THE FREQUENCY AND DISTRIBUTION OF THE FUNGI ASSOCIATED WITH WESTERN HARD RED SPRING WHEAT SEED DEGRADED DUE TO MILDEW

A Thesis Submitted to

The Faculty of Graduate Studies

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In Partial Fulfillment of the

Requirements for the Degree

Master of Science

by

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ABSTRACT

Hard red spring wheat (Triticum aestivum) seed samples with various levels of a degrading factor known in the trade as mildew were collected for 3 years from primary elevators in the prairie provinces. Samples were examined for viability and the presence of seed-borne fungi. Members of 32 fungal genera were isolated, with Alternaria spp. and Cladosporium spp. the most frequently isolated taxa. When the mycoflora of seeds displaying the mildew discoloration was compared with the mycoflora of the heterogenous mixture of seeds in the original sample from which they had been selected, only Cladosporium spp. showed a significant increase in frequency. The geographic origin of the samples influenced the frequency of a number of fungi. It appears prolonged wet weather at crop maturity bleaches the kernels and stimulates further growth of fungi, primarily Alternaria spp. and Cladosporium spp., at the exposed brush end of the seed. This growth discolors the kernels producing what is termed mildew. However, it is primarily the wet harvest weather which affects the actual end-use of the grain. The fungus induced discoloration and the bleaching of the kernels serve the grain inspectors as visual indicators of damp harvest conditions. Although the principle of degrading grain due to the presence of mildewed kernels is soundly based on actual quality reduction, it is suggested that a less ambiguous and more descriptive term such as grey-brush be used.

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GLOSSARY

BLACKPOINT

"Distinct discoloration of the germ and surrounding area" (Anonymous, 1985). The discoloration is caused in the field by Drechslera sorokiniana and Alternaria spp.

BRUSH END

The end opposite the germ where hair-like protuberances known as the apical hairs are found. This is the end exposed when the seed develops.

CREASE

The fold which runs the length of the wheat seed on the ventral side.

FIELD FUNGI

"Fungi which invade the seed while the plants are still growing in the field or after the grain is cut and swathed but before it is threshed". (Christensen, 1982)

GRAIN GRADES

"Divisions of quality, forming the basis on which grain is bought and sold" (Anonymous, 1985). HRS wheat is graded from #1 to #3, below which it is in the feed grade.

MILDEW

"A condition that develops in unthreshed kernels of grain caused by excessive moisture making the kernels greyish in color and affecting their quality" (Anonymous, 1985). However, to a plant pathologist, mildew refers to a group of parasitic diseases.

PRIMARY ELEVATOR

These elevators are "designed to receive grain from the producers' farm trucks, store the grain in bulk lots in separate bins according to kind and grade of grain, and transfer it quickly and efficiently into rail cars for shipment to domestic processing plants and export ports" (Anonymous, 1982).

PRIMARY STANDARD SAMPLES

"Used as guides for grading grain in Canada and, by definition, represent the minimum quality of the grade" (Anonymous, 1985).

RED SMUDGE

A reddish discolouration of amber durum wheat caused by the field fungus Drechslera tritici-repentis.

SMUDGE

"A dark brown or black discoloration or stain similar to blackpoint affecting more than one half of the kernel or extending onto the crease of the kernel, and includes the reddish discoloration associated with some plant diseases" (Anonymous, 1985).

STORAGE FUNGI

Fungi which invade the seed after harvest. They are able to grow at lower moisture levels than field fungi.

STREAK MOULD

"Kernels bearing unusually dark grey streaks on the sides of the kernels toward the brush may be affected by a very slow growing mould that is harmless in wheat, except that it affects kernel appearance" (Anonymous, 1985).

TERMINAL ELEVATOR

"An elevator, the principal uses of which are the receiving of grain upon or after the official inspection and official weighing of the grain and the cleaning, storing and treating of the grain before it is moved forward" (Anonymous, 1971).

TOMBSTONE

Refers to a chalky white, shrivelled wheat kernel resulting from the disease, fusarium head blight.

INTRODUCTION

Canada is a country which financially depends on exporting large quantities of agricultural produce. It is the second largest supplier of wheat on the world market, wheat that is grown mainly in the prairie provinces. Between 1978 and 1982, 78% of the wheat production was exported, bringing foreign capital into the country to help our net balance of payments. In the world market, Canada has a reputation second to none for supplying grain of a consistent high quality. This consistency is achieved by regulations controlling all aspects of the handling, grading and marketing of the crop.

In 1912, the Canada Grain Act gave control of grain inspection and grading to the Canadian Grain Commission. Grain grades represent divisions of quality defined by grading factors, and form the basis upon which grain is bought and sold in world markets. The grading of the grain is primarily visual, consequently the grade, and price received is affected by the appearance.

Mildew, after frost, is the second most common degrading factor which affects the appearance of the kernel. In 6 of the 18 years from 1961 to 1978, mildew was a major degrading factor in the prairies. In those same 6 years, sprouting was also a major degrading factor (Anonymous, 1979). The frequency with which mildew occurs, and the reduction in grade (and thus value) of one of our major exports emphasizes the need for a better understanding of the causes of mildew. At present, it is known that prolonged wet harvest weather re-

sults in the appearance of the mildewed kernels. However, the identity of the organisms which may cause this discoloration have not been examined.

The object of this study was to identify the fungi associated with this degrading factor, examine their geographical distribution, and compare these results with samples from the same area which were not mildewed.

For this 3-year study, samples were gathered from areas where mildew was known to be a problem each year. Levels of fungal infection were recorded under the categories of field and storage fungi as defined by Christensen and Kaufmann (1965). The moisture content of each sample was measured, and the health of the seeds determined by germination tests.

A sample graded as mildewed is a heterogenous mixture of kernels with and without the appearance of mildew. In 1985, a comparison was done between selected seeds displaying mildew symptoms and the original heterogenous sample from which they had been removed. By this approach, it was hoped to better define the differences, if any, between mildewed and symptomless seeds.

1.) **Mildew**

Mildew on Red Spring wheat is a degrading factor and considered in the statutory grade definition under "degree of soundness". Soundness for the No. 1 Grade of Red Spring wheat is defined by the Canadian Grain Act as being "reasonably well matured and reasonably

free from damaged kernels". The incidence and severity of mildewed kernels detract from the general appearance and end-use quality of wheat which results in the degrading of the wheat sample. The interpretation of soundness as it relates to mildew involves visually evaluating both the amount and the severity of the mildew in relation to a physical reference sample. This judgmental evaluation is performed by inspectors trained in the correct application of maximum limits of mildewed kernels.

Using experience gathered over many years, the grain inspector judges the percentage of kernels showing the discoloration and the intensity of the discoloration in the sample. In addition, primary grain standards are prepared each year to interpret visually the lowest limit of soundness acceptable for each numerical grade. In certain years, special guide samples may also be prepared which reflect only the maximum allowable limit of mildew for each grade.

The assessment of mildew is thus subjective. Levels of mildew affect the overall appearance of the sample and thus the grade. A sample with light mildew has a slight white haze (plate 1), whereas one with moderate mildew levels has a darker appearance (plate 2). Individual kernels can be described thus: a lightly mildewed kernel has a dull white brush end; moderately mildewed has a greyish brush end more deeply discolored and the bran of the kernel is a light grey; and in severely mildewed kernels, the brush end is dark grey to black. Severely mildewed kernels also exhibit dark grey to black discolorations on the entire surface of the kernel (plate 2). In extreme

Plate 1

Hard Red Spring Wheat Without Mildew

Hard Red Spring Wheat With Light Mildew

Plate 2

Hard Red Spring Wheat With Moderate Mildew

Hard Red Spring Wheat With Severe Mildew

cases, severely mildewed kernels may be soft, spongy and rotted.

Sprouting commonly occurs in association with mildew, although sprouting is usually found only in certain areas whereas mildew is widespread. Though certain varieties are resistant to sprouting, none is resistant to mildew. Consequently, significant economic loss can occur due to degrading of wheat for mildew. The mycoflora associated with blackpoint and other degrading factors has been investigated in detail, but not with mildew, the most common of the discolorations.

LITERATURE REVIEW

1) Effect on Quality

As far as can be determined, nothing has been published on the effect of mildew on quality of hard red spring wheat. Dexter and Matsuo (1982) however, have examined the effect on quality of mildewed kernels in farm samples of durum wheat. They reported significantly higher protein levels, alpha-amylase activities, and low pigment contents, while wheat ash levels and gluten properties were not significantly different from the controls. These changes resulted in weaker pasta dough mixing properties, more dough breakdown, and stickier dough. Shorter pasta dough mixing times also occurred and were attributed to a slightly higher protein content of the mildewed semolina, although enhanced enzymatic activity was also stated as a possible cause. Mildewed samples yielded undesirably high speck counts and spaghetti which was significantly duller and lower in yellow pigment content. The lower pigment level in the spaghetti was stated to be mainly due to lower pigment in the semolina. The bleaching of kernels also occurs on durum wheat graded mildewed; this may be a primary factor in the lower pigment levels of the mildewed semolina and spaghetti recorded by Dexter and Matsuo. Baker et al. (1958) found that growth of Cladosporium spp. at the brush end of wheat kernels resulted in poorer flour color. The fungus was not found in the endosperm of any of the seeds examined nor had it any direct effect on the endosperm. Rather it appeared the endosperm was not fully developed due to the

weather conditions. The authors did not do any baking tests with the flour, but if they had they would likely have found problems caused by high alpha-amylase levels.

2) Discolorations of Wheat Seed Caused by Seed-borne Fungi

There are many reports concerning the mycoflora of cereal grains. Some focused on the mycoflora present on and in grain in general, whereas others have directed their attention to fungi associated with visual abnormalities or seed health. Machacek et al. (1951) investigated seed-borne diseases of cereals from across Canada, identifying a total of 43 fungal genera and 102 species. Saprophytes such as Alternaria spp. were consistently the most frequent fungi. The most commonly isolated of the parasitic fungi was Drechslera sorokiniana (Sacc.) Subram. and Jain. These authors also reported variations in geographic distribution of a number of the fungi isolated. Other studies have concentrated on seeds with visual abnormalities. Terms such as blackpoint, smudge, red smudge, streak mould and tombstone are all grading terms referring to discolorations of wheat seed caused by the growth of field fungi.

Blackpoint has been attributed to the development of two field fungi, Alternaria spp. and D. sorokiniana. Drechsler (1923), Henry (1923), Weniger (1925), Machacek and Greaney (1938), Brentzel (1944), Hanson and Christensen (1953) and Simmonds (1968) all examined the fungi associated with this discoloration.

Smudge is merely an extension of the blackpoint discoloration

from the germ end along the crease of the kernel, and is caused by the same fungi. Apparently it is more common in durum than hard red spring wheats (Machacek and Greaney, 1938); (Greaney and Wallace, 1943).

Red smudge refers to a reddish discoloration of durum wheat seed due to infection by Drechslera tritici-repentis (Died.) Shoem., the causal agent of the foliar disease tan spot. It also occurs on HRS wheat seed, resulting in a purplish tinge to the seed (Clear and Cooke, 1985).

Tombstone kernels are shrivelled, chalky white seeds produced as a result of fusarium head blight infection. Fusarium graminearum Schwabe is the primary agent of this disease in Canada, although other Fusaria have also been found associated with the disease (Gordon, 1952; Simmonds, 1968; Sutton, 1982; Stack and McMullen, 1985; Clear and Abramson, 1986; Abramson et al, 1986).

Streak mould has been attributed to the growth of a slow growing fungus Aureobasidium pullulans (De Bary) Arnaud, but both Alternaria spp. and Cladosporium spp. also induce such a discoloration on wheat seed (Clear and Cooke, 1985).

Mildew has not been previously examined for the causal fungi. However, Baker et al. (1958) described symptoms on wheat seed typical of the degrading factor mildew, and attributed it to the development of Cladosporium spp. on the seed during wet harvest weather. No other references to this type of discoloration could be found.

3) Fungal Isolation Techniques

a) Surface Sterilization

Fungi occur on seeds as spores and mycelium. When examining seed for the causes of a discoloration, it is prudent to try to eliminate interference from those fungi which are present merely as contaminants and have not had any involvement in the discoloration. For this reason, seeds are often surface sterilized before being examined.

