Prevalence of some seedborne fungi on soft white winter wheat seed from Ontario, Canada

R.M. Clear and S.K. Patrick[1]

To determine the mycoflora of grain samples of white winter wheat *(Triticum aestivum),* 435 samples collected over three years were examined for the presence of fungi by plating surface disinfected seeds onto potato dextrose agar. At least 59 species representing 35 fungal genera were recovered from seed. *Alternaria alternata, Epicoccum nigrum,* and species of *Arthrinium, Aspergillus, Cladosporium, Drechslera* and *Nigrospora* infected more than 1% of the seeds every year. *Bipolaris sorokiniana, Drechslera tritici-repentis, Fusarium graminearum, F. poae,* and *Septoria nodorum* infected more than 1% of the seeds in one or two years. Yearly differences in the quantity and time of precipitation and the frequency of a number of fungi such as the pathogens *B. sorokiniana, D. tritici-repentis,* and *S. nodorum,* including a 100 fold increase in the frequency of *F. graminearum* between 1988 and 1989, were recorded. Whereas forty years ago *B. sorokiniana* was the most common pathogen recovered from Ontario wheat seed, *F. graminearum* was the most frequently detected pathogen in this study.

Can. Plant Dis. Surv. 73:2, 143-149, 1993.

Afin de determiner la mycoflore des echantillons de graines du ble tendre *(Triticum aestivum),* 435 echantillons recoltes durant trois annees ont ete examines pour verifier la presence de champignons en appliquant des semences sur des surfaces desinfectees d'agar compose de dextrose de pomme de terre. Au moins 59 especes appartenant a 35 genres de champignons ont ete récupérées de ces semences. *Alternaria alternata, Epicoccum nigrum* et des especes de *Arthrinium, Aspergillus, Cladosporium, Drechslera* et *Nigrospora* ont infecte plus de 1% des semences a chaque année. *Bipolaris sorokiniana, Drechslera tritici-repentis, Fusarium graminearum, F. poae* et *Septoria nodorum* ont infecte plus de 1% des semences en une ou deux années. Des differences annuelles ont ete enregistrées pour les quantites et les temps de precipitations et pour la frequence du nombre de champignons comme les pathogenes *B. sorokiniana, D. tritici-repentis* et *S. nodorum,* incluant une augmentation centuplee de la fréquence de *F. graminearum* durant les annees 1988 et 1989. Il y a quarante ans, *B. sorokiniana* était le pathogene le plus communement retrouve dans les semences de ble en Ontario, alors que *F. graminearum* a été le pathogene le plus frequemment detecte lors de cette etude.

Introduction

Fungi can be recovered from surface disinfected wheat seed (*Triticum aestivum L.*), even though their presence in the seed is usually not evident until the seeds have been placed in an environment conducive to prolific fungal growth. Extensive fungal development on grain may reduce the value because of seed discolouration, chemical changes, loss of dry matter, objectionable odours, and mycotoxin accumulation (Christensen and Kaufmann 1974). If infested grain is used as seed, the seedborne diseases can reduce yield and the grain will be a source of inoculum. Grain buyers sometimes set tolerance limits for specific organisms which, if exceeded, will result in either rejection of the shipment or demands for a price reduction.

A knowledge of the mycoflora and their frequency on particular types of grain provides regulatory agencies with a basis to assess the risk associated with undesirable organisms and their metabolites. Since the last surveys of the mycoflora of Ontario grown wheat seed in 1942 (Greaney and Machacek) and 1951 (Machacek *et al.*), the introduction of new varieties and cropping practices may have changed the frequency of various seedborne fungi. The purpose of this study was to record the fungi associated with soft white winter wheat seed grown in Ontario in recent years and to compare these results with those obtained 40 to 50 years ago.

