Development of Aster Yellows on crop and non-crop species from the Canadian Prairies

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Funding: Saskatchewan Canola Development Commission (CARP ADF 2017-203),

Abstract

Aster Yellows phytoplasmas (AYp) are a group of obligate parasites that infect a wide range of plant species including crops such as canola and cereals, and non-crops like dandelion and wild mustard. In the Canadian Prairies, these microorganisms are mainly transmitted by a migratory species of leafhopper (*Macrosteles quadrilineatus* Forbes). While a low incidence of the disease associated with this pathogen has been reported for most years in canola fields, several outbreaks have been documented in this region. A selection of crop and non-crop species commonly found in the Canadian Prairies and *Arabidopsis thaliana* was used to assess the suitability of these plant species as hosts for AYp (16SrI-B). Symptom expression and phytoplasma levels were examined at different time points following exposure to infective insects. *A. thaliana*, barley, and canola were susceptible to infection with AYp, yet symptoms differed among these plant species. *A.*

thaliana and canola exhibited symptoms of infection as early as 2 weeks following exposure to infected insects, whereas symptoms in barley were observed at 5 weeks. A lower incidence rate was observed in wheat and levels of AYp in phytoplasma-infected wheat plants were low. Dandelion and sowthistle tested negative for the presence of AYp at all time points, suggesting that these are unsuitable hosts for these microorganisms. Moreover, we observed a partial disassociation between the plant species that were suitable hosts for AYp and those that had been characterized as more suitable or suitable hosts for aster leafhopper oviposition and nymphal development in previous studies.

Keywords

Plant-host interaction, Aster yellows, phytoplasmas, disease development, *Macrosteles quadrilineatus*

Introduction

Among plant pathogenic bacteria, phytoplasmas (‘*Candidatus* Phytoplasma’ spp.) are particularly important due to the wide variety of plant species that can become infected with these microorganisms and the relatively poorly known specifics of the epidemiology of such diseases, which is related to the difficulties in the establishment of *in vitro* cultures (Namba 2019). These microorganisms are related to Gram-positive bacteria, and are characterized by the lack of a cell wall and a reduced genome size. Moreover, they are restricted to phloem tissue and can be transmitted by several insect groups such as leafhoppers, planthoppers, and psyllids (Alma et al. 2019). Phytoplasma transmission can also occur by seed and through vegetative propagation methods (Satta et al. 2019; Caglayan et al. 2019). A very wide range of plant hosts, including crop and non-crop species, are susceptible to infection by phytoplasmas from a diversity of putative species encompassing nearly 40 groups, which are defined based on sequence analysis of 16S rRNA-encoding genes (Wei and Zhao 2022).

Aster Yellows (AY) disease is associated with phytoplasmas (AYp) classified in ribosomal RNA group 16SrI (‘*Ca.* P. asteris’). At least fifteen distinct subgroups of AYp are associated with this disease, among which 16SrI-A, 16SrI-B, and 16SrI-C are of great importance given their worldwide distribution (Lee et al. 2004). While symptoms associated with phytoplasma infection can differ among phytoplasma subgroups and plant species, infected plants commonly exhibit stunting, yellowing, phyllody, witches’-broom, and virescence (Duduk et al. 2018; Ermacora and Osler 2019). Chlorosis and rolling of leaves were reported as common symptoms in phytoplasma-infected barley (Hollingsworth et al. 2008; Oliveira et al. 2018) and phyllody, witches’-broom, and virescence have been described in phytoplasma-infected rapeseed (Martini et al. 2018). Similar symptoms have been observed in non-domesticated plant species infected with strains

16SrI-B and 16SrI-C (Duduk et al. 2018). In some cases, however, plants can remain asymptomatic (Silva et al. 2004; Bertaccini et al. 2005; de Oliveira et al. 2018). While previous work on detection, classification, and symptomatology in a wide variety of domesticated and non-domesticated plant species has vastly contributed to our current understanding of these pathogens and associated diseases (Olivier et al. 2010; Hollingsworth et al. 2018; Martini et al. 2018; Oliveira et al. 2018), studies on phytoplasma transmission and disease development under controlled conditions using domesticated and non-domesticated plant species commonly found in a specific geographic region are limited (Bahar et al 2008; Olivier et al. 2014).

In the Canadian Prairies, AYp is mainly transmitted by migratory populations of aster leafhoppers (*Macrosteles quadrilineatus* Forbes). While a low incidence of AY has been reported for most years in canola fields (<1%), several outbreaks of this disease have been documented since the 1950s (Olivier et al. 2009, Alberta Agriculture and Forestry 2014). While symptoms of infection in canola can be severe, Olivier et al. (2008) reported a high proportion of cases in which plants exhibited no symptoms yet tested positive for the presence of this pathogen. Other plant species that can become infected with AYp include cereals (barley, wheat, oat), legumes (alfalfa, faba bean, and clover), umbellifers (parsley and wild celery), and asters (dandelion, lettuce, marigolds, and sowthistle) (Olivier et al. 2009). For this region, previous studies on this system have characterized the suitability of several plant species for the insect vector (Romero et al. 2020, 2022), yet the relationship between the host plant and the pathogen requires further exploration.

In this study, we examined the development of Aster Yellows on a variety of plant species, including crop and non-crop species commonly found in the Canadian Prairies and *Arabidopsis thaliana*, and determined the suitability of these plant species as hosts for AYp. Three taxonomic markers (16S rRNA, *cpn60*, and *rp*) were used to characterize the AY substrain. Symptom

expression and phytoplasma levels were examined at different time points following exposure to infective insects. When considered alongside previous findings by Romero et al. (2020, 2022), these results contribute to the understanding of the AY epidemiology in Canada and the biological aspects of the plant-insect-pathogen interactions involved in this system.

Materials and Methods

1.1. Plant species and growing conditions

Plants were grown according to procedures described by Romero et al. (2020, 2022), maintained under an 18-hour photoperiod, at 21ºC during the day and 17ºC during the night. 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, except for non-cultivated plant species, for which each pot contained 3-5 seedlings.

For this study, the following plant species were used: spring wheat (*Triticum aestivum* Linnaeus; cultivar AAC Brandon) (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), and *Arabidopsis thaliana* (Brassicales: Brassicaceae). Except for *A. thaliana*, non-crop plant seeds were initially collected from fields surrounding Saskatoon, SK and grown under laboratory conditions.

