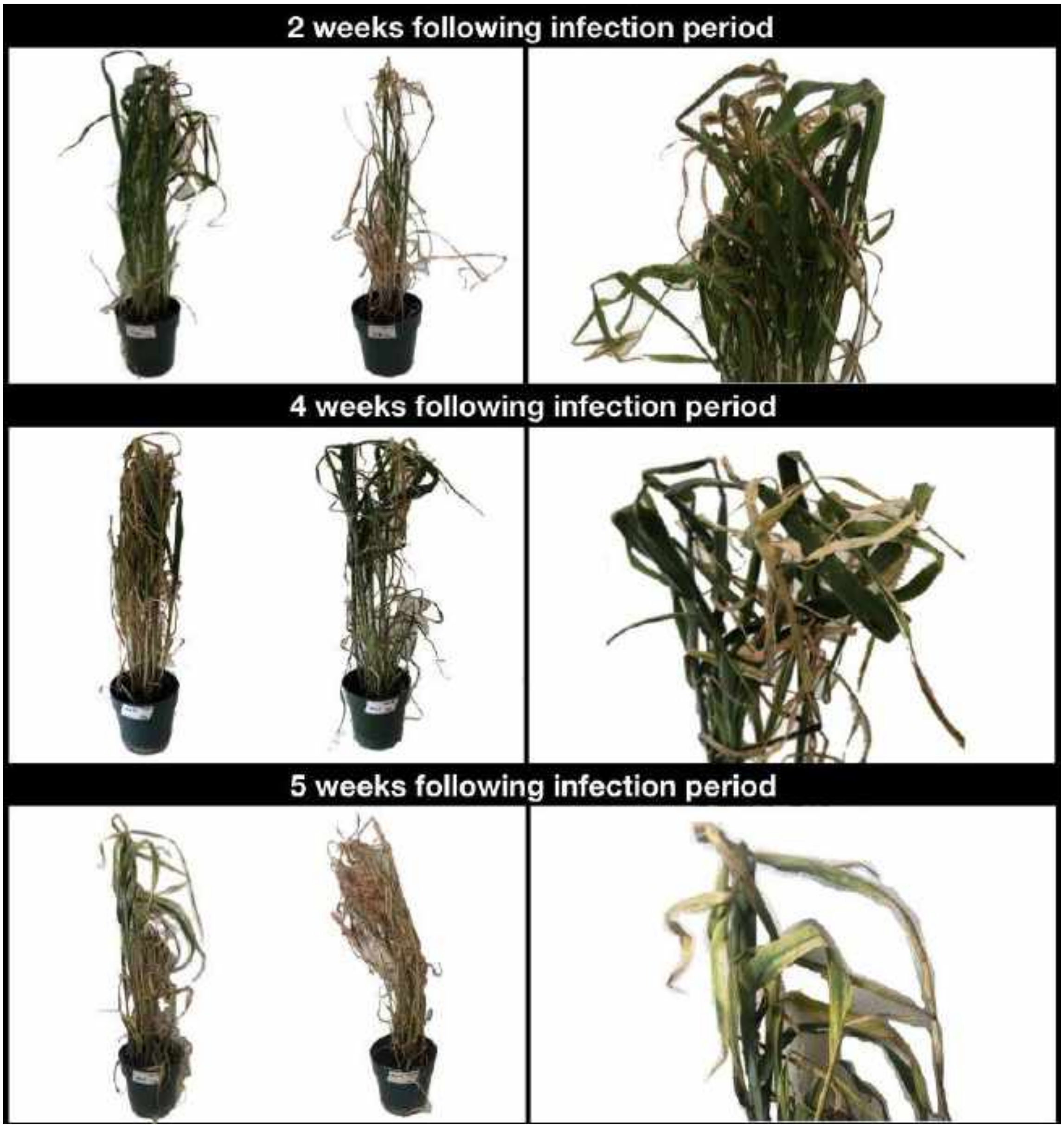

Figure 9: Examples of barley plants from the disease development assays at different sampling periods (2, 4, and 5 weeks following the last day of the infection period). Whole plant and close ups of some plants are provided.

Figure 10: Examples of canola plants from the disease development assays at different sampling periods (2, 4, and 5 weeks following the last day of the infection period). Whole plant and close ups of some plants are provided.

Figure 11: Examples of dandelion plants from the disease development assays at different sampling periods (2, 4, and 5 weeks following the last day of the infection period). Whole plant and close ups of some plants are provided.

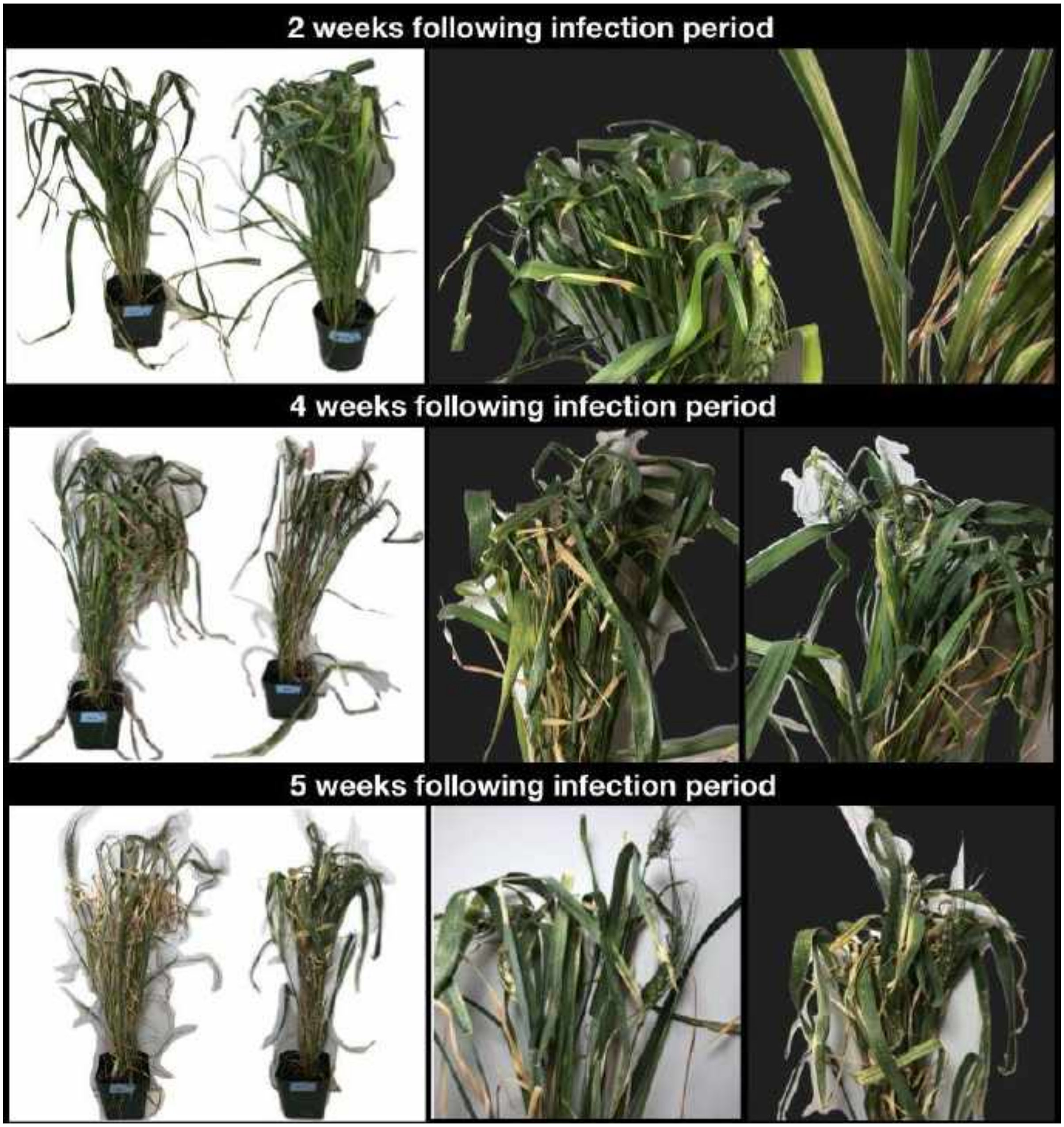

Figure 12: Examples of wheat plants from the disease development assays at different sampling periods (2, 4, and 5 weeks following the last day of the infection period). Whole plant and close ups of some plants are provided.

