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Aster Yellows in Leafhoppers and Field Crops in Saskatchewan, Canada, 2001–2008

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ABSTRACT

Leafhopper populations were sampled in canola and cereal crops over an eight year period at seven locations in Saskatchewan, Canada. Aster yellows (AY) disease incidence was determined at the same locations in canola in 2001–2008 and in cereals in 2005–2008. Over 20 leafhopper species tested positive for the presence of AY phytoplasma DNA at least once in the course of the study. Overall, the six-spotted aster leafhopper *Macrosteles quadrilineatus* was the most abundant species and had the highest infection rate. Most insects were infected with AY phytoplasma strain 16SrI-A; strain 16SrI-B was also detected. *Macrosteles quadrilineatus* was most abundant in barley and wheat fields. The AY phytoplasma vector *Amplicephalus inimicus* and leafhoppers belonging to the genus *Psammotettix* were found mostly in cereal crops and were infected with AY phytoplasma strain 16SrI-A or -B; strain 16SrI-C was also identified in *A. inimicus*. Twelve new potential AY phytoplasma vectors were identified. Strains 16SrI-A and -B were detected in tissue and seed of canola, barley and wheat. Strains 16SrI-A, -B, and -C were detected in oats. Strains 16SrI-A and -B were also detected in other crops, including chickpea, dill, caraway, coriander, and echinacea, and in weedy species, including goldenrod, dandelion, wild mustard, and stinkweed. The study confirmed that visual assessment of AY incidence in crops underestimates the number of plants infected with the AY phytoplasma.

Keywords: AY phytoplasma, canola, cereal crops, disease, nested PCR
Abbreviations: **AY**, aster yellows; **PCR**, polymerase chain reaction

INTRODUCTION

Phytoplasmas are a worldwide group of obligate parasites that are associated with yellows diseases in several hundreds of plant species (The IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group 2004; Firrao *et al.* 2005; Bertaccini 2007). The organisms have been classified in 28 major phylogenetic groups, of which the aster yellows (AY) group is the largest (Lee *et al.* 2004; Wei *et al.* 2007).

Symptoms indicative of AY infection in plants includes plant stunting, virescence (excessive greening of floral tissue), leaf yellowing and phyllody (leaf-like sepals and petals) (McCoy *et al.* 1989). In *Brassica* species, stems and leaves can also become purplish, and siliques are replaced by oval, bladder-like structures (Sackston 1953; Bailey *et al.* 2003; Olivier 2007). In cereals, other symptoms of AY infection include water-soak marks on leaves, abaxial leaf rolling (Westdal and Richardson 1968), and excessive branching (witches' broom) (Bailey *et al*. 2003). In North America, reports of AY infection in canola, barley, wheat, and oats date from the 1950's (Sackston 1953; Bantarri and Moore 1960; Chiykowski 1963; Westdal 1969), when the disease was thought to be caused by a virus. AY disease is generally considered of little significance, with an overall incidence less than 1% per year (Pearse *et al.* 2001; Bailey *et al.* 2003), despite occasional epidemics. However, the recent detection of large numbers of AY-infected asymptomatic plants in canola (Olivier *et al.* 2008) and cereal crops (Hollingsworth *et al*. 2008) suggests that AY incidence is underestimated.

Phytoplasmas are transmitted by phloem-feeding insects, mostly leafhoppers (McCoy *et al.* 1989; Weintraub and Beanland 2006). The primary vector of AY disease in western Canada is the aster leafhopper, *Macrosteles quadrilineatus* Forbes, which feeds and reproduces on many plant species (Kunkel 1926; Whitcomb and Tully 1979). *M. quadrilineatus* migrates to western Canada from May to July on winds originating from the southern USA (Chiykowski and Chapman 1965; Hoy *et al.* 1992; Valk and Stevenson 1994). In Saskatchewan, populations of *M. quadrilineatus* also include individuals hatched from overwintering eggs (Chiykowski 1981) and overwintering adults (Olfert *et al.* 2004). An Aster Yellows index based on the number and infectivity of *M. quadrilineatus* has been developed as a guidance tool for spraying decisions in vegetable crops (Chaput and Sears 1998), but no such system has been developed for field crops.

