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Biological Control of Cleavers (*Galium spurium and G. aparine*) with Pathogenic Fungi - Exploration and Discovery

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Abstract

A biological weed control research program has recently been initiated at the Alberta Research Council in collaboration with Agriculture and Agri-Food Canada, Saskatoon, to explore and develop indigenous fungal pathogens as bioherbicides for control of cleavers (*Galium spurium* and *G. aparine*). Diseased leaves, stems, flowers, and seeds of cleavers were collected from various crop fields, located at the districts of Peace River, Edmonton, Lamont, Vegreville, Vermillion, and Saskatoon. A total number of 161 fungal isolates were obtained. Among those isolates, 74 were pathogenic to cleavers after applying Koch's postulates. Pathogenic isolates were further assessed on weed control efficacy (virulence) using a 0 to 3 scale (0 - no symptoms, + - light infection, ++ - moderate infection, and +++ - severe infection to death). Results have shown that 47 isolates are virulent (with 2 or 3 virulence rating) to cleavers. Virulent isolates proceeded to crop safety test (preliminary host range) on nine major crops (wheat, barley, oats, canola, flax, safflower, field pea, lentil, and alfalfa). To date, laboratory and greenhouse experiments have demonstrated that several fungal isolates provide effective weed control and are safe to major crops. The potential of those fungal isolates as bioherbicides is being further assessed.

Keywords: bioherbicide, biological control, *Galium spurium*, *Galium aparine*.

Cleavers [false cleavers (*Galium spurium*) and cleavers (*G. aparine*)] are weeds of significant economic impact in western Canada, especially for canola (*Brassica napus* and *B. rapa*) producers. Heavy infestation of cleavers in canola causes severe yield losses (for false cleavers, 100 plants per square metre can cut yields in canola by 20%) (Terkington *et al.*, 1980); however, the main impact of cleavers is that its seed is similar in shape and size to canola, making mechanical seed separation difficult. Seed contamination leads to downgrading of canola, results in new infestation, and have implications for the crushing industry. Contamination of canola by over 1% cleavers by weight results in downgrading from #1 to #2 with a price deduction of $13/tonne. If costs of control, yield losses, and losses in other crops were also considered, total costs would be much higher. Cleavers are not only difficult to control in canola but are an increasing problem in other crops.

Cleavers are increasingly becoming problematic in the prairie provinces. The 1997 weed surveys in the prairie provinces of Canada revealed that cleavers populations have dramatically increased in the past 10 years. Overall, cleavers relative abundance over these years rose by 36, 27, and 19 rankings in Alberta, Saskatchewan and Manitoba,

respectively (Thomas *et al.*, 1998). Increasing cleavers infestation is threatening pedigreed canola seed industry. Under the Canada Seeds Act, no cleavers seed is allowed in pedigreed canola seed and thus, pedigreed seed of canola cannot be produced on land infested with cleavers.

Herbicide resistance in cleavers has recently been reported in Alberta, involving multiple resistance to chemicals in different groups (Group-2 and –4 herbicides, which are the major herbicides recommended for their control) (Hall *et al.*, 1998). Herbicides in Group 2 to which cleavers developed resistance include Sulfonylurea - triasulfuron (Amber, Unity), thifensulfuron/tribenuron (Champion Extra, Champion Plus, Crossfire, Express Pack, Harmony Total, Laser DF, Muster Gold, Refine Extra, Triumph plus), and sulfometuron (Oust) and Imidiazolinone - imazethapyr (Odyssey, Pursuit). Herbicides in Group 4 to which cleavers developed resistance contain Quinoline carboxylic acid - quinclorac (Facet) and Phenoxys (in Europe) - fluroxypyr and mecoprop. Increasing herbicide use against these weeds is likely to lead the development of more cases of multiple resistance.

Alternative or additional cleavers control strategies are need for both conventional and herbicide tolerant (HT) canola acreage. Conventional canola varieties are expected to maintain 20-40% of the canola acreage in Canada and cleavers populations resistant to commonly used chemical herbicides have been reported (Hall *et al.*, 1998), including cases of multiple resistance to herbicides in different chemical groups. Populations resistant to one of the products (i.e. Pursuit® or Odyssey®) for which herbicide-tolerant canola (SMART-canola) has been developed have been found, amplifying the need for additional methods of cleavers control on acreage occupied by SMART-canola. Other concerns over the use of HT canola have also arisen. Transfer of HT genes to related weedy species has long been a concern and recent evidence has shown this potential has become a reality (Seefeldt *et al.*, 1998). Research has demonstrated that glufosinate-resistant canola can outcross to birdsrape mustard producing glufosinate-resistant hybrids in both greenhouse and field studies (Brown and Brown, 1996). Effective control of volunteer HT canola in subsequent years has also been suggested as a problem of crop production systems which include HT canola in rotations. Concern over the general use of genetically modified (GM) crops, including many of the HT canola varieties, has also arisen in many countries world-wide, limiting the export of GM canola seed and products. A weed species (rigid ryegrass) resistant to the widely-used non-selective herbicide Roundup® has also been reported and confirmed in both Australia and the U.S (Powles *et al.*, 1998). This recent discovery is a timely reminder that weed control should not depend solely on a single weed management option and sound herbicide-resistance management strategies will remain essential regardless of whether HT or conventional crop varieties are used.