Materials and methods

Ninety-nine 1 kg weekly composite samples and vessel loading samples of soft white winter wheat from Ontario were collected from terminal elevators in 1988, 259 in 1989 and 77 in 1990. Samples were collected by inspectors of the Grain Inspection Division of the Canadian Grain Commission and sent to the Grain Research Laboratory where they were documented, mixed, subsampled, and then

[1] Contribution No. 698 of the Canadian Grain Commission, Grain Research Laboratory, 1404-303 Main Street, Winnipeg, Manitoba, Canada R3C 3G8.
Accepted for publication July 19, 1993.

stored at -15°C for up to 4 months. For mycological examination 100 seeds from each subsample were surface disinfected for 1 minute in a solution of 0.3% sodium hypochlorite then air dried in a laminar flow hood. The dry seeds were plated onto potato dextrose agar (10 seeds per plate) and incubated for 5 days on a 12 hr, 28°C light and a 12 hr 22°C dark cycle. Illumination was by a 4:1 mixture of fluorescent and long-wave ultraviolet lamps at 48 cm distance above the petri plates. The percentage of seeds in a sample which were infected by an organism and the average per year were recorded. Moisture and temperature conditions during the growing seasons were obtained from 11 Environment Canada weather stations within the white winter wheat growing areas of Ontario. The daily values were combined to obtain monthly averages for these environmental factors.

Results

During this 3 year study, 59 species representing 35 fungal genera were recovered from the grain samples (Table 1). Every year more than 1% of the seeds were infected by species from six genera, *Alternaria* Nees ex Fr. (represented solely by *A. alternata* (Fr.) Keissler), *Arthrinium* Kunze ex Fr. (primarily the *Arthrinium* state of *Apiospora montagnei* Sacc., but also including *Arthrinium phaeospermum* (Corda) M.B. Ellis), *Aspergillus* Mich. ex Fr. (primarily *A. glaucus* group species), *Cladosporium* Link ex Fr. (primarily *C. cladosporioides* (Fresen.) de vries), *Drechslera* Ito, *Epicoccum* Link ex Schlecht. (represented solely by *E. nigrum* Link), and *Nigrospora* Zimmermann (primarily *N. oryzae* (Berk. & Br.) Petch). More than 1% of the seeds in one or two of the test years were infected with *Bipolaris sorokiniana* (Sacc.) Shoemaker, *D. tritici-repentis* (Died.) Shoemaker, *Fusarium graminearum* Schwabe, *F. poae* (Peck) Wollenw., and *Septoria nodorum* (Berk.) Berk).

Maximum levels of seed infection found in the samples ranged from 98% for *A. alternata* to 1% for many other fungi recorded (Table 2). Only *A. alternata* was found in every sample tested.

A lower overall incidence of infection was recorded in 1988 than in 1989 or 1990, but not all fungi were found less often in 1988. Precipitation during the growing season prior to July, 1988 was considerably less than that during 1989 and 1990 (Table 3). The growing conditions of 1989 appeared to be especially suited to the commercially important pathogens *F. graminearum* and *B. sorokiniana.* Two other commercially important pathogens, *D. trifici-repentis* (causal agent of tan spot) and *S. nodorum* (causal agent of glume blotch), were observed most often during the moister growing seasons of 1989 and 1990. *Fusarium graminearum* had the greatest yearly fluctuation in infection levels, ranging from 0.08% in 1988 to 11.89% in 1989, over a 100 fold increase.

Discussion

During this three year study important fungal pathogens were isolated from the soft white winter wheat produced in Ontario. The diseases on the grain were similar to those recorded for seed in 1951 by Machacek *et al.* With the exception of species of *Pullularia Berk.,* seven of the eight most common fungal genera reported by Machacek *et al.* (1951) were among the most common ones in this study. In the present study *Arthrinium* spp. and *D. tritici-repentis* were frequently observed on seed. These two species were not common among the samples examined by Machacek *et al.* (1951). Species of *Arthrinium,* previously called *fapularia* Fr., were isolated at just above trace amounts by Machacek *et a/.* (1951), and *D. tritici-repentis* was not mentioned. During 1988-1990, *Nigrospora oryzae* was the dominant *Nigrospora* species isolated from Ontario winter wheat seed. Although Machacek *et al.* (1951) identified *N. sphaerica* (Sacc.) Mason as the only *Nigrospora* species observed, their spore size measurement of 15µ suggests that it most likely was *N. oryzae.*

Alfernaria species have been isolated from wheat seed in different regions of Canada (Greaney and Machacek 1942; Machacek *et al.* 1951), and their growth within wheat seed can cause the discolouratians known as blackpoint and smudge. However, those species along with *Arthrinium* spp., *Cladosporium* spp., *Epicoccum nigrum, F. poae,* and *N. oryzae,* appear to have a minimal effect on the health of wheat seed (Malone and Muskett 1964; Zillinsky 1983).