Figure 13: Examples of sowthistle plants from the disease development assays at different sampling periods (2, 4, and 5 weeks following the last day of the infection period). Whole plant and close ups of some plants are provided.

PCR testing of samples collected at different time points showed that *A. thaliana*, barley, and canola are suitable AY hosts and that AY titer generally increases over time (**Figure 15**). It is possible that the infection pattern observed in *A. thaliana*, in which AY titer is high in the early stages of the infection (2 weeks post infection period) and then decreases (**Figure 16**), might be related to the amount of sampling required for the analyses and the smaller size of this plant species. Given the smaller leaf size of *A. thaliana*, more leaves had to be collected to obtain a similar amount of tissue and have enough DNA material to work with, possibly removing a substantial amount of phytoplasmas that could have contributed to the infection and symptom expression. When compared to phytoplasma levels in *A. thaliana* and barley, canola was found to contain higher levels, suggesting that this plant species is more susceptible to AY infection than the aforementioned plant species (**Figure 15**). Moreover, while all canola plants were observed to be infected with AY, infection was only detected in 50% of *A. thaliana* and barley plants, providing further evidence that these plant species might be less susceptible to AY infection than canola (**Table 6**). Samples from dandelion, sowthistle, and wheat tested negative for AY infection (**Figures 15 and 16, and Table 6**), suggesting that these plant species might not be suitable AY hosts. However, PCR testing of some of the insects used for these experiments seems to indicate that Aster leafhoppers used for infecting wheat and dandelion (November 29, 2021) had lower levels of infection than those used in previous assays (September 20 and October 29, 2021) (**Tables 7 and 8**), possibly impacting the aforementioned results. To rule out this possibility, the remaining samples should be tested and disease development assays should be repeated using highly infective Aster leafhoppers.

A preliminary comparison of these six plant species suggests that a) barley, canola, and *A. thaliana* are more susceptible to infection with AYp than dandelion, sowthiste, and wheat, b) symptoms of AY infection appear sooner in canola and can compromise the development of floral structures, c) similarly to canola, infection of *A. thaliana* with AY can affect the formation of floral structures, and d) symptoms of AY infection in barley plants can be similar to abiotic stress, yet 50% of these plants were observed to be infected with AY, further complicating the detection of this disease by visual assessment only.

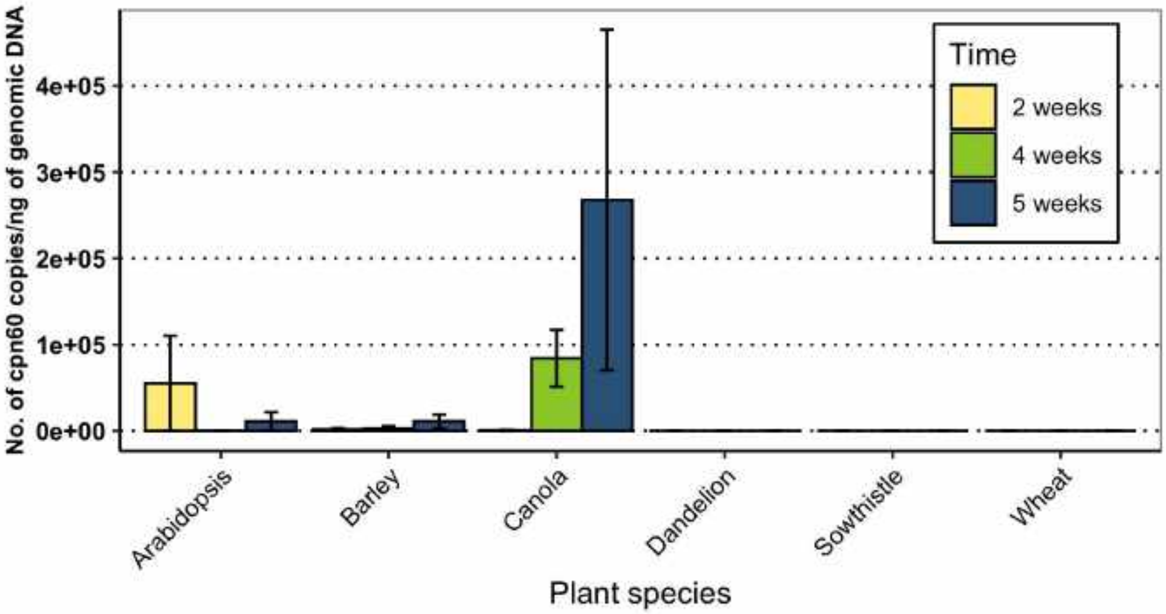

Figure 15: Mean AY titer (Number of *cpn60* copies/ng of genomic DNA) for each plant species and sampling time (2, 4, and 5 weeks post infection period). Error bars indicate the standard error of the mean (SEM). Yellow bars indicate the AY titer of samples collected 2 weeks following the infection period, green bars indicate the AY titer of samples collected 4 weeks following the infection period, and blue bars indicate the AY titer of samples collected 5 weeks following the infection period.

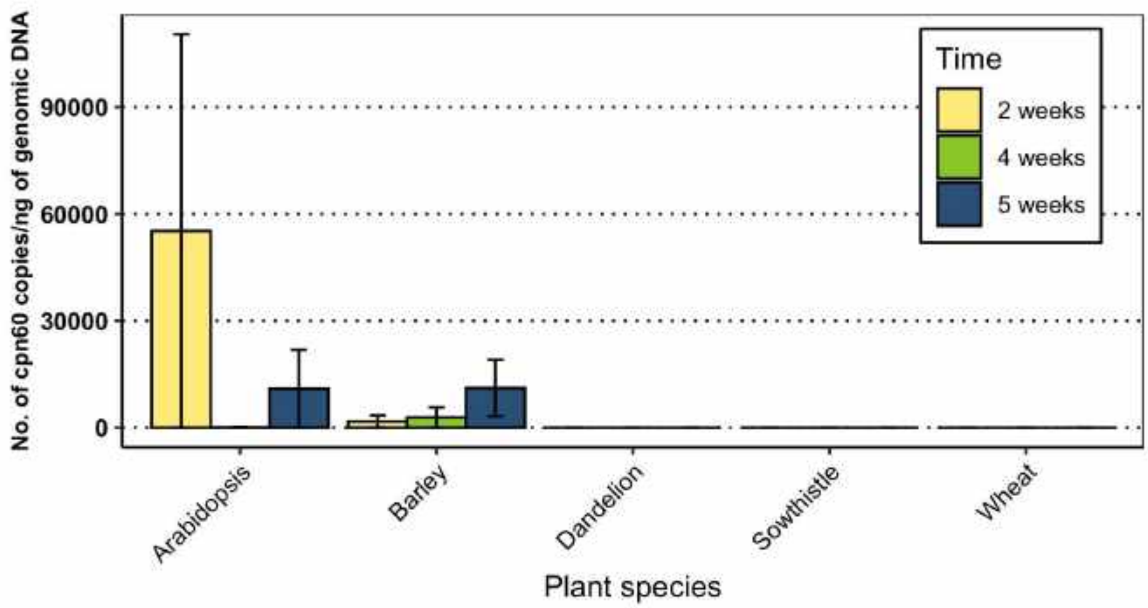

Figure 16: Mean AY titer (Number of *cpn60* copies/ng of genomic DNA) for each plant species and sampling time (2, 4, and 5 weeks post infection period). Canola was excluded from this figure to examine the other plant species under study (*A. thaliana*, barley, dandelion, sowthistle, and wheat). Error bars indicate the standard error of the mean (SEM). Yellow bars indicate the AY titer of samples collected 2 weeks following the infection period, green bars indicate the AY titer of samples collected 4 weeks following the infection period, and blue bars indicate the AY titer of samples collected 5 weeks following the infection period.

Table 6: For each plant species, the number of infected plants and AY titer (Number of *cpn60* copies/ng of genome DNA) have been provided. AY titer was calculated for each sampling period (2, 4, and 5 weeks post infection period), using both AY-uninfected and AY-infected samples. AY titer was additionally calculated using only AY-infected samples, for which the number of samples under analysis was included.