Other leafhopper species commonly found on the Canadian prairies and known to be phytoplasma vectors or infected with phytoplasma include *Ceratagalia humilis* Oman, *Amplicephalus inimicus* Say, *Neokolla hieroglyphica* Say, *Scaphytopius acutus* Say, *Gyponana* sp., *Euscelis maculipennis* DeLong & Davidson, *Diplocolenus configuratus* Uhler, *Sorhoanus uhleri* Oman, and *Psammotettix* sp. (Khadhair *et al.* 1997; Beanland and Wolf 2003; Rieddle-Bauer *et al.* 2006; Weintraub and Beanland 2006; Olivier *et al.* 2004, 2007). These species may play an important role in spreading AY in wild plants and field crops of Saskatchewan.

M. quadrilineatus, *A. inimicus* and *Scaphytopius* sp. are known vectors of AY phytoplasma strains 16SrI-A and -B (Chiykowski 1963; Weintraub and Beanland 2006). *E. maculipennis* is known to vector strain 16SrI-B (Beirne 1956; Lee *et al.* 2004). DNA of AY phytoplasma strains 16SrI-A and -B was detected in *C. humilis* (Khadhair *et al.* 1997; Olivier *et al.* 2007). *Athysanus argentarius* Metcalf is a known AY phytoplasma vector and *Elymana sulphurella* Zetterstedt has been found to be infected with the AY phytoplasma (Chiykowski 1979, 1983), but characterization of the AY phytoplasma strains was not performed.

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Fig. 1 Location of leafhopper and crop sampling sites in Saskatchewan, Canada, in 2001–2008. (**1**) Meadow Lake. (**2**) Turtleford. (**3**) Medstead. (**4**) Lanigan. (**5**) Brooksby. (**6**) Kelvington. (**7**) Canora.

AY phytoplasma strains 16SrI-A and 16SrI-B have been found in oats in Europe (Urbanavičienė *et al*. 2008). AY strain 16SrI-B has been found in barley, bromegrass and wheat in western Canada (Olivier *et al*. 2009). Aster yellows disease in canola crops in Alberta and Saskatchewan has been reported to be associated with AY phytoplasma strains 16SrI-A and 16SrI-B (Wang and Hiruki 2001; Olivier *et al*. 2006, 2007).

The current paper details the detection and strain identification of the AY phytoplasma in leafhoppers in field crops in Saskatchewan over an eight year period and in canola in 2001–2008 and cereal crops in 2005–2008.

MATERIALS AND METHODS

Sampling sites

The sampling sites were located in seven townships, namely Medstead (RM 497), Turtleford (RM 499), Meadow Lake (RM 588), Lanigan (RM 340), Brooksby (RM 458), Kelvington (RM 336), and Canora (RM 273), across three ecoregions (moist Mixed Grassland to subhumid Boreal Transition) in Saskatchewan, Canada (**Fig. 1**). There were on average two fields of canola and one field each of barley, wheat, and oats per sampling site.

Leafhopper sampling and identification

Leafhoppers were sampled twice a month from April to August in every field in each year of the study. Sampling consisted of taking 20 sweeps with a net at five points along a transect across the field: 0 (edge of the field), 5, 10, 20, and 50 m into the field away from any edge. Insects collected at each point were placed in a plastic bag, and kept on ice in a cooler until stored at –18°C.

