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Surveying for pathogens as potential weed biocontrol agents

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Abstract

Biological control of weeds with pathogens has mainly involved the inundative or bioherbicide approach. A major constraint in development of bioherbicides is the lack of knowledge of the pathogens that affect the most important weeds. It is therefore imperative that surveys be conducted to explore the availability of naturally-occurring weed pathogens. This can be done in two ways: (i) by conducting surveys during the growing season for disease symptoms on weeds; and (ii) by analyzing weed seeds from screening samples for seed-borne pathogens. Surveying during the growing season is time-consuming and expensive, as it involves extensive travel and tedious sample collection in the field. Analyzing weed seeds can be done on screening-samples requested from representative seed-cleaning operators. The latter method is less expensive, and it documents the occurrence and distribution of seed-borne diseases on weeds, indicating those weeds that were most prominent in field crops. However, it will only detect seed-borne diseases. Therefore, the two methods of surveying complement one another, and one cannot substitute for the other.

Introduction

Biological control of weeds with plant pathogens has mainly involved the inundative or bioherbicide approach, in which a microherbicide is applied in a similar manner to a chemical herbicide. Research on biological weed control with pathogens has been ongoing for over 25 years in the United States of America and Canada. In spite of significant research efforts in both countries, only two bioherbicie agents have been registered and sold commercially in the USA (TeBeest and Templeton 1985), and only one has been registered in Canada (Makowski and Mortensen 1992; Mortensen 1988). Obviously, there are constraints in the development of plant pathogens for biological control of weeds which

need to be overcome to ensure the future commercial success of bioherbicides (Auld and Morin 1995; Makowski 1996). One of the greatest constraints in bioherbicide research is the availability of effective biological control agents. Bioherbicide agents must be fast-acting, predictable, easy to use and provide a level of control comparable to chemical herbicides before they will have general acceptance from industry and users (Bowers 1982; Charudattan 1990). For private industry to become involved in the development of bioherbicides, weeds with high market potential must be targeted (Cross and Polonenko 1996). Information about diseases and the occurrence of pathogens on weeds is limited, compared to diseases of commercially important field crops. Therefore, exploration for new bioherbicide agents is an important part of bioherbicide research and has been an integral component of the biocontrol of weeds programme at Agriculture and Agri-Food Canada, Saskatoon Research Center. This has been pursued using two approaches: (i) surveying for weed diseases during the growing season; and (ii) analyzing weed seeds for seed-borne diseases.

Material and methods

Surveys during the growing season

In 1994, surveys for diseases of important weeds were conducted in Saskatchewan and Manitoba from the Regina location, during trips of 1-3 days duration, and diseased specimens were collected for diagnosis and isolations. Agreements were made with collaborators across Canada, to conduct surveys in their local areas and to submit diseased samples by courier service. Collaborators agreed to a flat fee of Canadian $50 per sample, up to a maximum of $1000 per season, for approximately five samples from each of four specified weeds, *Cirsium arvense* (L.) Scop. (Canada thistle), *Setaria viridis* (L.) Beauv. (green foxtail), *Avena fatua* L. (wild oats) and *Stellaria media* (L.) Vill. (chickweed).

These weeds were given priority in the surveys because they were regarded as prime targets for bioherbicide research from a market point-of-view (Cross and Polonenko 1996). If diseased specimens from these four weeds could not be collected, specimens from other important weeds in field crops were accepted. Surveyors were asked to look for disease symptoms such as necrotic leaf spots and, or, stem lesions and for severe cases of plants with top dieback. Pathogens causing these types of symptoms are the most promising as bioherbicides because many are facultative parasites and can be cultured on artificial media. Such pathogens often do not spread readily on their own, so diseased plants might occur as scattered single plants or in small patches.

Analyzing weeds seeds from screening-samples

Requests for screening-samples were mailed out in October 1994 to 48 selected seed-cleaning operators in Alberta, Manitoba, and Saskatchewan (based on lists from Inspection Memoranda, published by Agriculture and Agri-Food Canada, Food Production and Inspection Branch, Seed Section). Representative samples of weed seeds, collected by bulking small samples from various seed-lots in seed-cleaning operations, up to a total of 0.25 to 0.5 kg, were submitted by mail or by courier. The seed samples were sorted by size,

using sieves with different mesh sizes (2.34, 2.73 and 3.12 mm). The most common weeds were selected for analysis. One hundred seeds from each weed species were placed on moist filter paper in a petri dish and incubated for 14 days. Plates were inspected regularly, seed germination recorded, and isolations made from any disease symptoms occurring on the seedlings, using standard plant pathology procedures.

Pathogenicity testing

Fungi isolated from the samples and seedlings were cultured on potato dextrose agar (PDA) in an incubator with a 12-hour light period provided by fluorescent light (28 mol m^{-2} s^{-1}) at 24± 0.5° and a 12-hour dark period at 21± 0.5°C.