Plant selection was based on previous observations by Romero et al. (2020) and Romero et al. (2022). Barley and spring wheat had been characterized as suitable host plants for aster leafhopper reproduction and development, while canola had been described as a less suitable host. Dandelion

and sowthistle were identified as a suitable and less suitable hosts for aster leafhopper oviposition and development, respectively. *A. thaliana* was included given its close relatedness to canola and suitability as a host for aster leafhoppers.

1.2. AY strain molecular characterization and phylogenetic analysis

AYp was initially obtained from a symptomatic canola plant (*B. napus*, unknown cultivar) found at the Agriculture and Agri-Food Canada Saskatoon Research Farm in June 2021. The plant was dug out and transferred to the Controlled Environment Facility at the University of Saskatchewan, where it was placed within a cage and kept at 24 ºC and under an 18-hour photoperiod. Groups of AY-uninfected aster leafhoppers were forced fed on this plant for a total of 72-96 hr and later used for infecting periwinkle (*Catharanthus roseus*) plants. Periwinkle can be infected with AYp without any plant mortality and aster leafhoppers can readily acquire AYp from infected periwinkle plants. Plant tissue was collected and the AYp strains were determined by locus-specific PCR amplification and sequencing methods.

Three loci corresponding to taxonomic markers commonly used to characterize phytoplasmas were examined. Nested PCR targeting the 16S rRNA-encoding gene locus used primers P1(Deng and Hiruki 1991) and P7 (Schneider et al. 1995) to generate a product of ~1.8 kb. This product was diluted 1:30 and 2µl of the dilution used as template in a second PCR with primers R16F2n and R16R2 (Gundersen and Lee 1996), which provided an amplicon of ~1.2 kb (F2nR2). PCR conditions were as previously described (Pérez-López et al. 2017). A second nested PCR targeting the AYp ribosomal protein (*rp*) locus used primers rpF1/rpR1(Lim and Sears 1992), followed by rp(I)F1A/rp(I)R1 as described (Lee et al. 2004). The *cpn60* “universal target” (*cpn60* UT) was

amplified using a phytoplasma-specific primer cocktail as described (Muirhead et al. 2019). Amplicons were cloned using the vector pGEM-T Easy (Promega, Wisconsin, USA) following the manufacturer-recommended protocol. Recombinant plasmids were used to transform *E. coli* TOP10 competent cells (ThermoFisher, Massachusetts, USA), and the sequences of 5-6 clones from each amplicon were determined using a commercial DNA sequencing service (Eurofins Genomics, Toronto ON). Sequences corresponding to the 16S rRNA gene (F2nR2) were analyzed by *in silico* restriction fragment length polymorphism (RFLP) using the iPhyClassifier (Zhao et al. 2009) to determine the 16S group and subgroup. In addition, *cpn60* clone sequences were assigned to RFLP groups using the CpnClassiPhyR (Muirhead et al. 2019). For phylogenetic analysis, DNA sequences were manually trimmed to a common length, then aligned using ClustalW (Thompson et al. 1994). Phylogenetic relationships among the taxa were inferred using the Maximum Likelihood method and the Tamura-Nei model (Tamura and Nei 1993) in MEGAx (Kumar et al. 2018).

1.3. Aster leafhoppers

Aster leafhoppers were reared as previously described by Romero et al. (2020), with a few modifications. Colonies were maintained at 24 ºC and under an 18-hour photoperiod. 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 plants were added to supplement barley. Colonies (plants and insects) were periodically tested for AYp infection using quantitative Polymerase Chain Reaction (qPCR), as described below.

1.4. Latent period

2-week-old plants were transferred to a chamber at 24ºC and exposed to AY-infected aster leafhoppers. Insects were sorted into groups of 5 females and 5 males based on external genitalia (Romero et al. 2020), caged onto a plant, and allowed to feed on it for 1 week. Following the 1 week-exposure period, adults were removed, and plants were kept for further observations. In addition to this, a contact-acting foliar insecticide (Decis, Bayer CropScience, Leverkusen, Germany) was applied to each experimental unit to prevent any eggs from developing. Following the exposure period to AY-infected aster leafhoppers, plants were photographed, and tissue samples were taken at 2, 4, and 5 weeks. For *A. thaliana* plants, 4 to 5 leaves (mid-position along the stem) were sampled at each time point. For barley and wheat, a portion of the blade (3 to 4 cm along the longitudinal axis) was collected during each time point. For canola, dandelion, and sowthistle, a portion (2 cm x 3 cm) of a leaf was collected at each timepoint. Tissue samples were further processed and AYp was quantified using qPCR. Ten replicates were conducted for each plant species.

Control treatment plants were grown under the same conditions as previously described, with the exception that they were not exposed to AY-infected aster leafhoppers. Five replicates were conducted for each plant species.

1.5. Plant sampling and DNA extraction

For each plant species and each observation period (2, 4, or 5 weeks following the IAP), approximately 0.050-0.075 g of leaf tissue were collected in an Eppendorf tube and stored at -80 ºC until further processing. Plant DNA was isolated using the DNEasy Plant Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer's protocol, and DNA concentration was quantified

using a Nanodrop One Microvolume UV-Vis Spectrophotometer (ThermoFisher, Massachusetts, USA).

1.6. Quantitative PCR (qPCR)

Plant DNA samples were tested for the presence and titer of AYp using a probe-based qPCR. The phytoplasma *cpn60* gene was amplified using primers: 5'- TGGAGTTATTAATGTTGATG, 5'-GGAGAAGCATATCCTTTA (Pusz-Bochenska et al. 2022). Probe: FAM-ATCCTTCAACAACTTCTAATTCTG-BHQ1.

Each 20-µl qPCR contained: 10 µl of SsoAdvanced Universal Probes Supermix (Bio-Rad, California, USA), 0.3 µM of each forward and reverse primers (Integrated DNA Technologies, Iowa, USA), 0.2 µM probe (Integrated DNA Technologies, Iowa, USA), and 2 µl of DNA template. PCR cycling conditions were 95ºC for 3 min (1X), followed by 40 cycles of 95ºc for 30 seconds, 59ºC for 30 seconds, and 72ºC for 30 seconds. Amplification was carried out using a QuantStudio3 instrument (Thermo Fisher Scientific, Massachusetts, USA) and reactions were quantified using QuantStudio Design and Analysis Software v. 1.5.2.x (Thermo Fisher Scientific, Massachusetts, USA).

For each run, a positive control (DNA from a symptomatic plant, high titer), two negative controls (distilled water), and a set of standards with known copies of *cpn60* were included. Standards ranged from 10^7 to 10^1 copies of *cpn60* per reaction and were used to construct a calibration curve.