The objective of this study is to explore and discover pathogenic fungi as bioherbicides for the control of cleavers.

Materials and methods

Isolation of fungi. From 1998 to 1999, field trips were made to districts of Vermilion, Vegreville, Lamont, Edmonton, and Peace River in Alberta, Canada, to collect diseased cleavers plants. Diseased leaves, stems, flowers and seeds of *Galium* spp. were collected, air dried in a paper press, cut to appropriate size, and stored at 4 ºC in envelopes. Tissue pieces with lesions were surface sterilized with 0.5% sodium hypochlorite solution and incubated on fresh potato dextrose agar (PDA; Difco, Detroit, MI). Fungi that grew from

the lesions were isolated and Koch's postulates were performed for most samples shortly after each collection trip. Single conidial isolates of the recovered fungi were maintained in cryovials, each containing 2 ml of individual fungal isolate in 15% glycerol, and stored at -80ºC as stock cultures.

Preparation of inoculum. Stock cultures of each fungal isolate were thawed at 36ºC for 1.5 min, transferred onto PDA contained in 10-cm-diameter petri dishes, and incubated for 1 wk at 24ºC. A 12-h photoperiod was provided by fluorescent light with an intensity of 28 μmol m^{-2} s^{-1}. Single-conidium colonies was then be made for all fungal isolates by using standard single-spore-technique and incubated under the same conditions described above. Sufficient conidia for experiments was produced by transferring conidia from a single-conidium colony onto several PDA plates (spread plates). Plates were incubated as above for 1-2 wks. Conidia were harvested by gently scraping the surface of the agar and transferring into sterilized distilled water. Conidium concentrations were determined using a haemocytometer.

Preparation of plants. Seeds of false cleavers were collected at the Alberta Research Council research farm. Seeds of cleavers were obtained from Valley Seed Service, California, US. Differences of two targets were identified prior to experiments. Both false cleavers and cleavers and nine major crops (canola, wheat, barley, oats, flax, safflower, alfalfa, field pea, and lentil) were used to assess the fungal virulence and crop safety, respectively. A single batch of seeds of each weed or crop was used in all experiments. Seven seeds of each weed or crop were sown in 10-cm-diameter pots filled with pre-prepared soil mix. Seeded pots were incubated in greenhouse at 24/20ºC day/night temperature with 12-h photoperiod and watered daily. Prior to inoculation, five plants were established and maintained in each pot.

Inoculation procedure. False cleavers and/or cleavers seedlings at 2-3 whorl stage or crop seedlings at 2-3 leaf stage were inoculated with 5 x 10^5 to 5 x 10^6 conidia/ml to run-off with 0.05% gelatin as a wetting agent, using a airbrush at 100 kPa. Unless otherwise indicated, after spraying, pots were placed in a dark dew chamber with 100% relative humidity at 22ºC for 48h. Subsequently, pots were transferred back to the greenhouse with the same conditions mentioned as above. The control treatment were sprayed with distilled water containing only the wetting agent.

Fungal virulence to target weed. False cleavers was first used as target to test fungal virulence since it dominates over cleavers in western Canada. However, cleavers was also used to examine its responses to selected virulent fungi. Fungal virulence on false cleavers and/or cleavers were assessed 7 and 14 days after inoculation using a 0 to 3 scale (0 - no symptom, + - light infection, ++ - moderate infection, and +++ - severe infection to death). For each fungal isolate, there were four replications arranged in a completely randomized design. The experiments were repeated at least once. The virulent fungal species with a scale of +++ were selected and proceed to crop safety test.

Pathogenicity on crops. The above selected virulent fungal species were inoculated with each of nine crops. Pots of false cleavers and cleavers were also included as control to make sure the inoculum viable. After spraying, pots were placed in a dark dew chamber with 100% relative humidity at 22ºC for 24h. Disease severity on each crop were recorded daily for 2 wks following inoculation by using the 0-11 rating scale by Horsfall and Barrett. Selection of isolates for further study was based on those with the stringent host specificity. The experiment were repeated once.

Results and discussions

Isolation of fungi. From those collected plant materials, 138 fungal pathogens were isolated: 12 from Vermilion, 23 from Vegreville, 9 from Lamont, 11 from Edmonton, and 62 from Peace River. About 23 fungal pathogens were also obtained from Agriculture and Agri-Food Canada, Saskatoon. The majority of fungal pathogens belong to genera of *Phoma*, *Septoria*, *Colletotrichum*, *Plectosporium*, *Fusarium*, *Alternaria*, etc.