	No. of infected plants	No. of *cpn60* copies/ng of genomic DNA (Mean ± SEM)			No. of *cpn60* copies/ng of genomic DNA (Only AY-infected samples) (No. of obs)		
		2 weeks	4 weeks	5 weeks	2 weeks	4 weeks	5 weeks
A. thaliana	2/4	55228.7 ± 55228.7	109.4 ± 109.4	10932.2 ± 10932.2	220914.8 (1)	437.48 (1)	43728.6 (1)
Barley	2/4	1759.6 ± 1667.2	2875.3 ± 2875.3	11135.3 ± 7935.7	3519.1 ± 3238.0 (2)	5750.6 ± 5671.4 (2)	22270.5 ± 11395.9 (2)
Canola	4/4	584.6 ± 583.6	84343.7 ± 32825.2	267830.6 ± 197464.8	1169.2 ± 1169.1 (2)	112458.2 ± 23961.1 (3)	267830.6 ± 197464.8 (4)
Dandelion	0/4	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Sowthistle	0/4	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Wheat	0/4	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Table 7: Summary table of the AY titer from leafhopper samples from the disease development assays. Data from assays conducted on October 18, 2021 and November 17, 2021 has been excluded, as no leafhoppers were collected in these cases.

Disease development assay starting date	*cpn60* copies/ng of genome DNA (Mean ± SEM)			
September 20, 2021	*A. thaliana* 2004.1 ± 979.7	Barley 2971.6 ± 1141.8	Canola 1676.2 ± 569.0	*2195.7 ± 512.5
October 29, 2021	*A. thaliana* 0.0 ± 0.0	Barley 2688.1 ± 1080.3	Canola 53.5 ± 53.5	*913.9 ± 626.8
November 29, 2021		Wheat and dandelion		*16.82 ± 15.9
				**1830.1 ± 417.8
*Mean ± SEM for the entire period; **Mean ± SEM for all samples				

Table 8: Leafhopper adults from disease development assays were randomly collected following their exposure to the test plants and AY titer (Number of *cpn60* copies/ng of genomic DNA) was determined. For each sample, 2 Aster leafhopper adults were used. For assays conducted on October 18, 2021 and November 17, 2021, no leafhopper samples were collected. “Ara” = *A. thaliana*, “Ba” = Barley, “Ca” = Canola.

Sample ID (2 LH adults/sample)	Disease development assay starting date	No. of *cpn60* copies/ ng of genome DNA
DD Sep 20; col_ Sep 27, 2021 - Ara 1	Sep 20, 2021	8447.66
DD Sep 20; col_ Sep 27, 2021 - Ara 1	Sep 20, 2021	713.72
DD Sep 20; col_ Sep 27, 2021 - Ara 2	Sep 20, 2021	0
DD Sep 20; col_ Sep 27, 2021 - Ara 2	Sep 20, 2021	667.32
DD Sep 20; col_ Sep 27, 2021 - Ara 3	Sep 20, 2021	1144.01
DD Sep 20; col_ Sep 27, 2021 - Ara 3	Sep 20, 2021	1544.79
DD Sep 20; col_ Sep 27, 2021 - Ara 4	Sep 20, 2021	399.87
DD Sep 20; col_ Sep 27, 2021 - Ara 4	Sep 20, 2021	3115.64
DD Sep 20; col_ Sep 27, 2021 - Ba 1	Sep 20, 2021	7167.18
DD Sep 20; col_ Sep 27, 2021 - Ba 1	Sep 20, 2021	8797.75
DD Sep 20; col_ Sep 27, 2021 - Ba 2	Sep 20, 2021	936.34
DD Sep 20; col_ Sep 27, 2021 - Ara 2	Sep 20, 2021	667.32
DD Sep 20; col_ Sep 27, 2021 - Ara 3	Sep 20, 2021	1144.01
DD Sep 20; col_ Sep 27, 2021 - Ara 3	Sep 20, 2021	1544.79
DD Sep 20; col_ Sep 27, 2021 - Ara 4	Sep 20, 2021	399.87
DD Sep 20; col_ Sep 27, 2021 - Ara 4	Sep 20, 2021	3115.64
DD Sep 20; col_ Sep 27, 2021 - Ca 1	Sep 20, 2021	60.03
DD Sep 20; col_ Sep 27, 2021 - Ca 1	Sep 20, 2021	0
DD Sep 20; col_ Sep 27, 2021 - Ca 1	Sep 20, 2021	3365.59
DD Sep 20; col_ Sep 27, 2021 - Ca 2	Sep 20, 2021	4502.45
DD Sep 20; col_ Sep 27, 2021 - Ca 2	Sep 20, 2021	2626.37
DD Sep 20; col_ Sep 27, 2021 - Ca 2	Sep 20, 2021	45.59
DD Sep 20; col_ Sep 27, 2021 - Ca 3	Sep 20, 2021	2866.35
DD Sep 20; col_ Sep 27, 2021 - Ca 3	Sep 20, 2021	1343.85
DD Sep 20; col_ Sep 27, 2021 - Ca 3	Sep 20, 2021	275.92
Nov 5, 2021 - DD Oct 29 - Ara 7	Oct 29, 2021	0
Nov 5, 2021 - DD Oct 29 - Ara 2	Oct 29, 2021	0
Nov 5, 2021 - DD Oct 29 - Ba 10	Oct 29, 2021	1607.64
Nov 5, 2021 - DD Oct 29 - Ba 9	Oct 29, 2021	3768.52
Nov 5, 2021 - DD Oct 29 - Ca 3	Oct 29, 2021	107.06
Nov 5, 2021 - DD Oct 29 - Ca 4	Oct 29, 2021	0
Dec 6, 2021 - DD Nov 29 -Dec 6 2-2	Nov 29, 2021	32.70
Dec 6, 2021 - DD Nov 29 -Dec 6 2-1	Nov 29, 2021	0.94

Field survey of common weeds for the presence of aster leafhoppers and AYp - Insect surveys

In 2018, sweep samples came from field margins at 13 sites that were sampled in the last week of May and again in the first week of June, in an effort to collect the migrant leafhoppers. Sweep sampling did continue at research farms on the site list (Table 9) in cereal fields. A total of 356 aster leafhoppers were caught over the course of the spring sampling period (Table 9). Samples were tested with both conventional PCR using R16S primers (R16R2-N/R16F2-N) and fluorometric LAMP. Two leafhoppers tested positive for AYp using 16S Nested PCR (n=356; 0.56% of total samples), while LAMP tests indicated twelve insects were positive (3.37%). This discrepancy is common with the LAMP technique proving to be a more sensitive tool for detection of AYp than the "gold standard" 16S PCR (Pusz-Bochenska et al. 2020). Positive leafhoppers were found in ditches next to cropland near Yellow Creek with both conventional PCR (2 positive leafhoppers; 1.38%, n=144) and LAMP (9 positive leafhoppers; 6.25%) (Table 9). LAMP also detected AYp positive insects from AAFC Saskatoon Lowe Road (1 positive; 1.53%, n=65), and Llewellyn farm ditches near research fields (2 positive; 3.125%, n=64).

Table 9: Aster leafhopper adults testing positive for AYp in the 2018 spring migratory generation. The geographic location and testing method of each sample are provided.

	16S Nested Conventional		**LAMP**		**n**
Location	**# of positive insects**	**% positive**	**# of positive insects**	**% positive**	
Saskatoon	0	0	**1**	**1.53**	**65**
Grasswood	0	0	0	0	6
Aberdeen	0	0	0	0	19
Rosetown	0	0	0	0	6
Yellow Creek	**2**	**1.38**	**9**	**6.25**	**144**
Outlook	0	0	0	0	6
Melfort	0	0	0	0	1
Sovereign	0	0	0	0	3
Llewellyn	0	0	**2**	**3.125**	**64**
Clavet	0	0	0	0	2
Meachen	0	0	0	0	1
Wakaw	0	0	0	0	33
Alvena	0	0	0	0	6
Total	**2**	**0.56**	**12**	**3.37**	**356**

In 2019, sweep samples came from the field margins of 15 sites, including five sentinel sites where field margins were sampled in the last week of May and the first week of June, and cereal fields were sampled at sentinel sites thereafter. Both LAMP and 16S Nested PCR were used to test for AYp and the results are presented (Tables 10 and 11) as positive samples were found using both methods. A total of 20 leafhoppers tested positive for AYp (3.47%) from all of the 2019 sweep samples that were taken throughout the growing season (Tables 10 and 11). Aster leafhoppers collected in May and June were the migratory population and the detection of AYp in these samples indicates that leafhoppers that arrived in Saskatchewan were already infected with AYp. One AYp-infected aster leafhopper was found out of the nine that were collected in May 2019 (n_{May} = 9; 11.11%). More leafhoppers were caught through June (n--$_{\text{June}}$ = 269, Table 11), indicating more migratory events in early June. For this sampling period (June 2019), 10 aster leafhoppers tested positive for the presence of AYp (n--$_{\text{June}}$ = 269; 3.72%, Table 11). Even though July and August are outside the project parameters, results from aster leafhoppers collected in the fields in July and August are included to show how the AYp runs through the population once it establishes in Saskatchewan. Insects collected in fields in July were most likely migrant adults that entered cereal fields after staying in ditches. Of all aster leafhoppers collected in July, nine tested positive for AY (n_{July} = 213; 4.22%, Table 11), which is a relatively stable AY infection compared to June. Insects collected in fields in June are most likely adults of the same miratory generation as those collected in July, yet fewer insects were infected with AY for this sampling period (June). Aster leafhoppers collected in August are of the second generation, which would have been produced from eggs laid in the cereal fields where they were trapped. As no insects collected in cereal fields during August 2019 were positive for the presence of AYp (n_{August} = 14; 0%, Table 11), these results suggest that this second generation of aster leafhoppers was not infected with AY.