1.7.Statistical analysis

Statistical analyses were performed using R version 4.1.3 (R Core Team 2022). Quantification cycles (C_q) were converted to $\log_{10}$ copy numbers of *cpn60* in each reaction by interpolation of the

198 standard curve and results were corrected to account for the DNA concentration in each sample.
199 The $\log_{10}$ copy numbers of *cpn60* in each reaction were analyzed with a Generalized Linear Mixed
200 Model (GLMM), with a normal distribution and with "Plant species" and "Collection date" (2, 4,
201 or 5 weeks), and their interaction as categorical fixed effects. The "Experimental unit ID" was
202 incorporated as a random effect to account for the repeated measures. We used package "lme4" to
203 conduct the analyses (Bates et al. 2015) and package "emmeans" to perform post hoc tests (Russell
204 2020). Tukey's correction was used to adjust the obtained *p*-values for conducting multiple
205 comparisons.
206
207 **Results**
208 *Phytoplasma strain*
209 Phylogenetic analysis of the 16S rRNA-encoding gene sequences revealed that the AYp used for
210 this study was clustered with '*Ca.* P. asteris' related phytoplasma strains in the 16SrI group (Figure
211 1). Phylogenetic analysis also revealed that the clones were slightly distinct from one another and
212 clustered most closely with AYp strain SF1, which is a 16SrI-B strain originally isolated from
213 infected flax. The clone sequences also clustered with other 16SrI-B strains, including Maize
214 Bushy Stunt phytoplasma, and other strains identified with canola (Rapeseed Phyllody
215 Phytoplasma). RFLP analysis of the clone sequences showed that two subgroups within 16SrI
216 were represented in the infected tissue, since two clones typed as 16SrI-B ($F \leq 0.97$, suggestive of
217 a new subgroup within16SrI) and four typed as 16SrI-B ($F > 0.97$, suggestive of inclusion with
218 16SrI-B). Overall, the 16S clones shared >98.5% sequence identity with one another, which is
219 consistent with the presence of a single species of phytoplasma, '*Ca.* P. asteris'. The single 16S
220 rRNA-encoding sequence that was assembled from this strain using hybridization probes (Pusz-

Bochenska et al. 2022) was slightly distinct from both of the clone sequences and clustered most closely with Rapeseed Phyllody Phytoplasma (Figure 1).

Analysis of single-copy, protein-coding genes provided some clarity to the phylogenetic placement of this AYp strain. The *cpn60* UT sequences of five clones were nearly identical to one another, sharing >99.8% sequence identity, and all typed as *cpn60* I-IB using RFLP analysis. Furthermore, the *cpn60* clone sequences clustered with other *cpn60* I-IB strains and were identical to the sequences of Rapeseed Phyllody Phytoplasma *cpn60* (Supp. Fig. 1). Finally, the sequences of five *rp* clones were >99% identical to one another and clustered with 16SrI-B strains. 4 of the 5 sequences were identical to the *rp* sequence of Rapeseed Phyllody Phytoplasma (Supp. Fig. 2). Taken together, these results suggest that the AYp under study is a member of subgroup 16SrI-B, and shows evidence of 16S rRNA gene heterogeneity.

Symptom expression

Following exposure, symptom expression (yellowing) was observed at 2 weeks in some plant species such as *A. thaliana* and canola, while no symptoms associated with AY infection were observed in barley, wheat, dandelion, and sowthistle (Fig. 2 and Supp. Fig. 3). During this observation period, canola plants also exhibited distortion of flower buds (Fig. 2 and Supp. Fig. 3). At 4 weeks, symptoms were more pronounced in *A. thaliana* and canola, while the other plant species under study exhibited no symptoms (Fig. 2). Flower bud distortion was observed in *A. thaliana* plants; reddening and signs of phyllody were detected in canola plants. At 5 weeks, yellowing was observed in barley leaves, flower bud distortion was more pronounced in *A. thaliana*, and symptoms in canola were more severe (Fig. 2, Fig. 3, and Supp. Fig. 3). In wheat, however, no symptoms associated with phytoplasma-diseases were detected (Fig. 2, Fig. 3, and

Supp. Fig. 3). In sowthistle, most plants exhibited no symptoms, except for one plant, in which yellowing was observed in one leaf at 5 weeks post-infection period. While some dandelion plants exhibited reddening of leaf tips starting at 2 weeks following the exposure period to AY-infected leafhoppers, this was commonly observed in dandelion plants grown under laboratory conditions.

AY levels

AYp levels were quantified in tissue samples taken at 2, 4, and 5 weeks following exposure to AY-infected aster leafhoppers. When assessing the number of infected plants, dandelion and sowthistle samples tested negative for the presence of AYp during all sampling periods, while AYp was detected in only two wheat plants across the different sampling periods (Table 1). Contrary to these observations, a high proportion of barley, *A. thaliana*, and canola plants tested positive for the presence of AYp (Table 1).

Dandelion and sowthistle were excluded from the statistical analysis as all samples from these plant species tested negative for the presence of AYp. Analysis of the $\log_{10}$ copies of *cpn60* in each sample revealed no significant effect of the collection date (2, 4, or 5 weeks; $X^2 = 2.25$, df = 2, P = 0.324) and a significant effect of the plant species ($X^2 = 22.10$, df = 3, P < 0.001). There was a significant interaction between the collection date and the plant species ($X^2 = 13.42$, df = 6, P = 0.037). Overall, AY levels did not differ across the different plant species at 2 and 4 weeks, but were more variable during the last sampling period (Fig. 2 and Table 1). At 5 weeks, tissue samples from barley and canola plants had a higher number of *cpn60* copies than samples from wheat plants. In this case, while $1.26 \times 10^5 \pm 8.35 \times 10^4$ copies of *cpn60* per ng of genomic DNA were detected in barley samples (Mean ± SEM) and $8.22 \times 10^4 \pm 1.79 \times 10^4$ in canola, *A. thaliana* tissue samples were characterized by a value of $4.37 \times 10^3 \pm 4.37 \times 10^3$ copies of *cpn60* per ng of genomic

267 DNA and wheat samples tested negative (Fig. 2 and Table 1).

268 Tissue samples from control plants were collected and tested for the presence of AYp
269 during the last sampling period (5 weeks), with all samples yielding negative results.

