Aster yellows disease survey in Saskatchewan, Canada, 2001–2006

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Abstract

Phytoplasmas are wall-less prokaryotes belonging to the class Mollicutes. The organisms are obligate parasites and are transmitted by phloem feeding insects, mainly leafhoppers. Aster Yellows (AY) disease in canola is associated with the phytoplasma species '*Candidatus* Phytoplasma asteris' and mainly transmitted by the leafhopper *Macrosteles quadrilineatus*. Since the AY epidemic in canola crops in 2000, AY disease surveys have been conducted annually in oilseed, cereal, and pea crops throughout Saskatchewan, Canada. In 2000, AY disease incidence reached 12% in canola crops; a few fields in northern Saskatchewan had 30–45% infected plants. In 2001–2005, AY incidence in canola declined to less than 1%. In 2006, 19 canola fields, 20 cereal fields, four pea fields and two flax fields were surveyed to estimate AY incidence. Plants and populations of *M. fascifrons* were sampled to investigate the presence and identity of phytoplasmas using PCR technology and DNA sequencing. Results of the 2001–2006 surveys are presented.

Key words: Canola, cereals, aster yellows phytoplasma, leafhopper, *Macrosteles quadrilineatus*, survey

Introduction

Aster Yellows (AY) diseases are associated with the phytoplasma species '*Candidatus* phytoplasma asteris' (Firrao et al., 2004). Symptoms of AY infection in canola (*Brassica napus* L. and *B. rapa* L.) include plant stunting, virescence (excessive greening of floral tissue), leaf yellowing, phyllody (leaf-like sepals and petals) (McCoy et al., 1989) and bladder-like siliques (Bailey et al., 2003). In cereals, plants infected with AY phytoplasmas are dwarfed and chlorotic with distorted heads, twisted, thin and yellowed awns and reduced spikelets (Urbanaviciene et al., 2005). In western Canada, *Macrosteles quadrilineatus* Forbes is presumed to be the predominant vector for aster yellows (Chiykowski, 1965). The occurrence of AY-like symptoms in rapeseed was first reported in Saskatchewan in 1953 (Sackston, 1953) and until 2000 AY disease was considered to be of little importance, with the exception of an epidemic in 1957 (Vanterpool, 1963). In 2000, AY disease incidence reached 12% in canola crops (Pearse et al., 2001) and a few fields in northern Saskatchewan had 30–45% infected plants (Olivier et al., 2006). The objective of the research was to monitor AY in canola crops and leafhopper populations to generate information required for the development of early warning systems and pest management strategies.

Materials and Methods

On average, 40 fields of canola and 20 cereal (barley, oats and wheat) fields located throughout Saskatchewan were sampled for leafhoppers by sweeping twice a month from April to September in each year of the 6-year study (2001–2006). In the period 2004–2006, two to four fields of peas and two fields of flax were included in the surveys. Visual assessment of AY incidence (% of plants showing AY symptoms) in each field was made in mid-August and 20 plants were harvested randomly at the field's edge and at 5, 10, 20 and 50 m from the field's edge. Leaf tissue of each plant was freeze-dried. Twenty sweeps were made at each sampling distance to collect leafhoppers. Leafhoppers were identified, counted and stored at –20ºC. Phytoplasma DNA was extracted from leaf tissue and leafhoppers and amplified using nested PCR technology and universal phytoplasma-specific primer pairs R16R2/R16F2 (Lee et al., 1993) and P1/P6 (Schneider et al., 1995). Individuals of leafhopper species known to be phytoplasma vectors and carriers were tested singly and individuals belonging to other species were tested in groups of 5 to 10 specimens. RFLP analysis (Griffith et al., 1999) and/or DNA sequencing were conducted on phytoplasma DNA from canola plants that tested positive for the presence of phytoplasma DNA.

Results

Aster Yellows incidence. Visual assessments indicated that the frequency of canola plants showing AY symptoms in 2001–2006 was less than 1%, with the exception of a few fields in Northern Saskatchewan that had 3–5% infected plants in 2004. No cereal, pea and flax plants showing AY symptoms were observed during the 6-year period. However, PCR tests conducted on canola and cereal plants harvested in 2002–2006 showed that a large number of asymptomatic plants were infected with phytoplasma DNA (Table 1). None of the 2004 and 2005 plant samples from the pea and flax fields contained phytoplasma DNA. In 2006, six plants out of 260 pea plants were positive for the presence of phytoplasma DNA.

Table 1. Incidence of plants containing phytoplasma DNA in canola, cereal, pea and flax crops in Saskatchewan, Canada, 2002–2006.

Survey year	Canola fields[a]	Cereal fields[b]	Pea fields[c]	Flax fields[d]
2006	2.0%	11.1%	2.3%	0%
2005	1.8%	15.5%	0%	0%
2004	7.1%	60%	0%	0%
2003	0%	Not determined	Not determined	Not determined
2002	20%	Not determined	Not determined	Not determined

[a]600 plants tested per year.
[b]60 plants tested in 2004 and 200 plants tested in 2005 and 2006.
[c]200 plants tested in 2004–2005 and 260 plants tested in 2006.
[d]200 plants tested in 2004–2006.