Table 10: Number of Aster leafhopper adults testing positive for Aster Yellows by site/field in 2019.

Field location	No. of positive	No. of negative	n	% positive
Alvena	2	25	27	7.40
Casavant	1	1	2	50
Clavet	0	16	16	0
Corman Park	0	2	2	0
Meadowlake	0	1	1	0
Grasswood	0	5	5	0
J. Deere Road	0	6	6	0
Kare	1	4	5	20
Llewellyn	0	12	12	0
Mayerle	0	6	6	0
Melfort	7	188	195	3.59
Outlook	0	94	94	0
Saskatoon	4	90	94	4.25
AAFC Lower Rd	5	94	99	5.05
South Regina	0	2	2	0
Total	**20**	**546**	**576**	**7.24**

Table 11: Aster leafhopper adult samples from 2019. The number of insects collected each month and the percentage of AY-infected insects are provided.

Month	No. of positive	No. of negative	n	% positive
May	1	8	9	11.11
June	10	259	269	3.72
July	9	204	213	4.23
August	0	14	14	0
Unlabelled	0	6	6	0
Total	**20**	**556**	**576**	**3.47**

In 2020, aster leafhoppers were sampled in two alfalfa plots, field margins with grass, and forage grasses (the only green foliage at the end of May and beginning of June at the AAFC Saskatoon farms). Aster leafhoppers were present on the 19th of May, but no AYp positive leafhoppers were found until June 5th (1/10 tested) and June 11th (1/10 tested), which is enough time (17 and 23 days) for acquisition and increase of the AYp in a leafhopper that migrated on or before the 19th of May. It was possible that these leafhoppers acquired the AYp from reservoir plants at the farm, even though plant testing (see below) of two potential reservoir species did not result in any AYp positive reservoir plants of alfalfa or alsike clover. Sweep sampling in the AAFC Saskatoon alfalfa plots during this period (2020) was continued until after harvesting (September 9, 2020) and at this time, 16 adult aster leafhoppers/50 sweeps were found to be still active in alfalfa.

In 2021, aster leafhoppers from 14 sites in Saskatchewan were collected on three early season sample dates (May 22, June 1, and June 3, 2021). More sample dates and sites were examined during this period (2021), but sweep sample sorting followed by AYp testing has not caught up yet. Of those 32 leafhoppers collected in Saskatchewan, only one tested positive for the presence of AYp. Aster leafhoppers that appeared to be of the same Northward migration into Canada but landed in Carmen, MB, were also tested. 94 aster leafhoppers were collected from 3 sites around Carmen, but were all found to be negative for the presence of AYp. Similarly, leafhoppers that were collected on June 6 at Fork River, MB (n = 4) tested negative. Additional leafhopper samples collected on June 2, 2021 in Morris, MB were further inspected and only one insect was positive for the presence of AYp. In 2021, migratory aster leafhoppers arriving into Western Canada had lower levels of AY infection. Results from sweep samples later in the year are not yet available to know if the second generation of aster leafhoppers had higher levels of AY infection than the migratory generation.

Field survey of common weeds for the presence of aster leafhoppers and AYp - Plant surveys

In late summer, several sentinel sites were surveyed for the presence of AY-symptomatic plants. The reach of the AAFC weed survey (Sharpe and Leeson) in 2020 and 2021 was incorporated into these efforts, allowing the examination of additional AY-symptomatic plants along field margins. At this time, aster leafhoppers had been reproducing in the cereal fields and were not found in great numbers in the field margins. Plants were not inspected for the presence of eggs because, at this time, these insects do not reproduce in Saskatchewan.

Plants that displayed AY symptoms were returned to the Wist/Olivier laboratory for molecular testing for the presence of AYp and any of these species that were perennial plants and not already on our list were added to check for AYp the next spring. In addition, canola plants that exhibited AY symptoms from the Saskatchewan Ministry of Agriculture AY survey were tested and the prevalence of AYp strains in infected plants was determined. RFLP sequences have not been returned yet from the AY positive plants collected in 2021, so these results will not be included in this report.

In 2018, plants that exhibited symptoms later in the growing season were tested and few of them were found to be AY-symptomatic plants. One stinkweed (field pennycress) (*Thlaspi arvense*, Brassicaceae) from the AAFC Lowe Road farm looked symptomatic for AY and tested positive for the presence of AYp.

In 2019, alfalfa (*Medicago sativa*, Fabaceae) stands were evaluated as potential reservoirs for AYp. As a perennial plant, the AYp infection could return to new growth in the spring after overwintering in the roots. A stand of alfalfa that had been weakening over time was terminated in 2018 in Southern Alberta near Picture Butte. The field was replanted to canola in 2019 and many canola plants in mid-Aug (16th) in this field showed low-level AY infection symptoms. 15 of 17 (88%) of these plants tested positive for AYp (16S PCR). The alfalfa plants that had regrown as weeds in this field exhibited yellowing and all were positive for AYp by 16S PCR when tested (n=10) . This result prompted us to test the alfalfa plot at the AAFC Lowe Road farm where n=10 plants (roots, shoots and leaves were tested) were positive for AY.p These results prompted a colleague in Quebec to send us two yellowed alfalfa plants and both of these were positive for AYp. From these alfalfa plants, sections taken from four locations were tested (leaf, shoot, root and flowers tissue for a maximum sample of four/plant) and in the majority of the plants, all of the locations tested positive for AYp presence.

In 2020, several asymptomatic alfalfa (n=6) and clover (n=6) plants were tested from early June (4-5th) from the AAFC Lowe Road farm and the field margins of a nearby field, but all of these samples were negative. By the end of the season in 2020, AAFC staff were allowed to survey on day trips and alfalfa plants from 14 sites were collected, all of which tested negative (3 plants/site). These results, together with the low number of aster leafhoppers collected in 2019, are of special interest, as they suggest less potential for vectoring of AYp into plants in the fall that would then bridge into 2020.

In 2020 and 2021, canola plants exhibiting AY symptoms were sent to the Wist/Olivier AAFC laboratory facilities for further testing. These samples were collected as part of the efforts of field surveys for this project, during weed surveys, and from the provincial AY survey. In 2020, 24 canola plants from the Provincial Ministry survey were tested. 17 of these 24 symptomatic plants (71%) tested positive for the presence of AYp, with 13 of these 17 plants harboring the "B" strain of AYp (16SrI-B) and the remaining 4 being infected with the "A" strain (16SrI-A). Prior to 2020, samples were tested only for the presence of AYp, yet the strain was not identified. Noncrop samples from late August 2020 that appeared AY-symptomatic included: scentless chamomile

(*Tripleurospermum inodorum* (Asteraceae) (n=1; AYp positive, "A" strain), sowthistle (*Sonchus arvensis*, Asteraceae) (n=1, AY positive, but strain not determined), stinkweed (*Thlaspi arvense*, Brassicaceae) (n=1, AYp positive, "B" strain), foxtail barley (*Hordeum jubatum*, Poaceae) (n=1, AYp negative), kochia (*Bassia scoparia*, Amaranthaceae) (n=1, AYp negative), and prickly lettuce (Lactuca serriola, Asteraceae) (n=1, AYp negative). Interestingly, samples from a green ash tree (*Fraxinus pennsylvanica*, Oleacea) that had yellowed leaves were tested and were AYp positive ("A" strain), suggesting that not all of the yellow symptoms in ash trees are caused by the ash yellows phytoplasma.