Fungal virulence to target weed. With the supplement of 48 h dew, virulence of 163 fungal isolates against false cleavers varied and was categorized into 4 (0, +, ++, +++) responses (Table 1). Among those fungal isolates, 78 fungal isolates were non-pathogenic, 43 showed slight infections (+), 16 moderate infections (++), and 24 severe infections or 100% kill (+++), respectively, with the supplement of 48 h dew (Table 1).

Table 1.
Virulence of fungal isolates on false cleavers (*Galium spurium*) *

Isolates	Virulence	Isolates	Virulence	Isolates	Virulence
CL98101	++	CL98124	0	CL98147	+
CL98102	++	CL98125	0	CL98148	+
CL98103	+++	CL98126	0	CL98149A	+
CL98104	+	CL98127	0	CL98150	+
CL98105	+	CL98128	0	CL98151	+
CL98106	+	CL98129	0	CL98152	++
CL98107	++	CL98130	0	CL98153-1	+++
CL98108	0	CL98131	+++	CL98153-2	+
CL98109	+++	CL98132	0	CL98154	+++
CL98110	+	CL98133	0	CL98155-1	+++
CL98111	0	CL98134	++	CL98155-2A	+++
CL98112	0	CL98135	+++	CL98155-2B	++
CL98113	0	CL98136	++	CL98156-Y	+++
CL98114	0	CL98137	+++	CL98156-2	++
CL98115	0	CL98138	0	CL98156-B	+++
CL98116	0	CL98139	0	CL98157-1	+++
CL98117	+	CL98140	0	CL98157-2	+++
CL98118	0	CL98141	+	CL98158-1	+++
CL98119	0	CL98142	0	CL98158-2	+++
CL98120	0	CL98143	+++	CL98159	+++
CL98121	0	CL98144	++	CL98160-1	+
CL98122	0	CL98145	+	CL98160-2A	+++
CL98123	0	CL98146	+	CL98161	+
CL98162	0	CL98186	0	CL98A	+++
CL98163	++	CL98187	0	CL98B	+++
CL98164	+	CL98188	0	CL98C	+

Table 1 (cont.'d)

Isolates	Virulence	Isolates	Virulence	Isolates	Virulence
CL98165	0	CL98189	0	CL98D-1	+++
CL98166	0	CL98190	0	CL98D-2	+
CL98167	0	CL98191	0	CL98E-1	+
CL98168	+	CL98192	0	CL98E-2	++
CL98169-1A	+	CL98193	0	98-65A	+++
CL98169-1B	+	CL98194	0	98-57B	+++
CL98169-1C	+	CL98195	0	98-68A2	+
CL98169-1D	++	CL98196	0	98-53B2	+
CL98169-2A	+	CL98197	0	98-72B	+
CL98169-2B	+	CL98198	0	98-62	++
CL98169-3	+	CL98199	0	98-59A	0
CL98170	+	CL9819901	0	98-59B	0
CL98171	+	CL9819902	0	98-57A	+
CL98172	+	CL9819903	0	98-38A	+
CL98173-1	+	CL9819904	0	98-53B3	+
CL98173-2	+	CL9819905	+	98-56B	+
CL98174	+	CL9819906	+	98-65A1	0
CL98175	0	CL9819907	0	98-20E1	0
CL98176	0	CL9819908	0	98-51A	++
CL98177	0	CL9819909	0	98-39A	++
CL98178	++	CL9819910	0	94-196-B1	0
CL98179	+	CL9819911	0	94-196-C	0
CL98180	0	CL9819912	0	94-196-D	0
CL98181	0	CL9819913	0	1A SEED	0
CL98182	0	CL9819914	0	3 SEED	0
CL98183	0	CL9819915	0	96-58A	0
CL98184	0	CL9819916	0	94-196	0
CL98185	+++	CL9819917	0		

* Seedlings of false cleavers at the 2- to 3-whorl stage were inoculated with 10^5 to 10^6 conidia/ml, placed in a dew chamber at 22°C for 48 h and subsequently maintained in a greenhouse. Pathogenicity and virulence was rated 7 days after inoculation using a 0 to 3 grading system where 0 = no infection, + = light infection, ++ = moderate infection, and 3 = severe infection to death.

The virulence of twenty-four fungal isolates with a rating of +++ was further tested with the supplement of 24 h dew. The results demonstrated that only CL98103, CL98109, CL98158-1, and CL98158-2 killed false cleavers (Table 2). This finding suggested that these four isolates are better candidates for weed control because they require relatively short dew period.