Various agents have been used as surface sterilants, but none has been without drawbacks. Hoffer (1914) dipped seed in an alcoholic solution of mercuric bicholoride; Norton and Chen (1920) used the same solution but first presoaked the seed for 10-12 hrs in water. Henry (1924) also used Hoffer's solution, but then rinsed the seed with alcohol to remove the superfluous metallic salt adhering to the seed. However, fungi may respond differently to chemical seed treatments as demonstrated by Stakman (1920) who found D. sorokiniana was more sensitive to mercuric bichloride than Alternaria spp. Mead (1933), using silver nitrate as a sterilant, isolated fungi with pale mycelium more frequently than with dark mycelium. Simmonds (1930) avoided this apparent selective action by washing the seed in sterile water to remove seed-borne contaminants; he reported a greater variety of fungi was isolated from the water washed seeds than the chemically sterilized ones.

Machacek and Greaney (1938) used an ethyl alcohol-mercuric bichloride solution as a surface sterilant, but felt they avoided the

effects of the excess chemical on the seed when they partially enveloped the seed in potato dextrose agar.

Presently, the inherent health risk of mercury precludes its' use as a surface sterilant, so other methods have had to be developed. Flannigan (1969) used a sodium hypochlorite solution (NaOCl) to surface sterilize barley grains. The method is safe, easy, effective, and without any lingering effect of the chemical. This technique has not been without criticism however. Harmon and Pfleger (1974) found that not all spores of the Aspergillus glaucus group species inoculated onto seed were killed by NaOCl treatment. Most recently, Sauer and Burroughs (1986) examined the effectiveness of NaOCl surface sterilization of corn and wheat inoculated with spores of Aspergillus spp. and Penicillium spp. It was used both alone and in conjunction with other solutions but was not completely effective. The hydrophobic nature of these spores, their size, and great abundance in the specially inoculated seeds were likely factors in the failure to kill all the inoculum. Surface cracks, and the hairs at the brush end of the wheat seeds would serve to accentuate the effects of the above characters. Eckhoff et al. (1983) considered a water rinse after surface sterilization responsible for high and erratic counts of Penicillium spp. on samples of surface sterilized seed. They believed the rinse was spreading spores not contacted by the NaOCl treatment to clean seeds.

b) Media for Isolation of Field Fungi

The use of broad spectrum media is preferred over selective media when there is no specific target organism.

One of the most commonly used media for culturing saprophytic fungi is potato dextrose agar (PDA). It is frequently employed when identifying Fusarium spp. (Burgess and Liddell) and many of the dematiaceous hyphomycetes (Ellis, 1971).

However, selective media have been used to detect certain groups of fungi such as the Fusaria (Nash and Snyder, 1962; Gopinath and Shekara Shetty, 1984), dematiaceous hyphomycetes (Andrews and Pitt, 1986), Penicillia (Frisvad, 1983), and Aspergilli (Pitt et al, 1983).

c) Media for Isolation of Storage Fungi

A number of techniques have been used to isolate storage fungi from seed. Many involve agar media which, to increase selectivity, contain added sugar or salt to inhibit the development of non-target organisms. Mills et al. (1978) examined a few of these plus a method known as the salt filter paper (SFP) technique. They determined the SFP isolation technique was superior for the isolation and identification of storage fungi compared to malt salt agar, Czapek solution agar and filter paper soaked with Czapek solution.

d) Incubation Conditions

Temperature, light, moisture and nutrition are the four principal environmental factors influencing the growth and sporulation of

fungi. Changes in these can alter the size, shape and color of spores and cultures (Hawker, 1958; Carlile, 1965). These usually interdependent variables must be taken into account when deciding upon incubation conditions to optimize growth and sporulation of the target organisms. A fluctuating temperature and light regime is often incorporated into incubation conditions, as it is well recognized that such a regime is the most effective way to induce sporulation in many fungi (Houston and Oswald, 1946; Snyder and Hansen, 1947; Lilly, 1951; Hawker, 1958; Cochrane, 1958; Aragaki, 1961; Lukens, 1966; Leach, 1962, 1967).

Light is effective in encouraging sporulation, but the amount of exposure required or desirable largely depends on the wavelength used (Leach, 1967). With short wavelengths (such as 270 nm), less exposure time is required, but at these wavelengths dosage is critical; it is easy to overexpose, causing inhibition or death of the fungus. Longer wavelengths, e.g. 360 nm, are preferred since they have no adverse effects on fungi, most of the light is transmitted by plastics, and it is non-hazardous to the user (Leach, 1967). This type of light is best obtained by the use of a black light fluorescent lamp. As mentioned earlier, an alternating light regime is preferred. The one recommended by Leach (1967) and Burgess and Liddell (1983) is a 12 hr on/off cycle.

An organism's ability to grow at various moisture levels is a third major factor determining its' ecological niche. Koehler (1938) determined that field fungi require a relative humidity of at least

90-95% to grow. Christensen (1972) stated storage fungi can grow at relative humidities of 68% or more depending upon the species. In 1966, de Tempe and Limonard demonstrated high moisture levels in a blotter test can inhibit the development of some fungi. They noted an agar plate has a dry surface which thus avoids the selective action of excessive moisture.

Nutritional requirements vary for different fungi. As most of the seed-borne fungi are either obligate or facultative saprophytes, and only after seed has ripened does the mildew discoloration occur, it is important to use a medium which addresses the nutritional requirements of saprophytes. The seed itself serves as the ideal source of nutrients for development of fungi involved in the discoloration. The agar medium provides a larger surface on which to observe micro and macromorphology, and the production of pigments or other products which are important for identification. This is likely one reason Flannigan (1969) reported agar-plate tests detected a greater range of saprophytes than a blotter test. One of the drawbacks to the use of a medium which results in luxuriant fungal growth, is that slower growing species may be overgrown and masked.

4) Fungal Identifications

The identification of fungal genera, and even species, is commonly done by observing the organism *in situ* after growth and sporulation have occurred. The preparation of slides further facilitates the identification of fungi which are difficult to distinguish under the

dissecting microscope. In addition to micromorphology, colony characteristics such as growth rate, color, exudate, pigmentation and texture are useful identification tools.

The importance of standardized incubation conditions in fungal identifications has been demonstrated by a number of authors such as Hawker (1958), Leach (1962, 1963, 1964), de Tempe (1963) and Carlile (1965). Changes in the incubation conditions can alter pigmentation, spore morphology, sporulation, growth rate, colony characteristics and other features used for identifications.

a) Fusarium spp.

Many of the Fusaria in this study were identified from their macro- and micromorphology as defined by Burgess and Liddell (1983) and Nelson et al. (1983) without making monoconidial isolates each time (Abramson et al., 1986). The monoconidial isolation techniques of Booth (1971) and the use of carnation leaf agar (CLA) (Fisher et al., 1982) using leaf pieces sterilized by propylene oxide fumigation (Toussoun and Nelson, 1976) are frequently employed to check identities of Fusarium spp. (Burgess and Liddell, 1983).

b) Drechslera spp.

There are a number of methods used to induce conidiophore and conidial development in Drechslera spp.. Two methods are those of Shoemaker (1962) and Odvody and Boosalis (1981).

c) Other Fungi

Most of the other fungi one would expect to be present can be identified without subculturing using primarily descriptions given by Ellis (1971, 1976), Barnett et al. (1972), Carmichael et al. (1980), Domsch et al. (1980) Barron (1983), and for identifying the Aspergilli, Raper and Fennell (1977).

METHODS

1) Sample Collection, Moisture Determination, Storage and Grading

Samples were obtained with the assistance of the Inspection Division of the Canadian Grain Commission, and the Manitoba, Saskatchewan and Alberta Pool elevators. Four sturdy envelopes, each capable of holding 250 g of seed, were supplied to primary elevators in areas where mildewed wheat was reported. Two envelopes were for separate samples of non-mildewed wheat (their best grade if possible), and two were for separate samples of mildewed wheat. Spaces for notation of the elevator, grade, and variety were present on each envelope. Samples were also collected from the Inspection Division's program of screening primary elevator samples for use as grain standards, and from terminal elevators in Vancouver (for the 1985 Alberta samples).

The 1983 crop was represented by 75 Alberta and 58 Manitoba samples collected in the spring of 1984. In the fall of 1984, 123 samples were received from Alberta, mainly the Peace River area. In the fall of 1985, 66 samples were obtained from Manitoba, 135 from Saskatchewan and in January of 1986, 58 from Alberta. A total of 42,900 seeds were examined on PDA, and 38,700 by the SFP technique.

Samples received were documented, the moisture content determined to ±0.2% using a dielectric moisture meter (Halross Instruments, Model No. 919, Wpg.), then stored at -15°C until analyzed. After analysis, samples were officially graded and rated on the level of mildew (nil,

light, moderate, severe) by a grain inspector at the Canadian Grain Commission in Winnipeg.

2) Sample Preparation

Samples were thoroughly mixed, then a subsample was surface sterilized in a 0.3% sodium hypochlorite solution (Flannigan, 1969) for one minute with constant agitation and dried under a laminar flow hood. Using this surface sterilized subsample, tests were performed to determine fungal abundance and rates of germination .

3) Incubation Conditions for Field Fungi

Surface sterilized seeds (10 per plate), selected at random, were placed on potato dextrose agar (PDA, Difco Laboratories, Detroit, Michigan, USA) in sterile, disposable polystyrene petri plates, 100 mm x 15 mm (Maynard Scientific, Weston, Ontario). The plates were incubated for 5 days under an alternating temperature regime, 25°C day and 22°C night, and a 12-hr. photo period (henceforth called the normal incubation conditions). The light source consisted of a light bank 40 cm above the plates and 75 cm wide, containing four 40 W cool white fluorescent tubes and one 40 W black-light tube (General Electric F40BLB). They were contained in an incubation chamber assembled according to the specifications given by Burgess and Liddell (1983). In 1983 and 1984, 50 seeds/sample were examined, and in 1985, 100 seeds/sample.

4) Incubation Conditions for Storage Fungi

Levels and types of storage fungi were determined using the salt filter paper (SFP) method of Mills et al.(1978). Petri plates containing a filter paper disc (Whatman #3, 9 cm) moistened by 5 ml of a 7.5% NaCl solution had 25 seeds/plate distributed evenly on them, and then were incubated for 7 days at room temperature. Fifty seeds/sample were examined of the 1983 and 1984 crop, and 100 seeds of the 1985 crop.

5) Fungal Identification

Plates were examined under a dissecting microscope (Nikon SMZ-10) to identify the genera and, in some cases, the species present on the seeds. When necessary, slides were prepared for examination by means of a compound microscope with differential interference contrast 'NT' (Nikon Optiphot).

An attempt was made to identify all the Fusarium isolates encountered to species as well as a number of the Drechslera isolates.

Where a species name is definitely given, one or more isolates of the species will have been confirmed as such by workers at the B.R.I. in Ottawa.

a) Identification of Fusarium spp.

Five Fusarium spp. were frequently identified directly from seed; F. graminearum Schwabe, F. poae (Peck) Wollenw., F. sporotrichioides Sherb., F. culmorum (W.G. Smith) Sacc. and F. equiseti (Cda.) Sacc. Fusarium graminearum cultures were identified directly from seed if

they produced a red pigment in the agar, grew rapidly, produced perithecia, and if spore and colony characteristics were typical for that species. Typical colony characteristics are sparse to moderate aerial mycelium tinged with yellow, in which the more typical spore shape was found, and dry, red sporodochia on the agar containing spores not as uniform as those in the aerial mycelium. Spores in the aerial mycelium tended to be falcate to almost straight with 5 to 6 septa, a tapered apical cell and foot shaped basal cell. No microconidia are produced. Fusarium poae cultures were identified directly from seed if they produced a pink to red pigment in the agar, grew rapidly, produced tear drop shaped microconidia from doliform phialides, had a fruity odor, and if colony characteristics were typical for that species. The main colony characteristic noticed was the production of dense, white, floccose mycelium, invariably containing numerous microconidia. Macroconidia, if present, were formed in orange sporodochia and were short, often 3-septate with a nipple-like apical cell. Fusarium sporotrichioides was identified without transferring from seed if it produced a pink to red pigment in the agar, grew rapidly, produced microconidia from polyphialides in the aerial mycelium and if spore and colony characteristics were typical for that species. Fusarium sporotrichioides cultures produced less aerial mycelium than F. poae, and the mycelium was not as white. Macroconidia formed in orange sporodochia on the agar after 5 days of incubation, but microconidia were usually produced only after 7 days, usually near the seed. The microconidia were mainly spindle shaped, with tear drop-

shaped ones produced on occasion. Macroconidia were falcate, 3-5 septate, usually with a notched basal cell. Fusarium culmorum cultures grew more rapidly than F. graminearum, producing a red pigment in the agar and copious wet sporodochia containing short, stout macroconidia. Aerial mycelium, some tinged yellow, formed more densely than in F. graminearum. Fusarium equiseti cultures were identified by their moderately rapid growth rate, salmon color from beneath, the production of macroconidia with a strong dorsiventral curvature and tapering apical cell curved tightly inward and abundant chlamydospore production in the aerial mycelium after 7-10 days incubation. No microconidia were produced.