Fungal isolates were purified either by tip-culturing or single-spore separation, and tested for pathogenicity on the weed species from which they originated. Pathogenicity testing consisted of two steps: (i) bioassay; and (ii) inoculation, on live plants. In the bioassays, a small section of mycelium on PDA from pure cultures was transferred to a surface-sterilized leaf of the host-weed, placed on moist filter paper in a petri dish and incubated (as described above) for up to two weeks. If disease development did not occur on detached leaves, isolates were considered non-pathogenic and discarded. If disease development did occur, the pathogen was re-isolated and compared with the original cultures (Koch's postulate). For inoculation on live plants, spore suspensions of pathogenic isolates were sprayed on plants of their respective hosts, incubated in a dew chamber (Percival, Model E-54) at 18± 0.5°C for 48 hour (24 hours dark, 8 hours light, 16 hours dark), and returned to the greenhouse. Plants of the various weeds used in these tests were grown in soil:peat-moss:vermiculite (3:2:1), from seeds or root sections, under greenhouse conditions at 23± 4°C with ambient lighting extended to a 16-hour photoperiod with fluorescent and incandescent light (280 μmol in^{-2} S^{-1}**)**. A pathogen was regarded as having potential as a bioherbicide agent if disease symptoms occurred on the inoculated host plants within 2-3 weeks.

Results

Surveys during the growing season

A total of 411 diseased weed-samples were submitted and processed at Regina in 1994 (Table 1). Sixty-seven percent of these samples were from Saskatchewan, and 28% were front three eastern provinces, submitted by two collaborators in Ontario, and one collaborator in each of Quebec and New Brunswick. Four weeds, *C. arvense*, *S. viridis*, *A. fa*tua, and *S. media* made up 64% of the samples submitted. Seventy-four (18%) of the samples submitted were diagnosed with diseases caused by obligate parasites (Table 2). The latter were not tested further, because obligate parasites have little potential as bioherbicide agents. There were 272 facultative fungi (in eight genera) observed or isolated from the samples (Table 3). Several of these were identical, but from different locations. Not all of these fungi were isolated and tested for pathogenicity, because several had been tested in previous years. A total of 174 isolates were found to be pathogenic in the bioassay tests and

Table 1. Number of diseased weed samples submitted in 1994 from British Colombia (BC), Alberta (AB), Saskatchewan (SK), Manitoba (MB), Ontario (ON), Quebec (PQ) and New Brunswick (NB), Canada.

Weed species	BC	AB	SK	MB	ON	PQ	NB	Total
Cirsium arvense	1	5	105	1	11	9	7	139
Setaria viridis			34		10	9	5	58
Avena fatua		2	29		3	1	4	39
Stellaria media			12		12	1	1	26
Matricaria maritima			15					15
Euphorbia esula			7	2	1			10
Chenopodium album			3		4	2		9
Taraxacum officinale		3	4		2			9
Crepis tectorum			9					9
Sonchus arvensis		1	7					8
Galeopsis tetrahit			1		1		5	7
Amaranthus retroflexus						4	3	7
Echinochloa crusgalli			1		1	4		6
Other	2	1	50		8	5	3	69
Total	3	12	277	3	57	34	25	411

Table 2. Number of obligate parasites observed on weed samples submitted in 1994.

Weed species	Rust	White rust	Powdery mildew	Downy mildew	Smut	Nematodes
Cirsium arvense	9	7				12
Setaria viridis				14	1	
Avena fatua	9					
Chenopodium album				2		
Taraxacum officinale	2		1			
Euphorbia esula			1			
Crepis tectorum	5					
Amaranthus retroflexus		6				
Other	5					

were stored at -70°C. Testing of these isolates on live plants is in progress. Of the isolates tested to date, 17% were able to infect live plants (Table 3).

Analyzing weed seeds from screening-samples

A total of 22 screening-samples (a 46% response to the total number of requests that were sent out) were submitted from seed-cleaning organizations in the three prairie provinces. This was an increased response compared with the 27% obtained in a previous survey (Mortensen and Molloy 1993). Approximately 20 different weed species were obtained from the screening-samples, and the 15 most prevalent weeds were identified

Table 3. Number of facultative fungi observed and pathogens isolated from 1994 samples. '-' shows that isolates were not tested on live plants.

Weed species	No. of fungi observed	No. of isolates pathogenic	
		Bioassay	Live plants
Cirsium arvense	119	70	9
Setaria viridis	38	24	8
Avena fatua	31	8	-
Stellaria media	22	13	0
Taraxacum officinale	5	7	2
Matricaria maritima	9	12	-
Euphorbia esula	9	1	1
Chenopodium album	4	5	-
Crepis tectorum	3	2	-
Sonchus arvensis	6	2	-
Galeopsis tetrahit	7	3	-
Echinochloa crusgalli	5	1	-
Other	14	26	-

Table 4. Weed seeds obtained in submitted screening-samples from the 1994 growing season. The data are presented as percentage of the samples with seeds of weed species. Sask.-Saskatchewan.

