Frequency and distribution of seedborne fungi infecting canola seed from Ontario and western Canada – 1989 to 1993

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From 1989 to 1993, composite samples of canola from crop districts in western Canada and, from 1991 to 1993, individual producer samples of canola from Ontario were tested for the presence of seedborne fungi. Each year, 600 seeds from each western crop district and between 150 and 300 seeds from each Ontario sample were surface disinfected before plating onto 20% V-8 agar. Seventy species representing 36 genera were recovered. ***Alternaria alternata*** was the most common species recovered, followed by ***Alternaria brassicae*** and ***Alternaria raphani.*** The frequency with which ***Alternaria alternata*** was recovered from seed was higher in samples from the more easterly provinces, whereas that of ***Alternaria brassicae*** and ***Alternaria raphani*** were highest in samples from the more westerly ones.

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Entre 1989 et 1993, des echantillons composites de Canola provenant des districts agricoles de l'Ouest du Canada ont ete testes afin de détecter la presence de champignons transmis par la graine. Les mêmes tests ont été effectués entre 1991 et 1993 sur des Bchantillons de Canola provenant de differents producteurs ontariens. Chaque année, 600 graines de semence en provenance de chacun des districts agricoles de l'Ouest et entre 150 et 300 graines de semences puisées dans chacun des echantillons de l'Ontario ont été désinfectées en surface avant d'être plantées dans du V-8 agar **(20%)**. Soixante-dix espèces représentant 36 genres ont été retrouvées. Parmi les espèces retrouvées, ***Alternaria alternata*** s'est révélée la plus abondante suivie par ***Alternaria brassicae*** et ***Alternaria raphani.*** La frequence avec laquelle on a retrouvé ***Alternaria alternata*** dans les semences a été plus élevée dans les provinces plus a l'est, tandis que ***Alternaria brassicae*** et ***Alternaria raphani*** ont été retrouvées en plus grande quantite dans les echantillons provenant des provinces plus à l'ouest.

Introduction

Seedborne fungal pathogens are commonly found on canola seed harvested in Canada (Petrie, 1974; Martens ***et al.***, 1984). Three of the more important pathogens are ***Alternaria brassicae*** (Berk.) Sacc. and ***A. raphani*** Groves & Skolko, the causal agents of alternaria blackspot, and ***Leptosphaeria maculans*** (Desm.) Ces. & de Not., the causal agent of blackleg. In Canada, the most recent field surveys for these pathogens and the diseases they cause are those of Mathur and Platford (1994), Petrie (1994), Evans ***et al.*** (1994), Harrison and Kharbanda (1994), Turkington and Harrison (1994) and Jesperson (1994). However, much less is known about the frequency and distribution of other seedborne fungi infesting Canadian canola seed. The most recent information is in the report by Petrie (1974). The purpose of this survey was to obtain representative samples of canola seed harvested over several years in western Canada and Ontario, and to identify the fungi infecting these seeds.

Materials and methods

Between 1989 and 1993, 14,267 samples of canola seeds (grades 1 and 2) from 28 crop districts (Fig. 1) were submitted in envelopes capable of holding 500g of seed to the Grain Research Laboratory (GRL) (Table 1) by primary elevator managers, oilseed crushing companies and canola producers in Manitoba, Saskatchewan and Alberta. In addition, 112 samples (primarily spring, but some winter canola) were received from Ontario between 1991 and 1993. All samples used herein were graded as 1 or 2 by the Industry Services Division (formerly the Inspection Division) of the Canadian Grain Commission. Samples from the three western provinces were composited at the GRL according to grade and crop districts, whereas those from Ontario were maintained as individual samples. Subsamples of the seeds were surface disinfected by soaking in a 0.3% sodium hypochlorite solution for 1 min then air-dried in a laminar flow cabinet. Usually, 300 disinfected seeds from each Ontario sample and the No. 1 and No. 2 grade of canola from the western composites from each crop district were placed onto 20% V-8 agar in petri dishes, with 15 seeds per plate. The plates were incubated for 7 days at room