Fusarium avenaceum (Fr.) Sacc. and F. acuminatum Ell. and Everh. cultures frequently failed to sporulate after the seeds were incubated for 5 days. This required transferring a small portion of mycelium to a fresh PDA plate and incubating under the normal incubation conditions. Often a shock treatment was required before the transferred cultures would sporulate. This was done by slashing cultures with sterile inoculating needles. Macroconidia produced in the sporodochia after the shock treatment were very characteristic of the respective species. For example, F. avenaceum spores were very long and slender and F. acuminatum conidia displayed a strong dorsiventral curvature and pronounced foot cell typical of the Gibbosum section of Fusarium. Fusarium avenaceum isolates were typically faster growing and were able to grow to the edge of the petri plate unlike F. acuminatum. The reverse of F. acuminatum petri plate cultures were typically deep red

to burgundy in the center surrounded by a broad white border reaching to the edge of the culture. From below Fusarium avenaceum cultures were more pink with a much narrower white border. Fusarium acuminatum cultures usually produced chlamydospores in the aerial mycelium after about 14 days incubation; F. avenaceum does not produce chlamydospores.

Cultures not meeting the criteria used above to define individual species, were single spored and grown on PDA in the dark for 72 hours at 25°C to determine growth rate. Colony appearance, and often spore morphology, was determined by incubating the plates under the UV light bank, although some required shock treatment to induce sporulation (mainly F. avenaceum and F. acuminatum). Carnation leaf agar was also used to induce sporulation, with all but a very few isolates sporulating well and producing the macroconidia typical of a particular species on this medium.

Monoconidial cultures of all tentatively identified species were sent to Dr. G. Neish at the Biosystematics Research Institute (BRI) in Ottawa for confirmation of identity.

b) Identification of Drechslera spp.

In the first year, D. sorokiniana (Sacc.) Subram. and Jain was recorded separately from the other Drechslera species. In the other two years both D. sorokiniana and D. tritici-repentis (Died.) Shoem. were recorded separately. They possess distinctive characteristics which allow for accurate identifications. Other Drechslera species

were tabulated under the generic name, with 31 cultures being identified by Dr. Shoemaker of the BRI to species level.

Drechslera sorokiniana sporulated abundantly, producing shiny black colonies on the seed and on PDA. Colonies on PDA were observed to produce an inhibition zone at the periphery. On PDA, Drechslera tritici-repentis produced a grey colony consisting of sparse mycelium, and occasionally, long conidiophores arising from the agar. Seeds infected by D. tritici-repentis very frequently had dark circular areas of fungal material (possibly perithecia) under the pericarp after incubation. Colonies which grew to the edge of the plastic petri plates produced what appeared to be pseudothecia along their margins. This feature appeared to be characteristic of a number of Drechslera spp. which have Pyrenophora as the teleomorph state, but it was not observed in species where Cochliobolus was the perfect state. On the underside of the cultures D. tritici-repentis and most of the other Drechslera spp. produced a distinctive, dark, mottled coloration.

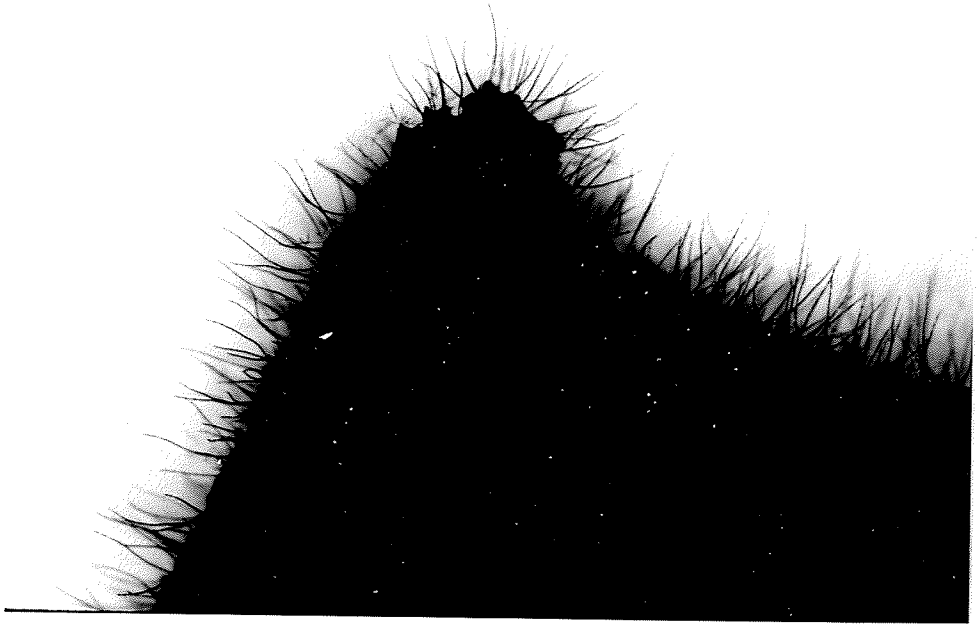
In this study, a novel method was developed to aid the identification of Drechslera spp. The procedure required transferring a small piece of aerial mycelium from the culture to a fresh plate of PDA and incubating for 3 days under the normal incubation conditions. A triangular wedge was then removed from this newly formed colony and placed in a polystyrene petri plate containing filter paper wetted with 5 ml of sterile distilled water. After 3 days incubation under the same conditions, many of the Drechslera species (i.e., D. teres

(Sacc.) Shoem., D. tuberosa (Atk.) Shoem., D. avenacea (Curtis ex. Cooke) Shoem., and D. graminea (Rab. ex. Schlecht.) Shoem. were sporulating profusely. Drechslera tritici-repentis required a further dark period of 18 hrs at 18°C to produce conidia. Spores produced by D. tritici-repentis were very typical of the species, with a prominent 'snakes head' basal cell produced first on conidiophores arising from the filter paper. This new method is easy, rapid, requires little preparation, and has the real advantage of allowing excellent observation of the conidia and conidiophores by virtue of having a white background which contrasts with the dark fungal structures and scant aerial mycelium (plate 3).

6) Germination Tests

Germination tests were performed according to the Canadian Methods and Procedures of Seed Testing (1965), except 50 to 100 surface sterilized seeds were used instead of 200 non-surface sterilized. Two to four petri plates with 25 seeds each on a Whatman #3 (9 cm) filter paper were moistened with 5 ml of sterile distilled water, chilled at 4°C for 4 days, then kept at room temperature for 6 days. Initially, only 2 recording categories were used, living or dead. However, in the 1984 and 1985 samples, 3 categories were used, normal, abnormal, and dead. Normal seedlings had good root and shoot development, abnormal seedlings lacked either good root or shoot development or both. During the 6 days at room temperature, the lids of the plates were lifted for a moment each day to avoid the occurrence of

PLATE 3



Conidiophores of Drechslera tritici-repentis

anaerobic conditions. Failure to do this would often retard seedling development.

7) Recording Data

Samples received from each elevator were labelled A,B,C, or D, with the results recorded accordingly. Fungal and bacterial infection levels and seed germination were expressed as percentages.

8) Statistical Analysis

The results for six of the most frequently isolated fungi; Alternaria spp., Cladosporium spp., Epicoccum nigrum Link ex Link, Drechslera sorokiniana, Nigrospora oryzae Hudson, and Septoria nodorum (Berk.) Berk. were analyzed using the SAS statistical software package (SAS Institute, 1985). As no data were available for Manitoba in 1984 or Saskatchewan in 1983 or 1984, it was decided to analyze each year individually. The effects of the sample source (province) and the presence of mildew on the above fungi were tested for significance.

Fixed effect ANOVA (analysis of variance) models were used because both province and mildew assessment were regarded as fixed factors (Neter and Wasserman, 1974). The 1983 and 1985 data were analyzed as for a Completely Randomized Design with a two-way treatment structure, the two factors being province and mildew assessment. As there are unequal numbers of data points for each subclass, the ANOVA analysis was based on the Type III sums of squares (Milliken and Johnson, 1984). The 1984 data were also analyzed as a Completely Ran-

domized Design, but with a one-way treatment structure (mildew assessment).

As the variance of the error terms was not constant in the data, the variance of error terms were stabilized using an arcsin or angular transformation prior to data analysis. As some of the proportions are zero or one, Bartlett's refinement of the arcsin transformation was used (Snedecor and Cochran, 1980; Zar, 1984).

A comparison of the results of the discolored vs randomly selected seeds in Table 9 was done using a student's t-test for significance.

RESULTS

1) Distribution of Samples

Table 1 shows the number of samples received graded as nil, lightly, moderately and severely mildewed. Few of the samples were rated as severely mildewed. Overall, 27 Manitoba samples were graded as having no mildew, 41 light, and 52 moderate. In Saskatchewan, only samples from 1985 were obtained, with 58 nil, 36 light, and 39 moderate. Alberta samples were gathered over 3 years, totalling 73 nil, 83 light, and 86 moderate. Altogether, a fairly equal distribution of nil, light and moderate samples were received (158, 160, 177 respectively), only severely mildewed were poorly represented, with 20 samples received. This is due to the infrequency with which severely mildewed samples are normally encountered.

Average moisture levels of the samples when received were below the 14.6% figure set by the Canadian Grain Commission (CGC) as the upper limit for red spring wheat samples in the dry category (CGC grading manual). Tough and damp levels are 14.6% - 17.0%, and over 17.0% respectively. Few individual samples received were tough, and none were damp.

2) Fungi Isolated

A total of 56 fungal genera and species were isolated. Of these, only a few were commonly found on the seed.

Alternaria spp.

Alternaria spp. were the most commonly isolated fungi from the seed of all three provinces and levels of mildew (Table 1). The frequency with which these fungi were detected decreased considerably from Manitoba to Alberta (Figures 1a, b, c), the difference being significant at the 99% confidence level. Alternaria spp. levels were also related to the presence of mildew in 1983 and 1984 (Table 2).

Cladosporium spp.

Species of this genus were the second most frequently isolated fungi in the study. They were found much more frequently in northern Alberta and northwestern Saskatchewan than in southeastern Saskatchewan and Manitoba (Figure 2a, b, c). In Manitoba in 1983 they were the sixth most frequently isolated fungi and in 1985 the fourth most common, whereas in Alberta they were the second most isolated fungi in all three sample years (Table 1). Differences between provinces were also significant, as was the relationship between Cladosporium spp. and the presence of mildew in two of the three years studied (Table 2).

Epicoccum nigrum

Overall, E. nigrum Link ex Link was the third most common fungus isolated, but the frequency with which it was isolated varied between provinces. E. nigrum was much more common in Alberta than Saskatchewan or Manitoba (Figure 3a, b, c), and it was significantly more frequent ($P<0.01$) in mildewed than non-mildewed samples in all 3

years (Table 2). The highest infection level observed was 30% in one lightly mildewed Alberta sample in 1985.

Drechslera spp.