Weed species	Alberta (8 sites)	Manitoba (5 sites)	Sask. (9 sites)	Total (22 sites)
Polygonum convolvulus	100	100	100	100
Avena fatua	63	60	100	77
Polygonum spp. (smartweed)	25	80	56	50
Setaria viridus	0	50	56	41
Thlaspi arvensis	50	20	33	36
Galium spp. (cleavers)	50	40	22	36
Galeopsis tetrahit	63	20	11	32
Chenopodium album	25	20	33	27
Brassica spp. (canola)	0	60	11	18
Amaranthus retroflexus	13	40	0	14
Medicago lupulina	13	0	22	14
Grass sp. (Not identified)	13	20	11	14
Sinapis arvensis	13	0	11	9
Lappula echinata	13	0	11	9
Trifolii spp.	13	0	11	9
Other (not identified)	13	20	44	27

Table 5. Number of seedings and fungi from screening-samples. Total - total number of seedlings (% germination); Infected - number of blighted seedlings (% of seedlings infected); Fungal isolates - number of isolates pathogenic in bioassay (isolates not yet tested).

Weed species	Total		Infected		Fungal isolates	
Polygonum convolvulus	976	(47)	65	(7)	16	
Avena fatua	725	(54)	37	(5)	17	
Polygonum spp. (smartweed)	732	(85)	51	(7)	17	(2)
Setaria viridus	611	(69)	14	(2)	4	(5)
Thlaspi arvensis	415	(46)	31	(8)	9	(5)
Galium spp. (cleavers)	284	(44)	69	(24)	5	(5)
Galeopsis tetrahit	66	(13)	10	(15)	3	(1)
Chenopodium album	355	(71)	14	(4)	3	
Brassica spp. (canola)	201	(57)	16	(8)	0	
Amaranthus retroflexus	160	(53)	9	(6)	0	
Medicago lupulina	58	(27)	7	(12)	2	
Grass sp. (Not identified)	185	(62)	0	(0)	0	
Sinapis arvensis	93	(47)	0	(0)	0	
Lappula echinata	109	(73)	17	(16)	0	(6)
Trifolii spp;	106	(53)	16	(15)	4	
Other (not identified)	194	(40)	15	(8)	0	(1)

(Table 4). *Polygonum convolvulus* L. (wild buckwheat) occurred in all screening-samples, and *A. fatua* occurred in all samples from Saskatchewan, but less frequently from Alberta and Manitoba. *Polygonum* spp. (smartweed) and *S. viridis* were most frequent in Manitoba, and *Thlaspi arvense* L. (stinkweed), *Galium* spp. (cleavers) and *Galeopsis tetrahit* L. (hemp nettle) were most common in Alberta. Seed-germination of the various weeds ranged from 13-85%, with 13 species above 44% germination. Seedlings became infected from nearly all weeds tested, but at a low frequency (Table 5). Disease-causing fungi were isolated from symptoms of several seedlings. However, in many cases saprophytic fungi were also isolated from diseased seedlings under these conditions.

Discussion and conclusion

Surveying during the growing season is the most effective way of detecting diseases of weeds. The best period to conduct weed-disease surveys is in the middle of the growing season; in Canada this is from early July to the middle of August. If surveys are conducted earlier, symptoms may not be sufficiently developed for easy detection. If surveys are delayed, secondary saprophytic fungi may invade plant tissues making it difficult to isolate the disease-causing organisms. In surveys during the growing season, specific weeds with high-market-value can be given priority. However, this is a very time consuming and costly method because it involves extensive travel and requires that surveys be completed in a short period of time. Samples collected during the growing season have to be processed soon after submission because diseased specimens decay relatively quickly, allowing secondary organisms to develop.

Analyzing weed seeds from screening-samples submitted by seed-cleaning organizations for seed-borne diseases, is a less expensive method and can be conducted outside of the growing season. This method gives information on the most prevalent weeds in a sample, indicating which weeds were most important in particular areas. However, it does not allow detection of some perennial weeds which are low seed-producers or have light seeds with a pappus. Due to the nature of the sampling technique, it would be difficult to trace the disease to a specific field, and it would not provide information about the severity of the disease under natural conditions. Not all diseases are seed-borne, so many diseases would not be detected.

The two methods of surveying complement one another, but one cannot substitute for the other. Both are necessary to obtain adequate information on naturally-occurring pathogens on major weeds.

Information obtained from the surveys that are described here is of the utmost importance to the future of biological control of weeds. If conducted on a regular basis, such surveys should lead to the detection of effective bioherbicide agents. However, because many weed pathogens are not sufficiently pathogenic on their host, it might not be possible to discover effective agents that occur naturally on important weeds. Collections obtained from such surveys will serve as a source of organisms, which might be manipulated into effective bioherbicide agents, through the formulation of combinations of one or more organisms and, or, genetic engineering.

Biological control with foliar pathogens has more potential for broad-leaved weeds than for grassy weeds. Infection can readily occur at the terminal meristems of broad-leaved weeds, resulting in top dieback, but is less likely in grassy weeds, where the meristem is less exposed (Makowski 1996). Therefore, in surveying for foliar pathogens for biological control of weeds, emphasis should be placed on broad-leaved weeds.

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