

Mycoflora and condition of grains from overwintered fields in Manitoba, 1977-78¹

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Cereal and oilseed crops that remained in the field over winter in an area east of the Red River in Manitoba had a high proportion of wrinkled seeds and black non-germinable embryos when sampled during 21 April to 3 May 1978. The low germination was due to late fall and winter weather conditions and molds rather than to dormancy. Corn cobs were frequently damaged by voles. Crops examined were severely weathered and were later either burnt or ploughed under rather than fed to animals. The mycoflora of cereal seeds consisted mainly of preharvest fungi, particularly *Alternaria alternata*, *Cladosporium cladosporioides* predominated on corn. The main *Fusarium* spp. isolated were *F. tricinctum*, *F. poae*, *F. sporotrichioides*, *F. avenaceum*, *F. acuminatum* and *F. sambucinum*. There was a much higher frequency of seed-borne *Fusarium* spp. isolated from overwintered crops than from crops collected from an adjacent area the previous wet fall. Thus, 12 to 52% (mean 33%) of wheat seeds from 9 fields were contaminated with *Fusarium* spp. compared to 0 to 20% (mean 2.5%) of wheat seeds from 21 swathed fields sampled in the fall. Similar high levels of *Fusarium* spp. occurred in seed samples from 16 overwintered fields of barley, oats, corn and flax.

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A l'occasion d'un échantillonnage réalisé du 21 avril au 3 mai 1978, les céréales et les oléagineux laissés sur pied tout l'hiver dans une région située à l'est de la rivière Rouge ont révélé une proportion élevée de grains ratatinés et d'embryons noircis non germables. La chute des capacités de germination s'expliquerait davantage par les conditions météorologiques survenues en fin d'automne et durant l'hiver que par des phénomènes naturels de dormance. En maintes circonstances, les épis de maïs étaient endommagés par les campagnols. La microflore des graines consistait essentiellement en champignons déjà présents avant la récolte normale, notamment *Alternaria alternata* (Fr.) Keissler. *Cladosporium cladosporioides* (Fres.) de Vries a été l'espèce prépondérante sur le maïs. Les principales espèces de *Fusarium* isolées des cultures hivernées étaient *F. tricinctum* Corda (Sacc.), *F. poae* (Peck) Wollenw., *F. sporotrichioides* Sherb., *F. avenaceum* (Fr.) Sacc., *F. acuminatum* Ell. et Ev. et *F. sambucinum* Fuckel. On a relevé une fréquence beaucoup plus forte de *Fusarium* spp. sur les grains hivernés que sur les grains prélevés sur les cultures non récoltées examinées au cours de l'automne pluvieux de 1977. Ainsi, de 12 à 52% (moyenne 33%) des grains de blé prélevés sur 9 champs étaient porteurs de *Fusarium*, contre seulement de 0 à 20% (moyenne 2.5%) pour les grains de blé obtenus de 21 champs andainés l'automne précédent. Des fréquences élevées de *Fusarium* ont été également observées chez des échantillons de grains hivernés provenant de 16 champs d'orge, d'avoine, de maïs et de lin.

It is not unusual for crops to be left in the field in Manitoba over winter due to an early snowfall. This happens in areas of up to 100 x 100 km, approximately once every 4 years and in larger areas about once every 8 years (personal communication, Hayden Tolton, Manager, Manitoba Crop Insurance Corporation). Other factors leading to unharvested overwintered crops include date of seeding, geographical location, and the soil and climatological conditions during the growing season and at harvest. The areas most likely to experience snow-covered unharvested crops are the Russell-Grandview-Roblin-Swan River and Interlake regions and the area east of the Red River (Fig. 1) although, if

lasting snow comes very early, most of the crop-growing area of the province can be affected. This last happened in 1959 when all crop land 300 m above sea level west of a line through The Pas, MacGregor, and the United States border was covered with deep snow in early October.