A number of species were isolated, two of which were routinely identified to species (D. sorokiniana and D. tritici-repentis), whereas the others were subsampled and sent to Dr. Shoemaker at the BRI for species identification. Of the cultures sent, the most common identity was D. tuberosa. Other species were D. avenacea, D. bicolor (Mitra) Subram. and Jain, D. biseptata (Sacc. and Roum.) Richardson and Fraser, D. catenaria (Drechs.) Ito., D. graminea, D. phlei (Graham) Shoem., D. teres, and D. victoriae (Meehan and Murphy) Subram. and Jain. One isolate was identified as a Bipolaris sp.. It did not match any species known to Dr. Shoemaker from wheat or the other major cereals, and he felt it was probably undescribed (DAOM 191556).

Drechslera sorokiniana

This species was most frequently isolated in Manitoba, less frequently in Saskatchewan, and was relatively uncommon in Alberta (Figure 4a, b, c). The highest recorded frequency was 76% in one sample from Manitoba in 1983.

Nigrospora oryzae

Nigrospora oryzae Hudson was one of the common fungi in Manitoba and Saskatchewan, but considerably less so in Alberta in all sample years (Figure 5a, b, c). Growth in culture is rapid but sparse, with the black conidia produced quickly and abundantly. A high of 22% was found in one Manitoba sample in 1983.

Fusarium spp.

Of the 11 Fusarium spp. isolated, the most frequently isolated was F. avenaceum. It was most common in northern Alberta where in 1984, one sample was found to have this species on 18% of the seeds. In comparison, it was considerably less common in Manitoba and Saskatchewan.

F. acuminatum was second in overall frequency, and was evenly distributed over all three provinces. F. equiseti was relatively common in samples from Manitoba and Saskatchewan in 1985, but was much less common in Manitoba in 1983 and Alberta in all three years. A maximum level of 15% was recorded in a moderately mildewed Saskatchewan sample.

In Manitoba, F. poae and F. sporotrichioides were both common. In 1985, F. sporotrichioides was the most common Fusarium spp. in Manitoba. Also in 1985, F. graminearum was isolated from samples originating from southeast Manitoba.

Septoria nodorum

Septoria nodorum (Berk.) Berk. was common on seed from Alberta in all three years, but especially in 1983 when more than one sample recorded a 40% infection rate. Much lower levels were found on Saskatchewan and Manitoba samples. The lowest level of this fungus was found in the 1985 samples, and the highest in the 1983 samples.

Drechslera tritici-repentis

D. tritici-repentis was isolated much more frequently from Manitoba seed than from the other provinces. This was particularly evident in 1985 when it was isolated from 3.61% of the seed from Manitoba, 0.87% from Saskatchewan and 0.26% from Alberta.

Other Fungi

Of the remaining fungi isolated, only Aspergillus glaucus group species, Penicillium spp., Arthrinium spp., Phaeoramularia spp., Ulocladium spp., Aureobasidium pullulans (DeBary) Arnaud and Botrytis cinerea Pers. ex. Pers; Persoon were detected on an average of over 1% of the seeds from any province or level of mildew (Table 1). However, some fungi occurred at considerably higher levels in individual samples.

Phaeoramularia spp. were found in one 1985 Alberta sample on 8% of the seeds. A. pullulans was detected on 9% of the seeds from another 1985 Alberta sample, this sample being mildewed. Also from Alberta, B. cinerea was present on 18% of a 1984 sample and Arthrinium

spp. on 14% of a 1983 sample. Ulocladium spp. occurred at 11% and Trichothecium roseum (Pers.) Link ex Gray at 14% from two different Saskatchewan samples.

The fungi whose environmental niche is the storage environment (Rhizopus spp., Mucor spp., Phycomyces spp., Scopulariopsis spp., Penicillium spp. and Aspergillus spp.) were found at low levels, save for the A. glaucus group species. The four other Aspergillus spp. isolated were very infrequent. The levels of storage fungi were found to be higher in the spring collected samples, those being the 1983 Manitoba and the 1983 and 1985 Alberta samples. Also, the spring collected Alberta samples had higher levels of Aspergillus spp. than the Manitoba samples (3.36% and 3.04% vs 1.17%).

3) Bacteria

A variety of bacterial colonies developed on seeds plated onto agar. The majority of the colonies were a golden yellow color, representatives of which were identified as Pseudomonas fluorescens, a species important in decomposition. Another bacterium identified was Bacillus cereus, an antibiotic producing species common on grain products. These cultures were white and produced a zone of inhibition in the agar which effectively repelled all fungi. A third species identified was Klebsiella pneumonia, which produced red colonies on PDA. The bacteria implicated in black chaff (Xanthomonas campestris pv. undulosa), and basal glume rot and bacterial blackpoint (Pseudomonas syringae pv. atrofaciens) were not detected in the representative subsampling used to determine some of the species present.

The frequency with which bacteria were recorded increased with severity of mildew on the kernels (Figures 6a, b, c).

4) Germination

The percentage of normal, abnormal and dead seeds was correlated with the severity of mildew in all provinces and years except for Manitoba in 1985, when lightly and moderately mildewed samples averaged a higher percentage of normal germinations.

5) Comparison of Selected Mildewed Kernels with the Original Sample

A comparison was made of the mycoflora present on seeds selected for the appearance of mildew and the original sample from which they had been removed. The original samples contained seeds with and without the mildewed appearance but had been degraded due to the presence of mildewed kernels. The selected seeds all displayed a grey discoloration of the brush end and were removed from the original sample only after all tests with the original sample had been completed. Fourteen 1985 samples which had been graded as moderately or severely mildewed were chosen at random from each province. One hundred seeds were selected from each sample, surface sterilized, plated onto PDA, and treated in the same fashion as were the seeds from the original sample.

Figures 7 a, b, c illustrate the large increase in the number of kernels with Cladosporium spp. present in the selected seeds. This increase was significant at the 0.01 level (Table 9). The levels of Epicoccum nigrum, D. sorokiniana and Alternaria spp. remained essentially the same, and consequently were not found to be significantly

different. Levels of the less common fungi remained low. Two fungi not encountered in the original samples, Acremoniella atra (Corda) Sacc. and Trichoderma sp., were isolated at low levels from the selected Manitoba and Alberta seeds respectively. In the selected seeds of all three provinces, the most common fungi were Alternaria spp. followed by Cladosporium spp., then D. sorokiniana in Manitoba and E. nigrum in Saskatchewan and Alberta. Frequency of bacteria dropped slightly in Manitoba and Saskatchewan selected seeds, but increased in Alberta seeds.

Figure 1A

Percent Infection by
Alternaria spp.
in Surface Sterilized Hard Red Spring Wheat Seed
Displaying Various Degrees of Mildew

1983 Number of samples examined

Degree of Mildew	Nil	Light	Moderate	Severe
Alberta	17	30	25	3
Saskatchewan		No data available		
Manitoba	20	11	23	4

Percent *Alternaria* spp.

Alberta	24.71	40.34	43.40	37.30
Saskatchewan	0.00	0.00	0.00	0.00
Manitoba	70.20	84.40	80.90	79.50

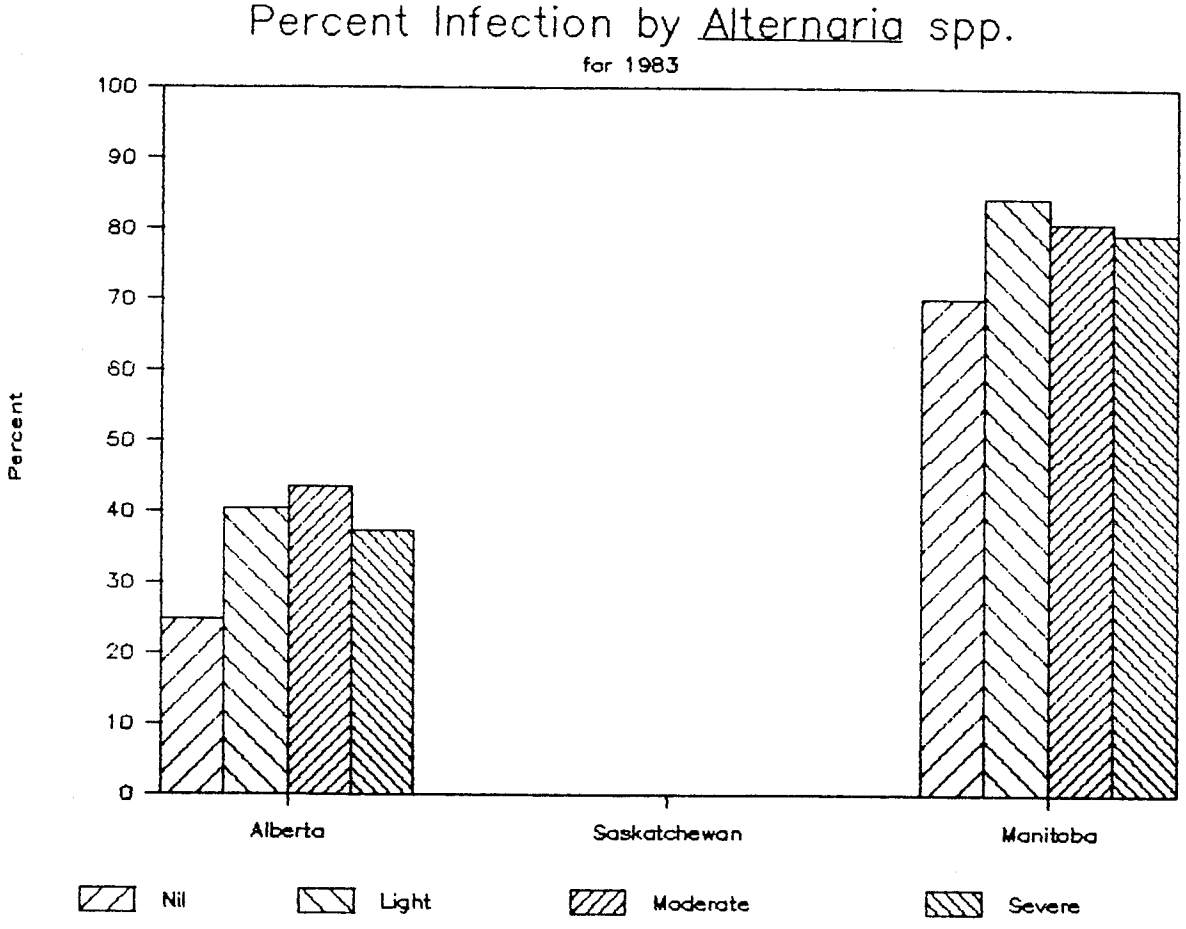


Figure 1B

Percent Infection by
Alternaria spp.
in Surface Sterilized Hard Red Spring Wheat Seed
Displaying Various Degrees of Mildew

1984 Number of samples examined

Degree of Mildew	Nil	Light	Moderate	Severe
Alberta	47	33	33	11
Saskatchewan		No data available		
Manitoba		No data available		

Percent *Alternaria* spp.