Aspergillus glaucus group species, *B. sorokiniana, D. tritici-repentis, F. graminearum,* and *S. nodorum* are all reported to affect seed health and occasionally seed appearance (Martens *et al.* 1984; Thorpe 1958; Valder and Shaw 1953). Machacek et al. (1951) and Greaney and Machacek (1942) found *B. sorokiniana* to be the most common pathogen recovered from wheat seed, and Machacek *et al.* (1951) reported yearly averages of seed infection to range from <0.1 to 12.0% of Ontario wheat. The results in this study show that *F. graminsarum* was the most common pathogen recovered from wheat seed. Although the monthly precipitation averages for both May and June of 1989 and 1990 (Table 3) were similar, the frequency of *F. graminearum* in 1989 was ten times that of 1990 (Table 1). It seems likely that the conditions at time of anthesis, which are critical for both the infection by *F. graminearum* (Sutton 1982) and the production of tombstone kernels (Atanasoff 1920), were more suitable for infection in 1989 than 1990. The observation that tombstone kernels were an important degrading factor in 1989 but not 1990 (Anonymous 1989, 1990) is consistent with these results herein. The abundance of *B. sorokiniana* in 1989 may also be due to epidemiological considerations similar to those which favoured *F. graminearum,* as both fungi were several times more common on the seed in 1989. Greaney and Machacek (1946) reported that the amount of

rain during the growing season was the most important factor influencing the epidemiology of *B. sorokiniana.* However, Jorgensen (1974) reported temperature after sowing and not the frequency of moisture influenced the incidence of *B. sorokiniana* on barley seed.

The frequency of *F. graminearum* is important since it is a causal agent of fusarium head blight as well as root and crown rot of cereals (Martens *et al.* 1984). It also lowers the value of the crop due to the production of the degrading factor known as tombstone kernels and the fungus also produces mycotoxins such as deoxynivalenol (Sutton 1982). Previously, *F. graminearum* was seldom isolated from Ontario wheat seed (Gordon 1952), and it was not among the four predominant species isolated from cereal seed (Greaney and Machacek 1942), even though almost all their *fusarium* isolates were from eastern Canada. This observation of increased recovery of *F. graminearum* from seed compared with 40 years ago is supported by recent surveys of Ontario wheat seed for *Fusarium* species by Duthie *et al.* (1986) and Clear and Patrick (1990). They found *F. graminearum* to be the most or second most common *Fusarium* species infecting soft white winter wheat seed grown in Ontario. The changes in the observed frequency of this pathogen may result from the same influences which resulted in several epidemics of fusarium head blight caused by *F. graminearum* since 1980.

Similarities in infection levels between years, such as for *S. nodorum* and *D. trifici-repenfis* in 1989 and 1990, may be due to comparable weather conditions. Wet periods at heading favour seed infection by S. *nodorum* (Shipton *et al.* 1971), and this, as well as the reported wet harvest conditions of 1989 and 1990 (Anonymous 1989, 1990), may have been factors in the frequency of S. *nodorum, D. tritici-repentis* and ***E.*** *nigrum.*

Aspergillus glaucus group species were the only storage fungi commonly isolated in this study while much less common were members of the *A. flavus* group species. These two group species were the ones most often isolated by Machacek *et al.* (1951), and reflect storage conditions prior to sampling. Fewer seeds infected by the Aspergilli were recorded by Machacek *et a/.* (1951), possibly because the samples they tested were destined to be used as seed and therefore may have been handled more carefully than grain. It is interesting that the highest observed incidence of the *A. glaucus* group species was in 1988, the year with the driest growing conditions. The higher incidence of this group and the higher bacterial levels observed during 1988 may have resulted from less overgrowth by other fungi masking their presence. Recovery of *A. glaucus* would likely have been higher if a media with a more optimal potential for their isolation had been used to culture the seeds. However, the scarcity of less xerophilic *Aspergillus* species shows the grain was still in good condition at time of sampling.