Leafhoppers were removed from the freezer, placed on a 4°C plate, identified using leafhopper identification keys and checklists (Beirne 1956; Maw *et al.* 2000), and enumerated. For leafhopper species known to be infected with or to vector phytoplasmas, specimens were placed in 2-ml Eppendorf tubes, one individual per tube. For leafhopper species not known to carry or vector phytoplasmas, specimens were measured and distributed into 2-ml Eppendorf tubes, up to ten individuals for insects measuring less than 0.3 mm, up to five individuals for insects measuring between 0.3 mm and 0.5 mm, and up to three individuals for insects measuring more than 0.5 mm. Eppendorf tubes were stored at –18°C.

AY disease incidence and plant sampling

AY incidence was assessed in mid-August in every canola field in each of the eight years of the study and in every barley, wheat and oat fields from 2005–2008. In total, 112 fields of canola, 26 fields of barley, 33 fields of wheat, and 18 fields of oats were sampled. For each field, 100 plants were examined for AY disease symptoms from the edge of the field towards the middle of the field in each of three rows (300 plants assessed in total per field). Following the assessment of AY disease incidence, 20 plants were harvested at random at 0, 5, 10, 20 and 50 m from the edge (total of 100 plants per field). Plants were stored at 4ºC until root, leaf, and stem tissue (1 g each) and 10 sets of 10 seeds were collected from each plant, placed into 2-ml Eppendorf tubes, freeze-dried and stored at –18°C. Leaf tissue of neighbouring crops and weeds was also collected, placed in 2-ml Eppendorf tubes, freeze-dried and stored at –18°C.

PCR detection of phytoplasma DNA in leafhopper and plant samples

Phytoplasma DNA was extracted from leafhoppers and plant tissues according to the methods described by Daire *et al.* (1997). DNA was amplified by nested PCR using a modified protocol of the technique of Tanne *et al.* (2001). Universal phytoplasma-specific primer pair P1/P6 (Deng and Hiruki 1991; Schneider *et al.* 1995) was used in the first amplification, followed by primer pair R16R2/R16F2 (Lee *et al.* 1993) in the second amplification. DNA amplification was performed using a Peltier thermal cycler (PRC-220, DNA Engine Dyad, MJ Research, Inc., San Francisco, California, USA) with *Taq* polymerase (New England Biolabs, Pickering, Ontario, Canada) at 1 U/20 µl. The first and second amplifications were performed in 20 µl mixtures containing 2 µl of 10X Invitrogen Thermopool buffer (Invitrogen Canada Inc., Burlington, Ontario, Canada), 200 µM dNTP, 5 µM of each primer and, for the first amplification, 4 µl of undiluted DNA and, for the second amplification, 2 µl of diluted PCR product (1/200) of the first amplification. Denaturation was performed at 92°C for 1 min 30 s, followed by 30 cycles of 45 s at 92°C, 45 s at 57°C and 1 min 45 s at 72°C, for the first amplification and at 92°C for 1 min 15 s, followed by 35 cycles of 30 s at 92°C, 30 s at 67.5°C and 1 min 15 s at 72°C for the second amplification. PCR products were separated by electrophoresis on 1.9% agarose gels followed by staining in ethidium bromide and visualization of the DNA bands with a UV transilluminator. The presence of phytoplasma DNA was indicated by the amplification of a 1200 bp band.

AY phytoplasma strain identification

AY phytoplasma strain identification was performed by sequencing the 1200 bp band. Sequencing was performed at the Plant Biotechnology Institute (National Research Council, Saskatoon, Saskatchewan, Canada). DNA sequences were then compared with sequences recorded in Genbank using the BLAST program. Sequence alignments were processed using the Vector NTI program.

Statistical analysis

Statistical analysis was carried out using t-tests to compare percent of infection in plants and leafhoppers and number of leafhoppers with $P \leq 0.05$ (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Identification and abundance of AY vector and AY-infected leafhopper species

Over 20 leafhopper species tested positive for the presence of phytoplasma DNA at least once in the course of the study (**Table 1**). The six-spotted aster leafhopper *M. quadrilineatus*, a well-known AY vector (Kunkel 1926) was the most abundant species with 11,166 specimens captured over the course of the study. This species showed the highest infection rate, with an average of 8.3% infected individuals (range: 1.1% to 19.8%) over the 8 year study (**Tables 1** and

Table 1 Leafhopper species containing Aster Yellows (AY) phytoplasma DNA, Saskatchewan, Canada, 2001-2008.