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temperature under a cycle of 12 hours darkness and 12 hours of mixed UV and fluorescent light. Although the seeds from the western crop districts were tested separately, the results in the grades 1 and 2 were combined and reported in the table. In the high quality crop for 1991, there was not enough grade 2 seed to prepare composites for the western crop districts, so 600 seeds of grade 1 per western crop district were tested. Also in 1991, 150 seeds per Ontario sample were plated. Between 1989 and 1993, 53,100 seeds from western Canada and between 1991 and 1993, 27,150 seeds from Ontario were examined in this study. For samples harvested in 1989 and 1990, the virulence of the ***L. maculans*** isolates was established by inoculating wounded cotyledons of 7-day-old canola seedlings of the cultivar Westar with 10 µL of a 1 x 10^6 spore suspension. After growth for **10** days at 22°C, the cotyledons were examined for signs of necrosis caused by virulent isolates. For isolates collected in the years after 1990, only the cultural characteristics of the ***L. maculans*** isolates on V-8 agar were used to classify them as virulent or non-virulent (McGee and Petrie, 1978).

Results and discussion

The number of samples within the composites from the three prairie provinces (Table 1) ranged from 2 for crop districts where little canola is grown to 712 for districts which are centers of production (DeClercq ***et al.***, 1989). Seventy species of fungi from 36 genera were recovered and identified as seedborne organisms (Table 2). The most common organism was ***Alternaria alternata*** (Fr.) Keissl., which was recovered from never less than 4.88% of the seeds in a crop district composite, and on average from 8.4% to 43.5% of the seeds from the four provinces (Table 2). Differences in the frequency of infection by several fungal species were recorded among the provinces. In an easterly direction from Alberta to Ontario, the frequency of seed infection by ***Alternaria alternata*** increased, whereas that of ***A. brassicae*** and ***A. raphani*** decreased (Table 2). ***Alternaria brassicae*** and ***A. raphani*** were ***8*** and more than 20 times more common, respectively, in Alberta than in Ontario. However, ***A. alternata*** was recovered over **5** times more often from Ontario seed than from Alberta seed. We observed that ***A. brassicae*** and ***A. raphani*** occurred more frequently on seeds from northern crop districts than southern ones, which agrees with the reports by Petrie (1974) and Clear (1992). The higher levels of these two ***Alternaria*** species on seeds in northern districts may be due to environmental conditions such as moisture (Tewari, 1985), and to the seeding of the earlier maturing ***Brassica rapa*** L. (syn. ***B. campestris*** L.) cultivars which are more susceptible to blackspot than are the ***B. napus*** L. varieties (Skoropad and Tewari, 1977; Conn and Tewari, 1989). In previous seed surveys, Petrie (1974) also found that ***B. rapa*** cultivars contained higher levels of ***A. brassicae*** and ***A. raphani.***

In the western provinces, the virulent strain of ***L. maculans*** was most commonly recovered from seeds grown in Saskatchewan crop district 6, one of the areas where it was first detected in Canada (McGee and Petrie, 1978; Petrie, 1978), and least common in Alberta seed, where it was found only in crop districts 2 and 6 in 1992 and 1993, respectively. Ontario also had a high percentage of seeds infected by the virulent (highly aggressive) strain, and in both Ontario and Saskatchewan, this strain was more frequent than the non-virulent (weakly aggressive) one. We noted that almost all of the virulent isolates from Ontario produced a greenish pigment in the agar, whereas the western isolates rarely produced a green pigment. Chigogora and Hall (1990) reported that **85%** of Ontario winter rapeseed samples they examined were contaminated by ***L. maculans*** and that the average seed infestation was 0.9%. Over the three years in our study of Ontario canola (primarily spring varieties), the average infection level was 0.1***8%.*** This seedborne pathogen is readily transported to uninfested fields, and even the use of seed with low infection levels can result in considerable numbers of infected seeds being sown (Clear 1992).

Eleven species of *Fusarium* were recovered, but only *F.* ***avenaceum*** (Fr.) Sacc., *F.* ***acuminatum*** Ell. & Everh., and *F.* ***equiseti*** (Corda) Sacc. were detected in each province. *Fusarium* ***avenaceum*** was the most common species of ***Fusarium*** recovered, and was found most often in Ontario seed samples (average of 0.54%), followed by samples from Alberta (0.14%), but particularly those from northern Alberta. One Ontario sample in 1992 had over 17% seed infection by *F.* ***avenaceum*** (Table 3). The maximum level of *Fusarium* spp. in any western crop district seed composite was 2.33% from Manitoba crop district ***8,*** which also was identified as *F.* ***avenaceum*** (Table 3). Petrie (1974) reported *F.* ***roseum*** Lk. emend Snyder & Hansen (largely the "Acuminatum" type) to be the most frequent ***Fusarium*** spp. infesting the seed of rape in western Canada, but that surface disinfestation eliminated almost all of the Fusaria. Likely the reduced recovery from seed of *Fusarium* spp. by Petrie after disinfecting is due to the elimination of Fusaria present merely as a surface contaminant. ***Fusarium graminearum*** Schwabe (mainly group II, but also group **I)** was found on seed collected in Ontario and from the area of southern Manitoba where this species has been responsible for fusarium head blight of cereals in recent years (Clear ***et al.,*** 1994). Its presence on canola seed is likely due to saprophytic ability and its abundance in the local environment.

