Do options matter? Settling behavior, stylet sheath counts, and oviposition of Aster leafhoppers (Hemiptera: Cicadellidae) in two-choice bioassays

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Abstract

Polyphagous insects are characterized by a broad diet comprising plant species from different taxonomic groups. Within these insects, migratory species are of particular interest, given that they encounter unpredictable environments, with abrupt spatial and temporal changes in plant availability and density. Aster leafhoppers (Hemiptera: Cicadellidae: *Macrosteles quadrilineatus* Forbes) arrive in the Canadian Prairies in spring and early summer and are the main vector of a prokaryotic plant pathogen known as Aster Yellows Phytoplasma (AYp). Host choice selection behaviour of 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. To assess possible

effects due to insect infection, two-choice bioassays were repeated using leafhoppers infected with AYp and a subset of plant species. 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-wild plant bioassays, 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.

Keywords

Leafhoppers, phytoplasmas, two-choice tests, stylet sheath, oviposition, preference-performance hypothesis

Introduction

Some of the most long-standing questions in insect ecology are associated with mechanisms of an insect´s choice of plant for herbivory or oviposition (Jaenike 1978, Miller and Strickler 1984, Jaenike 1990). Why some insects are polyphagous or generalists, feeding on a wide variety of plant species not necessarily from the same taxonomic group (Schoonhoven 2005), while others use one or just a few plant species, remains one of the key questions regarding host choice selection behavior. In polyphagous insects, we can additionally examine aspects associated to the acceptance and/or rejection of plant species and what sets the limit of such differences between them. Often, these matters are addressed and treated as either a practical problem, in which case the plants of interest are generally domesticated plant species, typically referred to as "crops" (Romero et al. 2019, Prager et al. 2014a), or as an ecological question, in which case the plant-insect pairing may not involve any domesticated plant species at all. However, domesticated plant species are often bred for traits other than insect resistance, while wild plant species may have been under severe selection leading to the evolution of traits that protect them from herbivory (Whitehead et al. 2017). Thus, comparing the interactions between one insect species and both domesticated and wild plant species may be an especially useful tool in establishing plant traits that influence insect host choice behaviors. This is especially the case when the insect is a polyphagous migratory insect reaching the limits of their migration, as they might not have their favorite host plants available depending upon the timing of migration. In these instances, suitable but not preferred plants may be chosen for feeding and even reproduction, until preferred host plants become available. In recent studies on gut content analysis of sucking-piercing insects, it has been shown that this might be the case for some insect species and that plant species other than common hosts can be incorporated into the diet until crop hosts become available (Cooper et al.

2016, Cooper et al. 2019, Barthel et al. 2020).

The relationship between a polyphagous insect and plant acceptance has been explained by multiple theories, incorporating aspects such as the availability of more suitable host plants, the presence of deterrent and/or defensive chemical compounds, the presence of enemies, and competition with conspecifics for the same resources (Jaenike 1978, Berdegue et al. 1996, Rosenthal and Dirzo 1997, Gratton and Welter 1999, Kaplan and Denno 2007). In the "enemy free space" theory, for example, novel plant species can be incorporated into an insect herbivores' diet if natural enemy species do not co-occur on those plant species (Berdegue et al. 1996, Gratton and Welter 1999). Other explanations focus on the relationship between adult female choices and offspring fitness. The "mother knows best" principle or "preference-performance hypothesis" proposes that female adults will choose reproductive hosts that will be more suitable for the development of their offspring (Jaenike 1978, Valladares and Lawton 1991, Johnson et al. 2006), whereas in the "optimal bad motherhood" scenario (Scheirs et al. 2000, Mayhew 2001), adult females will select plant hosts that maximize their own survival. Some explanations as to why there might be little to no association between female preference and offspring fitness include low genetic variability (Futuyma et al. 2005), the evolutionary history of the insect-plant interaction, and limitations in sensory structures and/or information processing (Bernays 1991, Bernays 1999). These hypotheses are particularly interesting in the context of insect migration, as the uncertainty and spatiotemporal changes of plant availability and abundance in the environment pose an additional level of complexity. For example, when weeds or natural habitat are embedded into a cropping matrix.

Over 1000 species of leafhoppers occur in Canada (Maw et al. 2000, Saguez et al. 2014), yet only approximately 30 of them are confirmed as vectors of phytoplasmas (Olivier et al. 2009).

Phytoplasmas are fastidious plant pathogenic microorganisms, closely related to gram-positive bacteria (Hogenhout and Musić 2010). Most phytoplasma vectors are in the families Cicadellidae, Psyllidae, Derbidae, and Delphacidae (Weintraub and Beanland 2006), with some Miridae and Pentatomidae also capable of transmitting these microorganisms (Olivier et al. 2009). In the Canadian Prairies, Aster leafhoppers (*Macrosteles quadrilineatus* Forbes) (Hemiptera: Cicadellidae) are the primary vector of phytoplasma subgroup 16SrI (AYp; *Candidatus* Phytoplasma asteris) (Olivier et al. 2009), which can affect approx. 200 plant species (Alberta Agriculture and Forestry 2014). In most years, Aster Yellows (AY) incidence and associated yield losses are minimal yet outbreaks of this disease in canola fields have been documented in 2001, 2007, and 2012 in Canada (Alberta Agriculture and Forestry 2014). While an extensive study of the host range and plant use of Aster leafhoppers in the Canadian Prairies exists (Romero et al. 2020), no complementary studies examining host preferences for these species have been performed. Aster leafhoppers are migratory and arrive on wind currents originating in the southern United States (Nichiporik 1965, Olivier et al. 2009). It is unclear, though, if upon arrival this insect species has preferred host plants for settling and oviposition or whether insect infection with AYp can influence these preferences.

In this study, we examined the settling behavior of Aster leafhopper adults using a two-choice bioassay approach and multiple domesticated and perennial wild plant species commonly found in the Canadian Prairies. These experiments provide complementary information to a previous characterization of the plant species under study by Romero et al. (2020) and allow for a better understanding of Aster leafhoppers´ host choice selection behavior in more complex environments where more than one plant species is available.

Materials and Methods

1.1.Plant species and growing conditions

All plants for these experiments were grown according to procedures described by Romero et al. (2020). Plants were watered every three days, with the addition of a 20-20-20 water-soluble fertilizer each time. After germination, additional seedlings were manually removed to ensure that each pot contained only one plant. The following plant species were used for this study: spring wheat (*Triticum aestivum* Linnaeus; cultivar AAC Brandon) (Poales: Poaceae), oat (*Avena sativa* Linnaeus; cultivar CS Candem) (Poales: Poaceae), barley (*Hordeum vulgare* Linnaeus; cultivar CDC Copeland) (Poales: Poaceae), canola (*Brassica napus* Linnaeus; cultivar AC Excel) (Brassicales: Brassicaceae), spiny annual sowthistle (*Sonchus asper* (L.) Hill) (Asterales: Asteraceae), dandelion (*Taraxacum officinale* (L.) Webber ex F.H. Wigg) (Asterales: Asteraceae), fleabane (*Erigeron annuus* (L.) Pers.) (Asterales: Asteraceae), and marigold (*Tagetes* sp. Linnaeus) (Asterales: Asteraceae). Non cultivated plant seeds were initially collected from fields surrounding Saskatoon, SK and grown under laboratory conditions. Plants from each plant combination were sown on the same day and used for two-choice bioassays after 30 days.

Plant selection was based on previous observations by Romero et al. (2020). Cereals such as oat and wheat had been described as preferred hosts for Aster leafhopper populations (Meade and Peterson 1962) and similar observations were made by Romero et al. (2020). Selected wild plant species included several members of the Asteraceae family, some of which were equally suitable to cereals for leafhopper reproduction and development (Romero et al. 2020).

1.2.Aster leafhoppers

Aster leafhoppers were reared as previously described by Romero et al. (2020). Colonies were maintained under an 18-hour photoperiod, at 21ºC during the day and 17ºC during the night. Barley was used as food and reproductive host and plants were changed on a weekly basis. At any given time, more than one cohort and generation were present in the colonies. To maintain AYp infection within AY-infected colonies, periwinkle (*Catharanthus roseus* (Gentianales: Apocynaceae)) plants were added to supplement barley. Periwinkle can be infected with AYp without any plant mortality and AY leafhoppers can readily acquire AYp from infected periwinkle plants. AY-uninfected colonies were reared on barley without the addition of AYp–infected periwinkle plants. Colonies were physically separated to prevent cross infection of AY and were periodically tested for AYp infection using nested Polymerase Chain Reaction (PCR). The phytoplasma 16S rRNA sequence gene was amplified using primers P1/P7 (Schneider et al., 1995) in the first round and primers R16F2n/R16R2 (Gundersen and Lee, 1996) in the second round of nested PCR. Only adult aster leafhoppers were used for the experiments.