In 2021, 123 plants from 26 locations were tested for the presence of AYp so far. Of these plants, 27 were positive by fluorometric LAMP and are in the process of confirming the phytoplasma strain through 16S PCR followed by sequencing. In the late summer, plants with AY symptoms that were tested included fleabane (*Erigeron canadensis*, Asteraceae) (AYp positive), cleavers (*Galium aparine*, Rubiaceae) (n=2, both AYp positive), flax (*Linum usitatissimum*, Linaceae) (n=8, 5 of the 8 plants were AYp positive), and buckwheat (*Fagopyrum esculentum*, Polygonaceae) (n=4, 2 of the 4 plants were AYp positive. From 32 sites in the spring 2021, potential perennial and biennial reservoir plants were collected based on the criteria that they had been confirmed as AYp hosts before, that the plant had the potential to bridge one growing season to the next (hence, perennial and biennial species), and that they were common in the prairie landscape. Plants selected included: alfalfa (*M. sativa*), common dandelion (*Taraxacum officinale*, Asteraceae), perennial (likely) sowthistle (first to emerge in spring) (*S. arvensis*), and stinkweed (*T. arvense*). Samples from these plants were collected as the arrival of the 2021 aster leafhopper spring migrants was detected (prior to May long weekend, May 18-19, 2021). Another set of samples was collected but not tested later in June (June 2-3, 2021), to ensure that any AY infections resulted from overwintering of the AYp in the plant itself and not from spring infection by migrating leafhoppers. Searching for eggs on leaves with a hand lens did not reveal any aster leafhopper eggs on the studied plants, potentially because leafhoppers had only just started migrating, and microscopic examination of all of the plant tissue in the lab would have been prohibitive. In each site, 10 plants of each weedy species were collected. Samples were mostly taken from fields margins (ditches), in which the dominant vegetation was alfalfa/brome grass, with stinkweed, sowthistle, and dandelion often present were soil was disturbed (stinkweed, dandelion) or everywhere (dandelion). Given the amount of samples to process at this point and limited resources (personnel, budget, and ability to test), 3 of the 10 plants per plant species per site were selected for further testing. With one full time technician and an FSWEP student helper working in winter 2021, the research team's personnel situation was better than during 2020. Results from plants collected in spring and summer 2021 were further delayed by several molecular biological setbacks and only partial results are provided here. Initial tests on the plants failed to extract DNA (suspected expiration issue with the DNAEasy extraction kit), but subsequent extraction worked well and we achieved results with the fluorometric LAMP (Jan 13, 2021). Subsequent 16S PCR to confirm results and sequence the AYp strain were unsuccessful and this will be continued after the end date of the final report. Results from the LAMP tests show that: from the first sample date (May 18-19, 2021), plants in all of the four species collected from sites within a two hour drive from Saskatoon tested positive for AYp; from five of the 14 sites, seven alfalfa plants tested positive for AYp (n=42 plants in total, 17% AYp-infected); of the 30 dandelions collected, five tested positive for AYp (n=30 plants in total, 16% AY-infected); of 15 sowthistle plants collected, one tested positive for AYp (n=15 plants total, 7% AYp-infected); and of 12 stinkweed plants collected, two tested positive for AYp (n=12 plants in total, 17% AY-infected).

Field survey of common weeds for the presence of aster leafhoppers and AYp - Overall results and observations

Several of the plants that were tested as potential AYp reservoirs have similar life histories: stinkweed, scentless chamomile, and sowthistle all germinate into rosette stage plants in the fall and then flower the following season, which makes these a potential green bridge plants for AY. Detection of the presence of AYp in some of these plants prior to the arrival of aster leafhopper migrants suggests that these plant species are acting as AY reservoirs in the Canadian Prairies. Interestingly, many of these plant species are in the Asteraceae family, which is fitting for a pathogen named 'Aster yellows' that gets its name from causing disease in Asteraceous plants. Stinkweed, as the only Brassicaceae member on this list, is an interesting plant as a disease reservoir and "green bridge" between seasons. It is considered an "annual" plant, but it germinates in the fall, grows into a small, green rosette in field margins and is some of the last green vegetation in the fall in Saskatchewan. The plant then completes the flowering cycle the following year and is likely hosting the AYp over the winter. Scentless chamomile also has the capacity of a similar lifestyle where it can be a winter annual, germinating in the fall and re-growing the following spring but it was far less common than stinkweed in our searches. Annual and perennial sowthistle follow a similar overwintering pattern, with a rosette in the fall followed by new growth in the spring. The most easily found plant reservoir, however, was alfalfa, which can be found in most ditches in Western Canada along with brome or other forage grasses. AYp does not replicate well in grasses and cereal crops (see above and Bahar et al. 2018) and are a fairly dead-end host for this pathogen. Similar results were observed above in the report (see ***Disease development*** results), as low AY titers were reported for barley. For this reason, we focused mainly on dicotyledonous plants. We successfully started a colony of aster leafhoppers on brome grass in the laboratory, indicating that this plant species is a viable food and reproductive host for the main AYp vector. This is also the case for fleabane, as a colony of aster leafhoppers on this plant species was successfully started in the Prager Lab in 2019.

The last generation of aster leafhoppers do not seem to attempt to migrate back south and are found in green plant patches in the early fall well after the main crops on the AAFC farms are harvested. For example, aster leafhopper adults were collected in alfalfa and other green patches such as wheat planted as green manure at the AAFC Research. Any AYp that this generation might be carrying then, can be transmitted into alfalfa or the aforementioned asteraceaen and brassicaceaen weeds, travel to the roots and return to be detectable and likely transmissible through aster leafhoppers in the spring. If the migratory aster leafhoppers arrive in the spring around the May long weekend with no crop plants available as food and reproductive hosts, these leafhoppers could be acquiring AYp from these reservoir plants around and in the fields and then moving the infection into crops. The small percentage of infected aster leafhoppers in the spring migrants, however, also suggests that a small percentage of the migratory leafhoppers are coming from locations in the South and bringing AYp into the province, indicating the possibility of two origins (migrant aster leafhopper populations and local reservoirs) for the AY infections in canola each year.

10. Conclusions and Recommendations: *Highlight significant conclusions based on the findings of this project, with emphasis on the project objectives specified above. Provide recommendations for the application and adoption of the project findings. (**Maximum of 500 words)***

To briefly summarize our results, we can conclude that:

a) When aster leafhoppers were exposed to one plant species, cereals and most noncrops were among the most suitable hosts for oviposition and development.
b) Unlike cereals and most noncrops, canola and sowthistle were unsuitable hosts for aster leafhopper oviposition and development.
c) In each plant species, AY-uninfected and AY-infected aster leafhoppers developed similarly, suggesting that AYp infection does not affect aster leafhopper development.
d) AY-uninfected aster leafhoppers were able to distinguish between a crop and a noncrop, exhibiting a preference for settling on the crops over the noncrops. However, this was not always the case when examining stylet sheath structures and egg counts, as numbers of stylet sheaths and eggs were similar between the crops and the noncrops.
e) In most crop-crop and some noncrop-noncrop combinations, distinctions between plant species became less clear, as similar numbers of AY-uninfected Aster leafhoppers were observed on both plant species.
f) Differences between AY-uninfected and AY-infected insect groups were observed in some cases, yet there was not a clear trend in these behavioural shifts.
g) Dandelion, sowthistle, and wheat are less susceptible to AY infection than *A. thaliana*, barley, and canola.
h) Dandelion, sowthistle, alfalfa, and stinkweed from field margins, cropping areas and established plots all had detectable levels of AYp in some of the plants from each species tested in the early spring . These plants were collected before aster leafhoppers could have brought the AYp into Saskatchewan and infected plants so these perennial and biennial plants are likely acting as AYp reservoirs and bridging AYp through the winter.

11. Is there a need to conduct follow up research?

Considering that canola is:

a) a less suitable host for leafhopper development (**no-choice bioassays**),

b) can be used for settling, probing, and/or ovipositing in the same manner as more suitable hosts when presented together with another plant species (**two-choice bioassays**), and

c) is more susceptible to AY infection than other plant species under study (**disease development assays**),

it would be recommended to examine and characterize the specific feeding behavior of Aster leafhoppers on canola and other selected plant species, as differences in the proportion of AY-infected plants and development of symptoms could potentially be related to the frequency and duration of salivation and/or phloem ingestion events. Such work has been funded by CARP and is currently ongoing (CARP 2021.27).

Furthermore, examining the gut content of leafhoppers collected in the field and ditches would provide additional information about the composition of their diet in the Canadian Prairies, allowing us to identify potential feeding hosts before and after crop emergence and potential plant species that could become infected with AY. This assay was tested in a pilot study with good results.

d) The dynamics of the alfalfa and other plants as reservoirs should be further investigated through lab experiments to test if the AYp can be vectored into and then out of these plant species by aster leafhoppers and into canola.