In Ontario, the ***Arthrinium*** state of ***Apiospora montagnei*** Sacc. was considerably more common (11%) than ***Arthrinium phaeospermum*** (Corda) M.B. Ellis (2%), but in the west they were recovered at about the same frequency (Table 2). ***Arthrinium*** was not identified to species in 1989. It is these ***Arthrinium*** spp. recovered from the 1989 samples

which comprise virtually all of the category entitled *Arthrinium* spp. not identified (Tables 2 and 3).

Stemphylium vesicarium (Wallr.) Simmons and S. *herbarum* Simmons were both found and the former was one of the more common fungi isolated from canola seed, especially from Ontario (Table 2). Although S. *vesicarium* is a destructive seedborne pathogen of onion (Aveling *et al.,* 1993), little is known of its effect on canola seed or its importance in canola production.

Cladosporium cladosporioides (Fres.) de Vries was most common in canola seed from Ontario and the eastern prairies, whereas *C. herbarum* (Pers.) Link ex Gray was more frequent in the western prairies. *Cladosporium macrocarpum* Preuss was only found on seed samples from the prairies. *Cladosporium cladosporioides* was identified as the dominant *Cladosporium* species infecting Ontario winter wheat seed (Clear and Patrick, 1993).

Although not usually infecting a great number of seeds, *Chaetomium* spp. were recovered from a number of samples. Species such as *C. erectum* Skolko & Groves, *C. funicola* Cooke, *C. globosum* Kunze ex Steud., *C. indicum* Corda, *C. perlucidum* Sergejeva, *C. reflexum* Skolko & Groves, and *C. spinosum* Chivers were isolated from the seed samples. They were recovered more often from canola seed than from Ontario winter wheat seed (Clear and Patrick, 1993). Their effect on canola seed quality is unknown, but soybean seed quality was not affected by *Chaetomium* sp. when artificially inoculated within the pods (Gupta and Schmitthenner, 1984).

On average, few storage fungi were detected in the samples, although a few individual Ontario samples had high infection rates (Table 3). *Aspergillus glaucus* Link ex Gray group species (teleomorph *Eurotium* Link ex Fr.) was the dominant group species, others being rarely recovered. The low levels of storage fungi reflect the short time the seed was in commercial storage before sampling.

Other species identified but not listed in the tables include *Preussia fleischhakii* (Auersw.) Cain, an unnamed *Preussia* species similar to *P. aemulans* Arx but of smaller dimensions, *Sordaria fimicola* (Rob.) Ces. & de Not., and *Verticillium nigrescens* Pethybr.

Infected seed can be an important source of disease spread. In Alberta, considerable effort has been expended to control the spread of the virulent form of *L. maculans.* Growers there are advised to purchase seed that has been tested for the virulent blackleg, and to treat all canola seed for planting with a recommended fungicide (Anonymous, 1994). Besides transporting pathogens of canola, it is evident that pathogens of other crops may be recovered from canola seed. The importance of this type of spread is unknown. However, the direct impact of seed infection on canola is likely minimal, although some seed samples may suffer germination or emergence problems due to seedborne pathogens. The most common fungi, the *Alternaria* species, have little effect on canola seed (Petrie, 1974), and in general seedborne fungi are rarely significant in rapeseed emergence problems (Martens *et al.*, 1984). There are reports of non-traditional pathogens such as *Epicoccum nigrum* Link ex Link affecting canola seed (Khulbe *et al.*, 1992), but the low frequency of this and other fungi suggests that their potential impact is minimal. However, soilborne diseases of the seedling and resulting plant are important (Tewari, 1985) and their occurrence on seed may be used as one indicator of the frequency and distribution of these organisms in the field as well as being a means of dispersal over time and space.