Species	Number of specimens collected	Number of samples tested with nested PCR	Samples positive for the presence of AY phytoplasma DNA (%)	AY phytoplasma strains
AY phytoplasma vectors				
Amplicephalus inimicus	640	287	3.8	16SrI-A, -B & C
Athysanus argentarius	347	42	1 positive sample	16SrI-B
Euscelis maculipennis	22	21	1 positive sample	16SrI-A
Macrosteles quadrilineatus	11,166	10,068	8.3	16SrI-A & -B
Scaphytopius sp.	12	12	1 positive sample	16SrI-A
Species infected with AY phytoplasma				
Ceratagalia humilis	248	132	2.2	16SrI-A
Diplocolenus configuratus	147	88	4.5	16SrI-A, B & -C
Elymana sp.	37	25	1 positive sample	ND
Sorhoanus uhleri	342	114	1.7	16SrI-A & -B
Psammotettix sp.	455	230	2.0	16SrI-A
New species infected with AY phytoplasma				
Balclutha neglecta	1,036	381	2.8	16SrI-A
Chlorotettix unicolor	98	52	1 positive sample	ND
Cosmotettix delector	7	7	1 positive sample	ND
Dikraneura sp.	58	34	1 positive sample	ND
Diplocolenus evansi	1,489	271	1.1	16SrI-A
Draeculacephala sp.	15	14	1 positive sample	ND
Empoasca sp.	69	27	1 positive sample	16SrI-A
Extrusanus extrusus	4	4	1 positive sample	ND
Hebecephalus truncatus	3	3	1 positive sample	ND
Idiocerus sp.	12	12	1 positive sample	ND
Latalus personatus	121	104	1.9	16SrI-A
Sorhoanus sp.	197	71	4.2	16SrI-A

ND: Not determined

Table 2 Aster yellows (AY) incidence in leafhopper species, Saskatchewan, Canada, 2001-2008.

Leafhopper species	2001	2002	2003	2004	2005	2006	2007	2008
AY phytoplasma vectors								
Amplicephalus inimicus	1.7	0	0	5/26[a]	1/7[a]	2.7	5.2	1/14[a]
Athysanus argentarius	0	0	0	2/19[a]	1/28[a]	0	0	0
Euscelis maculipennis	0	0	1/5[a]	0	0	0	0	0
Macrosteles quadrilineatus	8.6	16.0	4.3	5.2	7.6	1.1	19.8	5.1
Scaphytopius sp.	0	1/2[a]	0	0	0	0	0	0
Species infected with the AY phytoplasma								
Balclutha neglecta	2.5	0	1.2	4.5	0	1.7	2/22[a]	7.1
Ceratagalia humilis	2/22[a]	0	0	0	0	0	1/3[a]	0
Chlorotettix unicolor	0	0	0	2/6[a]	0	0	0	0
Cosmottetix delector	0	0	0	0	0	1/1[a]	0	0
Dikraneura sp.	2/13[a]	0	0	0	0	0	0	0
Diplocolenus configuratus	3/26[a]	3.8	0	0	0	0	0	0
Diplocolenus evansi	0	0	0	2/21[a]	2.6[a]	0	0	0
Draeculacephala sp.	1/11[a]	0	0	0	0	0	0	0
Elymana sp.	0	0	1/3[a]	0	0	0	0	0
Empoasca sp.	0	1/3[a]	0	0	0	0	0	0
Extrusanus extrusus	0	0	0	1/1[a]	0	0	0	0
Hebecephalus truncatus	0	0	0	1/1[a]	0	0	0	0
Idiocerus sp.	0	0	0	0	0	0	1/8[a]	0
Latalus personatus	1.6	0	0	0	0	0	0	0
Psammotettix sp.	1.2	3.3	0	0	0	1/14	0	1/13[a]
Sorhoanus sp.	0	1/10[a]	0	0	0	0	0	0
Sorhoanus uhleri	0	0	0	0	1/24[a]	0	0	0

[a] Number of samples containing AY phytoplasma/number of samples tested.

