

Survey for seed-borne diseases on weed species from screening samples obtained from seed cleaning plants across Canada in 1987/88

K. Mortensen and M.M. Molloy¹

In search for potential biological control agents for weeds, requests for samples of Screenings from seed cleaning were sent out to seed cleaning plants across Canada in order to analyze for seed-borne diseases of weeds. Seven samples of screenings were received: two from Alberta, and one each from British Columbia, Saskatchewan, Manitoba, Ontario, and Prince Edward Island. A large percentage of the seeds (varying from 10 to 80%) developed fungal growth, of which very few affected germinated seedlings. Pathogenic fungi were isolated from diseased seedlings of wild oats: *Drechslera avenacea*, cow cockle: *Alternaria alternata*, stinkweed: *Alternaria raphani*, green foxtail: *Bipolaris sorokiniana*, wild buckwheat: *Botrytis* sp., from western Canada, and from a grass sp.: *B. sorokiniana*, and red clover: *Colletotrichum trifoli*, from eastern Canada. These results show that surveys for weed diseases can be conducted from samples of screenings submitted by cooperators. It is a quick and a relatively inexpensive method for weed disease surveying. However, as not all weed diseases are seed-borne, it cannot substitute surveys during the growing season.

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Des installations de nettoyage de semences, situées un peu partout au Canada, ont reçu des demandes pour faire analyser des échantillons de tamisage. Les demandeurs voulaient faire une analyse des pathogènes transmis par les graines afin de trouver des agents biologiques de lutte contre les mauvaises herbes. Sept échantillons de tamisage ont été reçus : deux de l'Alberta, et un pour chaque province dont la Colombie-Britannique, la Saskatchewan, le Manitoba, l'Ontario, et l'Île-du-Prince-Édouard. La croissance de champignons a été décelée sur un grand pourcentage des graines (de 10 à 80 %), mais très peu de champignons ont affecté les plantules émergées. Des champignons pathogènes ont été isolés à partir de plantules malades de la folle avoine : *Drechslera avenacea*, de la saponaire des vaches : *Alternaria alternata*, du tabouret des champs : *Alternaria raphani*, de la setaire verte : *Bipolaris sorokiniana*, de la renouée liseron : *Botrytis* sp., provenant tous de l'ouest du Canada et à partir d'une graminée : *B. sorokiniana*, et du trefle rouge : *Colletotrichum trifoli*, provenant de l'est du Canada. Ces résultats montrent que des relevés pour les maladies de mauvaises herbes peuvent être menés à partir d'échantillons obtenus par des collaborateurs. C'est une méthode rapide et peu coûteuse d'inventorier les maladies des mauvaises herbes. Quoiqu'il en soit, puisque toutes les maladies de mauvaises herbes ne sont pas transmises par les graines, cette méthode ne peut remplacer des relevés menés pendant la saison de végétation.

Introduction

Biological control of weeds with plant pathogens has received much attention in recent years (3,4,21,23) because of the pressures to decrease our dependence on synthetic herbicides. At present two bioherbicides are registered in the United States, *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *aeschynomene* 'Collego' for control of northern jointvetch [*Aeschynomene virginica* (L.) B.S.P.] and *Phytophthora palmivora* (Butler) Butler 'De Vine' for control of strangler vine (*Morrenia odorata* Lindl.) (20). In 1992, *C. gloeosporioides* f. sp. *malvae* was the first bioherbicide

registered in Canada under the trademark 'BioMal' for the control of round-leaved mallow (*Malva pusilla* Sm.) (11). The fungus in 'BioMal' was discovered as a seedling blight originating from infected round-leaved mallow seed (15).

Explorations for new bioherbicide agents are an integral part of the program on biological control of weeds with plant pathogens at the Agriculture & Agri-Food Canada, Research Station in Regina. Surveying for diseases on weeds can be done during the growing season, but this is very time consuming and expensive. As many plant diseases are seed-borne, analyzing weed seeds for disease causing organisms, might be an effective method of identifying organisms that parasitize weeds. In addition to being quicker and less expensive, representative samples of weed seeds from across Canada would provide a broader sampling base than one derived from surveys.