Overwintered crops usually have a severely weathered appearance after exposure above or lying under the snow and subsequently lying in water for long periods, as might occur in the poorly drained soils of the Interlake region and east of the Red River. They are feed rather than seed material. However, farmers who feed such material to animals such as chickens, may encounter sickness in their stock if overwintering conditions are conducive to development of mycotoxigenic fungi (7). Snow-covered crops on the medium- to well-drained soils in the upland areas of northwest Manitoba including Russell, Grandview, Roblin, and Swan River usually overwinter in better condition. Cereals harvested in spring in those areas suffer only slight weight loss, show slight weathering but often grade dry and will meet commercial grade requirements.

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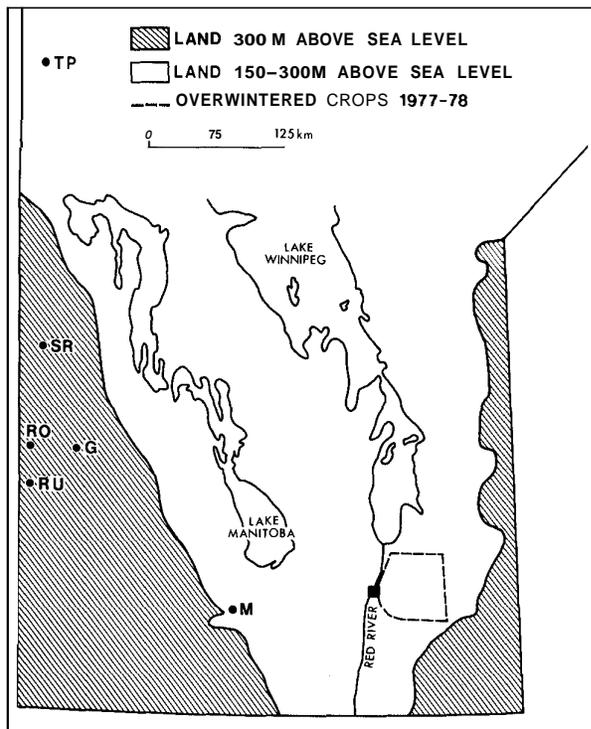


Fig. 1. Relief map of Manitoba showing locations with a history of overwintered crops. G = Grandview, M = MacGregor, Ro = Roblin, Ru = Russell, SR = Swan River, and TP = The Pas.

The normal harvest months of August and September 1977 in Manitoba were particularly wet, delaying harvesting operations until October and November. In mid November, a heavy snowfall occurred east of the Red River in an area of poorly drained soils. Crops of wheat, oats, barley, flax, and corn were covered with snow. In view of the paucity of information on overwintered crops under Manitoba conditions, the salient characteristics of these crops were documented the following spring.

Materials and methods

Collection of samples

Samples were taken from 25 fields in a 60 x 80 km area bounded by Winnipeg, Libau, Lac du Bonnet, Hadashville, and Blumenort, Manitoba (Fig. 2), during 21 April to 3 May 1978. The fields were located through reports received from farmers by the Manitoba Crop Insurance Corporation and included nine of wheat, five of oats, two of barley, five of corn and four of flax. Approximately the same number of standing and swathed fields were sampled. About 500 cereal stems with attached heads, 100 corn cobs or several large armfuls of flax were obtained at random after walking 150 paces into the fields. The crop appearance, vole damage, soil conditions and the occurrence of snow or standing water were noted.