Alberta	42.60	60.00	77.80	66.90
Saskatchewan	0.00	0.00	0.00	0.00
Manitoba	0.00	0.00	0.00	0.00

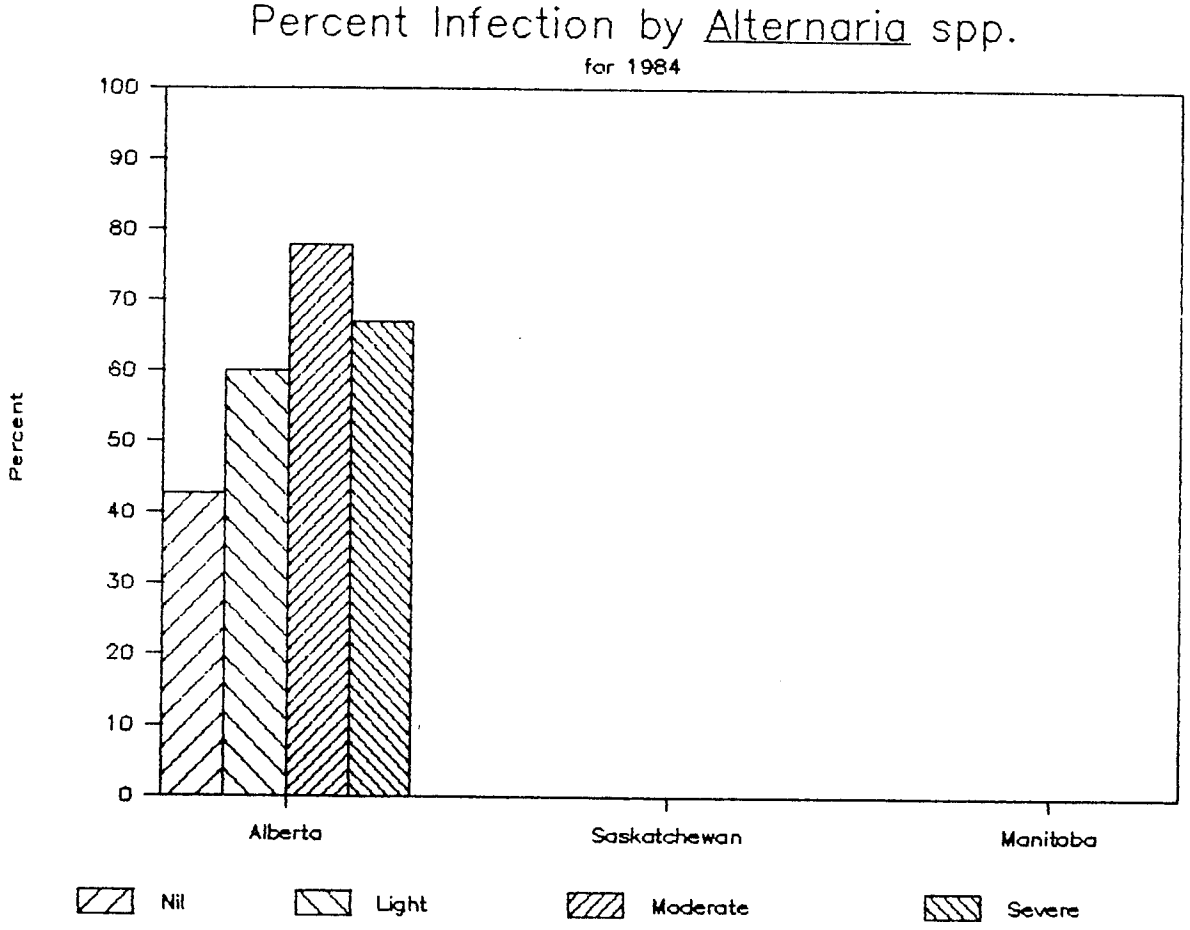


Figure 1C

Percent Infection by
Alternaria spp.
in Surface Sterilized Hard Red Spring Wheat Seed
Displaying Various Degrees of Mildew

1985 Number of samples examined

Degree of Mildew	Nil	Light	Moderate	Severe
Alberta	9	21	28	0
Saskatchewan	58	36	39	2
Manitoba	7	30	29	0

Percent *Alternaria* spp.

Alberta	70.20	55.00	49.30	0.00
Saskatchewan	66.40	89.00	92.10	95.50
Manitoba	95.70	92.10	92.80	0.00

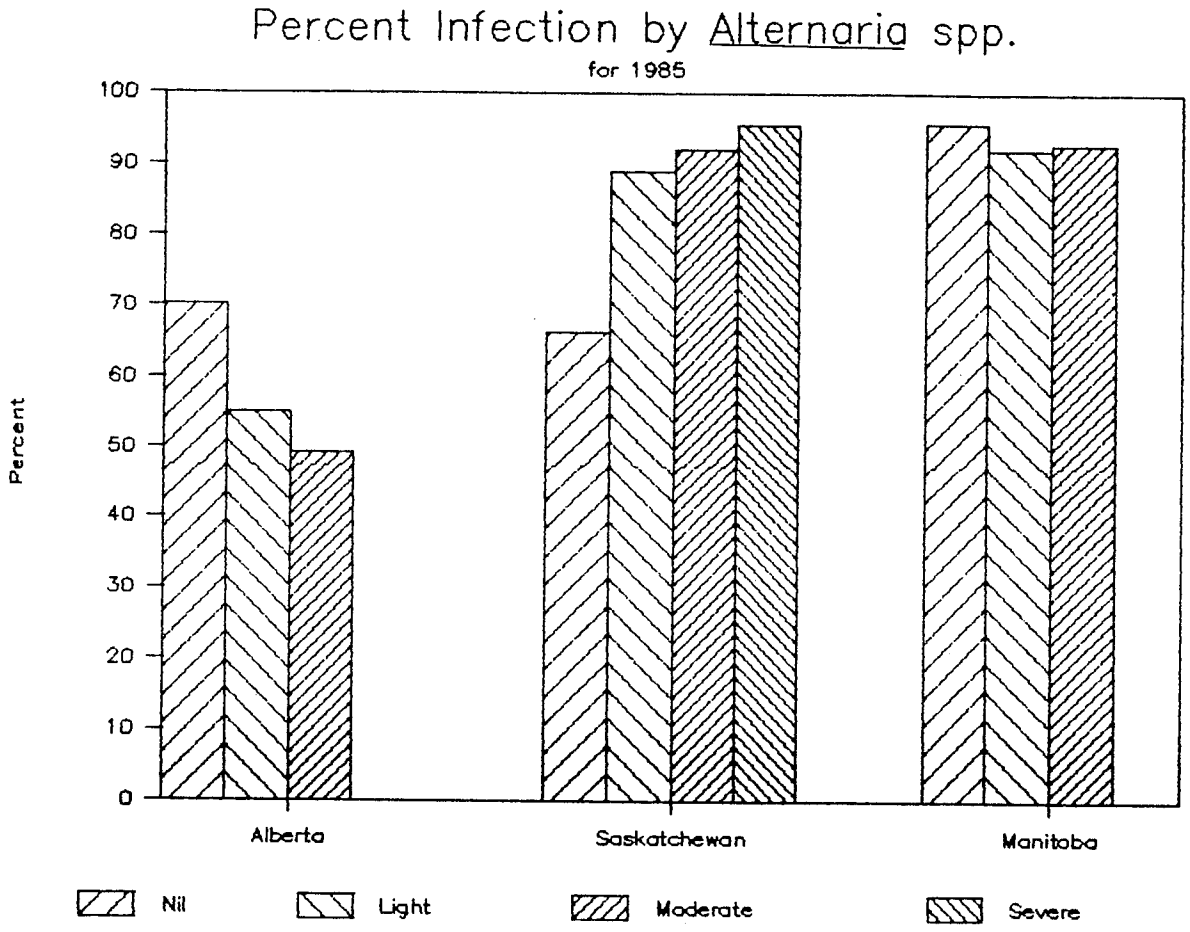


Figure 2A

Percent Infection by
Cladosporium spp.
in Surface Sterilized Hard Red Spring Wheat Seed
Displaying Various Degrees of Mildew

1983	Number of samples examined			
Degree of Mildew	Nil	Light	Moderate	Severe
Alberta	17	30	25	3
Saskatchewan		No data available		
Manitoba	20	11	23	4

Percent *Cladosporium* spp.				
Alberta	18.59	14.00	10.50	8.70
Saskatchewan	0.00	0.00	0.00	0.00
Manitoba	1.90	2.20	1.70	1.50

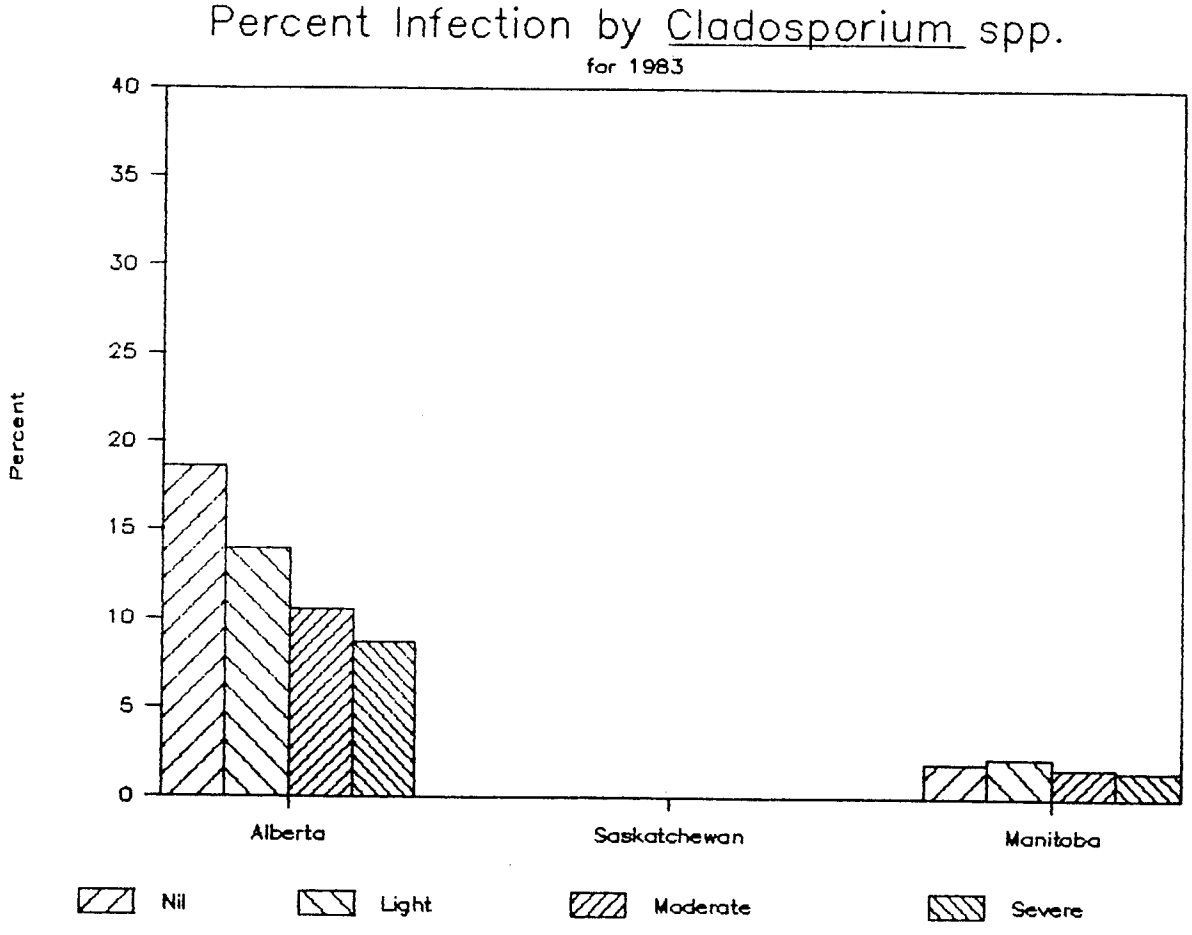


Figure 2B

Percent Infection by
Cladosporium spp.
in Surface Sterilized Hard Red Spring Wheat Seed
Displaying Various Degrees of Mildew

1984 Number of samples examined

Degree of Mildew	Nil	Light	Moderate	Severe
Alberta	47	33	33	11
Saskatchewan		No data available		
Manitoba		No data available		

Percent *Cladosporium* spp.