¹ Agriculture and Agri-Food Canada, Research Station, Regina, Saskatchewan, Canada S4P 3A2.

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The present work describes the results from an examination of seed samples from seed cleaning plants across Canada, the isolation of disease causing organisms from seedlings originating from weed seeds in the screening samples, and subsequently testing such organisms for their potential as bio-herbicide agents on the weeds from which they originated.

Materials and methods

Requests for screening samples were sent out to representative seed cleaning plants selected from those listed in Inspection Memorandum 1-2-57,86-08-07, Agriculture and Agri-Food Canada, Food Production and Inspection Branch, Seed Section. The requests were made in the fall of 1987 to three seed cleaning plants in British Columbia, three in Alberta, four in Saskatchewan, five in Manitoba, four in Ontario, four in Quebec, and one in each of New Brunswick, Prince Edward Island, and Nova Scotia. Screening samples from seed cleaning procedures were received from seven cooperating seed plants located at: Dawson Creek, British Columbia (received 23 Feb. 1988); Barrhead, Alberta (received 3 Mar. 1988); Camrose, Alberta (received 27 Jan. 1988); Wiseton, Saskatchewan (received 29 Dec. 1987); Ste. Rose du Lac, Manitoba (received 16 Mar. 1988); Belleville, Ontario (received 21 Dec. 1987); and Montague, Prince Edward Island (received 16 May 1988).

The screening samples were sorted into different sizes of seeds using screens with grid sizes, of 2.34 mm, 2.73 mm, 3.12 mm (6/64", 7/64, 8/64, respectively) and a pan sample. From each of the screens, 100 seeds, if present, of the most common species were selected for tests.

Seeds were placed on moist filter paper (MFP) (Whatman No. 3, 9 mm diam) in a petri dish and incubated for 4 to 7 days with a 12 h light period provided by fluorescent light ($28\mu\text{mol.m}^{-2}.\text{s}^{-1}$) at $24\pm0.5^\circ\text{C}$ and a 12 h dark period at $21\pm0.5^\circ\text{C}$. Seeds that germinated after four days were planted in autoclaved soil:peat moss:vermiculite (3:2:1) in 15 cm pots, covered gently with a thin layer of the same soil mixture and placed on greenhouse benches at $23\pm4^\circ\text{C}$ with ambient lighting extended to a 16 h photoperiod with fluorescent and incandescent light ($280\mu\text{mol.m}^{-2}.\text{s}^{-1}$). After seven days of incubation, the filter paper with the germinated or non-germinated seeds was placed in pots with soil as above and placed on the greenhouse benches. Pan samples from the screening samples were planted in soil in 34 cm x 48 cm steel flats and placed on the greenhouse benches. Plants were kept for one month and monitored daily for disease development. Seedlings that developed disease symptoms were surface sterilized (0.6% sodium hypochlorite for 1 min), placed on potato dextrose agar (PDA) and on MFP and incubated as described above. The fungal pathogens were isolated and increased on PDA. Spore suspensions of pure isolates were used to inoculate

seedlings of the plant species from which they originated. Inoculation was done by spraying the spore suspensions until runoff using an air brush [Paasche Airbrush (Canada) Ltd., Type H-5]. The inoculated plants were placed in a dew chamber (Percival, Model E-54) at $18\pm0.5^\circ\text{C}$ for 24 h in the dark, then returned to the greenhouse benches, and misted with water daily to maintain high humidity. If disease symptoms were not observed four weeks after inoculation, the fungal cultures were regarded as saprophytes and discarded. All material used in these tests was autoclaved before being discarded to prevent escape of weed seeds or diseases.