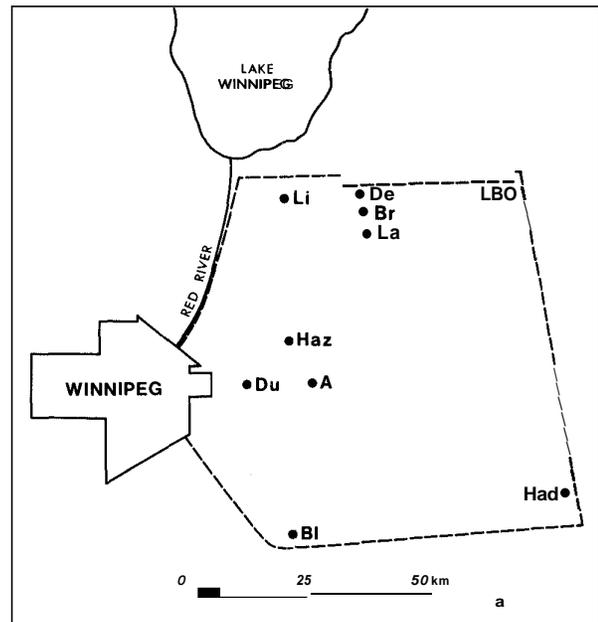


Fig. 2. Map of the east Red River region of Manitoba showing the area of overwintered crops under investigation during spring 1978. A = Anola, BI = Blumenort, Br = Brokenhead, De = Dencross, Du = Dugald, Had = Hadashville, Haz = Hazelwood, La = Ladywood, LB = Lac du Bonnet, and Li = Libau.

Processing of samples

A representative selection was made from each field sample on arrival at the laboratory and notes made on the extent of shattering of seeds and heads, brittleness of stems, visible fungi on the seed surface and on the glumes, lemmas, paleas or stems, and on the extent of vole damage, blackening of immature exposed grains on corn cobs, and splitting of bolls of flax. Samples were air dried before the seeds could be removed by threshing or hand rubbing and notes made on extent of embryo damage, seed shrinkage and wrinkling, and peeling of flax seeds. Seed samples were then examined by several physical and biological techniques to assess condition. The germinability of each sample was determined by standard seed testing methods (4); additional samples of wheat, oats, and barley were moistened with 0.2% KNO₃ solution, prechilled for 3 to 4 days at 10°C and were then germinated at 20°C. Both KNO₃ solution and prechilling are aids used for breaking seed dormancy. Further samples of each lot were examined by the tetrazolium test (1) which also permits separation into viable and non viable fractions.

Filter paper soaked in water (FP), filter paper soaked in 7.5% NaCl solution (SFP) (10), and potato dextrose agar (PDA) were used to determine types of fungi present on the seeds. One hundred seeds were placed on FP and PDA dishes and 50 on SFP dishes, with 25 cereal or flax seeds, or 10 corn seeds per dish. Five ml of solution were added per dish for corn and 4.5 ml for

cereal and flax seeds. The FP, SFP and PDA dishes were incubated and the fungi identified following the methods described earlier (10).

Results

Field and crop characteristics

Snow was 0 to 20 cm deep at sampling in two corn fields at Blumenort (Fig. 3). The remainder of the fields were sampled soon after the snow had melted. The soil was often very wet, with water sometimes lying on the surface. Most uncut cereals were badly weathered and severely lodged (Fig. 4). Standing uncut corn and flax stems often remained upright. The height of the lowest corn cobs above the ground varied from 10 to 60 cm. Damage to corn cobs by meadow voles [*Microtus pennsylvanicus* (Ord)] and prairie voles [*M. ochrogaster* (Wagner)] (2), was severe in fields with cobs close to the ground. The voles were abundant under cereal swaths and their nests and runways were in all the sampled fields.

Cereal stems from overwintered fields were usually brittle. Head breakage and kernel shattering was most common in oats and barley. Dried roots, 1 to 3 cm long, were observed emerging from heads of wheat from

swathed fields 4, 11, and 13. The tips of the corn cobs extending beneath the cob sheaths were often blackened, probably the result of immature kernels becoming frost damaged then invaded by fungi. The effect varied from field to field; blackening on cobs from fields 1, 16, 20, 24 and 25 extended on average 6, 12, 2.5, 2.5 and 0 cm from the tip, respectively. The extent of blackening could have been related to crop maturity. Vole damage to cobs was very severe in field 16 with many gnawed seeds, severe in fields 1 and 24, very slight in field 25 with only a few tooth marks on the cobs, and absent in field 20. The flax stem fibres varied in strength; those from fields 12 and 19 were very brittle whereas those from fields 6 and 23 were exceedingly strong.