Alberta	17.70	28.10	23.82	39.60
Saskatchewan	0.00	0.00	0.00	0.00
Manitoba	0.00	0.00	0.00	0.00

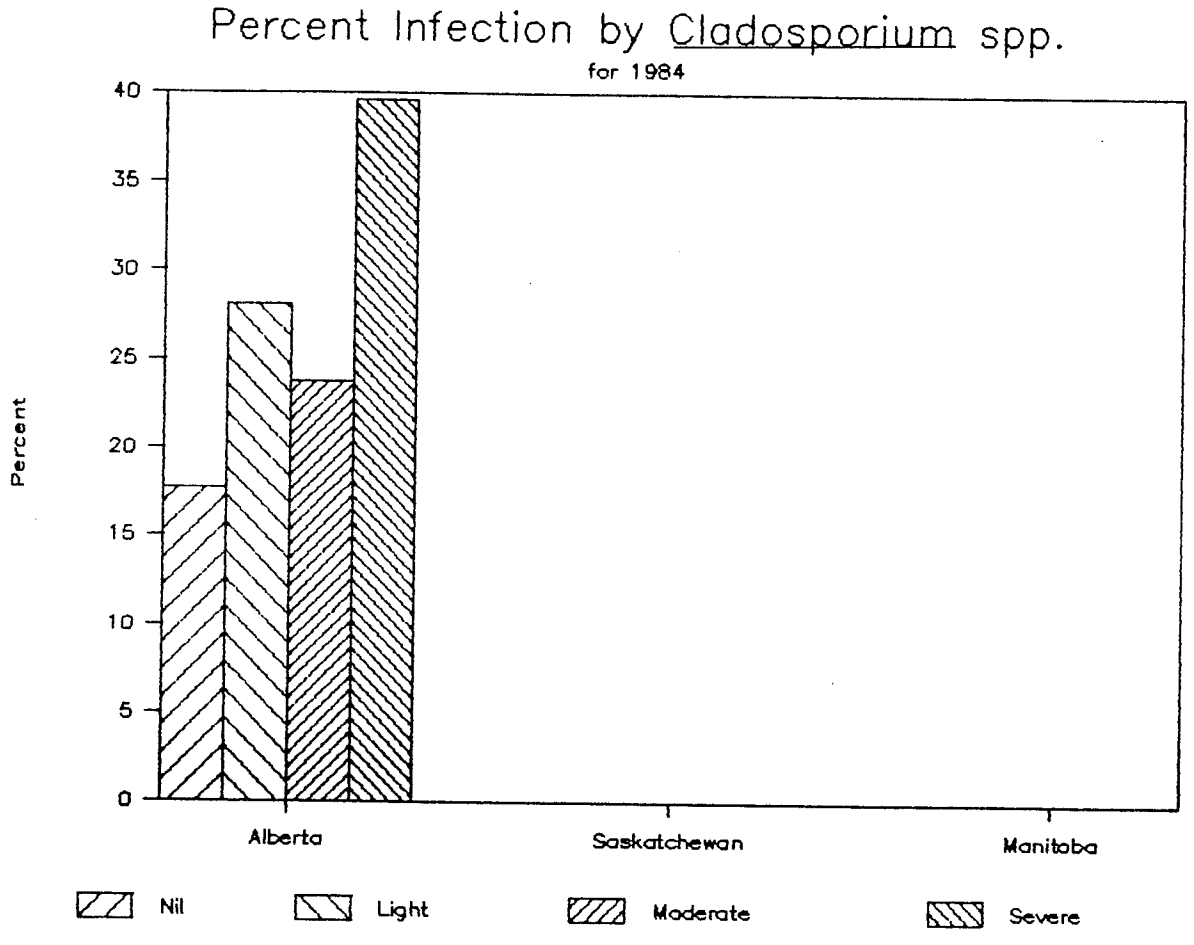


Figure 2C

Percent Infection by
Cladosporium spp.
in Surface Sterilized Hard Red Spring Wheat Seed
Displaying Various Degrees of Mildew

1985 Number of samples examined

Degree of Mildew	Nil	Light	Moderate	Severe
Alberta	9	21	28	0
Saskatchewan	58	36	39	2
Manitoba	7	30	29	0

Percent *Cladosporium* spp.

Alberta	7.44	13.14	17.39	0.00
Saskatchewan	11.19	15.33	10.92	4.00
Manitoba	2.71	3.13	3.83	0.00

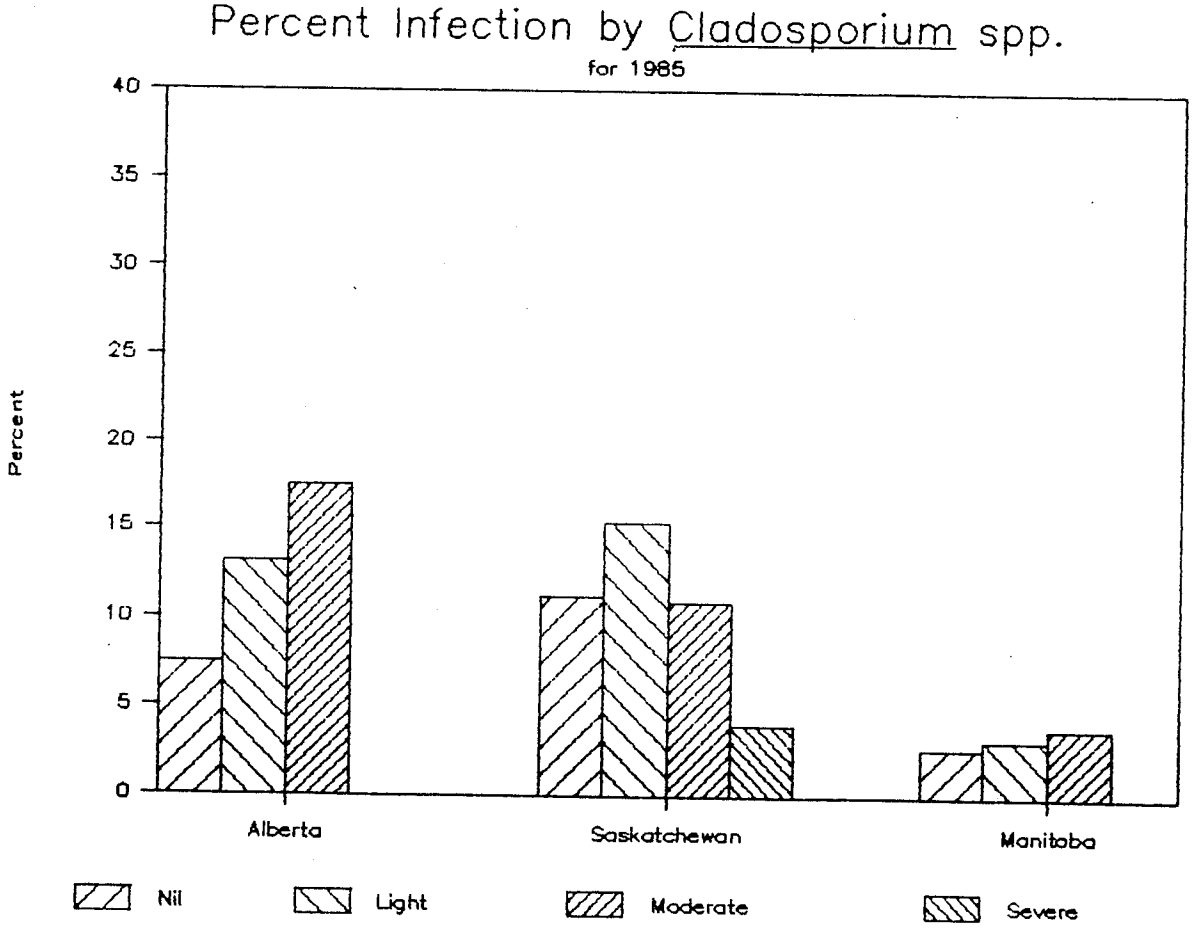


Figure 3A

Percent Infection by
Epicoccum nigrum
in Surface Sterilized Hard Red Spring Wheat Seed
Displaying Various Degrees of Mildew

1983 Number of samples examined

Degree of Mildew	Nil	Light	Moderate	Severe
Alberta	17	30	25	3
Saskatchewan		No data available		
Manitoba	20	11	23	4

Percent *Epicoccum nigrum*

Alberta	4.71	9.14	12.60	4.00
Saskatchewan	0.00	0.00	0.00	0.00
Manitoba	1.00	3.30	2.10	1.50

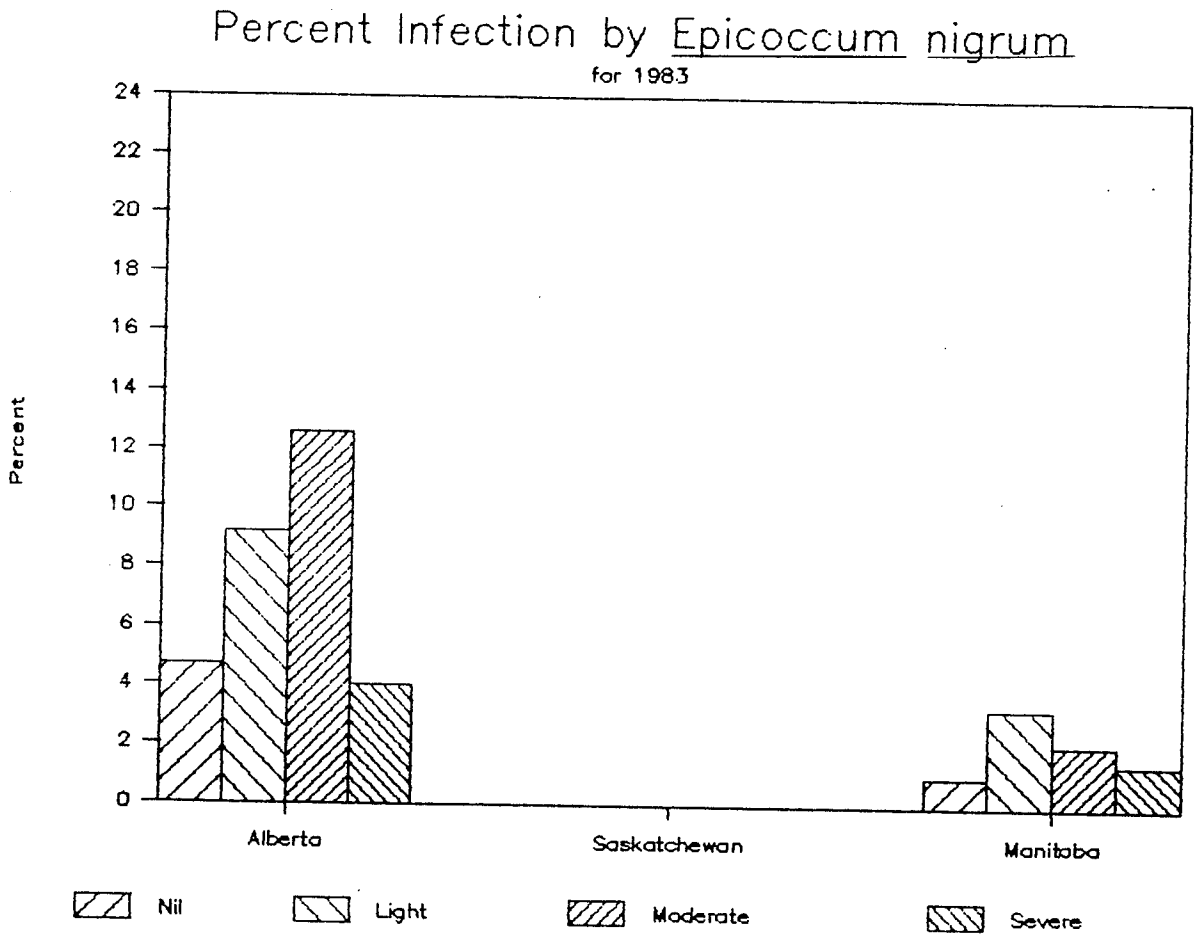


Figure 3B

Percent Infection by
Epicoccum nigrum
in Surface Sterilized Hard Red Spring Wheat Seed
Displaying Various Degrees of Mildew

1984 Number of samples examined

Degree of Mildew	Nil	Light	Moderate	Severe
Alberta	47	33	33	11
Saskatchewan		No data available		
Manitoba		No data available		

Percent *Epicoccum nigrum*

Alberta	2.80	15.30	18.91	22.20
Saskatchewan	0.00	0.00	0.00	0.00
Manitoba	0.00	0.00	0.00	0.00