Phytoplasma strains were identified in canola using RFLP analysis or DNA sequencing. Phytoplasma DNA found in plants of *B. napus* collected in 2001 and 2002 belonged to AY subgroup 16SrI–A or 16SrI–B. None of the plants were infected with more than one phytoplasma strain. Phytoplasma DNA in plants of *B. rapa* collected in 2003 belonged to AY subgroup 16SrI–A strain CHRY 16S (Gene Bank accession no. AY 180956) or AY subgroup 16SrI–B (Gene Bank accession no. AF 268407). Phytoplasma DNA in plants of *B. rapa* collected in 2004 belonged to AY subgroup 16SrI–A strain AY-WB (Gene Bank accession no. GI_45479367).

Leafhoppers. In general, there were five to seven times more leafhoppers in cereal crops than in canola, pea and flax crops. Of the 42 leafhopper species trapped in the sweeps, 15 species had individuals that tested positive for the presence of phytoplasma DNA at least once during 2001–2006 (Table 2). *Macrosteles quadrilineatus* Forbes, considered to be the main vector of the AY phytoplasma, was the most frequently trapped leafhopper. The frequency of infected individuals in this species ranged from 0.3% to 8.4%. *Amplicephalus inimicus* S., known to be a phytoplasma vector, and *Ceratagalia humilis* O., known to be a phytoplasma carrier, were found in low numbers. Fewer or similar numbers of infected individuals were found in these species than in *M. quadrilineatus*. The leafhopper species *Neokolla hieroglyphica* S., *Scaphytopius acutus* S., *Balclutha* spp., *Gyponana* spp. and *Psammotettix* spp., known to be phytoplasma carriers or vectors, and *Euscelis maculipennis* D&D., *Chlorotettix* spp., *Diplocolenus configuratus* U., *Extrusus extrusanus* Van D., *Sorhoanus ulheri* O. and *Verdanus evansi* A., which are not known to be phytoplasma carriers or vectors, showed fewer than 3 infected specimens per year, with the exception of *Balclutha* spp. where there were 11 infected insects in 2006.

RFLP analysis performed on infected individuals of *M. quadrilineatus* and *C. humilis* showed that the insects were infected with phytoplasmas belonging to either AY subgroup 16SrI–A or subgroup 16SrI–B. The phytoplasma strain(s) present in infected individuals of *A. inimicus* remains to be identified.

Discussion

The PCR studies revealed that phytoplasmas were widespread in canola and cereal crops in Saskatchewan, Canada and that the organisms were present in a large number of asymptomatic plants. Further research is required to determine the frequency of infection in pea and flax crops. Not unexpectedly, the AY strains observed in canola crops in Saskatchewan were identical to those that have been found in canola in Alberta, a neighbouring Canadian province (Wang and Hiruki, 2001).

Table 2. Leafhopper species and phytoplasma incidence in leafhoppers collected in canola and cereal crops, 2001–2006.

Leafhopper species	No. of infected leafhoppers/Total no. of tested leafhoppers					
	2001	2002	2003	2004	2005	2006
Amplicephalus inimicus	2/50	1/61	1/99	6/80	–[1]	1/85
Athysanus argentarius	0/83	0/132	0/193	2/55	0/51	0/14
Balclutha spp.	0/266	0/168	0/254	2/146	0/104	11/134
Ceratagalia humilis	6/61	1/282	6/335	1/42	0/21	0/2
Chlorotettix spp.	0/34	0/7	0/31	2/2	0/6	0/3
Diplocolenus configuratus	1/14	1/27	2/16	1/21	0/9	0/22
Euscelis maculipennis	3/10	0/2	–	–	–	–
Extrusanus extrusus	0/1	–	–	1/1	–	–
Gyponana spp.	1/2	–	–	–	–	0/3
Macrosteles quadrilineatus	305/3,625	78/1,752	43/2,228	63/1,395	32/1,335	8/2,548
Neokolla hieroglyphica	1/3	–	–	–	–	0/1
Psammotettix spp.	1/80	2/60	0/24	0/44	0/43	1/32
Scaphytopius acutus	0/15	1/5	0/1	0/2	–	–
Sorhoanus ulheri	0/81	1/37	0/23	0/25	0/39	0/121
Verdanus evansi	0/683	0/631	0/106	1/76	0/61	–

[1]–: No leafhopper trapped.

The study indicated that several leafhopper species contained phytoplasmas. Among these species, *M. quadrilineatus* was

the most commonly found in canola, cereal, pea and flax crops in Saskatchewan and generally had the largest number of phytoplasma-infected individuals. Infected insects contained phytoplasmas belonging to one of the AY subgroups. *Macrosteles quadrilineatus* is likely the major vector of Aster Yellows disease in Saskatchewan. The known phytoplasma carrier *C. humilis* was present in substantial numbers in 2001–2003 following the 2000 AY epidemic and individuals were shown to be infected with phytoplasmas belonging to one of the AY subgroups. It remains to be seen if *C. humilis* could vector the AY phytoplasma.

Conclusions

The results of the study revealed that the AY phytoplasma is present in a large proportion of asymptomatic plants in canola and cereal fields in Saskatchewan. Among the leafhopper species that were infected with the AY phytoplasma, *M. quadrilineatus* is likely the most important AY disease vector. The study also revealed that the same AY subgroups were found in canola plants and in the AY vector species *M. quadrilineatus* and the carrier species *C. humilis*. Further research is required to determine the phytoplasma strain(s) present in cereal and pea crops, as well as in the other leafhopper species.

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