1.3.Two-choice bioassays

To conduct two-choice bioassays, Aster leafhoppers were sorted into groups of 10 males and 10 females based on external genitalia, using similar procedures to those described by Romero et al. 2020. All 20 leafhoppers were then released in the middle of a choice cage containing two test plants and allowed to acclimate for 24 h. Following the acclimation period, leafhoppers´ positions (plant 1, plant 2, or off the plant) were determined each day, for a total of 96 hrs (Supp. Fig 1). Ten replicates were conducted for each plant combination, making observations between 9 and 11 am each day. Bioassays were conducted under similar light and temperature conditions to those for Aster leafhopper rearing.

Given that barley was used as a food and rearing host for colonies and also included as part of the two-choice bioassays, this could have represented a confounding factor. For this reason, we selected a subset of all plant species and repeated the two-choice bioassays using leafhoppers from a colony reared exclusively on fleabane for a minimum of four generations. Methods for these bioassays were identical as described above. The first generation of fleabane was field-collected during the summer of 2018, while later generations were obtained by continuous inbreeding under laboratory conditions.

To examine whether infection of leafhoppers with AYp would affect the insects' settling behavior and additional variables under examination, two-choice bioassays were also conducted using AY-infected leafhoppers and a subset of the plant species (barley, canola, bread wheat, and dandelion). Methods for these bioassays were otherwise identical as described above. To avoid potential confounding issues of generation and seasonality, bioassays with AY-uninfected and those with AY-infected leafhoppers were randomized rather than being performed sequentially.

1.4.Two-choice arenas

All bioassays were conducted in cages with the following dimensions: 34.29 cm x 34.29 cm x 60.96 cm (13.5 in x 13.5 in x 24 in) (BioQuip Products, California, USA). Cages had five mesh sides, which promoted air flow, and a clear vinyl window, which was placed face up to ensure that plants received correct amounts of light and to facilitate observations during the bioassays (Supp. Fig 1).

1.5. Quantification of stylet sheaths and oviposition

Following the bioassays, plants were retained in order to count the number of stylet sheaths and eggs. To facilitate counting, leaves were removed and stained. Given that leafhoppers had access to the entire shoot system of each plant species, yet were observed on leaf structures only, all blades from monocot species and leaves and petioles from dicot species were collected for staining. Stylet sheaths are structures produced by sucking-piercing insects during the early stages of probing activity which surround their mouthparts and provide mechanical stability and lubrication (Almeida and Backus 2004, Morgan et al. 2013, Will and Vilcinskas 2015). Staining methods followed those of Backus et al. (1988) with a few modifications. Two solutions were used: the McBride´s stain, consisting of a mixture of 95% ethanol and glacial acetic acid (1:1 vol/vol) (Commercial Alcohols and Fisher Chemical, respectively) and 0.2% acid fuchsin (5 ml per 100 ml of the ethanol and glacial acetic acid solution) (Fisher Chemical), and a second clearing agent, which consisted of distilled water, glycerol, and lactic acid (1:1:1 vol/vol/vol) (Fisher Chemical). Cut leaves were placed in glass Petri dishes and covered with the McBride´s stain for 48 hours. After this, they were placed onto paper towel to remove the remaining McBride´s stain and transferred to new Petri dishes containing the clearing agent. Samples were incubated for 4 hours at 75ºC and examined under a compound stereo microscope (Zeiss Stemi 305, Oberkochen, Germany) (Supp. Fig. 2).

1.6. Statistical analysis

Statistical analyses were performed using R version 3.6.2 (R Core Team 2019). In the two-choice bioassays, the number of leafhoppers on each plant was evaluated using the permutational multivariate analyses of variance technique (PERMANOVA) (Oksanen et al. 2019). In this

198 analysis, the plant species was the explanatory variable and the number of leafhoppers recorded
199 on each plant each day was the response variable. Insects recorded as being “off the plant” were
200 excluded from the analysis given that their values were low and consistent across all treatments.
201 Stylet sheath and egg counts were analyzed with a paired t-test for each combination of the plant
202 species being offered during the two-choice bioassay and the insects´ condition (AY-uninfected or
203 AY-infected). When residuals were not normally distributed, the Wilcoxon test was used instead.
204 For each plant species in each combination, the relation between the numbers of probing events
205 and eggs was examined by calculating Spearman´s correlation coefficient and the coefficient´s
206 significance. Additionally, for the subset of plant combinations for which both AY-uninfected and
207 AY-infected had been examined, differences in the total number of stylet sheaths between
208 bioassays with AY-uninfected and AY-infected were analyzed with a Mann-Whitney test for each
209 plant combination. The total number of stylet sheaths corresponds to the sum of stylet sheath
210 counts from each plant species present at each time in the two-choice arena.
211
212 **Results**
213 *Settling behavior of AY-uninfected Aster leafhoppers*
214 Following acclimation to the two-choice arena, we recorded the number of AY-uninfected
215 Aster leafhopper adults on each plant daily, for a total of 96 hrs. In most domesticated-
216 domesticated plant combinations, Aster leafhoppers exhibited no preference in terms of their
217 settling behavior. However, in bioassays where oat or wheat were presented along with canola,
218 leafhoppers preferred to settle on cereals over canola (Fig. 1A and Supp. Table 1). In canola-oat
219 bioassays, for example, while 2.7 ± 0.3 (mean ± SEM) leafhoppers were observed on canola, 8.6
220 ± 0.3 settled on oat. Similarly, in canola-wheat bioassays, 3.5 ± 0.4 leafhoppers were observed on

canola and 6.8 ± 0.5 leafhoppers on wheat. In domesticated-wild plant bioassays, Aster leafhoppers preferred to settle on domesticated plant species, except when oat and marigold were presented together, in which case similar numbers of leafhoppers were observed on both plants (2.2 ± 0.3 on marigold and 3.5 ± 0.2 on oat, mean ± SEM). In wild plant-wild plant bioassays, both preference and non-preference were observed, and this depended on the plant species combination being presented (Fig. 1A and Supp. Table 1). For example, Aster leafhoppers preferred to settle on dandelion over fleabane, but were found in similar numbers on both plants when dandelion and marigold were presented together.

To examine whether the rearing host plant could alter Aster leafhoppers´ settling behavior, a subset of plant species was selected for conducting additional bioassays and tested using Aster leafhoppers reared on fleabane (Supp. Table 3). When presented with barley and fleabane, uninfected Aster leafhoppers exhibited a similar settling behavior to those that had been reared on barley. Specifically, in bioassays with Aster leafhoppers reared on barley, 7.7 ± 0.5 (mean ± SEM) leafhoppers settled on barley and 2.7 ± 0.4 were observed on fleabane (Supp. Table 1). In bioassays with Aster leafhoppers reared on fleabane, those values were 6.7 ± 0.3 and 0.4 ± 0.1, respectively (Supp. Table 3). When oat and fleabane were offered, Aster leafhoppers reared on fleabane preferred to settle on oat and this was also the case for insects reared on barley. In bioassays with Aster leafhoppers reared on barley, 6.2 ± 0.3 leafhoppers were found on oat, while 2.3 ± 0.3 settled on fleabane (Supp. Table 1). In bioassays in which fleabane was the rearing host, 8.6 ± 0.3 Aster leafhoppers settled on oat, while 0.6 ± 0.1 were observed on fleabane (Supp. Table 3).

Stylet sheath counts

Leaves from bioassays performed with AY-uninfected Aster leafhoppers were stained and

stylet sheaths were recorded for each plant species in each plant combination (Fig. 1B and Supp. Table 1). In most domesticated-domesticated combinations, a similar number of stylet sheaths was observed in both plant species presented. However, when canola was presented along with oat or wheat, a higher number of stylet sheaths was observed in the cereals (Supp. Table 1). In some domesticated-wild plant bioassays, stylet sheath counts were similar between both plant species, while in others a higher number of stylet sheaths was observed in one of the plant species (Fig. 1B and Supp. Table 1). For example, when barley and dandelion were presented together, an average of 140.0 ± 23.2 (mean ± SEM) stylet sheaths were counted in barley and 8.8 ± 4.8 in dandelion. However, in barley-fleabane bioassays, stylet sheath counts were similar between both plant species, with 116.8 ± 45.5 stylet sheaths in barley and 42.6 ± 3.9 in fleabane. In most wild plant-wild plant combinations, a higher number of stylet sheaths was observed in one of the plant species, but in other bioassays, no differences were observed in the number of stylet sheaths between both plant species that were presented (Fig. 1B and Supp. Table 1).

Aster leafhoppers reared on fleabane appear to have behaved similarly to those reared on barley. When oat and fleabane were presented together, a higher number of stylet sheaths were present in oat in both cases (Supp. Tables 1 and 3). However, when insects were presented with barley and fleabane, differences were observed between both leafhopper colonies. In bioassays with Aster leafhoppers reared on barley, stylet sheath counts were similar between both plant species (116.8 ± 45.5 in barley and 42.6 ± 3.9 in fleabane, mean ± SEM). However, in bioassays with Aster leafhoppers reared on fleabane, a higher number of stylet sheaths was observed in barley (184.9 ± 50.2) when compared to fleabane (12.5 ± 4.5) (Supp. Table 1).