270

271 **Discussion**

272 In this study, the suitability of five crop and non-crop plant species commonly found in the
273 Canadian Prairies and *Arabidopsis thaliana* as hosts for the plant pathogen known as Aster
274 Yellows phytoplasma (AYp) was examined. Symptom expression and AYp levels were examined
275 at three different time points (2, 4, and 5 weeks post-infection period; Fig. 2). Plant species were
276 selected based on their economic importance for the ecoregion and/or previous reports of their
277 suitability for sustaining AYp infection and/or aster leafhopper populations (Lee et al. 2004;
278 Khadhair et al. 2008; Olivier et al. 2009, 2011; MacLean et al. 2011; Romero et al. 2020, 2022).
279 Molecular characterization of 16S, *cpn60*, and *rp* sequences indicated that the strain in this study
280 is a member of subgroup 16SrI-B. The observation that the 16S rRNA gene sequences typed as
281 distinct subgroups of 16SrI suggested the possibility of a mixed infection, or an infection with a
282 single strain that possesses 16S rRNA gene heterogeneity. To differentiate these possibilities, we
283 determined six amplicon sequences each of the single-copy loci *rp* and *cpn60*, both of which show
284 higher inter-strain sequence variability than 16S rRNA loci. We observed that the clone sequences
285 of *rp* and *cpn60* showed sequence identities >99.6% among them, which is evidence of infection
286 with a single strain that has two 16S loci that type distinctly. Zwolińska et al. (2019) noted that *B.*
287 *napus* and surrounding non-crop plants in Poland were infected with strains of phytoplasma
288 characterized as either 16SrI-B or a heterogeneous strain, 16SrI-(B/L)L (Rapeseed Phyllody
289 Phytoplasma strain RP166). The strain that was identified in this study also shows evidence of 16S

290 rRNA gene heterogeneity, although all clones typed as 16SrI-B but with varying similarity
291 coefficients above and below that used for defining novel groups (F>0.97). The single 16S rRNA
292 gene sequence that was provided by hybridization (Pusz-Bochenska et al. 2022) clustered with the
293 previously reported strains of phytoplasma that infect canola (subgroups B and L), including
294 Rapeseed Phyllody strain RP166. The sequences of the single-copy genes *cpn60* and *rp* were both
295 identical to the corresponding genes in strain RP166, suggesting that the strains infecting canola
296 are very similar across a wide geographic range.

297 Our results showed that crop species such as canola and barley can sustain AYp infections
298 with this strain, yet symptom expression differed between these two plant species. While canola
299 exhibited symptoms like yellowing and flower bud distortion in the early and mid- stages of the
300 infection, barley leaves showed chlorosis during the later stage of the infection and no other
301 symptoms were observed in this plant species. Wheat, however, had a lower incidence of the
302 pathogen than canola and barley (20%, 100%, and 80%, respectively; Table 1) and low levels of
303 AYp in infected plants. Most symptoms in *A. thaliana* were similar to those observed in canola
304 and levels of AYp did not differ between these two plant species over time. Both non-crop species
305 examined (dandelion and sowthistle) tested negative for the presence of AYp, suggesting that these
306 are unsuitable hosts for phytoplasmas belonging to subgroup 16SrI-B (Fig. 2 and Table 1).

307 When considered along with previous findings by Romero et al. (2020, 2022), observations
308 from this study indicated a partial uncoupling between the host suitability for aster leafhoppers
309 and the host suitability for AYp. Canola had been characterized as a less suitable host for aster
310 leafhopper oviposition and nymphal development (Romero et al. 2020, 2022), while this study and
311 work by Town et al. (2018) indicated that this plant species can become infected with AYp and
312 sustain high levels of infection with this pathogen. Conversely, wheat had been described as a

more suitable reproductive and food host for aster leafhoppers (Romero et al. 2020), yet almost no plants were infected with AYp, and AY levels were low in the few infected plants (Fig. 2 and Table 1). Interestingly, *A. thaliana* and barley could act as suitable hosts for both aster leafhoppers (Romero et al. 2020) and AYp, yet symptom expression differed between them, as symptoms in *A. thaliana* were more severe. In the case of sowthistle, little to no offspring had been observed on this plant species and it was described as an unsuitable host for AYp. Similar to sowthistle, dandelion tested negative for the presence of AYp, but was characterized as a suitable host for leafhopper oviposition and development (Romero et al. 2020). In a similar study by Batlle et al. (2008), *Macrosteles quadripunctulatus* individuals infected with Stolbur phytoplasma (strain 16SrXII-A) were allowed to feed on healthy periwinkle, tomato, carrot, lettuce, and grapevine plants for a total of 4 days. While periwinkle and tomato were highly susceptible to Stolbur phytoplasma, exhibiting symptoms as early as 15 days following the transmission period, other plant species such as lettuce and grapevine were not suitable hosts for this strain of phytoplasma (Batlle et al. 2008). Interestingly, while *M. quadripunctulatus* was capable of transmitting this plant pathogen to several host plants, most plant species were unsuitable hosts for the survival of this insect, suggesting a disassociation between the host ranges of the plant pathogen and the insect vector. Differences in the detection of phytoplasmas following transmission assays in a selection of plant species were also reported by Salehi et al. (2011), who observed that a strain of phytoplasmas related to Aster Yellows was successfully transmitted to plants such as periwinkle, rapeseed, and mustard, but was not detected in other plant species like sunflower, alfalfa, and wild mustard.

In the Canadian Prairies, several outbreaks of AY have been documented in previous years (1957, 2001, 2007, and 2012), with crops such as canola and flax being particularly susceptible to

the infection with this group of phytoplasmas. While the incidence of this disease is relatively low in most years (<0.01% in canola, Olivier et al. 2009), occasional early winds can carry populations of aster leafhoppers into the region in early spring and be correlated with the occurrence of an outbreak. Yield reduction in canola possibly associated with such events has been estimated to range between 10 and 15% and a similar trend of reduced yields in reported AY outbreak years has been observed in other crops such as barley, spring wheat, and flax (Statistics Canada 2022). While previous work on AY in barley has shown that the infection with this plant pathogen has little effect on that crop (Olivier et al. 2011), other studies have reported severe symptoms ranging from reduced spikelets to total plant collapse (Hollingsworth et al. 2008; Oliveira et al. 2019). In spring wheat, plants can be either asymptomatic or exhibit symptoms similar to those observed in plants infected with Barley Yellow Dwarf Virus (Olivier et al. 2011).