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Table 1. Number of canola samples (grades No. 1 and No. 2) in each western crop district composite from 1989 to 1993.

Crop District *	1989	1990	1991	1992	1993	Total
Manitoba						
1	60	54	39	37	49	239
2	60	117	122	119	74	492
3	60	119	77	62	81	399
4	33	53	33	17	27	163
5	51	69	44	20	31	215
6	32	43	77	35	47	234
7	60	105	112	85	105	467
8	60	116	135	56	110	477
9 & 10	54	38	77	35	49	253
11	46	37	78	17	26	204
12	21	25	10	6	16	78
Total	537	776	804	489	615	3221
Saskatchewan						
1	142	102	131	286	103	764
2	22	16	33	78	65	214
3	106	24	39	40	41	250
4	6	2	9	11	6	34
5	147	328	424	712	421	2032
6	171	145	148	319	233	1016
7	82	66	43	140	119	450
8	167	346	353	472	227	1565
9	128	430	271	612	221	1662
Total	971	1459	1451	2670	1436	7987
Alberta						
1	16	14	26	12	22	90
2	102	114	85	39	173	513
3	56	55	47	5	68	231
4	135	249	178	156	232	950
5	91	100	59	40	96	386
6	79	67	53	45	82	326
7	136	168	65	60	134	563
Total	615	767	513	357	807	3059

* See Fig. 1. Crop districts of western Canada.

Table 2. Average level of fungal infection (%) of Canadian canola seed between 1989 and 1993.

Fungi	Alberta*	Saskatchewan*	Manitoba*	Ontario**
Acremoniella atra	0	0	0.01	0.19
Acremonium spp.	0	0	tr	tr
Actinomycetes	0	0	0	0.01
Alternaria alternata	8.40	9.30	14.99	43.48
A. brassicae	3.40	1.20	1.15	0.42
A. raphani	1.66	0.48	0.34	0.07
Apiospora montagnei	0.19	0.14	0.16	0.60
Arthrinium phaeospermum	0.20	0.09	0.13	0.03
Arthrinium **not** *ID*	0.11	0.13	0.18	0.01
Ascomycetes	0	0	0.01	tr
Aspergillus candidus	0	0	0	0.01
A. flavus	0	0	0	0.06
A. glaucus	0.04	0.10	0.03	0.39
A. niger	0	0	0	tr
A. ochraceus	0	0	tr	0
A. terreus	0	0	0	tr
A. versicolor	tr	0	tr	tr
Aureobasidium pullulans	tr	0	0	0
Botrytis cinerea	tr	0	0	0.02
Cephalosporium spp.	0.02	0	tr	0
Chaetomium spp.	0.07	0.07	0.03	0.17
Cladosporium cladosporioides	0.05	0.03	0.07	0.32
C. herbarum	0.11	0.04	0.01	0.03
C. macrocarpum	0.03	0.05	tr	0
Cladosporium **not** *ID*	0	0	0	0.03
Cochliobolus bicolor	0	0	0	tr
C. sativus	0	0.1	0.03	0.03
C. spicifera	0	0	tr	0
Coelomycetes	0.01	0.02	0.02	0.04
Curvularia lunata	tr	0	tr	tr
Drechslera biseptata	0	0	0	0.26
Epicoccum nigrum	0.05	0.03	0.06	0.57
Fusarium acuminatum	tr	0.03	0.03	0.07
F. avenaceum	0.14	tr	0.10	0.54
F. culmorum	0	0	0	tr
F. dimerum	0	0	tr	0
F. equiseti	0.01	0.01	0.03	0.04
F. graminearum	0	0	0.02	0.11
F. oxysporum	0	0	0	tr
F. poae	tr	0.01	0	0.01
F. semitectum	0	0	0	tr
F. sporotrichioides	0	0	0.03	0.14
F. tricinctum	0	0	0	0.01
Fusarium spp. **not** *ID*	0	tr	0	0.01
Gonatobotrys **spp.**	0.09	0.07	0.15	2.23
Leptosphaeria maculans **(avirulent)**	0.10	0.05	0.09	0.01
L. maculans **(virulent)**	0.01	0.15	0.05	0.18
Microascus longirostrus	0	0	tr	0
Mucor spp.	tr	0.01	0.01	0.08

(cont'd.)