Egg counts

In addition to stylet sheaths, the number of eggs present in plant tissues was recorded for each plant species in each plant combination (Fig. 1C and Supp. Table 1). For some domesticated-domesticated combinations, similar numbers of eggs were observed in both plant species, while in others, the number of eggs differed between both plant species. For example, in barley-oat bioassays, 13.6 ± 4.3 (mean ± SEM) eggs were counted in barley and 41.6 ± 8.2 in oat. However, when barley and wheat were presented together, egg counts were similar between both plant species, with 18.4 ± 7.8 eggs in barley and 15.0 ± 7.3 in wheat (Supp. Table 1). In most domesticated-wild plant combinations, a greater number of eggs was observed in the domesticated plant species. However, in the remaining combinations, egg counts were similar between domesticated and wild plant species. In dandelion-domesticated bioassays, a greater number of eggs was consistently observed in domesticated plant tissues, with egg counts ranging from 13.3 ± 7.8 to 35.5 ± 7.0, in oat and wheat respectively. In barley-fleabane bioassays, however, egg counts were similar between both plant species, with 6.4 ± 4.2 eggs in barley and 0.8 ± 0.6 in fleabane. In most wild plant-wild plant bioassays, egg counts were similar between both plant species, such as when marigold (1.0 ± 0.5) and sowthistle (2.3 ± 1.1) were presented together; this was not the case with other bioassays involving marigold and other wild plant species, as a greater number of eggs was observed in marigold (18.6 ± 4.1 and 9.1 ± 1.5) when compared to egg counts in dandelion (6.0 ± 2.3) and fleabane (1.6 ± 0.8).

When assessing the number of eggs in plant tissues from bioassays with Aster leafhoppers reared on fleabane, a greater number of eggs was found in oat and barley when these were presented together with fleabane (Supp. Table 3). In oat-fleabane bioassays, for example, 10.7 ± 3.2 (mean ± SEM) and 0.6 ± 0.4 eggs were observed in oat and fleabane, respectively (Supp. Table 1). This was also the case for bioassays with Aster leafhoppers reared entirely on barley, with an

290 average of 29.6 ± 7.6 eggs in oat and 3.9 ± 1.7 in fleabane (Supp. Table 1). However, when insects
291 were offered barley and fleabane, differences were observed between Aster leafhoppers reared on
292 fleabane and those reared on barley (Supp. Tables 1 and 3). In bioassays with leafhoppers reared
293 on fleabane, a greater number of eggs was observed in barley (5.9 ± 2.6) when compared to
294 fleabane (0.3 ± 0.3) (Supp. Table 3). In bioassays with insects reared on barley, egg counts were
295 similar between both barley and fleabane (6.4 ± 4.2 eggs in barley and 0.8 ± 0.6 in fleabane).
296
297 *Are settling, probing and egg laying behaviors affected by insect infection with AYp?*
298 To examine whether infection of Aster leafhoppers with AYp would affect their settling
299 behavior, we repeated the bioassays using AY-infected leafhoppers and a subset of all
300 aforementioned plant species (Fig. 2A and Supp. Table 2). In plant combinations such as barley-
301 dandelion, canola-wheat, and wheat-dandelion, settling behavior was similar between AY-
302 uninfected and AY-infected Aster leafhoppers (Fig. 1A and Fig. 2A). In other cases, however,
303 settling behavior differed between these insect groups. When barley and canola were presented
304 together, for example, a similar number of uninfected Aster leafhoppers were observed on both
305 plant species (Fig. 1 and Supp. Table 1). When AY-infected Aster leafhoppers were offered this
306 plant combination, leafhoppers preferred to settle on barley (Fig. 2A and Supp. Table 2). This was
307 also the case for bioassays with barley and wheat, as AY-uninfected Aster leafhoppers exhibited
308 no settling preference (Fig. 1 and Supp. Table 1), while AY-infected insects preferred to settle on
309 barley (Fig. 2A and Supp. Table 2). In dandelion-canola bioassays, AY-uninfected leafhoppers
310 preferred to settle on canola, while AY-infected insects preferred to settle on dandelions.
311 When examining stylet sheath and egg counts from bioassays with AY-infected Aster
312 leafhoppers, AY-uninfected insects exhibited similar probing and oviposition behavior to AY-

313 infected leafhoppers in most plant combinations (Fig. 1B-C and Fig. 2B-C). In barley-canola
314 bioassays with AY-uninfected Aster leafhoppers, for example, similar numbers of stylet sheaths
315 were observed on both plant species (85.0 ± 26.1 in barley and 36.0 ± 31.6 in canola, mean ± SEM)
316 (Fig. 1B and Supp. Table 1). This was not the case with AY-infected leafhoppers, as leaves from
317 barley contained a greater number of stylet sheaths (196.6 ± 48.3) when compared to canola leaves
318 (9.5 ± 3.7) (Fig. 2B and Supp. Table 2). The opposite behavioral shift could be observed with
319 barley-dandelion bioassays. While more stylet sheaths were found in barley leaves (104.0 ± 23.2
320 stylet sheaths in barley and 8.8 ± 4.8 in dandelion) when AY-uninfected leafhoppers were
321 examined (Fig. 1B and Supp. Table 1), stylet sheath counts were similar between both plant species
322 when AY-infected insects were used (155.6 ± 39.5 stylet sheaths in barley and 96.7 ± 29.7 in
323 dandelion) (Fig. 2B and Supp. Table 2). Comparison of egg laying behavior between groups of
324 AY-uninfected and AY-infected Aster leafhoppers revealed that insects exhibited similar patterns
325 of preference/no preference in most plant combinations, including barley-wheat, barley-dandelion,
326 canola-wheat, and dandelion-wheat combinations (Figs. 1C and 2C). In barley-canola bioassays,
327 however, a similar number of eggs was found on both plant species with AY-uninfected Aster
328 leafhoppers (20.0 ± 8.4 in barley and 10.0 ± 8.1 in canola) (Fig. 1C and Supp. Table 1), while a
329 greater number of eggs was observed on barley when AY-infected insects were examined (11.8 ±
330 2.3 in barley and 1.9 ± 0.8 in canola) (Fig. 2C and Supp. Table 2). In canola-dandelion bioassays
331 with AY-uninfected leafhoppers, egg counts were greater on canola (26.8 ± 7.7) when compared
332 to dandelion (4.1 ± 1.4) (Fig. 1C and Supp. Table 1). With AY-infected leafhoppers, however,
333 similar numbers of eggs were observed on both plant species (1.1 ± 0.7 in canola and 0.9 ± 0.5 in
334 dandelion) (Fig. 2C and Supp. Table 2).
335

Comparison of stylet sheath counts between AY-uninfected and AY-infected insect groups

To provide additional information about the effect of insect infection with AYp on probing behavior, the stylet sheath counting procedure was conducted on the subset of plants used in bioassays with infected Aster leafhoppers and compared to those from the bioassays performed with AY-uninfected leafhoppers. 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 (Supp. Fig. 3). When comparing the total number of stylet sheaths in bioassays in which barley and dandelion were presented, more total stylet sheaths were observed in the AY-infected Aster leafhopper treatment ($p = 0.021$, Supp. Fig. 3). The opposite effect was observed in bioassays with canola and dandelion, in which samples from AY-infected Aster leafhoppers had a fewer stylet sheaths when compared to those from AY-uninfected insects ($P = 0.012$, Supp. Fig. 3).

Correlation between stylet sheath and egg counts

To partially evaluate the specific use of each plant species by Aster leafhoppers, we analyzed the relationship between the stylet sheath and egg counts for each plant species in each plant combination by calculating Spearman´s correlation coefficient (Supp. Table 4). In most cases, no correlation was observed between these variables ($P > 0.05$), yet for 13 plant species in some bioassays, a strong positive correlation between the number of stylet sheaths and the number of eggs was found ($P < 0.05$, Supp. Table 4).