In this study, dandelion and sowthistle were selected for examining host suitability of non-crop species for AYp. This selection was based on previous findings about the suitability of dandelion for sustaining aster leafhopper nymphal development (Romero et al. 2020) and reports of phytoplasma-infected dandelion and various thistle species that had been collected near sampling sites (Wang and Hiruki 2001; Lee et al. 2004; Khadhair et al. 2008; Olivier et al. 2011). Interestingly, our results showed that these plant species tested negative for the presence of AYp and did not exhibit symptoms commonly associated with an AY infection. Such differences between previous studies and our findings could be related to the strain of phytoplasma that was identified or used in each case. In this study, the strain of AYp was identified as belonging to the 16SrI-B subgroup. In a previous study by Wang and Haruki (2001), in which dandelion plants were collected nearby a phytoplasma-infected canola field, it was reported that the strain of AYp was 16SrI-A. This same strain was identified in phytoplasma-infected spiny annual sowthistle

samples examined by Lee et al. (2004) and perennial sowthistle (*Sonchus arvensis* L.) samples collected by Khadhair et al. (2008). Olivier et al. (2011) reported that sequences similar to subgroups 16SrI-A and 16SrI-B had been identified in a variety of plant species collected at different sampling sites, among which dandelion was found, yet did not indicate if this plant species had a mixed infection with both strains, if different dandelion samples were infected with one strain or the other, or if only one strain was detected in this plant species. Interestingly, Lee et al. (2004) have described canola (*Brassica* spp.) as a natural host of subgroup 16SrI-A, while in a more recent study by Olivier et al. (2010), subgroup 16SrI-B was detected in this plant species. Other members of the Brassicaceae family such as wild mustard (*Brassica rapa* Linnaeus) and false flax (*Camelina sativa* Linnaeus) can also function as hosts for the 16SrI-A strain, while members of the Poaceae family act as hosts for the 16SrI-B subgroup (Olivier et al. 2010). While both strains 16SrI-A and 16SrI-B have been found in hosts such as canola, wild mustard, and China aster (Olivier et al. 2009), this might not be the case for dandelion and sowthistle, which could possibly be suitable hosts for strain 16SrI-A but not for strain 16SrI-B. Taking this possibility into consideration, examining the symptomatology and AYp titer on various plant species including those examined in this study using '*Ca*. P. asteris' strain 16SrI-A would provide valuable information regarding the host range of different strains. In addition to this, some authors have observed that high densities of the main insect vector in areas with phytoplasma-infected plants do not always correlate with a high incidence of the disease (Batlle et al 2000) and that other insect species present in the ecosystem can successfully acquire phytoplasmas and transmit them to non-domesticated plant species, for example, which can act as wild reservoirs of the pathogen and contribute to the epidemiology of the disease (Chuche et al. 2016). In the Canadian Prairies, other leafhoppers species present in canola fields include *Amplicephalus inimicus*, *Balclutha* spp., and

382 *Ceratagalia humilis* (Olivier et al. 2007), yet their role in the transmission of AYp and contribution
383 to outbreaks of the disease are unknown.

384 The aims of this study were to characterize the suitability of various plant species as hosts
385 for AYp and to examine the symptom expression associated with this infection. Overall, our results
386 showed that plant species that had been characterized as more suitable or suitable hosts for aster
387 leafhopper oviposition and nymphal development were not necessarily suitable hosts for AYp. For
388 example, canola was highly susceptible to AYp infection and exhibited severe symptoms
389 associated with this disease (Fig. 2, Fig. 3, and Table 1), yet had been described as an unsuitable
390 host for aster leafhopper reproduction and nymphal development (Romero et al. 2020).
391 Conversely, almost no wheat plants tested positive for the presence of AYp and no distinguishable
392 symptoms were observed (Fig. 2, Fig. 3, and Table 1), yet this plant species was reported as a
393 suitable host for aster leafhoppers (Romero et al. 2020). This disassociation between the host
394 suitability for the insect vector and that for the plant pathogen requires further study. Moreover,
395 possible differences in host susceptibility to phytoplasma infection and symptomatology between
396 phytoplasma strains should be further investigated. Findings from this study have serious
397 implications for the management of AY as they provide insights into what plant species can harbor
398 high levels of AYp, what symptoms will be observed in each plant species, and whether symptoms
399 will appear during the early, middle-, or later stages of the infection. Furthermore, results from this
400 study highlight the importance of the relationship between the host plant and the pathogen and
401 how the interplay between them can lead to an unsuitable environment for the pathogen (absence
402 of pathogen) or different levels of tolerance and resistance to the pathogen. Additionally, it should
403 be noted that AY infection of plants can only occur by exposing plant species to infective
404 leafhoppers and that the feeding behavior of these insects can be altered by characteristics of the

host plant, which can in turn affect the initial amount of inoculum in the plants and possibly explain some differences in the AY levels observed across all plant species examined in this study. While no acquisition experiments were conducted to examine if aster leafhoppers could acquire AYp from several phytoplasma-infected hosts in a similar manner, this is another aspect of the epidemiology of this disease that deserves further study.

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 and AY-infected periwinkle. We thank Farrah Fischer for helping with the DNA extractions. 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, TD, TW, BR and CO conceived the ideas and designed the methodology; BR and TD 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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Table 1

	No. of infected plants	No. of *cpn60* copies/ng of genome DNA (**Figure 2**) 2 weeks		4 weeks		5 weeks		Host suitability to vector
A. thaliana	8/10	4.92 ± 2.99 §	abc	1.41 ± 1.39 ¶	abc	4.37 ± 4.37 †	bc	Most suitable
Barley	8/10	1.63 ± 1.03 §	abc	3.79 ± 2.21 §	ab	8.22 ± 4.25 §	a	Most suitable
Canola	10/10	3.80 ± 3.42 †	abc	3.65 ± 1.79 §	ab	1.26 ¶ ± 8.35 §	ab	Least suitable
Dandelion	0/10	0.00 ± 0.00		0.00 ± 0.00		0.00 ± 0.00		Suitable
Sowthistle	0/10	0.00 ± 0.00		0.00 ± 0.00		0.00 ± 0.00		Least suitable
Wheat	2/10	1.60 ± 1.60 *	c	7.00 ± 6.40 *	bc	0.00 ± 0.00	c	Most suitable

* These values are multiplied by 10^1

† These values are multiplied by 10^3

§ These values are multiplied by 10^4

¶ These values are multiplied by 10^5

Supp. Table 1

	No. of infected plants	No. of *cpn60* copies/ng of genome DNA in AY-infected samples 2 weeks		4 weeks		5 weeks		Host suitability to vector
A. thaliana	8/10	9.83 ± 5.29 §	(5)	2.36 ± 2.31 ¶	(6)	4.37 §	(1)	Most suitable
Barley	8/10	2.32 ± 1.41 §	(7)	5.42 ± 3.0 §	(7)	1.03 ¶ ± 5.10 §	(8)	Most suitable
Canola	10/10	4.75 ± 4.26 †	(8)	4.56 ± 2.13 §	(8)	1.45 ± 1.03 ¶	(8)	Least suitable
Dandelion	0/10	-		-		-		Suitable
Sowthistle	0/10	-		-		-		Least suitable
Wheat	2/10	1.57 *	(1)	3.16 ± 2.62 *	(2)	-		Most suitable

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574 **Figures**

575 **Fig. 1**: Phylogenetic analysis of 16S rRNA-encoding gene sequences (F2nR2 fragment) of the
576 AYp obtained in this study, in the context of reference phytoplasma sequences. The tree was
577 constructed using the Maximum Likelihood method and bootstrapped 100 times, with the
578 percentage of trees in which the associated taxa clustered together shown next to the branches. The
579 sequence of clones 5 and 6 have been denoted with a black circle and can be found in GenBank
580 (OP806521 and OP806522).