Although Machacek *et al.* (1951) used potato sucrose agar and both Greaney and Machacek (1942) and Machacek *et al.* (1951) used an ethyl alcohol-mercuric bichloride solution for surface disinfection, it seems quite likely that these earlier studies and the present one provide a good estimate of the pathogens prevalent in soft white winter wheat seed over the survey periods. Even with some differences in methodology it still appears that the procedures used then and now would yield valuable data on the frequencies of seedborne fungi in this crop.

This study presents the principle species infecting soft white winter wheat seed produced in Ontario and shows some of the yearly and sample variation in infection levels that can occur over several survey years. The most abundant pathogenic species on the wheat seed appears to be *F. graminearum,* causal agent of fusarium head blight as well as diseases of the roots and crown. This pathogen appeared to be uncommon in Ontario wheat forty years ago, when *B. sorokiniana* was the most frequently identified seedborne pathogen.

Literature cited

1. Anonymous. 1989. Eastern Standards Reports. Canadian Grain Commission, Winnipeg, Manitoba.
2. Anonymous. 1990. Eastern Standards Reports. Canadian Grain Commission, Winnipeg, Manitoba.
3. Atanasoff, D. 1920. Fusarium-blight (scab) of wheat and other cereals. J. Agric. Res. 20:1-32.
4. Christensen, C.M., and H.H. Kaufmann. 1974. Microflora. Pages 158-192 in Christensen, C.M. ed. Storage of Cereal Grains and Their Products. American Association of Cereal Chemists, St. Paul, Minnesota. 549 pp.
5. Clear, R.M., and S.K. Patrick. 1990. *Fusarium* species isolated from wheat samples containing tombstone (scab) kernels from Ontario, Manitoba, and Saskatchewan. Can. J. Plant Sci. 70:1057-1069.
6. Duthie, J.A., R. Hall and A.V. Asselin. 1986. *Fusarium* species from seed of winter wheat in eastern Canada. Can. J. Plant Pathol. 8:282-288.
7. Gordon, W.L. 1952. The Occurrence of *Fusarium* species in Canada. II. Prevalence and taxonomy of Fusarium species in cereal seed. Can. J. Botany 30:209-251.
8. Greaney, F.J., and J.E. Machacek. 1942. Prevalence of seedborne fumgi on cereals in certain seed inspection districts of Canada. Sci. Agric. 22:419-437.
9. Greaney, F.J., and J.E. Machacek. 1946. The Prevalence and control of seed-borne diseases of cereals in Manitoba. Sci. Agric. 26:59-78.

10. Jorgensen, J. 1974. Occurrence and importance of seed-borne inoculum of *Cochilobolus sativus* on barley seed in Denmark. Acta Agriculture Scandinavica 24:49-54.

11. Machacek, J.E., W.J. Cherewick, H.W. Mead and W.C. Broadfoot. 1951. A study of some seed-borne diseases of cereals in Canada. II. Kinds of fungi and prevalence of disease in cereal seed. Sci. Agric. 31:193-206.

12. Malone, J.P., and A.E. Muskett. 1964. Seed-borne fungi. Description of 77 fungus species. Proc. Int. Seed Test. Ass. 29:385.

13. Martens, J.W., W.L. Seaman and T.G. Atkinson. 1984. Diseases of field crops in Canada - An Illustrated Compendium. The Canadian Phytopathological Society. 160p.

14. Shipton, W.A., W.R.J. Boyd, A.A. Rosielle and B.I. Shearer. 1971. The common Septoria diseases of wheat. Bot. Rev. 37:231-262.