2). The abundance of this leafhopper has been related to its high numbers during migration (Chiykowski and Chapman 1965; Hoy *et al.* 1992; Valk and Stevenson 1994), its polyphagous habit, allowing it to feed and reproduce on many plant species (Kunkel 1926; Whitcomb and Tully 1979), and its nature as a very good flyer that can disperse very efficiently (Zhou *et al.* 2003).

Six and 10 times more specimens of *M. quadrilineatus* were found in wheat and barley fields, respectively, than in canola fields (**Fig. 2**). Among the cereal crops, barley was the crop most attractive to *M. quadrilineatus* (**Fig. 2**), as observed by Westdal *et al.* (1961). In contrast, McClanahan (1962) reported that wheat was preferred over oats and barley and Westdal and Richardson (1968) concluded that oats were a preferred food source.

Specimens of *A. inimicus* and *Psammotettix* sp. positive for the presence of phytoplasma DNA were mostly found in cereal crops (**Fig. 2**). *A. inimicus* is an AY phytoplasma vector (Chiykowski 1963) and species of *Psammotettix* have been shown to vector the AY phytoplasma in Europe (Sabate *et al.* 2003). Chiykowski (1963) suggested that *A. inimicus* plays a role in maintaining AY disease in cereal crops in North America. The relatively high numbers of specimens of *Psammotettix* sp. and *A. inimicus* that were collected and their percent AY phytoplasma infection (2.0% and 3.8%, respectively) (**Table 1**) suggest that these leaf-