581

582 **Fig. 2**: Examples of symptom expression in all plant species under study during each observation
583 period (2, 4, and 5 weeks following exposure to AY-infected aster leafhoppers). Close-ups of
584 symptoms have been provided for *A. thaliana*, barley, canola, and wheat. The average number of
585 copies of *cpn60* per ng of genomic DNA has been indicated for each plant species and observation
586 period. Details about the number of infected plants and host suitability can be found in Table 1.

587

588 **Fig. 3**: Symptom expression in *A. thaliana*, barley, canola, and wheat at 5 weeks following
589 exposure to AY-infected aster leafhoppers. Examples of control plants (AY-Uninfected) have been
590 placed next to symptomatic plants for comparison.

591

Tables

Table 1: AY concentration on selected crop and non-crop species over time. The avg. no. of copies of *cpn60* per ng of genomic DNA for each combination of plant species and sampling period (2, 4, and 5 weeks) is presented. The "no. of infected plants" corresponds to the number of experimental units that tested positive for the presence of AYp in at least one of the sampling periods. Different letters indicate statistically significant differences in the number of copies of *cpn60* per reaction (GLMM followed by Tukey's test with adjustment for multiple comparisons, with an α-value of 0.05). Based on findings by Romero et al. (2020), the suitability of each plant species for sustaining leafhopper development has been provided; plant species have been classified as "most suitable", "suitable", and "least suitable". Mean and standard error of the mean (SEM) values are provided. Samples that tested positive for the presence of AYp were analyzed separately in Supp. Table 1.

Supplementary material

Supp. Fig. 1: Molecular phylogenetic analysis of the ribosomal protein (*rp*) gene sequence of the AYp obtained in this study, in the context of reference phytoplasma sequences. The tree was constructed using the Maximum Likelihood method and bootstrapped 100 times. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The sequence of clone 3 has been denoted with a black circle and can be found in GenBank (OP820497).

Supp. Fig. 2: Molecular phylogenetic analysis of chaperonin cpn60/groEL Universal Target gene sequences of the AYp obtained in this study, in the context of reference phytoplasma sequences. The tree was constructed using the Maximum Likelihood method and bootstrapped 100 times. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The sequence of clone 3 has been denoted with a black circle and can be found in GenBank (OP820496).

Supp. Fig. 3: Examples of control treatment plants during each observation period. Close-ups of leaves or floral structures have been provided for *A. thaliana*, barley, canola, and wheat.

Supp. Table 1: **AY concentration in positive samples of crop and non-crop species over time**. For those samples that tested positive for the presence of AYp, the avg. no. of copies of *cpn60* per ng of genomic DNA for each combination of plant species and sampling period (2, 4, and 5 weeks) is presented. The number of observations (N) used for calculating each average has been indicated between brackets. Based on findings by Romero et al. (2020), the suitability of each plant species for sustaining leafhopper development has been provided; plant species have been classified as

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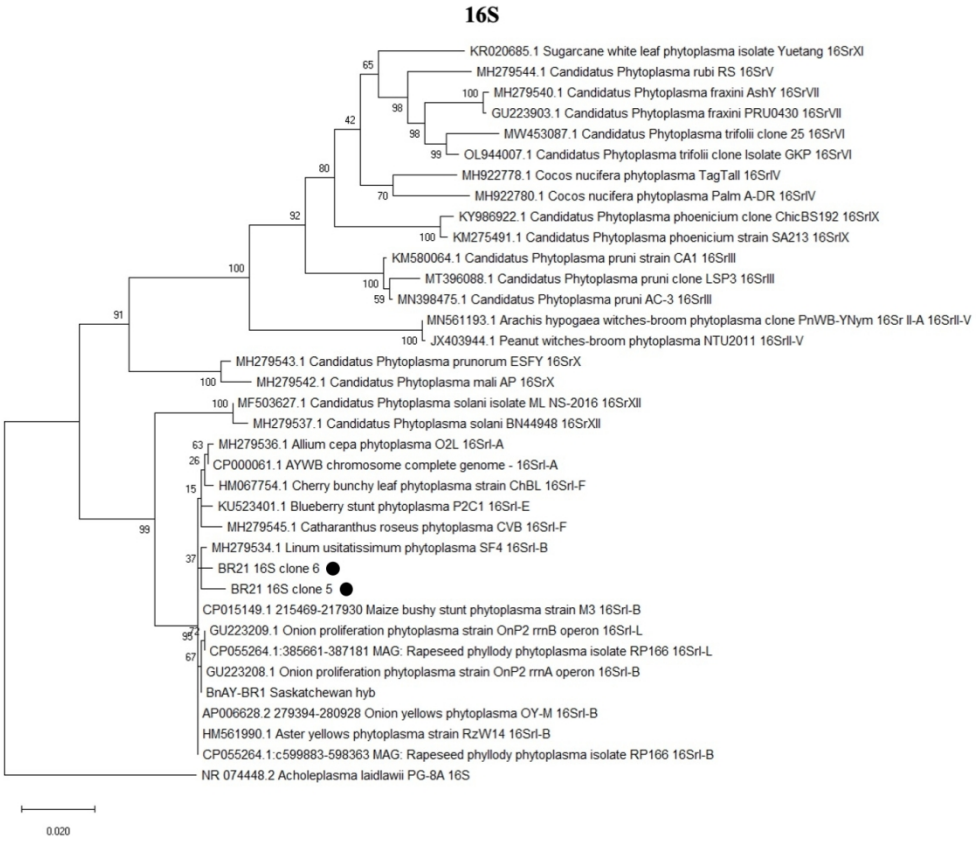