12. Patents/ IP generated/ commercialized products:

This project did not result in any commercialized product.

13. List technology transfer activities: *Include presentations to conferences, producer groups or articles published in science journals or other magazines*.

Romero B, Olivier C, Wist T, Prager SM (2020) Oviposition Behavior and Development of Aster Leafhoppers (Hemiptera: Cicadellidae) on Selected Host Plants From the Canadian Prairies. J Econ Entomol 113:2695–2704. doi: https://doi.org/10.1093/jee/toaa243

Romero, B., Olivier, C., Wist, T., and Prager, S. M. Do options matter? Settling behavior, stylet sheath counts, and oviposition of Aster leafhoppers (Hemiptera: Cicadellidae) in two-choice bioassays. Environmental Entomology. *Submitted on October 21, 2021. Accepted on December 27, 2021.*

Romero, B. and Prager, S. M. *Host-preference and performance in a plant pathosystem*. Oral presentation at the ESC, CSEE, and AES 2019 Joint Meeting. Fredericton, New Brunswick. August 18 to August 21, 2019.

Romero, B. and Prager, S. M. *Of leafhoppers and phytoplasmas: host-choice behaviour of Macrosteles quadrilineatus in the Canadian Prairies*. Oral presentation at the ESS and ESA 2019 Joint Meeting. Elkwater, Alberta. October 3 to October 5, 2019.

Romero, B., Wist, T., Olivier, C., and Prager, S. M. *Investigating the role of plant hosts in the outbreak of the aster leafhopper-vectored aster yellows*. Report presented at the Western Committee on Crop Pests Annual Meeting. Kelowna, British Columbia. October 24, 2019.

Romero, B., Wist, T., Olivier, C., and Prager, S. M. *Aster Yellows: effects of phytoplasmas on Aster leafhoppers´ development and preference*. Oral presentation at Soils and Crops 2020, Saskatoon, Saskatchewan. March 10, 2020.

Romero, B., Wist, T., Olivier, C., and Prager, S. M. *Aster Yellows: insights into Aster leafhoppers´ behavior and development in the Canadian Prairies.* Oral presentation at the 36th Annual Plant Sciences Graduate Students´ Symposium, Saskatoon, Saskatchewan. March 14-15, 2020. *This event was cancelled due to the global coronavirus situation.*

Romero, B. and Prager, S. M. *A tale of leafhoppers, Aster Yellows, and crops in the Canadian Prairies.* Oral presentation at the XXVI International Congress of Entomology, Helsinki, Finland. July 19-24, 2020. *This event has been postponed due to the global coronavirus situation.* New dates: July 17-22, 2022.

Romero, B., Olivier, C., Wist, T., and Prager, S. M. *Evaluating aster leafhopper performance and preference on a variety of crop and non-crop host plants*. Oral presentation at the ESBC Annual General Meeting and Symposium. Virtual meeting. October 26-28, 2020.

Romero, B., Wist, T., Olivier, C., and Prager, S. M. *Examining crop and non-crop species from the Canadian Prairies as reproductive and food hosts for aster leafhoppers*. Report presented at the Western Committee on Crop Pests Annual Meeting. Virtual meeting. October 29-30, 2020.

Romero, B., Olivier, C., Wist, T., and Prager, S. M. *Do´s and don´ts in the Canadian Prairies: a study about oviposition and host choice selection behavior of Aster leafhoppers*. Oral presentation at the Fall Meeting of the Entomological Society of Saskatchewan. Virtual Meeting. November 20, 2020.

Romero, B., Olivier, C., Wist, T., and Prager, S. M. *One or too many choices? Oviposition, development, and settling of Aster leafhoppers on a variety of plant hosts*. Oral presentation at the 1st International Electronic Conference of Entomology (IECE). Virtual meeting. July 1-15, 2021. – *Withdrawn*

Romero, B., Olivier, C., Wist, T., and Prager, S. M. *One or too many choices? Oviposition, development, and settling of Aster leafhoppers on a variety of plant hosts*. Oral presentation at the ESC/ESO 2021 JAM. Virtual meeting. November 15-18, 2021

Romero, B., Olivier, C., Wist, T., and Prager, S. M. *What no-choice and two-bioassays can tell us about Aster leafhoppers´ host choice selection behavior*. Oral presentation at the ESS Fall Meeting. Virtual meeting. December 3, 2021.

Romero, B., Olivier, C., Wist, T., and Prager, S. M. *Aster Yellows: symptom expression and disease reservoirs*. Oral presentation at Soils and Crops 2022. Virtual meeting. March 8-9, 2022.

14. List any industry contributions or support received.

- The survey for leafhoppers and plant reservoirs and consumable materials for aster yellows phytoplasma testing of plants and leafhoppers in Objective four in the Wist/Oliver lab was funded through WGRF project AGR 1817 to Dr. Wist as a complementary project to this one.

15. Acknowledgements. *Include actions taken to acknowledge support by the Ministry of Agriculture and the Canada-Saskatchewan Growing Forward 2 bilateral agreement (for projects approved during 2013-2017) or Canadian Agriculture Partnership (For projects approved beyond 2017).*

Support by the Saskatchewan Ministry of Agriculture as well as SaskCanola is gratefully acknowledged with logos in the acknowledgments section of all of the above-mentioned presentations in section 13. We thank our benevolent funding agencies also in the acknowledgments section of the two publications that Ms. Romero has produced from this project.

16. Appendices: *Include any additional materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications, literature cited (Use a consistent reference style throughout).*

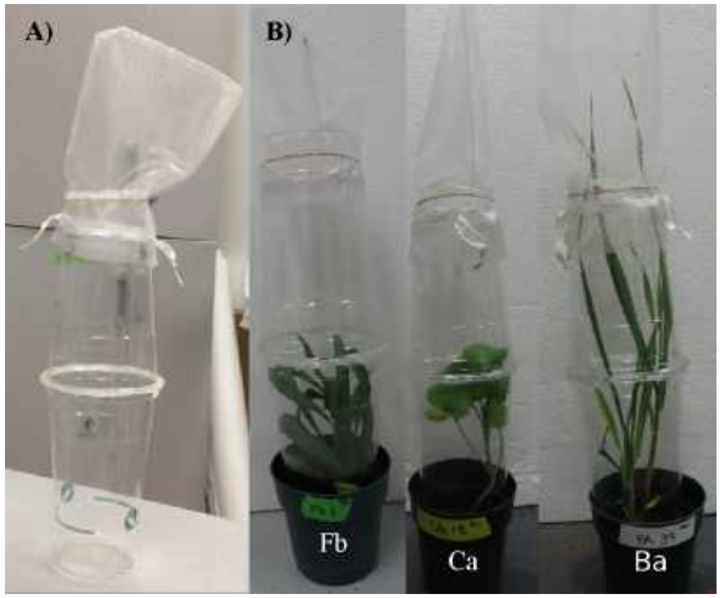

Figure 11: A) Plastic cups used to cage the leafhoppers onto a plant. A rubber band has been used to attach the organza bag around the top of the cups. B) Examples of no-choice bioassays with different plant species. Fb = fleabane, Ca = canola, Ba = barley.

Figure 12: Example of an adult female dissection. The content of the abdomen was exposed using a dissecting needle. Numbers have been used to indicate how many eggs there were and where they can be observed.

Figure 13: A) Example of one two-choice bioassay where marigold and barley were the choice plants. The star indicates the position within the cage where leafhoppers were initially released. B) Aster leafhoppers (white circles) on a canola test plant.