Most wheat seeds were bleached and had gray or darkened brush ends; frequently frost wrinkles (11) were also visible. Low amounts of sprouted seeds were present in samples from fields 7 and 13 and of shrunken kernels in field 14. Most barley seeds examined had loose hulls and the dehulled seeds were dark and wrinkled. A high proportion of oat seeds had medium to heavy discoloration, especially at the apices; embryos of samples from fields 21 and 22 were black and the dorsal part of the seed including the embryo was mummified. Corn seeds were often wrinkled with wrinkling most



Fig. 3. Overwintered corn (field 24) north of Blumenort, Manitoba on 21 April 1978, showing broken plants in deep snow.



Fig. 4. Overwintered wheat (field 2) north of Anola, Manitoba on 25 April 1978, showing lodging after snow melt

prevalent in samples from fields 1 and 16; moderate amounts of small shrivelled seeds were present in samples from fields 1 and 20, discolored seeds from fields 1 and 16, and dark embryos from fields 1, 16, 20, and 24. Most flax seeds were discolored and exhibited probable frost damage consisting of blistered, cracked and split seeds with the lining of the pods often adhering to the seeds.

Germination

Seed germination was generally low (Table 1). Normal germination varied from 1 to 47% of non-prechilled seeds from the nine sampled wheat fields. Corresponding figures for oats were 3 to 13% of seeds with normal germination, for barley 7%, for corn 3 to 24% and for flax 16 to 37% of seeds. Similar figures were obtained in the tetrazolium test with 0 to 32% of wheat seeds germinable, 4 to 14% of oat seeds, 7 to 10% of barley seeds, 4 to 25% of corn seeds and 19 to 45% of flax seeds. Dormancy, as shown by the presence of ungerminated seeds and increased normal germination after prechilling, was demonstrated only in seeds from field 18 of barley. It is likely, therefore, that the low figures obtained for normal seed germination in most other lots are the result of severe late fall and winter weather conditions and molds and are not due to dormancy.

Microflora

Preharvest fungi were visible on the surface of cereal heads particularly on the paleas and lemmas of oats. Spores of *Alternaria alternata* (Fr.) Keissler and of the imperfect state of *Cochliobolus sativus* (Ito and Kurib.) Drechsl. ex Dastur were common on cereal seeds, and spores of *Epicoccum purpurascens* Ehrenb. ex Schlecht and *Ulocladium atrum* Preuss on dehulled oat seed. *Cladosporium cladosporioides* (Fres.) de Vries commonly occurred on the stems, cob sheaths, and kernels of corn. Conidial heads of the *Aspergillus glaucus* group and of *Monilia sitophila* (Montagne) Sacc. were present between the kernels of cobs from field 24. The bolls and seeds of flax were free of obvious fungal contamination but several fungi, including *Pleospora herbarum* (Pers. ex Fr.) Rabenh., *Phoma* spp., and rust [*Melampsora lini* (Ehrenb.) Lev.] urediospores occasionally were observed on the stems.

Stem rust (*Puccinia graminis* Pers. f. sp. *avenae* Eriks. and E. Henn) urediospores and teliospores were often seen on the dehulled seeds, inside the glumes and on the stem and leaf sheaths of oats. The prevalence of this fungus reflects the widespread nature of an epidemic of the disease which resulted in loss of much of the late oat crop during 1977. Affected crops had a weathered appearance caused by premature senescence of the plants and a grey-white discoloration of the heads.

Table 1. Germinability of seeds from overwintered fields.