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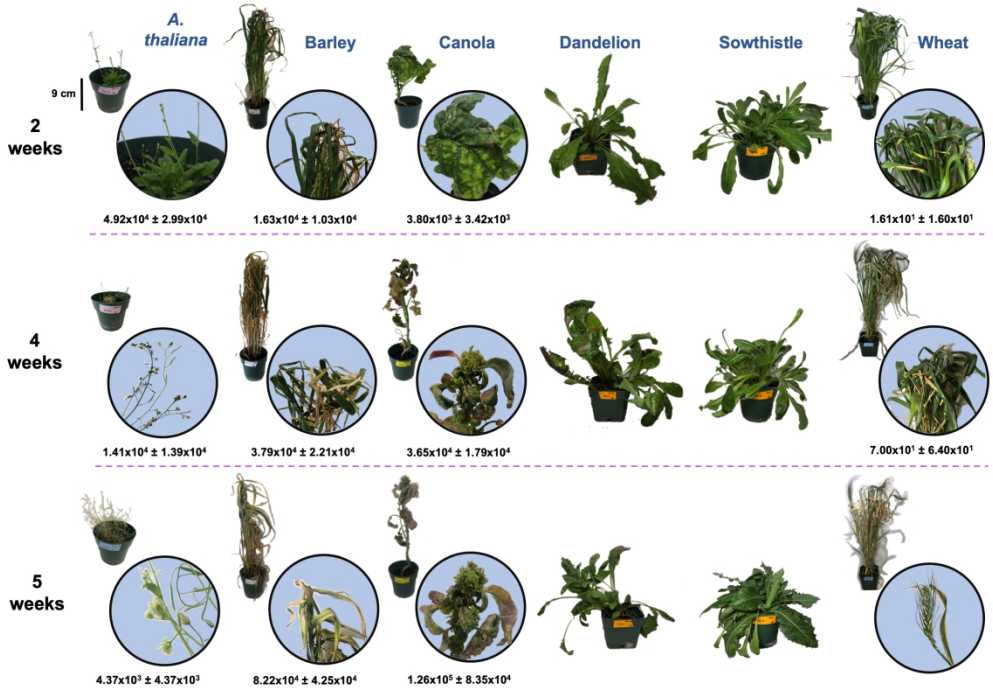

Fig. 2: Examples of symptom expression in all plant species under study during each observation period (2, 4, and 5 weeks following exposure to AY-infected aster leafhoppers). Close-ups of symptoms have been provided for A. thaliana, barley, canola, and wheat. The average number of copies of cpn60 per ng of genomic DNA has been indicated for each plant species and observation period. Details about the number of infected plants and host suitability can be found in Table 1.

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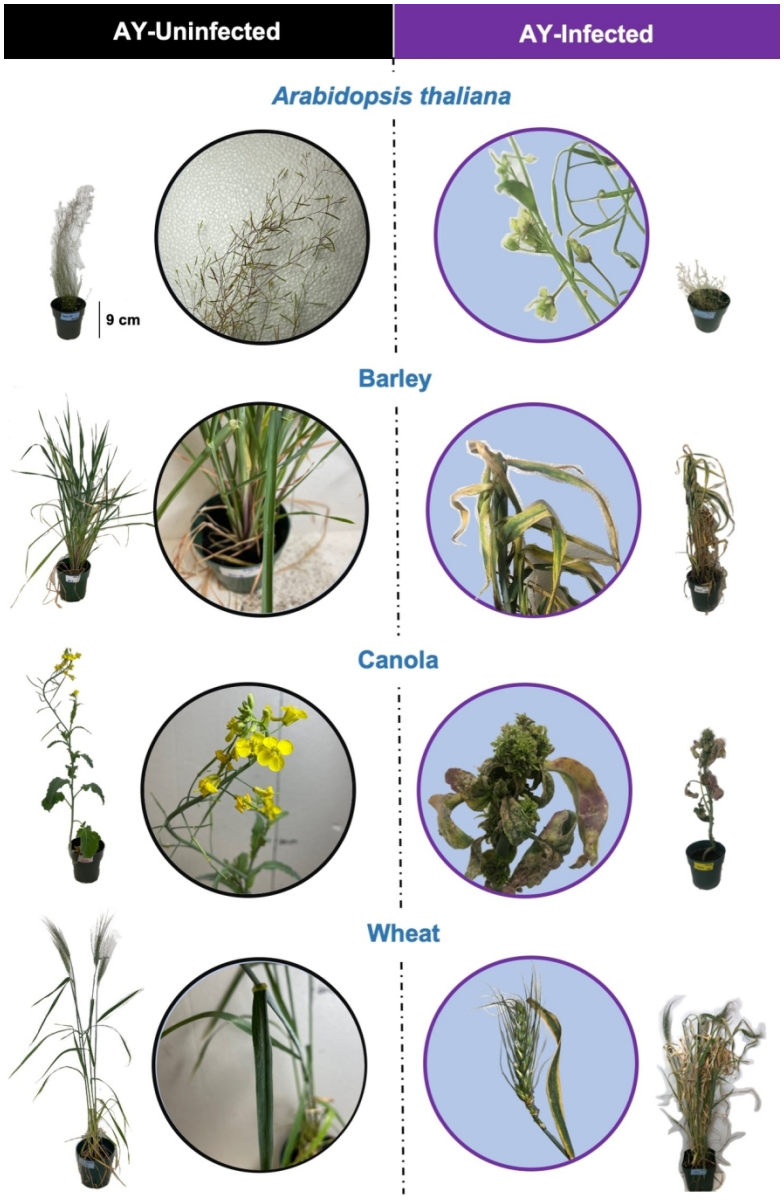