Pathogenicity on crops. Pathogenicity of CL98103, CL98109, CL98158-1, CL98158-2, and other virulent fungal isolates (+++) on nine crops was significantly different from each other (Table 2). The results showed that CL98109 infected all nine crops tested. Thus, CL98109 was not selected as bioherbicde candidate. CL98103 was non-pathogenic to most of crops including canola. Although it caused cotyledon chlorosis of flax and safflower but did not infect any leaves sprayed with CL98103 inoculum. The disease severity on both flax and safflower was rated at 5%. Similarly, CL98158-1 and CL98158-2 did not infect majority of crops except alfalfa. Small flecks were observed on lower leaves treated with CL98158-1 and CL98158-2, with a disease severity of 2%.

Table 2.
Pathogenicity of selected virulent isolates on various crops*

Fungal	False	Crops								
Isolates	Cleavers	Wheat	Barley	Oats	Canola	Alfalfa	Flax	Lentil	Safflower	Pea
CL98103	+++	0	0	0	0	0	+	0	+	0
CL98109	+++	++	++	++	+	+	+++	+++	++	++
CL98131	++	0	0	0	0	0	0	+	+++	+
CL98135	+	0	0	0	0	0	0	0	+	0
CL98137	++	0	0	0	0	0	0	+	+	+
CL98143	+	0	0	0	0	+	+	+	+++	0
CL98153-1	+	0	0	0	0	0	0	0	+	0
CL98154	++	0	0	0	0	0	0	+	0	0
CL98155-1	+	+	0	+	0	0	0	+	++	0
CL98155-2A	+	0	0	+	0	0	0	+	++	0
CL98156-Y	++	+	0	0	0	0	0	0	+++	0
CL98156-B	+	0	0	0	0	0	0	+	+++	+
CL98157-1	+	0	0	0	0	0	0	+	+++	+
CL98157-2	+	0	0	0	0	0	0	+	+++	+
CL98158-1	+++	0	0	0	0	+	0	0	0	0
CL98158-2	+++	0	0	0	0	+	0	0	0	0
CL98159	+	0	0	0	0	0	0	+	+++	+
CL98160-2A	++	0	0	0	0	+	0	0	0	0
CL98185	++	0	0	0	0	0	0	0	+++	+
CL98A	+	0	0	0	0	0	0	0	++	0
CL98B	+	0	0	0	+	0	0	+	++	+
CL98D-1	++	0	0	0	0	0	0	+	++	+
98-57B	+	0	0	0	0	0	+	+	++	0
98-65A	++	+	+	+	+	0	0	++	+++	0

* Seedlings of crops at the 2- to 3-leaf stage were inoculated with 10^5 to 10^6 conidia/ml, placed in a dew chamber at 22°C for 24 h and subsequently maintained in a greenhouse. Pathogenicity and virulence was rated 7 days after inoculation using a 0 to 3 grading system where 0 = no infection, + = light infection, ++ = moderate infection, and 3 = severe infection to death. False cleavers at the 2- to 3-whorl stage were also inoculated with the same inoculum as a control.

Since the development of bioherbicides against cleavers mainly targets on canola fields, seven different varieties of canola with different genetic background were sprayed with CL98103 and CL98158. The data demonstrated that no variety of canola was infected by either CL98103 or CL98158.

The potential of CL98103 and CL98158 as bioherbicides should be further characterized.

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References

Brown, J., and A.P. Brown. 1996. Gene transfer between canola (*Brassica napus* L. and *B. campestris* L.) and related weed species. Ann. Appl. Biol. 129:513-522.

Hall, L.M, K.M. Stromme, G.P. Horsman, and M.D. Devine. 1998. Resistance to acetolactate synthase inhibitors and quinclorac in a biotype of false cleavers (*Galium spurium*). Weed Sci. 46:390-396.

Poweles, S.B., D.F. Lorraine-Colwill, J.J. Dellow, and C. Preston. 1998. Evolved resistance to glyphosate in rigid ryegrass (*Lolium rigidium*) in Australia. Weed Sci. 46:604-607.

Seefeldt, S.S., R. Zemetra, F.L. Young, and S.S. Jones. 1998. Production of herbicide-resistant jointed goatgrass (*Aegilops cylindrica*) x wheat (*Triticum aestivum*) hybrids in the field by natural hybridization. Weed Sci. 46:632-634.

Thomas, A.G., B.L. Frick, L.T. Juras, L. Hall, R. van Acker, and D. Joose. 1998. Changes in weed distributions indicated by quantitative surveys in the prairie provinces of Canada over 10 years. Proceedings of the 38th Annual WSSA Conference, Chicago, IL.

Turkington, R., N.C. Kenkel, and G.D. Krankko. 1980. The biology of Canadian weeds. 42. *Stellaria media* (L.) Vill. Can. J. Plant Sci. 60:981-992.