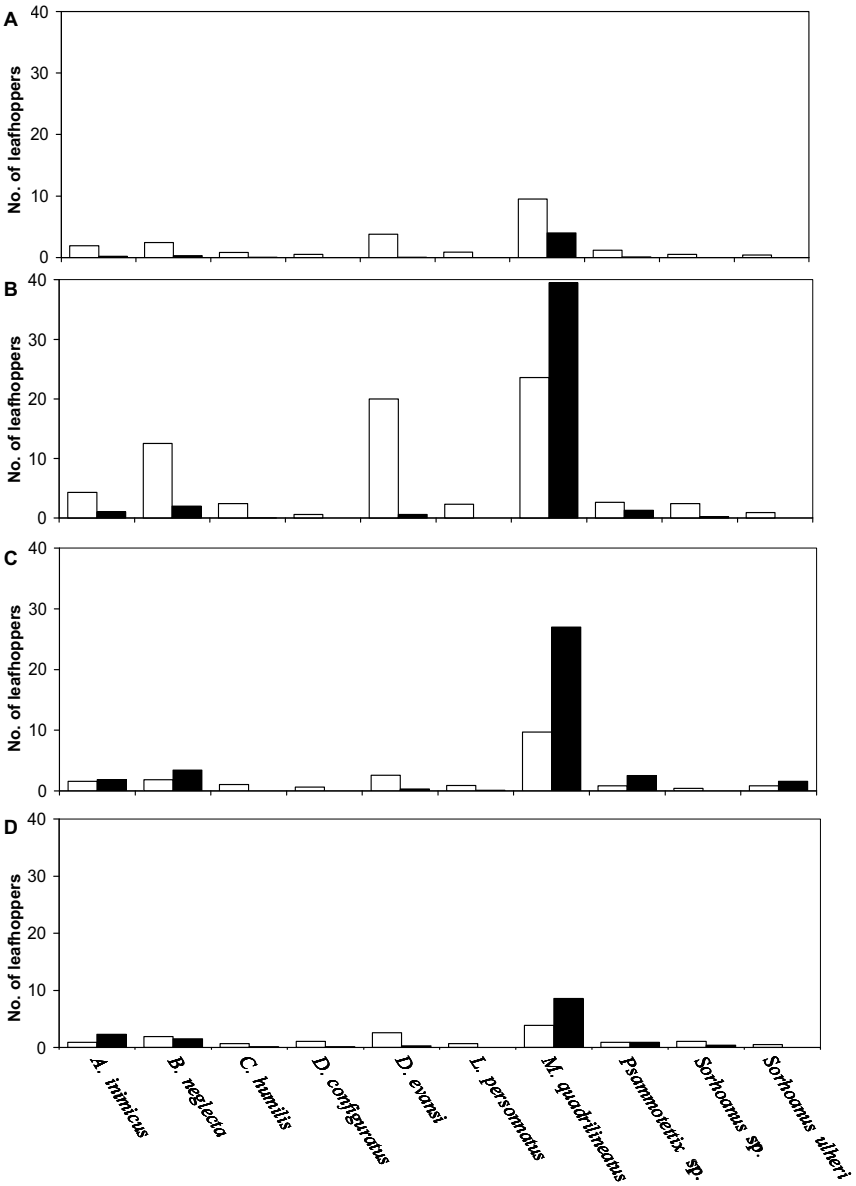

Fig. 2 Average number of leafhoppers captured per year and sampling location at the edge of the crop (white columns) and inside the crop (black columns) for species that had more than one insect positive for AY phytoplasma DNA. (**A**) Canola. (**B**) Barley. (**C**) Wheat. (**D**) Oat.

hoppers play a role in maintaining AY disease in cereal crops.

The leafhoppers *B. neglecta* and *D. evansi* are the next two most abundant species after *M. quadrilineatus* (**Table 1**) and are found mostly in cereal crops (**Fig. 2**). AY phytoplasma DNA was detected almost every year in *B. neglecta* but only in 2004 and 2005 for *D. evansi* (**Table 2**). This is the first report of these leafhoppers being infected with the AY phytoplasma. *Balclutha* species have been found to be infected with the stolbur phytoplasma in Europe (Rieddle-Bauer *et al.* 2006), but there is no report of this leafhopper being able to be infected by or to vector the AY phytoplasma.

C. humilis was found mostly at the edge of the fields (**Fig. 2**) and 2.2% of the *C. humilis* samples contained AY phytoplasma DNA (**Table 1**). Preliminary research with AY phytoplasma-infected *C. humilis* placed in cages containing a mixture of barley, wheat and canola plants did not provide evidence of AY transmission (data not shown). In similar experiments, Khadhair *et al.* (1997) were unable to observe the transmission of the alfalfa witches' broom phytoplasma by *C. humilis*. It is unlikely that *C. humilis* plays a role in the AY disease complex in Saskatchewan.

The remaining species that tested positive for the AY phytoplasma were found mostly in small numbers at the edge of the fields (**Table 1, Fig. 2**). Of these species, *A. argentarius*, *Elymana* sp., *E. maculipennis*, and *Scaphytopius* sp. are known phytoplasma vectors (Weintraub and Beanland 2006; Olivier *et al.* 2009), suggesting that, as a group, they might play a role in maintaining AY disease in reservoir plants, as indicated by Chiykowski (1979) for *A. argentarius*. *Empoasca* sp. has been found infected with '*Ca.* phytoplasma *caricae*', a phytoplasma not present in Saskatchewan (Arocha *et al.* 2005). However, this the first report of AY phytoplasma infection in the leafhoppers *Chlorotettix unicolor* Fitch, *Cosmotettix delector* Sanders & DeLong, *Dikraneura* sp., *Diplocolenus evansi*, *Draeculacephala* sp., *Empoasca* sp., *Extrusanus extrusus* Van Duzee, *Hebecephalus truncatus* Beamer & Tuthill, *Idiocerus* sp., *Latalus personatus* Beirne, and *Sorhoanus* sp.