Crop	Field No	Location	Standing or Swathed (St) (Sw)	Not prechilled				Prechilled				Tetrazolium test		
				% Germination		% Dead	% Fresh ungerminated (dormant)	% Germination		% Dead	% Fresh ungerminated (dormant)	% Germinable	% Abnormal	% Non-germinable
				Normal	Abnormal			Normal	Abnormal					
Wheat	2	Anola	sw	2	1	97		2	1	97		2	2	95
	4	Ladywood	sw	24	6	69	1	13	6	80	1	13	13	74
	7	Dencross	St	17	2	81		11	3	85	1	12	8	80
	8	Brokenhead	St	29		71		21	4	75		11	13	76
	11	Libau	sw	22	3	74	1	22	7	71		12	8	80
	13	Libau	sw	10		90		10	1	89		3	5	92
	14	Libau	sw	9		91		9	1	90		8	3	89
	15	Richtot	sw	1		99		1		99			1	99
	17	Oakbank	sw	47	8	45		48	14	38		32	30	38
Oats	3	Ladywood	St	5		95		6	1	93		5	3	92
	5	Ladywood	Sw	13	2	85		11		89		11	6	83
	9	Dencross	St	11	1	88		19	3	78		14	5	81
	21	Ladywood	sw	3	1	96		6	2	92		4	6	90
	22	Ladywood	St	9	4	87		6	1	93		4	4	92
Barley	10	Libau	St	7		93		13	5	82		10	14	76
	18	Hazelridge	St	7	2	80	11	19	2	79		7	11	82
Corn	1	Anola	St	4		96						6	16	78
	16	Dugald	St	21	5	74						25	24	51
	20	Anola	St	3	3	94						4	63	33
	24	Blumenort	St	24	2	74						20	25	55
	25	Blumenort	St	12	5	83						18	62	20
Flax	6	Ladywood	sw	37	4	57	2					33	25	42
	12	Libau	sw	29	3	68						45	21	34
	19	Dugald	St	16	4	80						19	10	71
	23	Ladywood	Sw	35	8	37						43	24	33

The frequency of occurrence of seed microfloral components after plating seeds on various media is given in Table 2. Preharvest fungi predominated on wheat, oat, and barley seeds and included *Alternaria alternata*, *Cochliobolus sativus*, *Fusarium* spp., including *F. tricinctum* (Corda) Sacc., *F. poae* (Peck) Wollenw., *F. sporotrichioides* Sherb., *F. avenaceum* (Fr.) Sacc., *F. acuminatum* Ell. & Ev., *F. sambucinum* Fuckel, and *Epicoccum purpurascens*. *Alternaria*, *Fusarium*, *Cochliobolus*, and *Epicoccum* were present on an average of 87%, 35%, 12%, and 2% of the sampled cereal seeds respectively. The preharvest fungus *Cladosporium cladosporioides* and all harvest fungi, including *Gonatotryps simplex* Corda and *Cephalosporium acremonium* Corda, occurred at low levels. Trace amounts of *Acremonia atra* Sacc., *Papulospora* sp., and *Trichoderma viride* Pers. ex S.F. Gray also occurred. Myxomycetes and nematodes were seen occasionally. Postharvest fungi, including *Mucor*, *Penicillium*, and *Rhizopus*, occurred in trace amounts.

Preharvest fungi also predominated on corn but the frequency of occurrence of the species differed markedly from that on cereals. *Cladosporium cladosporioides* was the dominant fungus followed by *A. alternata*, *Fusarium* spp., *E. purpurascens*, and *Nigrospora oryzae* (Berk. and Br.) Petch. *Cladosporium*, *Alternaria*, *Fusarium*, *Epicoccum*, and *Nigrospora* were present on an average of 81%, 51%, 35%, 9%, and 7% of the sampled corn

seeds, respectively. Harvest fungi, including *Cephalosporium*, *Gonatotryps*, *Paecilomyces bacillisporus* Onions and Barron, and *Papulospora* sp. occurred in trace amounts. *Penicillium* spp. and *Mucor* sp. were commonly identified from corn but *Aspergillus* spp. were rarely isolated.

The preharvest fungi on flax seed consisted mainly of *Alternaria* and *Fusarium* together with low amounts of *Cladosporium*, *Cochliobolus*, and *Epicoccum*. *Alternaria* and *Fusarium* were present on an average of 78% and 34% of the flax seeds sampled, respectively. Harvest and postharvest fungi were negligible on flax seed.

Discussion

The poor condition of seeds from overwintered fields in the region east of the Red River in the spring of 1978 was determined partly by the status of the crops the previous fall and partly by the ecological factors which affected the crops during winter and early spring.