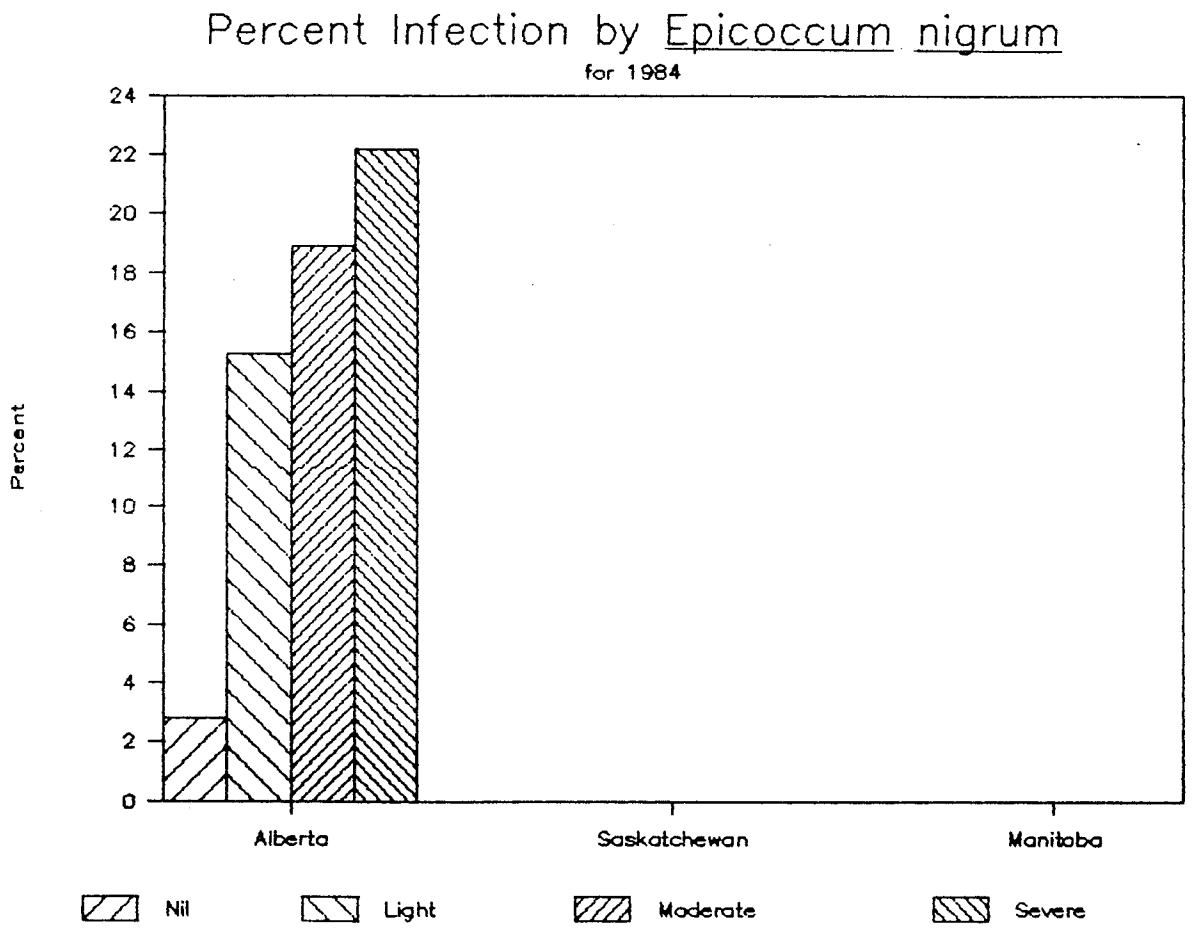


Figure 3C

Percent Infection by
Epicoccum nigrum
in Surface Sterilized Hard Red Spring Wheat Seed
Displaying Various Degrees of Mildew

1985 Number of samples examined

Degree of Mildew	Nil	Light	Moderate	Severe
Alberta	9	21	28	0
Saskatchewan	58	36	39	2
Manitoba	7	30	29	0

Percent *Epicoccum nigrum*

Alberta	11.30	14.40	11.90	0.00
Saskatchewan	4.40	9.70	7.10	3.00
Manitoba	3.60	5.60	6.60	0.00

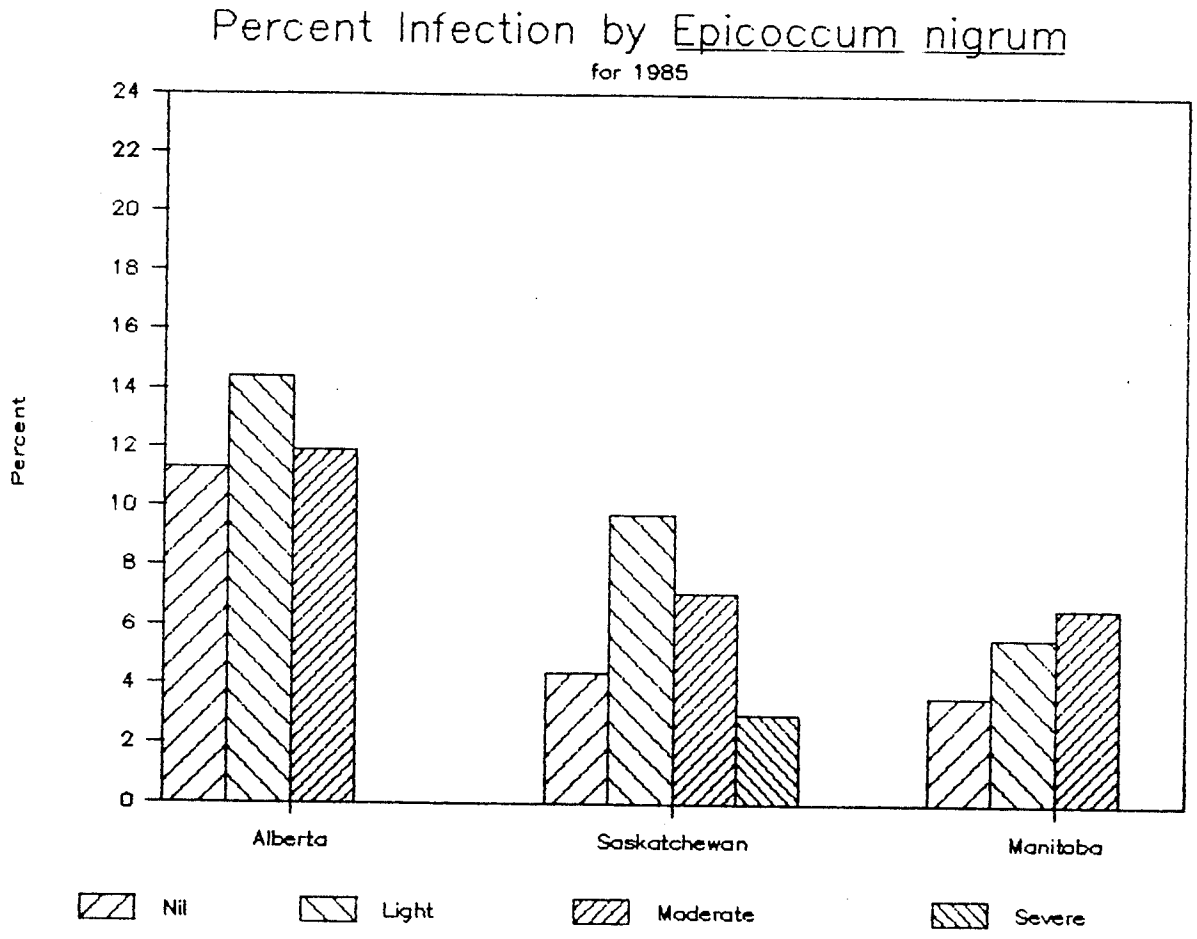


Figure 4A

Percent Infection by
Drechslera sorokiniana
in Surface Sterilized Hard Red Spring Wheat Seed
Displaying Various Degrees of Mildew

1983 Number of samples examined

Degree of Mildew	Nil	Light	Moderate	Severe
Alberta	17	30	25	3
Saskatchewan		No data available		
Manitoba	20	11	23	4

Percent Drechslera sorokiniana

Alberta	1.20	1.00	2.00	1.30
Saskatchewan	0.00	0.00	0.00	0.00
Manitoba	5.10	7.80	14.60	10.50

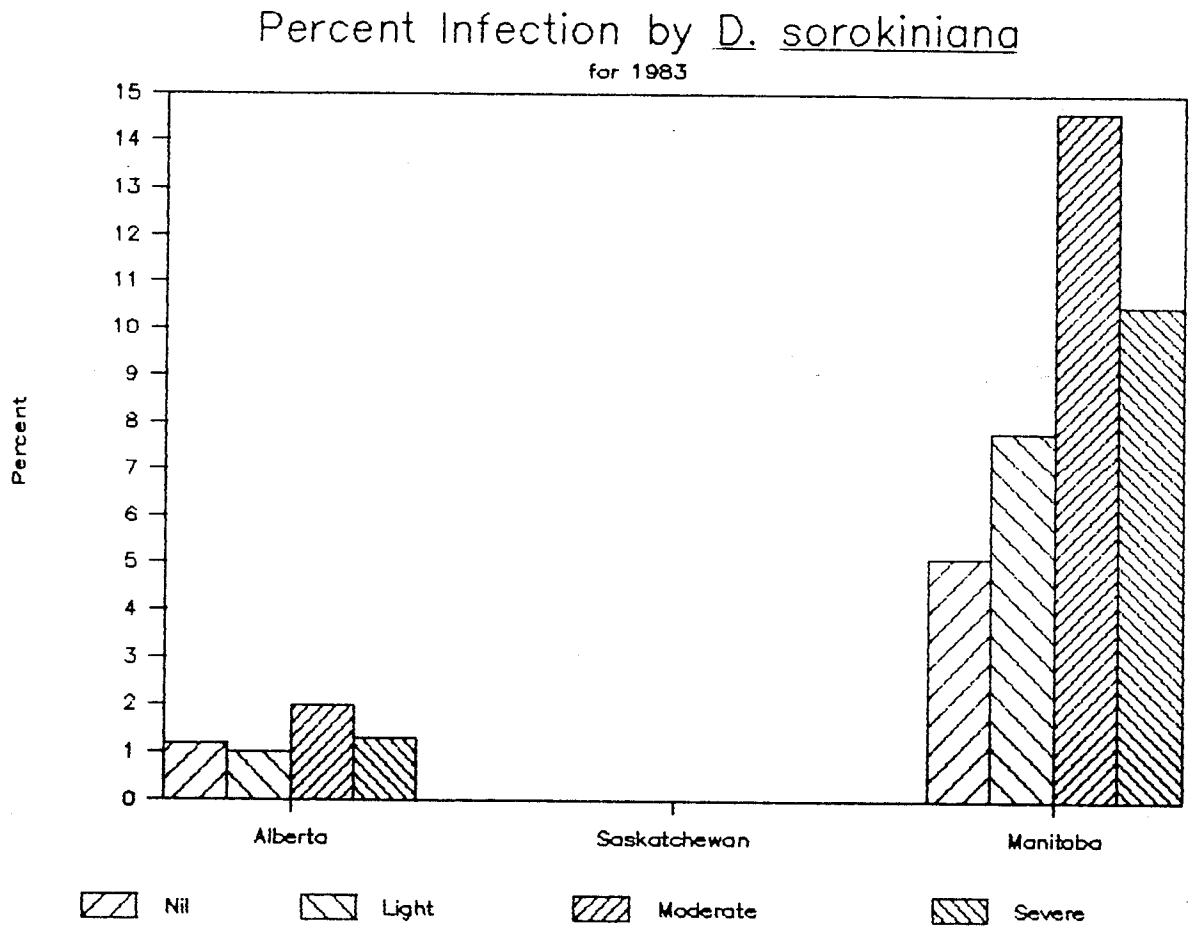


Figure 4B

Percent Infection by
Drechslera sorokiniana
in Surface Sterilized Hard Red Spring Wheat Seed
Displaying Various Degrees of Mildew

1984 Number of samples examined

Degree of Mildew	Nil	Light	Moderate	Severe
Alberta	47	33	33	11
Saskatchewan		No data available		
Manitoba		No data available		

Percent *Drechslera sorokiniana*

Alberta	0.70	0.40	0.40	0.70
Saskatchewan	0.00	0.00	0.00	0.00
Manitoba	0.00	0.00	0.00	0.00