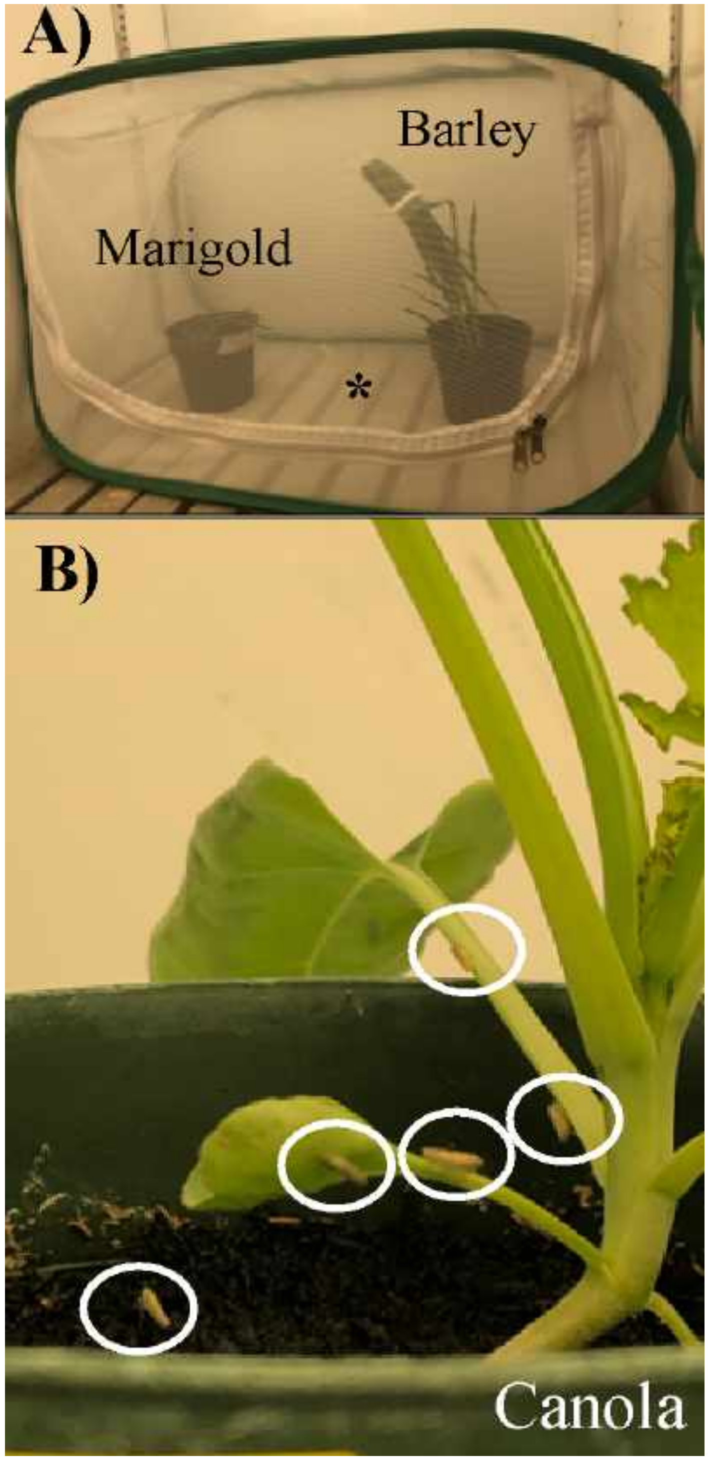

Figure 14: Plant leaves stained with the McBride solution. Black arrows and circles show where stylet sheaths can be observed. An "E" indicates the presence of an egg.

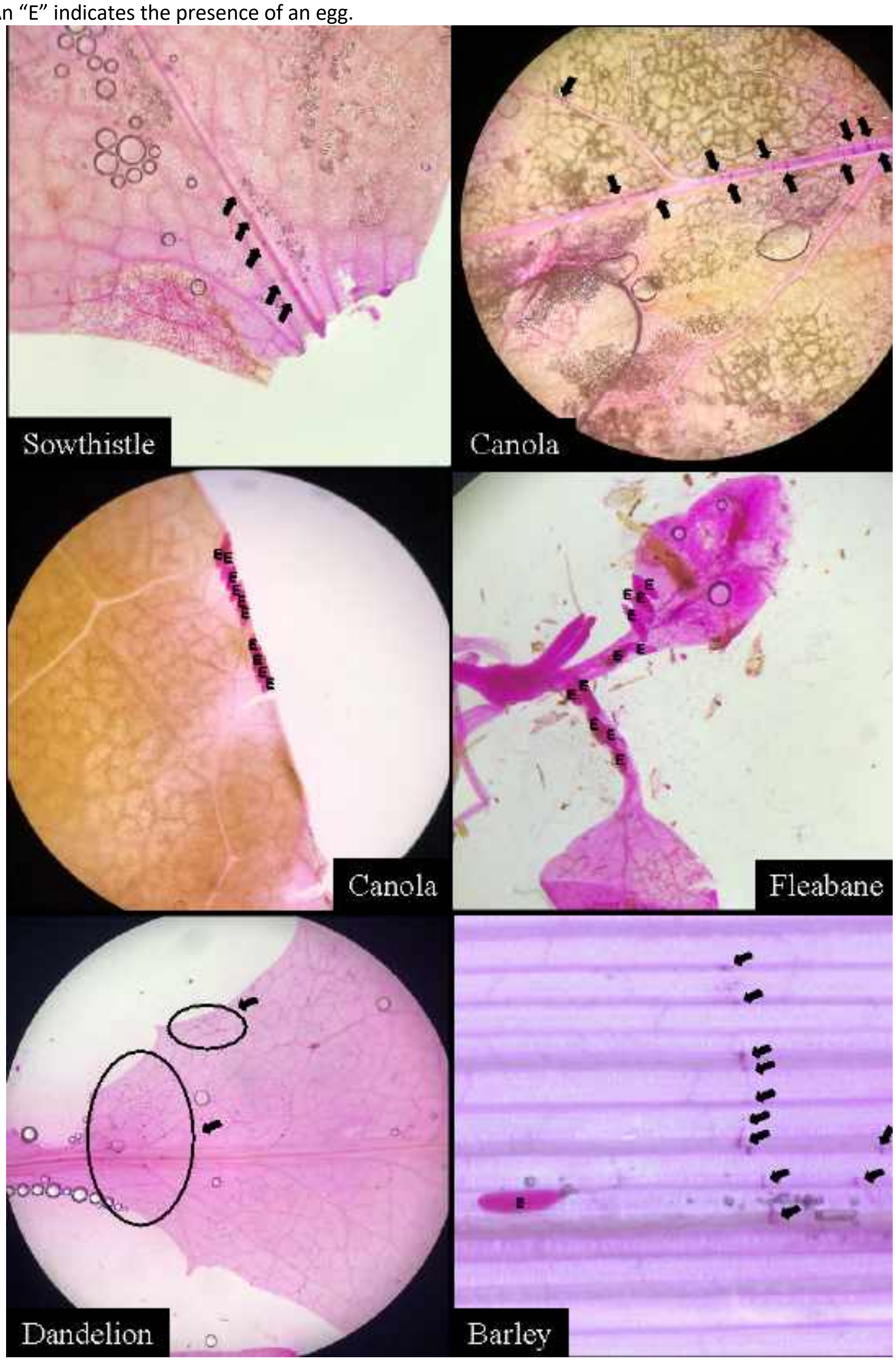

Table 4: Results from two-choice bioassays using AY-uninfected Aster leafhoppers reared on fleabane. The following abbreviations have been used: “Ba” = barley, “Fb” = fleabane, and “O” = oat. For each plant combination, the first plant is referred to as “Plant 1”, while the second plant is “Plant 2”. The average percentages and number of leafhoppers on each plant, the p-values from PERMANOVA analyses, the number of probing events and eggs on each plant, and the p-values from paired-t tests have been provided. “(W)” indicates that residuals were not normally distributed and a Wilcoxon test was used instead. In these cases, refer to Table 5 for median and interquartile range values. A significance level (α-value) of 0.05 was used.

Plant combination Plant 1 – Plant 2	Plant 1 Avg. % of leafhoppers	Plant 2 Avg. % of leafhoppers	Plant 1 Avg. no. of insects	Plant 2 Avg. no. of insects	PERMANOVA p-value (Figure 1)
Ba – Fb	95.1 ± 1.5	4.9 ± 1.5	6.7 ± 0.3	0.4 ± 0.1	**0.001**
O - Fb	94.2 ± 1.3	5.8 ± 1.3	8.6 ± 0.3	0.6 ± 0.1	**0.001**

Table 4 (Continuation): Results from two-choice bioassays using AY-uninfected Aster leafhoppers reared on fleabane. The following abbreviations have been used: “Ba” = barley, “Fb” = fleabane, and “O” = oat. For each plant combination, the first plant is referred to as “Plant 1”, while the second plant is “Plant 2”. The average percentages and number of leafhoppers on each plant, the p-values from PERMANOVA analyses, the number of probing events and eggs on each plant, and the p-values from paired-t tests have been provided. “(W)” indicates that residuals were not normally distributed and a Wilcoxon test was used instead. In these cases, refer to Table 5 for median and interquartile range values. A significance level (α-value) of 0.05 was used.