Fungi	Alberta*	Saskatchewan*	Manitoba*	Ontario**
Myrothecium spp.	0.02	tr	0.01	0
Nigrospora oryzae	tr	0.02	0.05	0.01
N. sphaerica	0	0	0	0.01
Papulospora spp.	0	0.03	0.01	tr
Penicillium spp.	0.01	0.03	0.02	0.55
Phaeoramularia spp.	0.01	0.03	0	0
Plectosphaerella cucumerina	tr	tr	0.02	0.39
Preussia spp.	tr	0	0.01	0
Pseudomicrodochium spp.	0	0	tr	0
Pyrenophora teres	tr	0	0	0
Rhizoctonia solani	0	0.01	0.01	0.01
Sclerotinia sclerotiorum	tr	0	0.01	0.07
Scopulariopsis spp.	0	0	tr	0
Sphaeronaemella fimicola	0	0	0	0.13
Stemphylium herbarum	tr	0.01	tr	tr
S. vesicarium	0.12	0.07	0.16	1.38
Stemphylium not ID	0	0	0.01	0
Trichothecium roseum	0	0	0	tr
Ulocladium atrum	0.03	0.01	tr	0
Verticillium spp.	0	0.01	0.02	0.03
Yeast	0	tr	0	0.01

* Five-year average, 1989 to 1993.
** Three-year average, 1991 to 1993.
tr Trace (<0.01%).

Table 3. Maximum percentage of fungal infection of Canadian canola seed in any one sample between 1989 and 1993.

Fungi	Alberta*	Saskatchewan*	Manitoba*	Ontario**
Acremoniella atra	0	0	0.33	4.67
Acremonium spp.	0	0	0.33	0.33
Actinomycetes	0	0	0	0.33
Alternaria alternata	33.00	46.00	61.67	96.00
A. brassicae	24.33	8.67	11.33	6.00
A. raphani	13.67	4.33	8.00	1.67
Apiospora montagnei	2.67	1.33	2.67	11.33
Arthrinium phaeospermum	1.33	1.00	1.33	2.00
Arthrinium not ID	4.00	2.00	12.00	10.0
Ascomycetes	0	0	0.33	0.33
Aspergillus candidus	0	0	0	0.33
A. flavus	0	0	0	0.33
A. glaucus	0.67	3.67	2.00	9.00
A. niger	0	0	0	0.33
A. ochraceus	0	0	0.33	0
A. terreus	0	0	0	0.33
A. versicolor	0.33	0	0.33	0.67
Aureobasidium pullulans	0.33	0	0	0
Botrytis cinerea	0.33	0	0	0.67

(cont'd.)

Fungi	Alberta*	Saskatchewan*	Manitoba*	Ontario**
Cephalosporium spp.	0.67	0	0.33	0
Chaetomium spp.	0.67	1.00	0.67	11.00
Cladosporium cladosporioides	0.67	0.67	1.00	14.67
C. herbarum	1.00	1.00	0.33	0.67
C. macrocarpum	.67	1.00	0.33	0
Cladosporium not *ID*	0	0	0	2.00
Coelomycetes	0.33	0.67	0.33	1.00
Cochliobolus bicolor	0	0	0	0.33
C. sativus	0	0.33	0.33	1.33
C. spicifera	0	0	0.33	0
Curvularia lunata	0.33	0	0.33	0.67
Drechslera biseptata	0	0	0	22.67
Epicoccum nigrum	0.33	1.00	1.00	3.67
Fusarium acuminatum	0.17	1.00	0.67	1.00
F. avenaceum	2.00	0.33	2.33	17.33
F. culmorum	0	0	0	0.33
F. dimerum	0	0	0.33	0
F. equiseti	0.33	0.33	1.00	1.00
F. graminearum	0	0	0.67	1.33
F. oxysporum	0	0	0	0.33
F. poae	0.33	0.33	0	0.67
F. semitectum	0	0	0	0.33
F. sporotrichioides	0	0	1.00	2.00
F. tricinctum	0	0	0	0.33
Fusarium spp. not *ID*	0	0.33	0	0.33
Gonatobotrys spp.	1.33	1.00	4.00	24.00
Leptosphaeria maculans (avirulent)	1.00	0.67	1.67	0.67
L. maculans (virulent)	0.67	1.33	1.00	5.33
Microascus longirostrus	0	0	0.33	0
Mucor spp.	0.33	0.33	0.33	3.33
Myrothecium spp.	1.00	0.17	0.67	0
Nigrospora oryzae	0.33	0.33	0.33	1.33
N. sphaerica	0	0	0	0.33
Papulospora spp.	0	0.67	0.33	0.33
Penicillium spp.	0.33	0.67	0.67	41.33
Phaeoramularia spp.	0.33	0.33	0	0
Plectosphaerella cucumerina	0.33	0.33	1.00	18.00
Preussia spp.	0.33	0	0.33	0
Pseudomicrodochium spp.	0	0	0.33	0
Pyrenophora teres	0.17	0	0	0
Rhizoctonia solani	0	0.33	0.33	0.67
Sclerotinia sclerotiorum	0.33	0	0.33	1.33
Scopulariopsis spp.	0	0	0.33	0
Sphaeronaemella fimicola	0	0	0	11.33
Stemphylium herbarum	0.33	0.33	0.33	0.33
S. vesicarium	1.00	1.00	1.33	21.00
Stemphylium not *ID*	0	0	0.33	0
Trichotheciumroseum	0	0	0	0.33
Ulocladium atrum	0.33	0.33	0.33	0
Verticillium spp.	0	0.33	0.33	1.67
'feast	0	0.17	0	0.33