Many Manitoba crops were in swath for prolonged periods during the very wet summer and early fall of 1977 and were in contact with the moist soil, resulting in sprouting, proliferation of preharvest fungi, and even development of slime molds or myxomycetes. Myxomycetes isolated from overwintered crops were considered to be remnants of high levels the previous fall. Similarly, splitting of flax bolls occurred the previous wet fall, not

Table 2. Mycoflora of cereal and oilseeds from overwintered fields.

Crop	Field No	% Frequency of Occurrence of microfloral components+ on seeds																																	
		FP (filter paper with added water)														SFP (filter paper with added NaCl solution)							PDA (potato dextrose agar)												
		Al	As ca	As fl	Ce	Cl	Co	Ep	Fu	Go	Mu	Ni	Pe	Rh	My	Al	As ca	As gl	Ce	Cl	Fu	Ni	Pe	Al	Cl	Co	Ed	Fu	Go	Mu	Ni	Pe	Rh		
Wheat	2	100				4	1	1	6	7					62											95	6	3	4			1			
	4	100				24	2	23	8						70											100	39	13	29	3					
	7	87		9		13		39	1				1		68			2		6						82	27		2	12			4		
	8	80				18	1	22	3						46											68	55	18	10	1					
	11	53		1		9		52							48											61	27	17	55				7		
	13	79		2		6		45	8						62					10						100	82	29	43	7					
	14	75		2		11	1	46	4						66											100	64	17	48	14					
	15	84		1		4	3	3	8	6					56											100	86	6	29	13					
	17	89				26	2	12	2						34											100	87	10	18	10			2	9	
Oats	3	100				5	2	19	25						90											96	20	50	46	11					
	5	100			1	0	8	9	7	5					94					2						100	52	39	23	8					
	9	91		2		9	6	5	4	4				2	72					6						100	2	49	28	41	1				
	21	94		7		1		68			7				100					12			2			100	63	48	45	37					
	22	98		9	3	16	3	58	13			3		2	100											100	92	42	25	33					
Barley	10	75			1	26		46	5						56											95	84	19	24					15	
	18	87		2		11	2	20	7						100											90	60	12	24	21				39	
Corn	1	92	1		90		11	18		1	3	2	1		6			4	86		10	4			99	100		4	88			42	2	5	10
	16	56			63		1	34	3	12	2	45	3		6				18	8	2	96	100	100		100	100	1	72		100	100	100	100	
	20	42			90	2	10	39	2		19	1							80		6	16	100	100		100	15	23		32	10	1	11		
	24	49			78		28	33			9	3			4		2	8	84	6	12	40	100	100	100	100	100	14	40		30	2	1		
	25	17			87		5	53	7	7					2	2	8	4	8	2	12	4	56	100	100		100	7	29		37	3	10		
Flax	6	89	1	1				46	4						88											4	100		6	4	2			6	20
	12	68		1				34	8						88											100	17	6	15	7	2			10	
	19	62						28	29						70											92	50	65						1	
	23	92						28	7						62		2									100	1	10	7					6	10

+ Al = *Alternaria alternata* As ca = *Aspergillus candidus* Link ex Fr As fl = *Aspergillus flavus* Link ex Fr As gl = *Aspergillus glaucus* group Ce = *Cephalosporium acremonium* Cl = *Cladosporium cladosporioides* Co = *Cochliobolus sativus* imperfect state Ep = *Epicoccum purpurascens* Fu = *Fusarium* spp including *F. tricinctum* (Corda) Sacc *F. poae* (Peck) Wollenw *F. sporotrichioides* Sherb *F. avenaceum* (Fr) Sacc *F. sacchari* Butler (W Gams) *F. oxysporum* Schlecht *F. acuminatum* Ell & Ev *F. sambucinum* Fuckel and *F. sulphureum* Schlechtendahl Go = *Gonatotryps simplex* Mu = *Mucor* spp Ni = *Nigrospora oryzae*, Pe = *Penicillium* spp mainly *P. verrucosum* var *cyclopium* Rh = *Rhizopus arrhizus* Fischer and My = myxomycete spp