15. Stakman, L.J. 1920. A *Helminthosporium* disease of wheat and rye. Minn. Agr. Exp. Sta. Tech. Bull. 191 pp.

16. Sutton, J.C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum.* Can. J. Plant Pathol. 4:195-209.

17. Thorpe, H.C. 1958. Article in: Rev. Appl. Mycol. 37(3): 136.

18. Valder, P.G., and D.E. Shaw. 1953. Article in: Rev. Appl. Mycol. 33:21

19. Zillinsky, F.J. 1983. Cornmon Diseases of Small Grain Cereals. A Guide to Identification. CIMMYT. 141 pp.

Table 1. Average level of seed infection (%) by various microorganisms on surface disinfected white winter wheat seed produced in Ontario during 1988, 1989, and 1990.

	Percentage of Seeds Infected*		
Year	1988	1989	1990
no. of seed samples	99	259	77
Microorganisms			
Acremoniella atra	0.00	0.09	0.00
Acremonium spp.	0.00	0.01	0.00
Alternaria alternata	58.99	71.99	84.05
Arthrinium spp.	2.59	1.35	1.43
Aspergillus candidus	0.04	0.01	0.04
A. clavatus	0.09	0.01	0.01
A. flavus	0.45	0.30	0.45
A. fumigatus	0.01	0.00	0.00
A. glaucus	6.33	1.02	1.86
A. nidulans	0.03	tr	0.00
A. niger	0.05	0.02	0.03
A. ochraceus	0.01	tr	0.00
A. terreus	0.01	0.01	0.00
A. wentii	0.02	0.00	0.00
Aspergillus not ID	0.05	0.01	0.00
Aureobasidium pullulans	0.16	0.18	0.20
Bacteria	5.41	2.79	3.84
Bipolaris bicolor	0.01	0.08	0.06
B. sorokiniana	0.42	2.67	0.50
Botrytis cinerea	0.00	0.02	0.01
Cephalosporium spp.	0.05	tr	0.00
Chaetomium spp.	0.02	0.02	0.02
Cladosporium spp	1.08	3.38	2.41
Coelomycetes	0.15	0.23	0.24
Curvularia spp	0.15	0.05	0.09
Drechslera biseptata	0.23	0.24	0.38
D. tritici-repentis	0.74	1.70	1.43
D. teres	0.07	0.06	0.07
Epicoccum nigrum	4.26	8.49	8.99
Fusarium acuminatum	0.04	0.18	0.12
F. avenaceum	0.05	0.43	0.25
F. crookwellense	0.00	0.01	0.01

Microorganisms	Year / no. of seed samples	Percentage of Seeds Infected* 1988 99	1989 259	1990 77
continued				
F. culmorum		0.00	0.01	0.04
F. equiseti		0.21	0.05	0.13
F. graminearum		0.08	11.89	1.83
F. oxysporum		0.04	0.03	0.00
F. pallidoroseum		0.00	0.01	0.00
F. poae		0.16	0.98	1.26
F. proliferatum		0.00	0.01	0.01
F. sporotrichioides		0.16	0.47	0.23
F. subglutinans		0.00	0.01	0.00
Fusarium not ID		0.01	0.07	0.01
Gonatobotrys spp.		0.02	0.07	0.05
Microdochium bolleyi		0.01	0.06	0.02
M. nivale		0.00	0.01	0.00
Mucor spp.		0.15	0.13	0.15
Nigrospora oryzae		2.11	1.62	1.07
N. sphaerica		0.02	0.04	0.02
Penicillium spp.		0.49	0.17	0.36
Phaeoramularia		0.00	0.01	0.01
Phomopsis spp.		0.00	0.11	0.12
Pithomyces spp.		0.01	0.01	0.00
Pseudomicrodochium spp.		0.03	0.07	0.00
Rhizopus spp.		0.34	0.11	0.07
Scopulariopsis spp		0.04	0.00	0.00
Septoria nodorum		0.26	5.75	5.47
Sordaria fimicola		0.00	0.02	0.00
Stemphylium spp		0.35	0.21	0.17
Syncephalastrum racemosum		0.01	0.02	0.00
Trichoderma spp.		0.02	0.01	0.00
Trichotheciumroseum		0.01	tr	0.00
Ulocladium spp		0.02	0.00	0.01
Verticillium spp.		0.00	tr	0.00

tr = <0.01%

* Results based on 100 seeds per sample plated onto potato dextrose agar at room temperature.