Results and discussion

The screening samples received contained representative crop and weed seeds from an area near the seed cleaning plant. Five of the screening samples, Dawson Creek, British Columbia; Barrhead, Alberta; Camrose, Alberta; Ste. Rose du Lac, Manitoba; and Montague, Prince Edward Island; contained wheat seed; the Wiseton, Saskatchewan, screening sample had lentil seed; and the Belleville, Ontario, sample had rye grass (*Lolium* sp.) seed. The weed species varied among the screening samples. Table 1 shows the weeds identified in the western Canadian samples from selected seed samples and Table 2 shows weeds from the two eastern Canadian selected seed samples. The most common weeds from western Canada were wild oats (*Avena fatua* L.), wild buckwheat (*Polygonum convolvulus* L.), lamb's-quarters (*Chenopodium album* L.), green foxtail (*Setaria viridis* (L.) Beauv.), and stinkweed (*Thlaspi arvense* L.). Mustard spp. (not identified to species) were observed in pan samples planted in flats under greenhouse conditions from all western Canadian locations. Green smartweed (*Polygonum scabrum* Moench.), Russian thistle (*Salsola pestifer* Nels.), hemp nettle (*Galeopsis tetrahit* L.), lady's-thumb (*Polygonum persicaria* L.), cow cockle (*Saponaria vaccaria* L.), white cockle (*Lychnis alba* Mill.), and Russian pigweed, (*Axyris amaranthoides* L.) were observed from one or two locations in seeds samples, as well as in the pan samples. Chickweed (*Stellaria media* (L.) Vill.) was only observed in the pan sample from Barrhead (Table 1). From the Ontario screening sample, ragweed (*Ambrosia* spp.), a grass (*Lolium* sp. ?), and red clover (*Trifolium pratense* L.) occurred, red clover and grasses (not identified) also occurred in the pan sample. From the Prince Edward Island screening sample, grass (not identified), wild radish (*Raphanus raphanistrum* L.), wild buckwheat, barnyard grass (*Echinochloa crusgalli* (L.) Beauv.) and *Convolvulus* sp. (not identified to species) were most common, and corn spurry (*Spergula arvensis* L.), lamb's-quarter and *Polygonum* sp. (not identified to species) grew from the pan samples (Table 2).

Fungal growth developed on the coats of a large percentage of the seeds from most samples placed on MFP (Tables 1 and 2). Fungi growing on the seed coats and causing disease symptoms on the seedlings, were identified and used in pathogenicity tests. *Alternaria* spp. were most prominent on the seed coats. However, spores of *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem. occurred on some seeds from all of the weed species in the Prince Edward Island screening sample (Table 3). Since *B. sorokiniana* is a known pathogen on graminaceous species across Canada (5,12) and it occurred so frequently on seeds of non-graminaceous species (Table 3), one isolate from each weed species was inoculated on plants from four of the Prince Edward Island weed species in order to see if they were host specific. Pathogenicity did not vary significantly among the five isolates. They were most pathogenic on the unidentified grass sp., less so on barnyard grass, and only slight or no symptoms were observed on the wild buckwheat and wild radish (Table 3). *B. sorokiniana* was isolated from brown spots on cotyledons and first leaves of two grass seedlings from the Prince Edward Island location (Table 7), but the seedlings outgrew the disease. Symptoms were not observed on any of the other seedlings from weed species where *B. sorokiniana* spores occurred. Although the *B. sorokiniana* isolates originated from dicotyledonous weed seeds, they were not significantly different from isolates originating from graminaceous species.

Germination of weed seeds from the western Canadian samples was good with averages of 65% or higher for green foxtail, lamb's-quarter, stinkweed, cow cockle, and green smartweed; fair with averages of about 45% or slightly above for wild oats, wild buckwheat, lady's-thumb, and Russian pigweed; and poor with less than 25% germination for white cockle, Russian thistle, and hemp nettle (Table 4). Germination from the eastern Canadian screening samples were low, less than 16% for barnyard grass, grass sp. (not identified), wild radish, wild buckwheat, and ragweed; somewhat better (36%) for *Polygonum* sp. (not identified to species); while the red clover sample from Ontario had 71% germination (Table 5).