Discussion

Aster leafhoppers (Forbes) (Hemiptera: Cicadellidae) are a migratory species that are

359 introduced into the Canadian Prairies by wind currents originating in the United States in spring
360 and early summer (Nichiporik 1965; Olivier et al. 2009; Alma et al. 2019). This leafhopper species
361 can transmit phytoplasma subgroups 16SrI-A, -B, and -C (Olivier et al. 2009), which are associated
362 with aster yellows (AY) disease. While Aster leafhoppers have been described as a polyphagous
363 species (Olivier et al. 2009; Weintraub & Beanland 2006), biological aspects such as their host
364 range and host selection behaviour in the Canadian Prairies remain largely unknown. In a previous
365 study by Romero et al. (2020), the suitability of three cereals and an oilseed crop, and several non-
366 crops from the Asteraceae family were evaluated as hosts for Aster leafhopper reproduction and
367 development. Cereals were found to be among the most suitable hosts for both reproduction and
368 development, as a higher number of offspring and higher proportion of adults were found on these
369 plant species (Romero et al. 2020). In canola, however, almost no offspring were observed and
370 development was greatly impaired. Among wild plants, fleabane was similarly suitable to cereals,
371 while marigold and dandelion had fewer eggs. As with canola, almost no offspring were observed
372 on sowthistle and development on this wild plant was slower than in other plant species.
373
374 *Settling behavior of AY-uninfected Aster leafhoppers reared on barley*
375 In this study, both preference and non-preference were observed when assessing AY-
376 uninfected Aster leafhoppers´ settling behaviour. When two domesticated species were presented
377 together, similar numbers of leafhoppers were found on both plant species in most plant
378 combinations, suggesting that they might be perceived as potential hosts with similar chemical
379 characteristics, containing a similar array of cues mediating recognition and plant acceptance
380 (Larsson and Ekbom 1995), or that leafhoppers might be insensitive to differences between them.
381 This is supported by previous observations by Romero et al. (2020), who noted similar numbers

of offspring in barley, oat, and wheat. Canola, however, was less preferred to settle on when presented together with oat or wheat. This pattern was also reflected in recent findings by Romero et al. (2020), who observed almost no offspring and very limited development in Aster leafhopper on canola, while oat and wheat were described as suitable reproductive and food hosts for this insect. When a domesticated and a wild plant were offered together, Aster leafhoppers exhibited a preference for settling on the domesticated plant species over the wild plants (Fig. 1A and Supp. Table 1). This finding does not fully support previous observations by Romero et al. (2020), who observed a higher number of offspring and faster insect growth in wild plants such as fleabane, dandelion, and marigold when compared to canola. It is unclear why a plant species on which Aster leafhoppers lay few eggs and have developmental difficulties might still be a preferred host to settle on. One possibility is that the same processes by which specific traits are selected for in domesticated species result in trade-offs that reduce the ability of those plants to respond to insect attack when compared to their wild relatives (Rosenthal and Dirzo 1997; Bellota et al. 2013; Dávila-Flores et al. 2013), consistent with the resource allocation and growth-differentiation balance (GDB) hypotheses (Herms and Mattson 1992, Mole 1994). In a study by Rosenthal and Dirzo (1997), field experiments with naturally occurring relatives of *Zea* sp., a land-race cultivar and a modern cultivar were conducted. These authors assessed infestation rates and plant damage from *Diatraea grandiosella* (Dyar) (Lepidoptera: Crambidae) larva, reporting lower levels for annual and perennial wild relatives when compared to the modern cultivar. Bellota et al. (2013) and Dávila-Flores et al. (2013) made similar observations when examining development, mass, survivorship, and oviposition preferences of *Dalbulus maidis* (DeLong & Wolcott) (Hemiptera: Cicadellidae) on wild and domesticated maize species. When two wild plants were presented together, there were some cases in which Aster leafhoppers distributed similarly between both

plant species being offered and some cases in which a higher number of insects was observed on one of the plant species. Overall, this presents a complex scenario in which plant species preferred for settling might not always be the most suitable ones for other biological aspects such as oviposition and nymphal development, and also suggests that plant acceptance and use will be highly dependent on the context in which that plant species is encountered. Another explanation for these patterns would be that the presence of two potential host plants would generate a more complex stimuli to process and chemical signals would get mixed up, suggesting some constraints or limitations of the neural system and reducing the ability to make a "good" decision (Bernays 1998, Gripenberg et al. 2010).

Settling behavior of AY-uninfected Aster leafhoppers reared on fleabane

Aster leafhoppers reared entirely on fleabane exhibited a similar settling behavior to leafhoppers reared on barley, suggesting that previous exposure and feeding on a certain plant species might not influence Aster leafhoppers´ host choice selection. This is consistent with previous observations by Prager et al. (2014b), who conducted three-choice bioassays with *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae) reared on tomato and pepper and reported that insects from both colonies exhibited a similar settling behavior. However, these results are in contrast with those of Chuche et al. (2016), who found differences between *Scaphoideaus titanus* (Ball) (Hemiptera: Cicadellidae) nymphs reared on grapevine and on broadbean. While nymphs reared on grapevine preferred to settle on grapevine infected with flavescence dorée (FD) phytoplasma over healthy grapevine, the opposite behavioral shift was reported for nymphs maintained on broadbean for 15 days before the experiment. Adults of *S. titanus* had a similar settling behavior to nymphs maintained on broadbean and preferred to settle on healthy grapevine

over a phytoplasma-infected one (Chuche et al. 2016). It should be noted, however, that the authors used neonate nymphs and do not report sex ratios, which are important considerations since some studies comparing male and female insects demonstrate differences in feeding activity and localization on plant parts between males and females (Brodbeck et al. 1993; Gruenhagen and Backus 1999; Joost and Riley 2008; Cornara et al. 2018).

Probing behavior of AY-uninfected Aster leafhoppers

To characterize AY-uninfected Aster leafhoppers´ probing behavior and examine the relationship between stylet sheath counts and settling behavior, we quantified stylet sheath structures present in leaf tissues from both plant species offered simultaneously during two-choice bioassays. In domesticated-domesticated combinations, plant species which had been preferred for settling were found to contain a higher number of stylet sheath structures, suggesting that for this subset of plant species, probing and settling behaviors seem to be associated with one another. This was not the case, however, with domesticated-wild plant and wild plant-wild plant combinations, as we observed cases in which plant species were preferred for settling and contained fewer stylet sheaths, such as with oat-dandelion, oat-sowthistle, and canola-sowthistle combinations. In other plant combinations, Aster leafhoppers preferred to settle on one plant species, yet stylet sheath counts between both plant species being offered were similar. This was the case of barley-fleabane, canola-marigold, and fleabane-sowthistle combinations. Overall, this might suggest that different cues are involved in the recognition and acceptance of a plant species as a host for settling and for probing. It is also possible that number of stylet sheaths is a poor indicator of the palatability or nutritional value of a plant species, as plant species described as unsuitable hosts for Aster leafhopper oviposition and development (Romero et al. 2020) such as

canola and sowthistle were observed to have similar or higher numbers of stylet sheaths than more suitable host plants like barley, wheat, or fleabane. This result is consistent with previous observations by Zeilinger et al. (2011), who found no relationship between stylet sheath structures and food consumption in *Nezara viridula* (Linnaeus) (Hemiptera: Pentatomidae).

When Aster leafhoppers were presented with barley and fleabane, differences in the number of stylet sheaths were observed between insects that had been reared on barley and those reared on fleabane. While this difference 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. It is possible that the high inbreeding in the 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.

Oviposition behavior of AY-uninfected Aster leafhoppers

To characterize Aster leafhoppers´ oviposition behavior when two plant species were presented together, we counted the number of eggs laid on both plant species from each pair. Overall, oat and wheat were among the most preferred plant species for oviposition, fleabane was less or equally preferred to other plant species, and barley was more or equally suitable for laying eggs when presented together with another plant species. These patterns observed for wheat and oat are in accordance with previous work by Romero et al. (2020), who characterized these two domesticated species as suitable reproductive hosts for Aster leafhoppers. However, these authors also reported similar findings for barley and fleabane (Romero et al. 2020), in contrast to the observations from this study, whereby little to no preference for ovipositing on barley or fleabane

was observed. It is interesting to note that while canola had been characterized as an unsuitable reproductive host for Aster leafhoppers (Romero et al. 2020), here oviposition on canola was found to be similar to multiple other species.

Changes in food quality and availability can also affect reproductive behavior, as nutrient deficiency can lead to decreased egg production and/or affect discrimination against less suitable host plants (Brodbeck et al. 1993, Rosenheim et al. 2008, Jaumann et al. 2019). AY-uninfected Aster leafhoppers laid on average between 0.1 ± 0.1 and 41.6 ± 8.2 (mean ± SEM) eggs on leaf tissues, while AY-infected Aster leafhoppers laid between 0.5 ± 0.1 and 49.2 ± 18.3 eggs. This broad range of eggs laid suggests that Aster leafhopper females might exhibit a differential oviposition behavior when more than one plant species is available and that the number of eggs to be laid on a certain plant species might not be determined by the identity of such plant species, but by the comparison with the other plant species available for ovipositing. It is possible that oviposition decision making in Aster leafhoppers is dependent on context. While one plant species might be a suitable or preferred host when encountered alone, the addition of another potential host plant might influence plant acceptance and create a more complex scenario. Perhaps such behavioral shifts are attributable to the ability to compare potential hosts in combination, while in the absence of alternate choices, a female is faced with a more binary decision of whether to accept the plant or not.

In accordance with our observations about the acceptance and use of plant species such as canola, fleabane, or barley as reproductive hosts in no-choice and two-choice bioassays, Brodbeck et al. (2007) observed that *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) preferred to reside or oviposit on plant hosts on which its survivorship and development were greatly impaired, and conversely, not oviposit or settle on more suitable hosts for leafhopper development.