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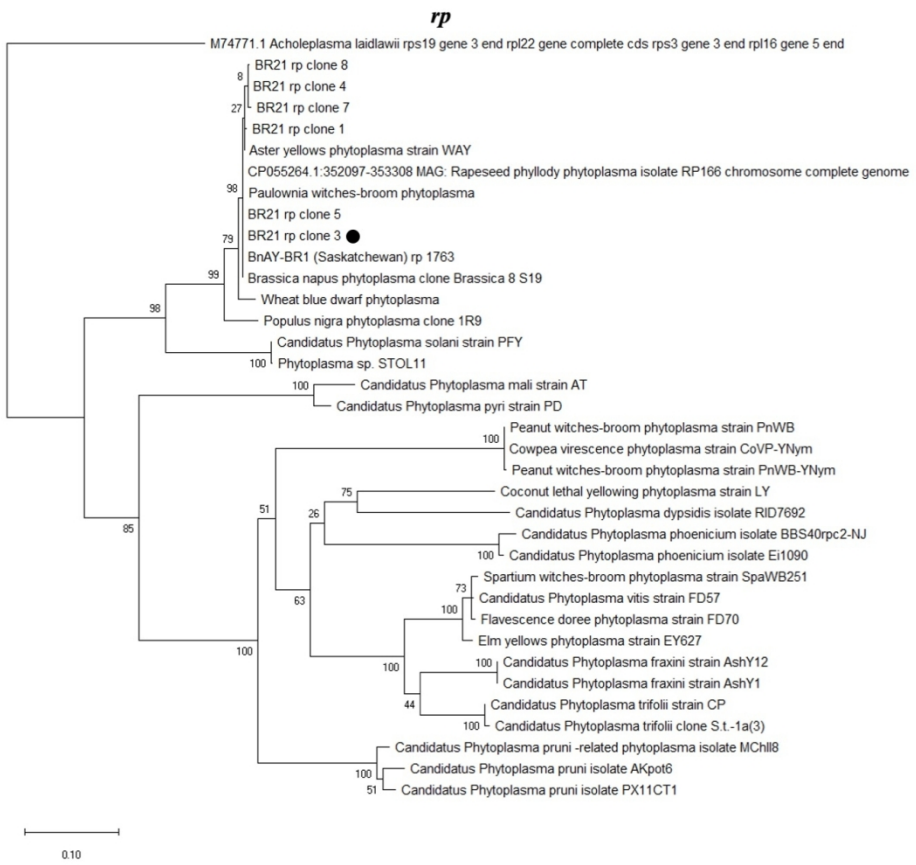

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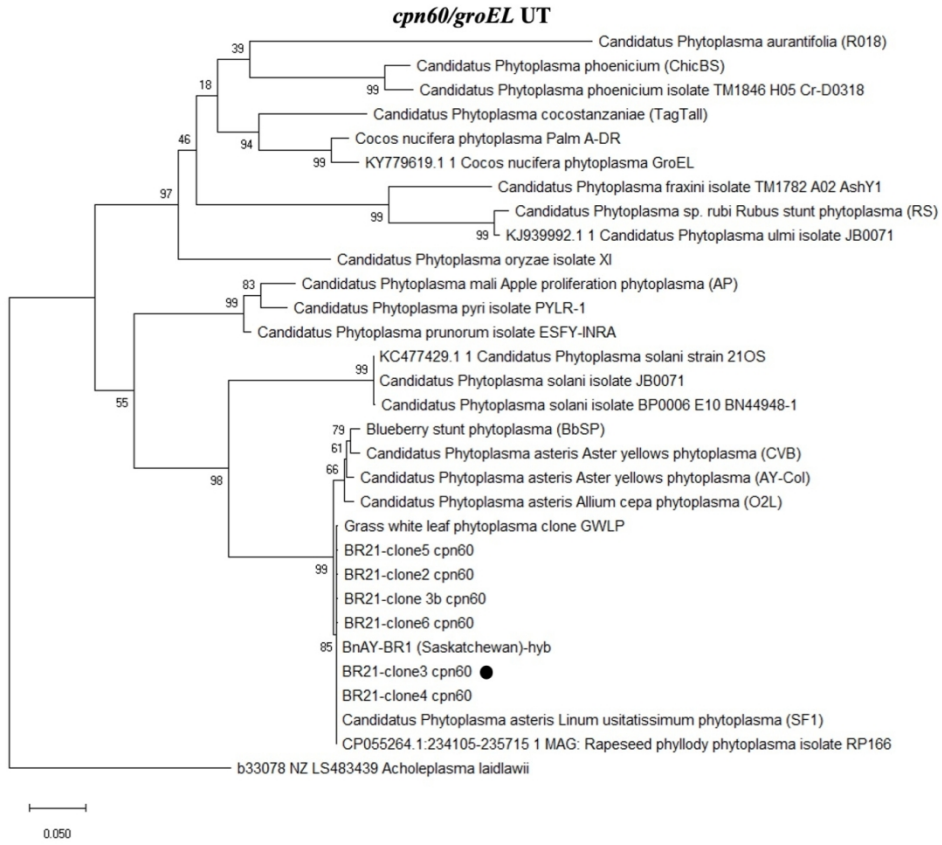

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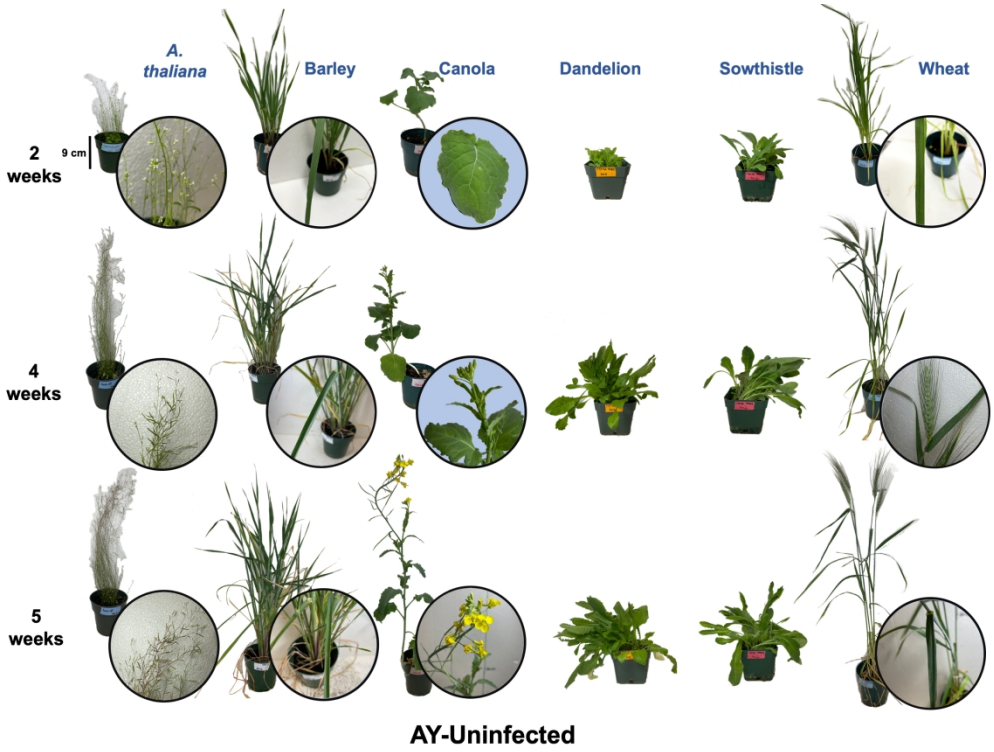

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