Disease symptoms developed on about 13% of wild oats seedlings from the five western samples (Table 4), which is 7% of all seeds tested. The symptoms on wild oats seedlings from these locations were caused by *Drechslera avenacea* (Curtis ex Cooke) Shoem. (Table 6). This is in agreement with other data showing that *D. avenacea* was isolated from 7.5% of wild oats seeds from 59 locations in the prairie provinces (unpublished data). Avenacea leaf blotch or leaf stripe (*D. avenacea*) occurs commonly on cultivated and wild oats in all provinces (5,12, 19). The seedling-blight stage has rarely been observed on cultivated oats under natural conditions (6). The present results showed that wild oat seedlings originating from infected

seeds can become severely infected under greenhouse conditions, but often only a streak was observed on the coleoptile and the first leaves and the plants remained alive. Under natural conditions these lesions would be sufficient to allow for secondary spread by spores and cause the common avenacea blotch often observed on upper leaves later in the season.

Seedling blight developed on 30% of the cow cockle seedlings from the Saskatchewan sample (Table 4) which is 21% of total seeds tested. All of the observed symptoms on cow cockle seedlings were caused by an *Alternaria* sp. Earlier observations indicated that seedling blight caused by *Alternaria alternata* (Fr.) Keissl. affected up to 65% of seedlings from a Regina seed lot of cow cockle (unpublished data). *A. saponaria* (Pk.) Neerg. has been reported from cow cockle from Manitoba (2), from several states in the United States (7), and from western Europe (17). Another species, *A. dianthi* Stevens & Hall, was reported on cow cockle from Montana (13). Perhaps, several *Alternaria* species may attack cow cockle.

Disease symptoms developed on 7.9% of stinkweed seedlings (Table 4) which represents about 6% of all seeds tested. A pathogenic *Alternaria* sp. was isolated from five of these diseased seedlings, which were all from the Saskatchewan screening sample (100 seeds) (Table 6). This *Alternaria* sp. was submitted to National Identification Service, Ottawa, and identified as *Alternaria raphani* Groves & Skolko (Daom NO. 211978). *A. raphani* has previously been observed on stinkweed (18).

Disease symptoms developed on 1.7% of green foxtail seedlings (Table 4). The causal agent *B. sorokiniana* was isolated from two seedlings in the Manitoba sample (Table 6). Both isolates were rated as weakly pathogenic on green foxtail. Although inoculated tissues showed leaf spots, the plants outgrew the disease. In another study, *B. sorokiniana* was isolated from green foxtail at Regina, and a spore suspension was inoculated back onto green foxtail. Under optimum conditions these plants developed leaf spots but outgrew the disease. Under field conditions very little or no effect was observed when inoculated with this isolate of *B. sorokiniana* (unpublished data). *B. sorokiniana* has previously been isolated from crowns of green foxtail from Saskatchewan but to a much lesser extent than from cereal crops (10). These results indicate that *B. sorokiniana* has little potential as a biological control agent for green foxtail.

Disease symptoms were observed on wild buckwheat seedlings from three of the western locations, and *Botrytis* sp. was isolated from two seedlings originating near Barrhead, Alberta (Table 4). Both of these isolates caused slight leaf spotting when wild buckwheat was inoculated with these isolates. The symptoms were not regarded as suffi-

ciently pathogenic to warrant further study. *Rhizoctonia* sp. was isolated from diseased seedlings from two locations (Camrose, Alberta and Ste. Rose du Lac, Manitoba), but neither isolate was pathogenic.

The cause of the other seedling symptoms (Table 4) was not diagnosed. Species of *Alternaria*, *Pythium*, *Fusarium*, and *Phoma* (not identified to species) were isolated from the diseased seedlings, but in pathogenicity tests the injury was negligible. Perhaps a combination of stress in the greenhouse, including the activity of fungus gnats (Mycetophilidae) and these saprophytic fungi resulted in death of small seedlings.

From the two eastern Canadian locations, only two grass seedlings from the Prince Edward Island location showed disease symptoms which were attributed to *B. sorokiniana* and one seedling of red clover from the Ontario location showed symptoms (Table 5). A *Colletotrichum* sp. isolated from the red clover seedling (Table 7) caused typical anthracnose symptoms when red clover seedlings were inoculated with this isolate. This fungus was submitted to National Identification Service, Ottawa, and was identified as *Colletotrichum trifolii* Bain. Anthracnose of red clover caused by *C. trifolii* is a severe disease of red clover in the southern and mid-Atlantic United States, hence the name southern anthracnose (1,8,14). It has been recorded as far north as southern Canada, but is of little importance in the northern clover areas (9,24). Because *Colletotrichum* spp. have shown good potential as mycoherbicide agents (11,15,20,22) further studies were conducted for comparison with other *Colletotrichum* spp., and to determine its potential for biological control of black medick (*Medicago lupulina* L.), a serious weed in Canada (16).