In another study, Bellota et al. (2013) assessed oviposition preferences of *Dalbulus maidis* when released in a cage containing a maize cultivar or a wild relative and reported that leaves from the domesticated species contained a higher density of eggs, while a high proportion of leaves from the wild relative species contained no eggs at all. These authors suggested that a plant trait associated with these preferences was leaf toughness, meaning the "work for penetration" of a stylet or ovipositor was higher in perennial and wild relatives (Bellota et al. 2013). Interestingly, Antolinez et al. (2017) assessed settling and oviposition preferences of two psyllid species and found that the settling and oviposition preferences were similar when presenting plant species individually or together.

Settling, probing, and oviposition behavior of AY-infected Aster leafhoppers

Phytoplasmas are a group of obligate vector-borne microorganisms, which can circulate and replicate within insect and plant tissues in a similar manner to other persistently-transmitted plant pathogens (Hogenhout et al. 2008, Bosco and D´Amelio 2010). Previous studies with vector-borne pathogens have indicated that pathogen infection can influence host-plant selection behavior, feeding activity, and oviposition behaviour (Stafford et al. 2011, Ingwell et al. 2012, Mauck et al. 2012, Tack and Dicke 2013, Carmo-Souza et al. 2015, Lei et al. 2016, García González et al. 2018, Ramos et al. 2020), possibly enhancing disease transmission. In the context of a migratory insect species, the possibility of behavioral differences between uninfected and infected insects would be a major aspect to be examined, given that their host range could overlap to a greater or lesser extent or include novel plant species, ultimately influencing disease dynamics. For this reason, we examined whether Aster leafhopper infection with AYp would affect settling, probing, and oviposition preferences. Differences between AY-uninfected and AY-infected insect

groups were observed in some cases, yet there was not clear trend in these behavioral shifts. Similarly, the total number of stylet sheaths differed between AY-uninfected and AY-infected Aster leafhoppers for two plant combinations (barley-dandelion and canola-dandelion). Given that bioassays with both insect groups were not conducted simultaneously but in different weeks, and that we did not control for the age of the insects used, it is possible for these factors to have introduced additional variability in our results and partly explain why we did not observe differences in the total number of stylet sheaths in all cases. In a study by Ingwell et al. (2012), it was shown that while uninfected aphids were more attracted to infected wheat, aphids infected with Barley yellow dwarf virus preferred to settle on uninfected plants. Ramos et al. (2020) and García González et al. (2018) observed that uninfected *Dalbulus maidis* preferred to settle and oviposit on maize exhibiting advanced disease symptoms of Maize bushy stunt phytoplasma. Other authors compared feeding behavior between uninfected and infected insects, observing that infected insects were characterized by higher probing rates and/or longer ingestion times when feeding on uninfected plants (Stafford et al. 2011, Carmo-Sousa et al. 2015, Lei et al. 2016). In our study, we only noticed differences between AY-uninfected and AY-infected Aster leafhoppers when insects were exposed to barley and dandelion.

Relationship between stylet sheath and egg counts

We examined the relationship between stylet sheath and egg counts for each plant species in each combination and our results suggested that stylet penetration and egg laying behaviors are independent from one another, as a strong positive relationship between these variables was observed in few plant species. Likewise, Horton and Krysan (1990) assessed the probing and oviposition behaviours of *Cacopsylla pyricula* (Förster) (Hemiptera: Cicadellidae) and observed

543 little correspondence between the percent of psyllids probing on different plant species and the
544 percent of psyllids engaged in preoviposition activity. Similar findings were reported by Prager et
545 al. (2014a), who observed a lack of association between the number of eggs laid and the number
546 of stylet sheaths of *Bactericera cockerelli* in potato plants.
547
548 *Theories about host plant choice and implications for AY epidemiology*
549 The results of this study when considered alongside with those of Romero et al. (2020),
550 who used the same plant species and insects, allow us to examine different theories about host
551 plant choice. Namely the "mother knows best" (Valladares and Lawton 1991, Johnson et al. 2006)
552 or "preference-performance" hypothesis (Mayhew 2001; Brodbeck et al. 2007). While there is
553 agreement between adult preferences and immature development in some of the plant species
554 examined such as barley, wheat, and oat, we did not observe this for all the suitable hosts described
555 by Romero et al. (2020). The number of offspring and amount of nymph development reported on
556 fleabane (Romero et al. 2020) suggested that this might be another plant species preferred for
557 settling and oviposition, yet our results here suggest otherwise. Similarly, canola was a mostly
558 unsuitable host plant for Aster leafhopper oviposition and nymphal development (Romero et al.
559 2020) and was still preferred over more suitable reproductive host plants such as fleabane and
560 dandelion. One explanation is that, in contrast to "good mothers", Aster leafhopper females might
561 not be capable of discriminating against unsuitable hosts or are more willing to take risks on
562 species of questionable quality. This lack of discrimination may be associated with their
563 polyphagous and migratory character, as Aster leafhoppers encounter very distinct and diverse
564 plant communities as they migrate and may not always encounter suitable or optimal host plants
565 to reproduce and feed on in these different environments. In fact, potato psyllids, another migratory

566 species of Sternorrhyncha, also exhibit little to no correlation between performance and preference
567 (Prager et al. 2014b). Some migratory insects may be less choosy as a response to uncertainty in
568 early season host availability or due to limitations of the neural system to process complex stimuli
569 (Bernays 1998, Gripenberg et al. 2010). Interestingly, settling and oviposition preferences of AY-
570 infected leafhoppers support previous observations by Romero et al. (2020), as higher numbers of
571 insects and eggs were observed on more suitable plant species such as barley and wheat. The
572 choice to oviposit on more suitable or highly ranked plant species would be in accordance with the
573 "Preference-performance hypothesis" (Mayhew 2001, Brodbeck et al. 2007), suggesting that AYp
574 might affect neural mechanisms involved in information processing and ultimately host choice
575 selection behavior. Behavioral shifts due to insect infection with a pathogen have been reported
576 for other plant pathosystems such as Tomato spotted wilt virus and *Frankliniella occidentalis*
577 (Pergande) (Thysanoptera: Thripidae) (Stafford et al. 2011) and Barley yellow dwarf virus and
578 *Rhopalosiphum padi* (Linnaeus) (Hemiptera: Aphididae) (Ingwell et al. 2012).

579 The aims of this study were to characterize Aster leafhopper host choice selection behavior
580 in a complex environment and to examine whether insect infection with AYp influenced
581 behavioral preferences. We additionally quantified stylet sheath structures and eggs laid on leaf
582 tissues of both plant species presented during bioassays to provide a better understanding of plant
583 use. Overall, our results showed that AY-uninfected Aster leafhoppers were able to distinguish
584 between a domesticated and a wild plant, exhibiting a preference for settling on the domesticated
585 over the wild plant species. However, this was not always the case when examining stylet sheath
586 structures and egg counts, as numbers of stylet sheaths and eggs were similar between the
587 domesticated and the wild plant species. Moreover, in most domesticated-domesticated and some
588 wild plant-wild plant combinations, these distinctions between plant species became less clear, as

similar numbers of AY-uninfected Aster leafhoppers were observed on both plant species. In most domesticated-domesticated combinations, similar numbers of stylet sheaths were observed, suggesting that many of these plant species provide a similar array of cues mediating probing behavior and/or that Aster leafhoppers are not capable of discriminating between them. Oat and wheat, followed by barley, had the highest egg abundance on leaf tissue when presented together with another plant species. In wild plant-wild plant combinations, marigold was preferred when presented together with dandelion or fleabane, while other combinations of two wild plant species would result in similar number of eggs in both leaf tissues. In most cases, there is no correlation between the number of stylet sheaths and the number of eggs in each plant species, suggesting that cues mediating plant acceptance for probing might not necessarily be the same for oviposition. The pattern observed when a domesticated and a wild plant were offered simultaneously has serious implications in AY epidemiology as it would suggest that after the germination of domesticated plant species, Aster leafhoppers may move from nearby wild plants into the domesticated plant species and settle on them. While only one wild plant was used for characterizing settling behavior of AY-infected Aster leafhoppers, these insects exhibited a preference for barley and wheat over dandelion. When offered canola and dandelion, insects preferred to settle on dandelion. Overall, this preference to settle on the domesticated plant species could represent a higher risk of AYp infection, but additional studies would be required to examine this possibility. Moreover, Aster leafhoppers´ feeding behavior on plant species such as those selected for this study has not been previously characterized. Using the electropenetrography (EPG) technique, specifics of feeding activity such as salivation, ingestion periods, and probing frequency have been described for other sucking-piercing insect species, including those capable of transmitting a plant pathogen like a virus or a bacteria (Backus and Shih 2020, Jiménez et al.

2020, Roddee et al. 2021). Characterizing the feeding activity of Aster leafhoppers on various plant species would provide a better understanding of stylet sheath counts and would help examine possible differences between AY-uninfected and AY-infected Aster leafhoppers that might contribute to a lower/higher risk of AYp infection for certain plant species.