AY phytoplasma strain identification in leafhoppers

Nested PCR products obtained from 86 individuals of *M. quadrilineatus* and 42 samples of other leafhopper species were sequenced and compared with sequences recorded in Genbank using the BLAST program. Forty-two individuals of *M. quadrilineatus* contained AY phytoplasma DNA showing at least 99% nucleotide sequence identity with subgroup 16SrI-A and 26 individuals of *M. quadrilineatus* contained AY phytoplasma DNA showing at least 98% nuc-

Table 3 Aster yellows (AY) disease incidence (%) based on field observations and nested PCR detection of AY phytoplasma DNA.

Year	Canola		Barley		Wheat		Oats	
	Field	PCR	Field	PCR	Field	PCR	Field	PCR
2001	(-)[a]	ND[b]						
2002	2.5	8.1						
2003	(-)	1.2						
2004	0.1	5.1						
2005	(-)	1.8	(-)	9.4	(-)	6.4	(-)	7.2
2006	(-)	2.0	(-)	9.3	(-)	24.5	(-)	6.9
2007	2.5	11.2	(-)	66.6	(-)	38.8	(-)	25.4
2008	(-)	1.7	(-)	14.3	(-)	30.3	(-)	7.3

[a] (-): Lower than 0.001% incidence.
[b] Not determined.

leotide sequence identity with subgroup 16SrI-B; ten individuals of *M. quadrilineatus* contained DNA sequences belonging to both strains (**Table 1**).

DNA sequences similar at 99% to strain 16SrI-A were found in samples from most of the other leafhoppers and for the first time, in samples of *E. maculipennis*, *Balclutha neglecta*, *Psammotettix* sp. and *Empoasca* sp. DNA sequences similar at 99% to strain 16SrI-B were found in seven samples of leafhoppers belonging to the species *D. configuratus* and *S. uhleri* and for the first time in one sample of *A. argentarius*. Two samples of *A. inimicus* and 1 sample of *S. uhleri* contained DNA sequences from both strains, 16SrI-A & -B. The occurrence of different phytoplasma strains has been described in several leafhopper species, including *M. quadrilineatus* (Beanland and Wolf 2003; Lee *et al.* 2003), *Euscelidius variegatus* Kbm (D'Amelio *et al*. 2007), and *Neoaliturus fenestratus* Herrich-Schäffer (Orenstein *et al.* 2003). DNA sequences similar at 99% to the AY phytoplasma strain 16SrI-C was found in one sample of *A. inimicus* and one sample of *D. configuratus.* This is the first report of this strain in these species.

Strains 16SrI-A, -B and -C are the most widely distributed strains of AY phytoplasma (Lee *et al.* 2004). Strains 16SrI-A and -B are reported worldwide in herbaceous plants (Lee *et al*. 2004) and are common in canola crops in Alberta (Wang and Hiruki 2005) and Saskatchewan (Olivier *et al.* 2007, 2008). Therefore it is not surprising to find these strains in leafhopper species that feed on these crops.

AY phytoplasma disease incidence in canola, barley, wheat and oats

AY disease incidence in canola fields over the eight years of the study was less than 0.1% (**Table 3**), similar to the provincial average (Pearse *et al*. 2008). In the 2007 epidemic, AY incidence in the canola sampling sites was 2.5%, similar to the 2.0% provincial average for 2007 (Pearse *et al*. 2008). On average, in cereal crops, the frequency of plants with stunting and yellowing symptoms was less than one plant per 1000 at all sampling sites.