Plant combination Plant 1 – Plant 2	Plant 1 No. of probing events	Plant 2 No. of probing events	Paired t-test p-value (Fig. 2)	Plant 1 No. of eggs	Plant 2 No. of eggs	Paired t-test p-value (Fig. 3)
Ba – Fb	184.9 ± 50.2	12.0 ± 4.5	(W) **0.003**	5.9 ± 2.6	0.3 ± 0.3	(W) **0.035**
O - Fb	132.4 ± 25.9	24.9 ± 6.3	**0.004**	10.7 ± 3.2	0.6 ± 0.4	(W) **0.021**

Table 5: Results from all two-choice bioassays. The following abbreviations have been used: “Ba” = barley, “Ca” = canola, “O” = oat, “Wh” = wheat, “Da” =dandelion, “Fb” = fleabane, “Ma” = marigold, and “Th” = sowthistle. The “rearing host” indicates the plant species on which Aster leafhoppers had been reared, while the “Insect infection status” refers to the group of insects used in each case (Uninfected or AY-infected). For each plant combination, the first plant is referred to as “Plant 1”, while the second plant is “Plant 2”. The median and interquartile range (IQR) of probing events and eggs on each plant have been provided.

Rearing host	Insect infection status	Plant combination Plant 1 – Plant 2	Plant 1 No. of probing events Median (IQR)	Plant 2 No. of probing events Median (IQR)	Plant 1 No. of eggs Median (IQR)	Plant 2 No. of eggs Median (IQR)
Ba	Uninfected	Ba – Ca	67.0 (59.0 – 86.0)	2.0 (0.0 – 16.0)	17.0 (8.0 – 18.0)	1.0 (0.0 – 7.0)
Ba	Uninfected	Ba – O	70.0 (49.0 – 90.0)	128.0 (54.0 – 150.0)	11.0 (7.0 – 14.0)	45.0 (23.0 – 57.0)
Ba	Uninfected	Ba – Wh	65.0 (23.5 – 143.5)	7.0 (3.0 – 29.0)	7.0 (4.0 – 28.5)	0.0 (0.0 – 31.0)
Ba	Uninfected	Ba – Da	63.0 (58.2 – 138.2)	5.0 (2.2 – 6.0)	31.0 (18.0 – 41.5)	0.0 (0.0 – 0.0)
Ba	Uninfected	Ba – Fb	91.0 (49.0 – 105.0)	38.0 (37.0 – 51.0)	3.0 (1.0 – 5.0)	0.0 (0.0 – 1.0)
Ba	Uninfected	Ba – Ma	52.5 (10.2 – 85.7)	0.0 (0.0 – 3.7)	20.5 (13.5 – 21.7)	0.0 (0.0 – 1.0)
Ba	Uninfected	Ba - Th	125.0 (101.5 – 258.5)	140.0 (43.0 – 203.0)	20.0 (15.5 – 23.5)	1.0 (0.5 – 1.5)
Ba	Uninfected	Ca – O	5.0 (0.0 – 23.0)	363.0 (274.5 – 577.0)	1.0 (0.0 – 3.5)	17.0 (13.0 – 21.0)
Ba	Uninfected	Ca – Wh	30.5 (6.2 – 64.7)	370.5 (124.7 – 572.0)	3.5 (1.0 – 5.7)	11.5 (8.0 – 19.5)
Ba	Uninfected	Ca – Da	66.5 (57.2 – 122.7)	215.0 (110.7 – 313.7)	18.5 (12.7 – 31.5)	2.0 (1.0 – 6.5)
Ba	Uninfected	Ca – Fb	8.5 (0.0 – 46.7)	58.5 (33.2 – 86.5)	3.0 (0.0 – 13.2)	0.5 (0.0 – 3.7)
Ba	Uninfected	Ca – Ma	27.5 (1.7 – 80.5)	31.0 (18.5 – 57.7)	3.0 (0.2 – 12.2)	1.5 (0.0 – 8.5)
Ba	Uninfected	Ca – Th	0.0 (0.0 – 4.5)	17.0 (10.2 – 34.5)	1.0 (0.0 – 2.70.5)	0.5 (0.0 – 1.0)
Ba	Uninfected	O – Wh	33.0 (3.2 – 58.0)	43.0 (26.5 – 53.7)	11.0 (6.5 – 32.2)	22.0 (10.2 – 27.0)
Ba	Uninfected	O – Da	309.5 (212.5 – 575.2)	136.5 (31.0 – 240.2)	5.5 (2.0 – 26.5)	0.0 (0.0 – 0.7)
Ba	Uninfected	O – Fb	580.5 (413.5 – 756.2)	112.5 (80.5 – 221.0)	21.0 (15.5 – 40.0)	2.0 (0.2 – 5.5)
Ba	Uninfected	O – Ma	254.5 (53.5 – 421.5)	54.0 (42.5 – 69.5)	27.5 (13.5 – 58.5)	11.0 (10.0 – 24.5)
Ba	Uninfected	O – Th	144.0 (84.0 – 352.0)	531.0 (290.0 – 543.0)	7.0 (2.0 – 13.0)	3.0 (3.0 – 8.0)
Ba	Uninfected	Wh – Da	239.5 (134.7 – 400.2)	40.5 (6.7 – 140.7)	24.0 (19.7 – 53.5)	0.0 (0.0 – 0.0)
Ba	Uninfected	Wh – Fb	157.5 (51.0 – 214.0)	8.5 (5.2 – 15.7)	31.5 (31.0 – 51.7)	1.5 (0.0 – 3.0)
Ba	Uninfected	Wh – Ma	271.5 (93.0 – 445.7)	29.5 (2.5 – 58.7)	22.0 (16.7 – 38.0)	4.0 (0.0 – 9.7)
Ba	Uninfected	Wh – Th	45.0 (28.0 – 165.0)	265.0 (118.0 – 321.0)	12.0 (10.0 – 18.0)	0.0 (0.0 – 1.0)
Ba	Uninfected	Da – Fb	254.0 (177.7 – 319.0)	58.0 (45.5 – 95.2)	2.5 (1.0 – 4.7)	3.0 (1.0 – 5.5)
Ba	Uninfected	Da – Ma	224.0 (155.7 – 264.2)	50.0 (25.2 – 72.2)	3.5 (1.2 – 6.5)	18.5 (6.7 – 30.0)
Ba	Uninfected	Da – Th	292.0 (205.2 – 412.0)	306.0 (238.0 – 424.2)	2.0 (0.5 – 3.7)	2.0 (0.2 – 3.0)
Ba	Uninfected	Fb – Ma	119.0 (78.0 – 187.5)	39.0 (24.7 – 54.7)	0.5 (0.0 – 1.7)	7.5 (5.2 – 13.2)
Ba	Uninfected	Fb – Th	51.0 (18.5 – 105.0)	137.0 (122.0 – 182.0)	1.0 (0.0 – 7.0)	0.0 (0.0 – 4.5)
Ba	Uninfected	Ma - Th	0.0 (0.0 – 5.0)	212.0 (190.0 – 356.0)	0.5 (0.0 – 1.0)	1.0 (1.0 – 2.5)
Fb	Uninfected	Ba – Fb	118.0 (67.5 – 265.2)	7.0 (0.0 – 20.2)	3.5 (1.0 – 5.7)	0.0 (0.0 – 0.0)
Fb	Uninfected	O - Fb	113.0 (87.5 – 171.5)	28 (5.2 – 38.5)	8.5 (2.5 – 15.0)	0.0 (0.0 – 0.7)
Ba	AY-infected	Ba – Ca	203 (70.0 – 316.7)	6.5 (2.5 – 9.5)	13.0 (7.0 – 15.7)	1.0 (2.5 – 9-5)
Ba	AY-infected	Ba – Wh	111.0 (54.0 – 163.0)	82.0 (76.0 – 100.0)	34.0 (24.0 – 61.0)	8.0 (8.0 – 19.0)
Ba	AY-infected	Ba – Da	114.5 (81.7 – 212.0)	60.5 (28.0 – 144.0)	12.0 (6.5 – 28.0)	1.0 (0.0 – 3.7)
Ba	AY-infected	Ca – Wh	0.0 (0.0 – 27.7)	269.5 (203.5 – 340.0)	0.5 (0 – 4.7)	19.0 (6.5 – 24.7)
Ba	AY-infected	Ca – Da	0.0 (0.0 – 3.25)	105.5 (65.0 – 186.0)	0.0 (0.0 – 1.5)	0.0 (0.0 – 1.0)
Ba	AY-infected	Wh – Da	221.0 (154.7 – 373.0)	75.5 (73.3 – 89.7)	7.5 (2.3 – 9.7)	0.0 (0.0 – 0.0)