Acknowledgments

We thank Pierre Hucl, Aaron Beattie, and Ellen Misfeldt for providing us with seeds. We thank Dana Leedahl for supplying additional AY-infected leafhoppers for some of the experiments and AY-infected periwinkle. Adam Jewiss-Gaines, Abigail Cohen, Tim Sharbel, and Eric Lamb provided helpful comments and advice on this manuscript. This work was funded by a grant from the Saskatchewan Canola Development Commission (CARP ADF 2017-203). BR was funded partly by the Western Grains Research Foundation Endowment Fund Graduate Scholarship.

Declarations

The authors declare no conflict of interest.

SMP, TW, BR and CO conceived the ideas and designed the methodology; BR collected and analyzed the data; BR and SMP led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

This work was funded by a grant from the Saskatchewan Canola Development Commission (CARP ADF 2017-203). BR was funded partly by the Western Grains Research Foundation Endowment Fund Graduate Scholarship.

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Figures

Figure 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 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. Domesticated-domesticated combinations are indicated by a white background, domesticated-wild plant combinations by a light grey background, and wild plant-wild plant 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 Supp. Table 1. B) Probing events were used as proxy for feeding activity and results were evaluated using a paired t-test for each combination. If residuals were not normally distributed, the Wilcoxon test was used instead. Details about the number of stylet sheaths on each plant can be found in Supp. Table 1. C) Oviposition event results were evaluated using a paired t-test for each combination. If residuals were not normally distributed, the Wilcoxon test was used instead. Details about the number of eggs on each plant can be found in Supp. Table 1.

Figure 2

Results from two-choice bioassays using AY-infected 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. Domesticated-domesticated combinations are indicated by a white background, domesticated-wild plant combinations by a light grey background, and wild plant-wild plant 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 Supp. Table 2. B) Probing events were used as proxy for feeding activity and results were evaluated using a paired t-test for each combination. If residuals were not normally distributed, the Wilcoxon test was used instead. Details about the number of stylet sheaths on each plant can be found in Supp. Table 2. C) Oviposition event results were evaluated using a paired t-test for each combination. If residuals were not normally distributed, the Wilcoxon test was used instead. Details about the number of eggs on each plant can be found in Supp. Table 2.

Appendix (Supplementary material)

Supp. Fig. 1

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

Supp. Fig. 2

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.

Supp. Fig. 3

Boxplot of the total number of stylet sheaths for the subset of plant combinations for which both AY-uninfected and AY-infected insects were examined. Boxes are drawn between the 25th and 75th percentiles, with the median marked with a horizontal black line. Whiskers indicate the largest and smallest values within 1.5 times interquartile range from the ends of the boxes. 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 above the treatments being compared. A significance level (α-value) of 0.05 was used.

Supp. 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 Supp. Table 5 for median and interquartile range values. A significance level (α-value) of 0.05 was used.

Supp. 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, “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 Supp. Table 5 for median and interquartile range values. A significance level (α-value) of 0.05 was used.

Supp. Table 3

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 Supp Table 5 for median and interquartile range values. A significance level (α-value) of 0.05 was used.

Supp. Table 4

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.

Supp. 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 (AY-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.

Do options matter? Settling behavior, stylet sheath counts, and oviposition of Aster leafhoppers (Hemiptera: Cicadellidae) in two-choice bioassays

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	↑	↑	↑	↑	↑		0.070	**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 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 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. Domesticated-domesticated combinations are indicated by a white background, domesticated-wild plant combinations by a light grey background, and wild plant-wild plant 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 Supp. Table 1. B) Probing events were used as proxy for feeding activity and results were evaluated using a paired t-test for each combination. If residuals were not normally distributed, the Wilcoxon test was used instead. Details about the number of stylet sheaths on each plant can be found in Supp. Table 1. C) Oviposition event results were evaluated using a paired t-test for each combination. If residuals were not normally distributed, the Wilcoxon test was used instead. Details about the number of eggs on each plant can be found in Supp. Table 1.

Do options matter? Settling behavior, stylet sheath counts, and oviposition of Aster leafhoppers (Hemiptera: Cicadellidae) in two-choice bioassays

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**

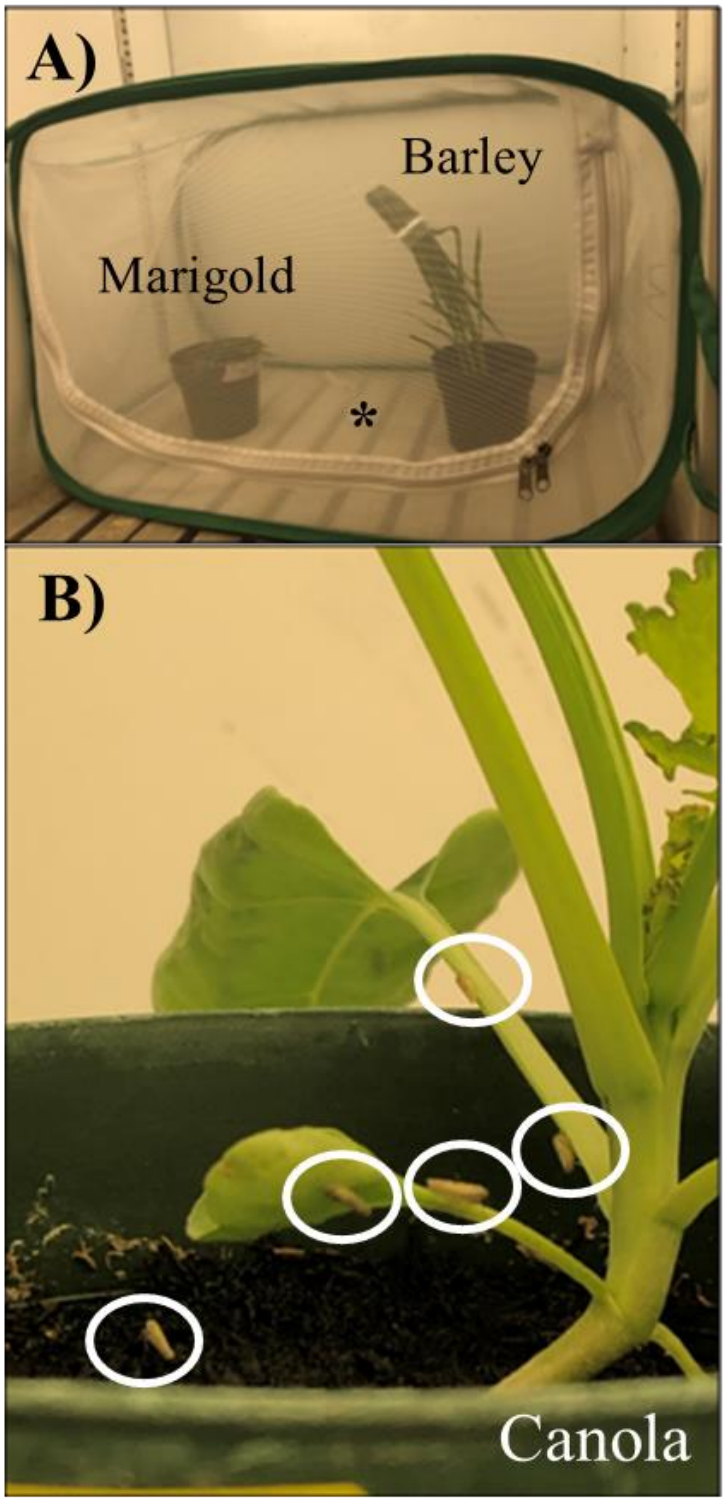
	Ba	Ca	Wh	Da
Ba		**0.002**	0.326	0.239
Ca	⬆		**0.002**	**0.002**
Wh	=	⬅		**0.011**
Da	=	⬅	⬆	

C) **Oviposition**

	Ba	Ca	Wh	Da
Ba		**0.013**	0.123	**0.001**
Ca	⬆		**0.011**	0.577
Wh	=	⬅		**0.010**
Da	⬆	=	⬆	