* Samples collected from 1989 to 1993.
** Samples collected from 1991 to 1993.

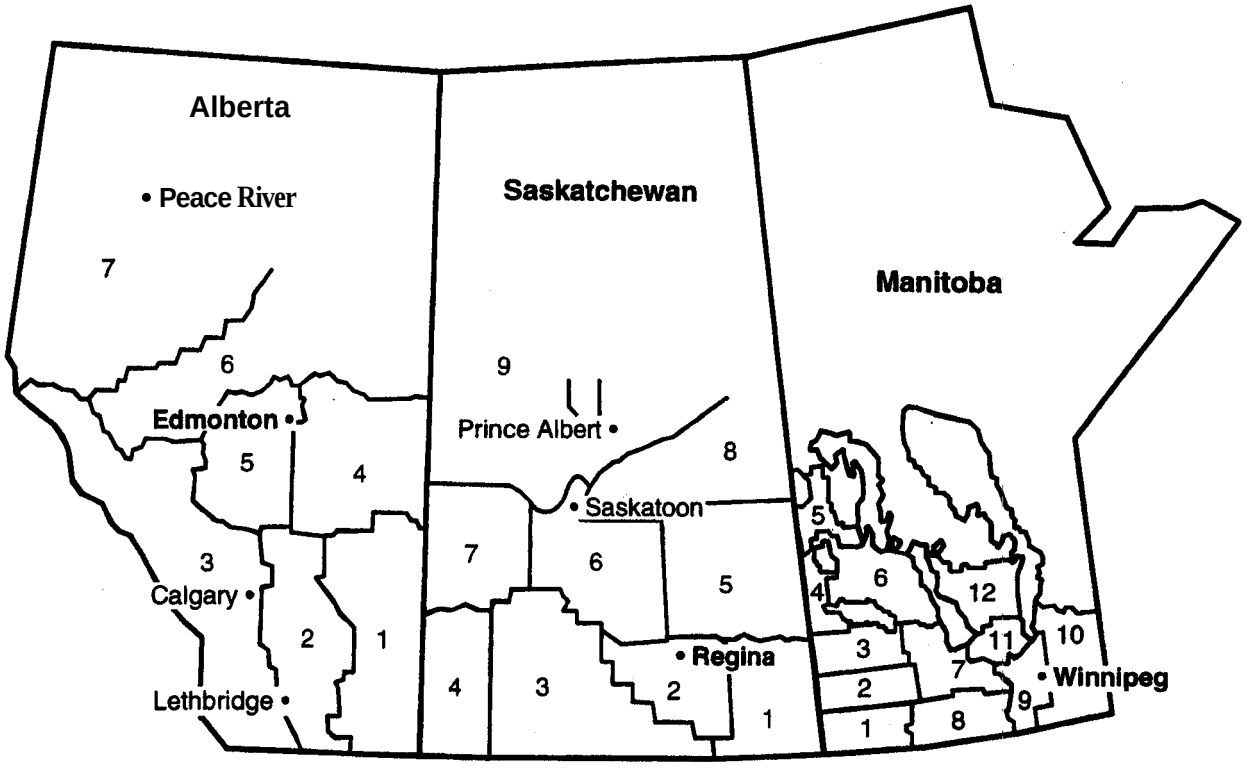

Fig. 1. Crop districts of western Canada.