The seven screening samples we received for this study during 1987-88 are not sufficient to give any accurate information on occurrence of seed-borne diseases of weeds. However, the present results show that surveys for diseases of weeds can be conducted from screening samples submitted by cooperators. This is a relatively inexpensive way of conducting weed disease surveys as it does not involve travel and accommodation expenses. A large geographical area can be covered and it can be done outside the growing season, at less busy periods of the year. This survey method works well for diseases that are seed-borne. However, it will not detect all diseases and, therefore, cannot substitute disease surveys during the growing season.

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Table 1. Weeds seeds identified in screening samples from western Canadian provinces and occurrence of fungi on seed coat when plated out on moist filter paper.

Weed species	Locatipns				
	Dawson Creek BC	Barrhead AB	Camrose AB	Wiseton SK	Ste. Rose du Lac MB
Wild oats	100*/46**	175116	43/81	75/77	71/31
Wild buckwheat	300/10	300147	300166	225170	200139
Lamb's-quarter	200111	(+)	100118	100181	
Green foxtail	(+)		100/19	(+)	200113
Stinkweed	100119	(+)	25/32	10116	
Mustard spp.	(+)	(+)	(+)	(+)	(+)
Green smartweed		100110		(+)	10018
Russian thistle		(+)		100184	
Hemp nettle		100162	50184		
Lady's-thumb			110128		
White cockle		100147			
Cow cockle				10014	
Chickweed		(+)			
Russian pigweed			201110		

* indicates number of seeds selected from screening sample, plated on moist filter paper (MFP) then planted in pots,

** indicates percent of seeds with fungi (not identified) on seed coats, and

(+) indicates that seeds were not selected, but that seedlings developed from pan sample, plated on MFP or in flats under greenhouse condition.

Table 2. Weeds seeds identified in screening samples from eastern Canada and occurrence of fungi on seed coat when plated out on moist filter paper.

Weed species	Locations	
	Belleville ON	Montague P.E.I.
Ragweed (not identif.)	225*/100**	
Grass (<i>Lolium</i> sp.)	3001100	
Grass (not identified)	(+)	100155
Red clover	250199	
Wild radish		100193
Wild buckwheat		300186
Corn spurry ?		(+)
Barnyard grass		100176
lambs-quarter		(+)
<i>Polygonum</i> sp.		(+)
<i>Convolvulus</i> sp.		3001100

* indicates number of seeds selected from screening sample, plated on moist filter paper (MFP) then planted in pots,

** indicates percent of seeds with fungi on seed coat (not identified) and

(+) indicates that seeds were not selected but that seedlings developed from pan sample, plated on MFP or in flats under greenhouse condition.

Table 3. Effect of *Bipolaris sorokiniana* isolates, originating from seed coats of five weeds, on plants from four of these weeds. Seed from screening sample received from Montague, Prince Edward Island.

Weed species	% seeds with fungus	Effect ¹ of <i>B. sorokiniana</i> isolated from					Mean rating
		WB	G.sp.	WR	BG	C.sp.	
Wild buckwheat (WB)	3.0	1	0	2	2	1	1.6
Grass sp. (G.sp.)	10.0	2	3	4	4		3.3
Wild radish (WR)	14.0	0	0	0	1		0.3
Barnyard grass (BG)	15.0	1	1	1	2	2	1.4
<i>Convolvulus</i> sp. (C.sp.)	19.0						

¹ Disease rating using a scale from 0 - 9: 0 = no symptoms; 9 = plants dead

Table 4. Germination of weed seeds and percentages of seedlings with disease symptoms from western Canadian screening samples.