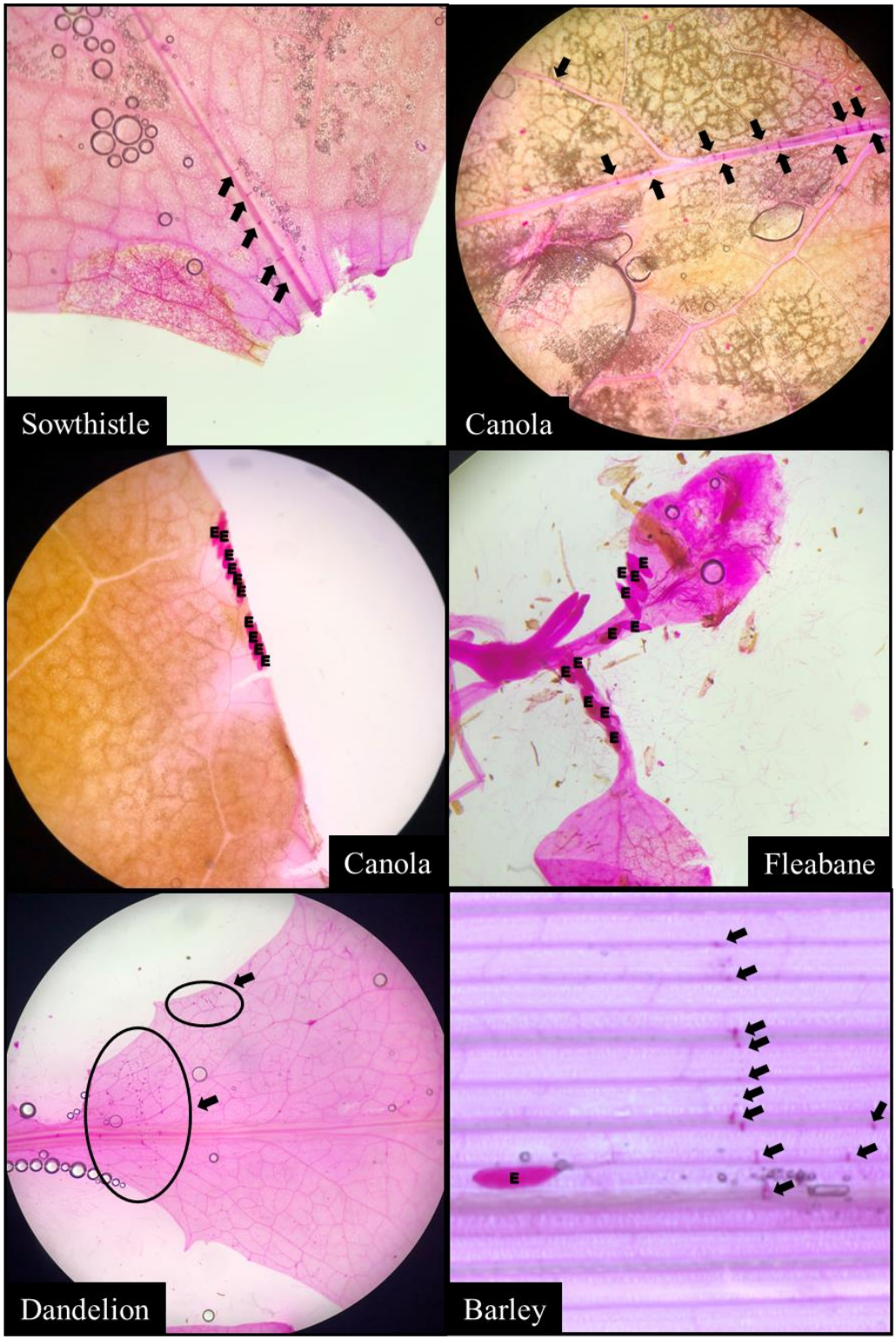
Figure 2 Results from two-choice bioassays using AY-infected Aster leafhoppers reared on barley. The following abbreviations have been used: “Ba” = barley, “Ca” = canola, “O” = oat, “Wh” = wheat, “Da” =dandelion, “Fb” = fleabane, “Ma” = marigold, and “Th” = sowthistle. For all panels (A-C), P-values 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. Domesticated-domesticated combinations are indicated by a white background, domesticated-wild plant combinations by a light grey background, and wild plant-wild plant 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 Supp. Table 2. B) Probing events were used as proxy for feeding activity and results were evaluated using a paired t-test for each combination. If residuals were not normally distributed, the Wilcoxon test was used instead. Details about the number of stylet sheaths on each plant can be found in Supp. Table 2. C) Oviposition event results were evaluated using a paired t-test for each combination. If residuals were not normally distributed, the Wilcoxon test was used instead. Details about the number of eggs on each plant can be found in Supp. Table 2.

Do options matter? Settling behavior, stylet sheath counts, and oviposition of Aster leafhoppers (Hemiptera: Cicadellidae) in two-choice bioassays



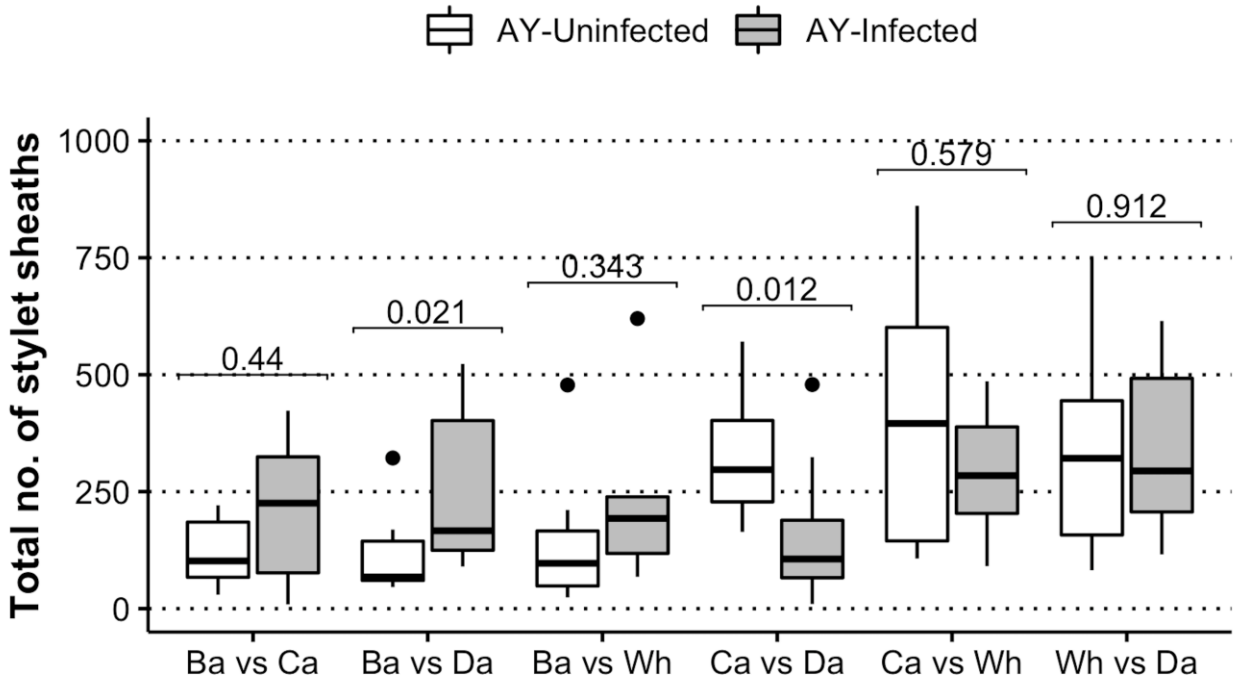
Supp. Fig. 1 a) Example of one two-choice bioassay where marigold and barley were the choice plants. The asterisk indicates the position within the cage where leafhoppers were initially released. b) Aster leafhoppers (white circles) on a canola test plant.

Do options matter? Settling behavior, stylet sheath counts, and oviposition of Aster leafhoppers (Hemiptera: Cicadellidae) in two-choice bioassays



Supp. Fig. 2 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.

Do options matter? Settling behavior, stylet sheath counts, and oviposition of Aster leafhoppers (Hemiptera: Cicadellidae) in two-choice bioassays



Supp. Figure 3 Boxplot of the total number of stylet sheaths for the subset of plant combinations for which both AY-uninfected and AY-infected insects were examined. Boxes are drawn between the 25th and 75th percentiles, with the median marked with a horizontal black line. Whiskers indicate the largest and smallest values within 1.5 times interquartile range from the ends of the boxes. 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 above the treatments being compared. A significance level (α-value) of 0.05 was used.