Table 2. Maximum incidence (%) of seed infection by various microorganisms on surface disinfected white winter wheat seed from Ontario during 1988, 1989, and 1990.

Microorganisms	Year / no. of seed samples	Percentage of Seeds Infected* 1988 99	1989 259	1990 77
Acremoniella atra		0	3	0
Acremonium spp.		0	1	0
Alternaria alternata		95	96	98
Arthrinium spp.		14	13	8
Aspergillus candidus		1	1	1
A. clavatus		2	2	1
A. flavus		8	4	7
A. fumigatus		1	0	0
A. glaucus		48	14	15
A. nidulans		2	tr	0
A. niger		2	1	1
A. ochraceus		1	1	0
A. terreus		1	1	0
A. wentii		2	0	0
Aspergillus not ID		1	1	0
Aureobasidium pullulans		3	2	2
Bacteria		56	17	18
Bipolaris bicolor		1	2	1
B. sorokiniana		3	16	2
Botrytis cinerea		0	1	1
Cephalosporium spp.		1	1	0
Chaetomium spp.		1	1	2
Cladosporium spp.		7	14	23
Coelomycetes		2	3	3
Curvularia spp.		2	1	1
Drechslera biseptata		2	2	2
D. tritici-repentis		6	13	7
D. teres		1	1	1
Epicoccum nigrum		17	27	25
Fusarium acuminatum		1	2	1
F. avenaceum		1	4	3
F. crookwellense		0	1	1
F. culmorum		0	1	1
F. equiseti		3	1	1
F. graminearum		3	85	6
F. oxysporum		1	1	0
F. pallidoroseum		0	1	0
F. poae		2	5	5
F. proliferatum		0	1	1
F. sporotrichioides		3	7	3
F. subglutinans		0	1	0
Fusarium not ID		1	3	1
Gonatobotrys spp.		1	4	1
Microdochium bolleyi		1	2	1
M. nivale		0	1	0
Mucor spp		3	2	2
Nigrospora oryzae		9	11	4
N. sphaerica		1	1	1

Microorganisms	Year / no. of seed samples	Percentage of Seeds Infected* 1988 99	1989 259	1990 77
continued				
Penicillium spp.		3	3	3
Phaeoramularia		0	1	1
Phomopsis spp.		0	2	1
Pithomyces spp.		1	1	0
Pseudomicrodochium spp.		1	2	0
Rhizopus spp.		5	3	1
Scopulariopsis spp.		1	0	0
Septoria nodorum		3	24	11
Sordaria fimicola		0	2	0
Stemphylium spp.		2	3	3
Syncephalastrum racemosum		1	2	0
Trichoderma spp.		1	1	0
Trichothecium roseum		1	1	0
Ulocladium spp.		2	0	1
Verticillium spp.		0	1	0

* Results based on 100 seeds per sample plated onto potato dextrose agar at room temperature.

Table 3. Average of the daily temperature and monthly rainfall recorded at eleven weather stations within the white winter wheat growing areas of Ontario in 1988, 1989, and 1990.

	April		May		June		July		August	
	C	mm	C	mm	C	mm	C	mm	C	mm
1988	6.3	59.3	14.3	50.1	17.8	17.3	22.4	106.0	21.4	75.5
1989	4.9	58.7	12.6	104.9	18.2	94.8	21.1	48.0	19.5	61.8
1990	8.2	67.4	11.7	106.5	18.0	85.4	20.1	98.3	19.5	97.5
30yr avg*	6.4	78.6	12.5	67.1	17.9	78.7	20.3	72.8	19.6	84.1

* 1950-1980