during the winter or wet spring. Splitting of the bolls, which exposes the seeds to weathering, was reported during the wet harvest of 1941 (Winnipeg Free Press, September 26) and was also observed in samples of the 1977 crop obtained before the arrival of the permanent snow. The adverse weather conditions in the fall of 1977 severely affected seed weight of flax, with many Manitoba growers reporting low bushel weight (G. Platford, Manitoba Department of Agriculture, personal communication). The conditions favored development of pasmo disease, causal organism *Septoria linicola* (Speg.) Garov., which bands the stems and affects the pedicels of flax. Although the problem was widespread, we did not observe spores of this fungus on flax stems from sampled fields.

Certain features exhibited by the overwintered crops can be attributed to winter conditions. Many of the kernels showed surface wrinkling resembling frost damage (11). Whitcomb and Johnson (12), in a study of overwintered wheat in stooks under snow, also observed that kernels had a crinkled appearance. They observed more crinkled kernels in wheats which had been subjected to alternate freezing and thawing than in wheats subjected to continuous cold, the number of crinkled kernels increasing as the exposure progressed from December 18 to April 16. Also, many corn cobs were damaged by meadow voles the breeding and survival of which was favored by ample food from unharvested crops and a protective blanket of snow. Breeding of the meadow vole

normally terminates in October but may continue until February under ideal conditions (2). Judging by the prevalence of voles and signs of their activity, ideal breeding conditions occurred in some of the fields in the winter of 1978.

Germination of seed samples, collected the previous fall from 21 swathed and standing wheat fields in an adjacent 60 x 60 km area west of the Red River, averaged only 10% (9). The low germination was probably due to postharvest dormancy accentuated by the cool moist conditions. In the present work, overwintered seeds were either of low germination (Table 1) or dead or ungerminable with mummified black embryos, as occurred in oats and barley. Many of the seeds probably started to germinate the previous fall but were killed by the severe cold or by preharvest fungi, e.g. *Fusarium* spp. and *Cochliobolus sativus*, which can continue to grow at seed moisture contents above 20-21% (5). Strains of these fungi are known to reduce seed germination (6). In our survey, no correlations were evident between the number of dead seeds in a sample (Table 1), the occurrence of a particular preharvest fungus (Table 2), the seed or soil moisture, or other field conditions. The microflora of overwintered cereal seeds was similar to that of seeds collected from unharvested fields in an adjacent area the previous wet fall (9) except for a much higher frequency of *Fusarium* spp., particularly *F. poae* and *F. sporotrichioides*, in overwintered samples. Thus, 12 to 52% (mean 33%) of wheat seeds

from 9 fields were contaminated with *Fusarium* spp. compared to 0 to 20% (mean 2.5%) of wheat seeds from 21 swathed fields sampled in the fall. Similar high levels (mean 35%) of *Fusarium* spp. occurred in seed samples from 16 overwintered fields of barley, oats, corn and flax. *Fusarium poae* and *F. sporotrichoides* were shown by Joffe (7) to cause toxicoses in humans in Russia after ingestion of overwintered cereal grains. Joffe further showed that only grain in contact with the soil during the winter-spring period developed toxicity and that toxin formation was favored by deep snow cover and relatively high temperatures which favored repeated thawing and freezing (8). In the affected area of Manitoba, heavy snowfalls of up to 42 cm occurred during the 15th-20th November at Beausejour and

other points (3), temperatures were below normal from late fall to early spring and the ground remained covered with snow until April. Presently, we do not know whether conditions in the sampled overwintered fields were conducive to production of *Fusarium* toxins. No cases of animal toxicoses were reported in Manitoba during the early summer of 1978 probably because most of the affected crops, largely insured, were either burnt or ploughed under rather than fed to animals. What we have ascertained is that more *Fusarium* species, strains of which are known to be toxigenic, developed on seed in spring than in the previous fall. Extreme caution must therefore be exercised before feeding overwintered grains to livestock and poultry.

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