Supp. 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 Supp. 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)	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	53.2 ± 3.0	46.8 ± 3.0	4.5 ± 0.3	4.3 ± 0.3	0.478	85.0 ± 26.1	36.0 ± 31.6	0.437	20.0 ± 8.4	10.0 ± 8.1	(W) 0.543
Ba – O	53.0 ± 2.6	47.0 ± 2.6	5.9 ± 0.5	5.2 ± 0.4	0.087	84.6 ± 25.1	142.8 ± 52.9	0.428	13.6 ± 4.3	41.6 ± 8.2	**0.048**
Ba – Wh	60.6 ± 3.3	39.4 ± 3.3	4.7 ± 0.4	2.7 ± 0.3	0.058	124.6 ± 60.1	22.3 ± 11.7	(W) 0.110	18.4 ± 7.8	15.0 ± 7.3	(W) 0.830
Ba – Da	83.0 ± 2.2	17.0 ± 2.2	7.9 ± 0.3	1.8 ± 0.3	**0.001**	104.0 ± 23.2	8.8 ± 4.8	(W) **0.002**	29.2 ± 7.9	0.2 ± 0.2	(W) **0.002**
Ba – Fb	75.9 ± 3.4	24.1 ± 3.4	7.7 ± 0.5	2.7 ± 0.4	**0.001**	116.8 ± 45.5	42.6 ± 3.9	(W) 0.125	6.4 ± 4.2	0.8 ± 0.6	(W) 0.269
Ba – Ma	88.4 ± 2.6	11.6 ± 2.65	6.5 ± 0.3	0.9 ± 0.2	**0.002**	65.5 ± 23.2	3.3 ± 1.7	(W) **0.009**	23.0 ± 5.8	0.9 ± 0.5	(W) **0.009**
Ba - Th	62.2 ± 3.6	37.8 ± 3.6	6.2 ± 0.4	3.8 ± 0.4	**0.002**	190.7 ± 62.9	140.8 ± 43.7	0.598	18.0 ± 3.1	1.7 ± 0.9	(W) **0.016**
Ca – O	24.9 ± 2.6	75.1 ± 2.6	2.7 ± 0.3	8.6 ± 0.3	**0.001**	13.6 ± 5.8	407.6 ± 63.4	(W) **0.001**	1.9 ± 0.7	16.1 ± 2.1	(W) **0.003**
Ca – Wh	34.6 ± 3.4	65.4 ± 3.4	3.5 ± 0.4	6.8 ± 0.5	**0.004**	35.9 ± 10.5	384.9 ± 88.8	(W) **0.004**	4.1 ± 1.3	14.9 ± 3.5	**0.019**
Ca – Da	62.5 ± 3.2	37.5 ± 3.2	4.7 ± 0.3	2.9 ± 0.3	**0.011**	101.4 ± 24.2	221.3 ± 35.9	(W) **0.037**	26.8 ± 7.7	4.1 ± 1.4	(W) **0.002**
Ca – Fb	90.8 ± 1.7	9.2 ± 1.7	5.9 ± 0.3	0.7 ± 0.1	**0.001**	28.8 ± 11.8	67.5 ± 17.1	(W) **0.001**	6.6 ± 2.6	3.4 ± 1.7	(W) 0.294
Ca – Ma	63.1 ± 4.2	36.9 ± 4.2	2.9 ± 0.3	1.5 ± 0.2	**0.013**	52.9 ± 22.2	40.6 ± 9.4	(W) 0.813	6.3 ± 2.2	4.3 ± 1.8	(W) 0.309
Ca – Th	75.1 ± 3.3	24.9 ± 3.3	5.7 ± 0.3	2.0 ± 0.3	**0.001**	3.5 ± 2.3	24.0 ± 6.1	(W) **0.030**	1.6 ± 0.6	0.6 ± 0.2	(W) 0.150
O – Wh	54.0 ± 3.8	46.0 ± 3.8	4.3 ± 0.4	3.6 ± 0.4	0.310	43.0 ± 17.4	81.4 ± 33.1	(W) 0.359	22.6 ± 7.7	18.9 ± 3.8	(W) 0.999
O – Da	75.4 ± 3.6	24.6 ± 3.6	5.8 ± 0.4	2.1 ± 0.3	**0.001**	423.4 ± 98.1	174.2 ± 57.1	(W) **0.014**	13.3 ± 4.2	0.5 ± 0.3	(W) **0.006**
O – Fb	75.1 ± 3.2	24.9 ± 3.2	6.2 ± 0.3	2.3 ± 0.3	**0.001**	642.3 ± 106.5	140.9 ± 29.1	**0.001**	29.6 ± 7.6	3.9 ± 1.7	(W) **0.037**
O – Ma	47.9 ± 3.1	52.1 ± 3.1	3.5 ± 0.2	2.2 ± 0.3	0.132	255.4 ± 70.9	60.4 ± 7.8	**0.021**	40.8 ± 11.3	16.3 ± 3.7	(W) **0.037**
O – Th	63.4 ± 3.8	36.6 ± 3.8	7.1 ± 0.6	3.5 ± 0.3	**0.006**	187.8 ± 72.2	466.0 ± 115.6	**0.047**	9.4 ± 4.4	5.6 ± 2.1	0.474
Wh – Da	74.3 ± 3.2	25.7 ± 3.2	6.7 ± 0.4	2.3 ± 0.3	**0.001**	274.5 ± 61.1	66.9 ± 22.2	(W) **0.006**	35.1 ± 7.0	0.1 ± 0.1	(W) **0.006**
Wh – Fb	75.6 ± 2-9	24.4 ± 2.9	8.1 ± 0.4	2.2 ± 0.4	**0.001**	188.2 ± 59.0	28.7 ± 14.0	(W) **0.002**	39.0 ± 4.9	2.4 ± 0.9	(W) **0.006**
Wh – Ma	67.4 ± 2.8	32.6 ± 2.8	5.5 ± 0.4	2.6 ± 0.2	**0.001**	277.9 ± 66.8	37.3 ± 12.4	**0.003**	25.9 ± 4.1	5.3 ± 1.8	**0.001**
Wh – Th	64.4 ± 2.8	35.6 ± 2.8	7.1 ± 0.4	3.9 ± 0.3	**0.001**	87.4 ± 39.6	260.0 ± 87.1	**0.048**	14.2 ± 5.1	1.0 ± 0.8	(W) **0.004**
Da – Fb	63.7 ± 4.2	36.3 ± 4.2	5.55 ± 0.5	2.6 ± 0.3	**0.002**	241.5 ± 36.6	98.4 ± 30.4	(W) **0.018**	3.2 ± 1.1	4.0 ± 1.3	(W) 0.726
Da – Ma	46.6 ± 3.7	53.4 ± 3.7	3.5 ± 0.4	3.7 ± 0.3	0.984	224.5 ± 34.5	65.6 ± 17.9	(W) **0.004**	6.0 ± 2.3	18.6 ± 4.1	**0.004**
Da – Th	46.9 ± 3.5	53.1 ± 3.5	3.9 ± 0.4	4.3 ± 0.4	0.706	301.2 ± 47.9	323.6 ± 35.6	0.767	3.5 ± 1.5	2.6 ± 0.9	(W) 0.999
Fb – Ma	37.4 ± 3.1	62.6 ± 3.1	2.8 ± 0.3	4.6 ± 0.3	0.070	129.2 ± 30.2	46.1 ± 10.3	**0.037**	1.6 ± 0.8	9.1 ± 1.5	(W) **0.011**
Fb – Th	34.3 ± 4.2	65.7 ± 4.2	1.9 ± 0.2	4.3 ± 0.4	**0.001**	81.4 ± 35.6	149.3 ± 27.4	0.229	3.7 ± 1.8	2.0 ± 0.9	(W) 0.527
Ma - Th	26.6 ± 3.1	73.4 ± 3.1	2.4 ± 0.3	6.2 ± 0.3	**0.001**	14.7 ± 12.2	252.7 ± 42.1	(W) **0.005**	1.0 ± 0.5	2.3 ± 1.1	(W) 0.359

Supp. 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, "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 Supp. 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 (Fig. 1)	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	68.6 ± 3.0	31.4 ± 3.0	5.5 ± 0.4	2.4 ± 0.2	**0.001**	196.6 ± 48.3	9.5 ± 3.7	(W) **0.002**	11.8 ± 2.3	1.9 ± 0.8	(W) **0.013**
Ba – Wh	60.3 ± 3.9	39.7 ± 3.9	5.2 ± 0.4	3.7 ± 0.5	**0.015**	142.6 ± 61.3	105.0 ± 39.7	0.326	49.2 ± 18.3	13.2 ± 5.1	0.123
Ba – Da	65.5 ± 3.7	34.5 ± 3.7	5.7 ± 0.4	3.4 ± 0.4	**0.001**	155.6 ± 39.5	96.7 ± 29.7	0.239	17.6 ± 4.5	1.9 ± 0.7	(W) **0.001**
Ca – Wh	19.4 ± 2.5	80.6 ± 2.5	1.3 ± 0.2	5.4 ± 0.3	**0.001**	16.5 ± 7.9	273.4 ± 36.5	(W) **0.002**	2.3 ± 0.9	15.9 ± 3.1	(W) **0.011**
Ca – Da	21.9 ± 3.3	78.1 ± 3.3	1.4 ± 0.2	4.6 ± 0.2	**0.001**	1.7 ± 0.9	154.4 ± 45.5	(W) **0.002**	1.1 ± 0.7	0.9 ± 0.5	(W) 0.577
Wh – Da	79.3 ± 3.7	20.7 ± 3.7	4.7 ± 0.2	1.2 ± 0.2	**0.001**	256.7 ± 54.8	82.4 ± 12.2	**0.011**	7.9 ± 2.3	0.5 ± 0.4	(W) **0.010**

Supp. Table 3: 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 Supp 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)	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	95.1 ± 1.5	4.9 ± 1.5	6.7 ± 0.3	0.4 ± 0.1	**0.001**	184.9 ± 50.2	12.0 ± 4.5	(W) **0.003**	5.9 ± 2.6	0.3 ± 0.3	(W) **0.035**
O - Fb	94.2 ± 1.3	5.8 ± 1.3	8.6 ± 0.3	0.6 ± 0.1	**0.001**	132.4 ± 25.9	24.9 ± 6.3	**0.004**	10.7 ± 3.2	0.6 ± 0.4	(W) **0.021**

Supp. Table 4: 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

Supp. 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 (AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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	AY-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)
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)
Fb	AY-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	AY-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)