Weed species	Locations				
	Dawson Creek BC	Barrhead AB	Camrose AB	Wiseton SK	Ste. Rose du Lac MB
Wild oats					
Germination (%) ¹	24.0	86.3	44.2	24.0	43.7
Seedl.w.sympt.(%) ²	8.3	13.9	15.8	5.5	19.4
Wild buckwheat					
Germination (%)	66.3	28.0	51.6	23.6	43.5
Seedl.w.sympt.(%)	0	2.3	0.6	0	11.5
Lamb's-quarter					
Germination (%)	73.5		67.0	81.0	
Seedl.w.sympt.(%)	0.7		1.5	2.5	
Green foxtail					
Germination (%)			87.0		76.0
Seedl.w.sympt.(%)			1.1		2.0
Stinkweed					
Germination (%)	64.0		76.0	60.4	
Seedl.w.sympt.(%)	3.1		5.3	18.0	
Green smartweed					
Germination (%)		66.0			63.0
Seedl.w.sympt.(%)		0			7.9
Russian thistle					
Germination (%)				10.0	
Seedl.w.sympt.(%)				0	
Hemp nettle					
Germination (%)		7.0	8.0		
Seedl.w.sympt.(%)		0	0		
Lady's-thumb					
Germination (%)			59.1		
Seedl.w.sympt.(%)			2.1		
White cockle					
Germination (%)		26.0			
Seedl.w.sympt.(%)		0			
Cow cockle					
Germination (%)				71.0	
Seedl.w.sympt. (%)				29.6	
Russian pigweed					
Germination (%)			51.7		
Seedl.w.sympt. (%)			0		

¹ (%) germinated of total number of seeds selected from screening sample (Table 1), plated on moist filter paper (MFP) then planted in pots.

² (%) of seedlings with disease symptoms, from which isolations were done.

Table 5. Germination of weed seeds and percentages of seedlings with disease symptoms from eastern Canadian screening samples.

Weed species	Locations		Weed species	Locations	
	Belleville ON	Montague P.E.I.		Belleville ON	Montague P.E.I.
Ragweed (not identif.)			Wild buckwheat		
Germination(%) ¹	2.6		Germination(%)		8.3
Seedl.w. sympt.(%) ²	0		Seedl.w. sympt.(%)		0
Grass (not identif.)			Barnyard grass		
Germination (%)	17.3	12.0	Germination (%)		16.0
Seedl.w. sympt.(%)	0	16.7	Seedl.w. sympt.(%)		0
Red clover			Polygonum sp.?		
Germination (%)	71.2		Germination(%)		36.0
Seedl.w. sympt.(%)	0.6		Seedl.w. sympt.(%)		0
Wild radish			Convolvulus sp.?		
Germination (%)		9.0	Germination (%)		0
Seedl.w. sympt.(%)		0			

¹ (a)germinated of total number of seeds selected from screening sample, plated on moist filter paper (MFP) and planted in pots.² (%) seedlings with disease symptoms, from which isolations were done.

Table 6. Fungi isolated from diseased seedlings from screening samples from western Canada.

Fungi isolates	Host species	Locations				
		Dawson Creek BC	Barrhead AB	Camros AB	Wiseton SK	Ste. du Lac MB
<i>Drechslera avenacea</i>	Wild oats	2(3.1%) ¹	21(12%)	3(6.9%)	1(1.3%)	6(8.4%)
<i>Alternaria raphani</i>	Stinkweed				5(5.0%)	
<i>Alternaria alternata</i>	Cow cockle				18(18%)	
<i>Bipolaris sorokiniana</i>	Green foxtail					2(1.0%)
<i>Botrytis</i> sp.	Wild Buckwheat		2(0.7%)			
<i>Rhizoctonia</i> sp.	Wild Buckwheat			1(0.3%)		1(0.5%)

¹ Number of seeds from which fungus has been isolated from selected seed sample (% of seeds)

Table 7. Fungi isolated from diseased seedlings from screening samples from eastern Canada.

Fungi isolated	Host species	Locations	
		Belleville ON	Montague P.E.I.
<i>Colletotrichum trifolii</i>	Red clover	1(0.4%) ¹	
<i>Bipolaris sorokiniana</i>	Grass sp.		2(2.0%)

¹ Number of seeds from which fungus has been isolated from selected seed sample (% of seeds).