On average, from 2002 to 2008, phytoplasma DNA was detected in 4.7% of canola plants. The PCR analyses revealed a significantly higher AY incidence than the incidence based on visual observations. In the 2007 epidemic, 11.2% of the plants were infected with the AY phytoplasma. In cereals, AY incidence ranged from 6.4 to 30.4% in years with no AY epidemics, while in 2007, AY incidence reached 66.6% in barley, 38.8% in wheat and 25.4% in oats (**Table 3**).

On average, phytoplasma DNA was found in seed of 11% of canola plants, seed of 16.3% of wheat plants, 8.6% of barley plants, and 13.1% of oat plants. This is the first report of the presence of AY phytoplasma DNA in seeds of these cereals.

Field observation of disease symptoms in canola and cereal crops gave significantly lower AY incidence than incidence determined by PCR testing (**Table 3**). This is because AY-infested plants can be asymptomatic (Olivier *et al.* 2007; Hollingsworth *et al*. 2008) or as in the case of barley, AY symptoms are undistinguishable from those caused by the Barley Yellow Dwarf Virus (Bantarri 1965).

AY phytoplasma strains in canola and cereals

DNA sequences that were similar at least at 98% to phytoplasma DNA belonging to strains 16SrI-A or -B was found in canola plants (N= 457), with 42% of plants infected with strain 16SrI-A. One canola plant was infected with strain 16SrI-C. The presence of both 16SrI-A and -B strains was observed in five plants. Mixed phytoplasma infection has been reported for a variety of plant species, including fruit trees (Lee *et al.* 1995), ornamentals (Bertaccini *et al.* 2002), potato (Khadhair *et al.* 2003), and herbs and spices (Lee *et al*. 2003).

Strains 16SrI-A and -B were equally distributed in barley (N= 569 plants) and wheat (N= 1054), while strain 16SrI-B was predominant in oats (N= 175). The present study is the first report of strain 16SrI-C in canola and strain 16SrI-A in barley and wheat, and the first report of strains 16SrI-A and -B in oats in Canada.

AY phytoplasma DNA strains in other plant species

AY phytoplasma strains were also identified in plants collected near the sampling sites. DNA sequences similar to 99% to phytoplasma DNA belonging to strains 16SrI-A and -B was detected in species of Apiaceae, including *Anethum graveolens* L. (dill), *Carum carvi* L. (caraway), *Coriandrum sativum* L. (coriander), and *Daucus carota* L. (carrot); Fabaceae, including *Cicer arietinum* L. (chickpea); Asteraceae, including *Cirsium* sp. (thistle), *Crepis* sp. (hawk's-beard), *Echinacea angustifolia* DC (echinacea), *Iva xanthifolia* Nutt. (false ragweed), *Solidago canadensis* L. (goldenrod), and *Taraxacum officinale* Weber (dandelion); Rubiaceae, including *Galium* sp. (bedstraw); Brassicaceae, including *Capsella Bursa-pastoris* L. (shepherd's purse), *Sinapis arvensis* L. (wild mustard), *Thlaspi arvense* L. (stinkweed); and Poaceae, including bromegrass (*Bromus* sp.). DNA sequence similar at 100% to strain 16SrI-C was found only in shepherd's purse.

CONCLUSIONS

The study confirmed the presence of the AY phytoplasma in several leafhopper species known to vector or to be infected with the AY phytoplasma. The AY phytoplasma was also detected in several other leafhopper species for the first time. *M. quadrilineatus* was the most abundant AY-infected species from year to year and appears to be the main AY vector in canola and cereal crops in Canada. In cereals, the next most abundant and consistently AY-infected species were the vectors *B. neglecta* and *A. inimicus*; these species may play a role in maintaining AY disease in cereal crops. The remaining species were found to be infected with the AY phytoplasma only occasionally. Further studies are required to determine if these species can vector the AY phytoplasma. The concurrent presence of AY phytoplasma

strains 16SrI-A, -B, and -C in a large number of leafhopper species and plant hosts may provide opportunities for genetic recombination among these strains, possibly resulting in the development of pathogenic strains able to occupy new ecological niches.

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