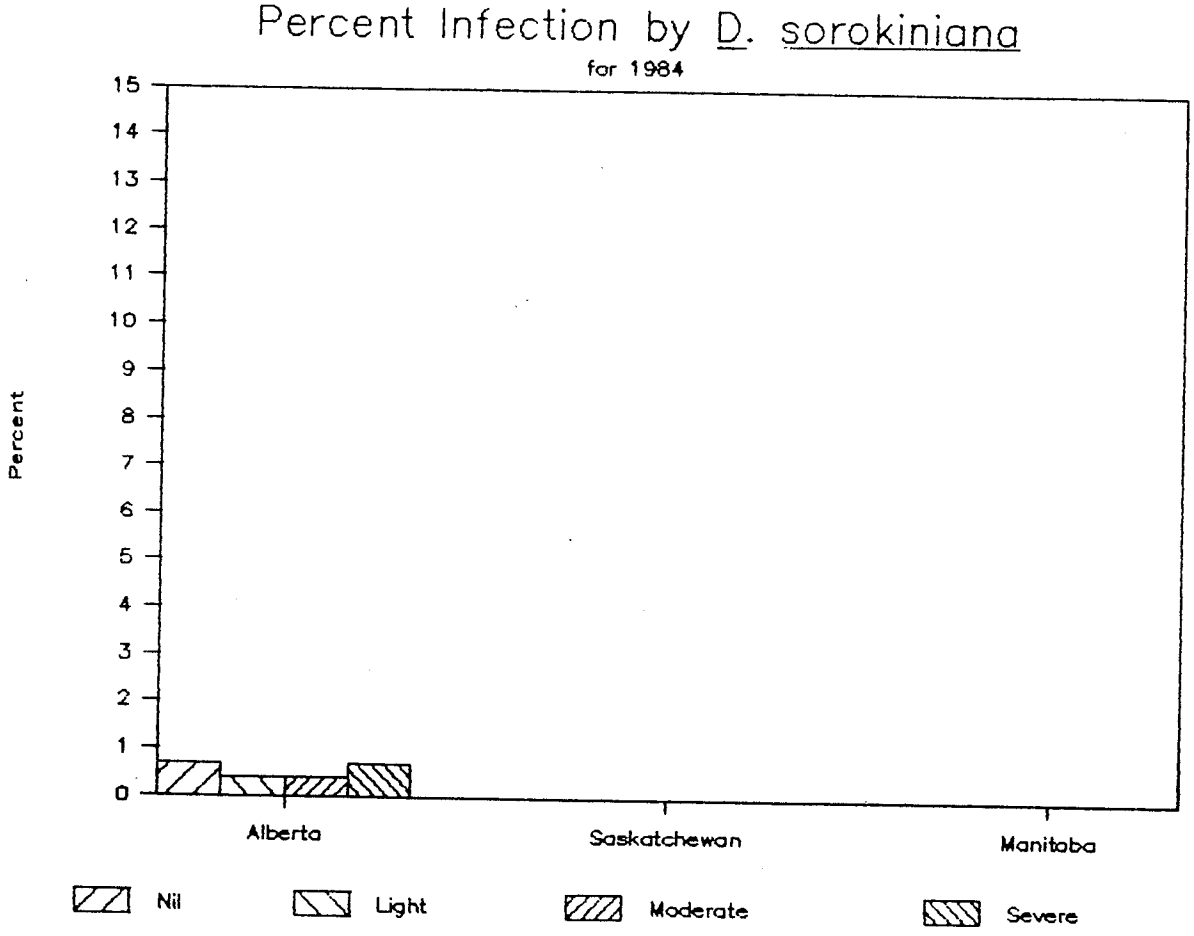


Figure 4C

Percent Infection by
Drechslera sorokiniana
in Surface Sterilized Hard Red Spring Wheat Seed
Displaying Various Degrees of Mildew

1985 Number of samples examined

Degree of Mildew	Nil	Light	Moderate	Severe
Alberta	9	21	28	0
Saskatchewan	58	36	39	2
Manitoba	7	30	29	0

Percent *Drechslera sorokiniana*

Alberta	0.40	0.30	0.50	0.00
Saskatchewan	1.10	2.40	4.40	6.00
Manitoba	6.30	5.80	8.20	0.00

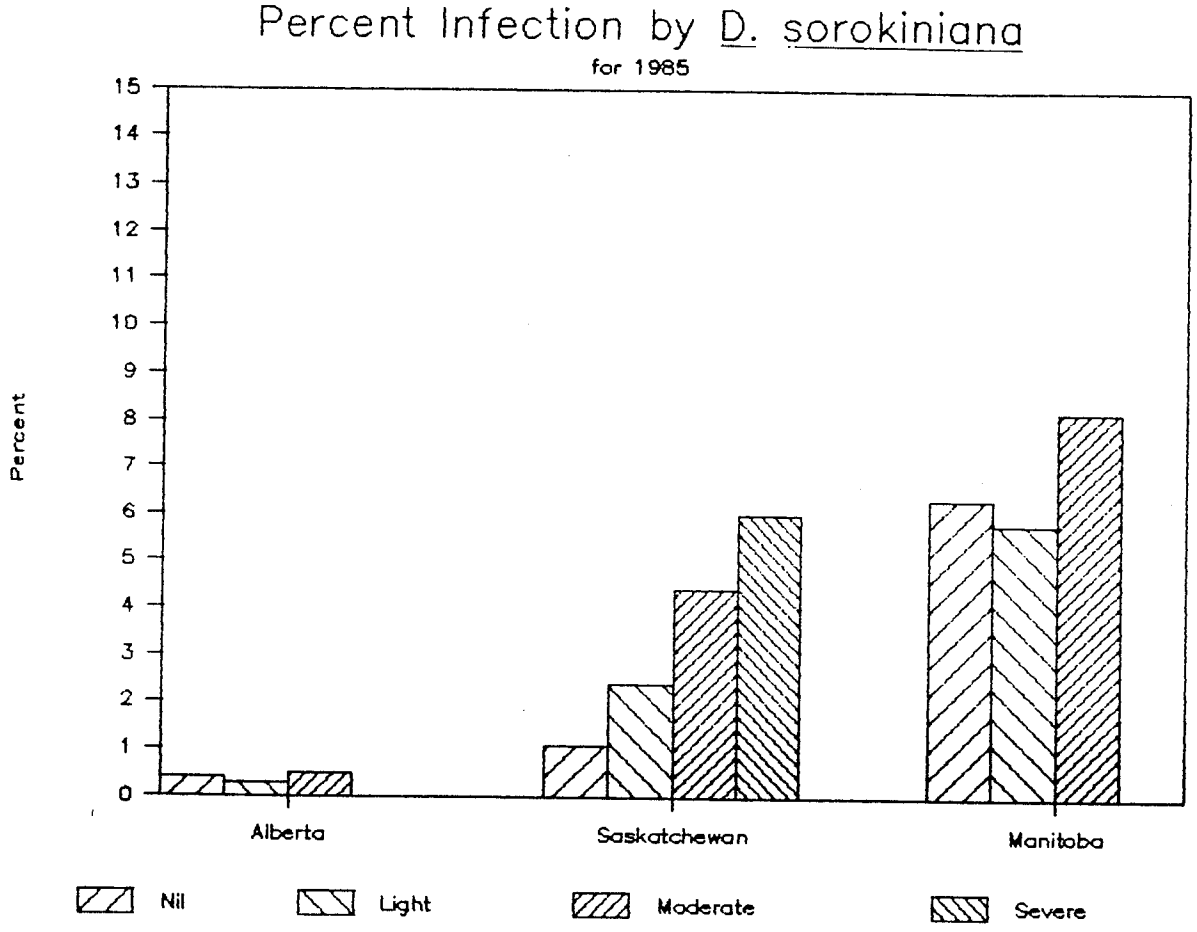


Figure 5A

Percent Infection by
Nigrospora oryzae
in Surface Sterilized Hard Red Spring Wheat Seed
Displaying Various Degrees of Mildew

1983	Number of samples examined			
Degree of Mildew	Nil	Light	Moderate	Severe
Alberta	17	30	25	3
Saskatchewan		No data available		
Manitoba	20	11	23	4

Percent Nigrospora oryzae				
Alberta	0.60	0.50	0.20	0.00
Saskatchewan	0.00	0.00	0.00	0.00
Manitoba	4.80	5.50	3.60	3.00

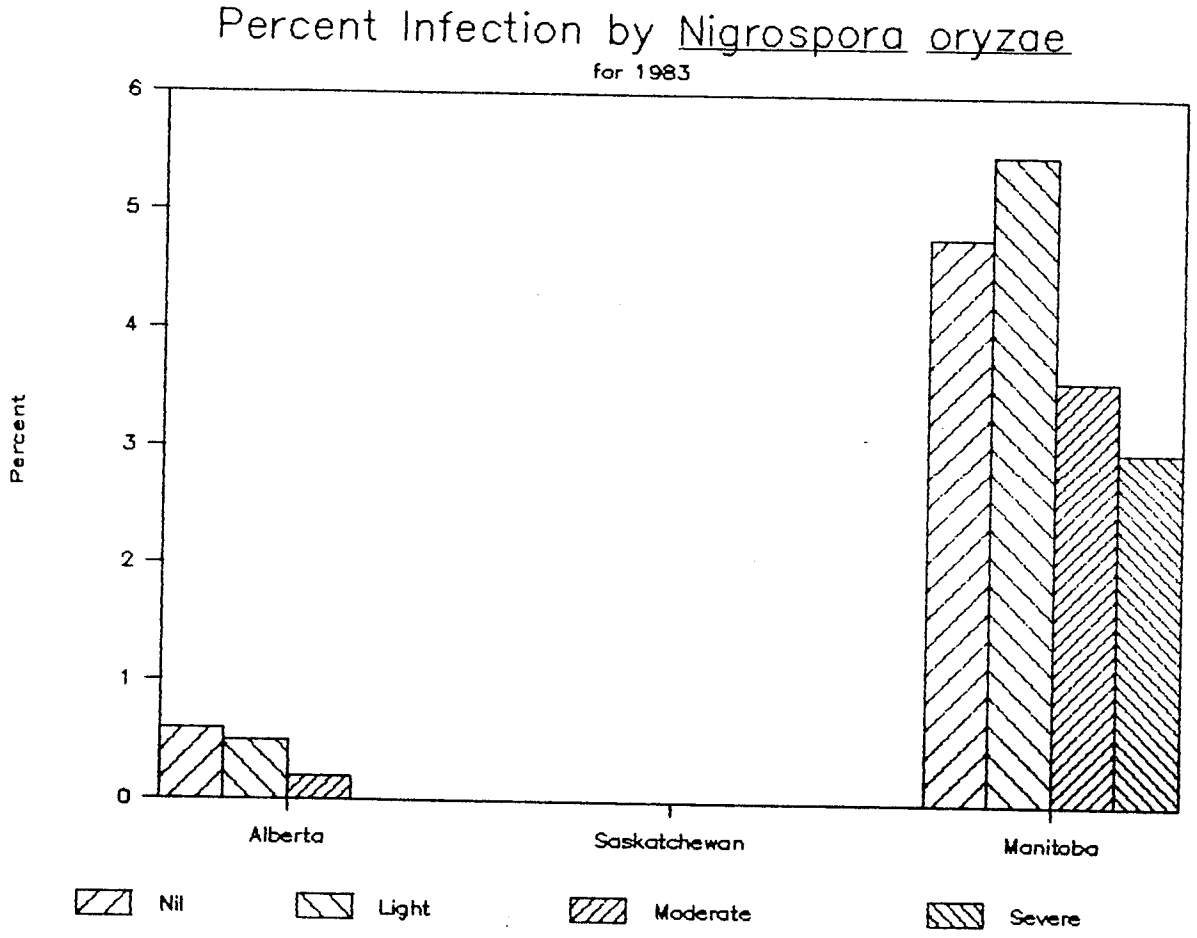


Figure 5B

Percent Infection by
Nigrospora oryzae
in Surface Sterilized Hard Red Spring Wheat Seed
Displaying Various Degrees of Mildew

1984

Number of samples examined				
Degree of Mildew	Nil	Light	Moderate	Severe
Alberta	47	33	33	11
Saskatchewan		No data available		
Manitoba		No data available		

Percent *Nigrospora oryzae*

Alberta	1.50	1.20	1.40	0.40
Saskatchewan	0.00	0.00	0.00	0.00
Manitoba	0.00	0.00	0.00	0.00

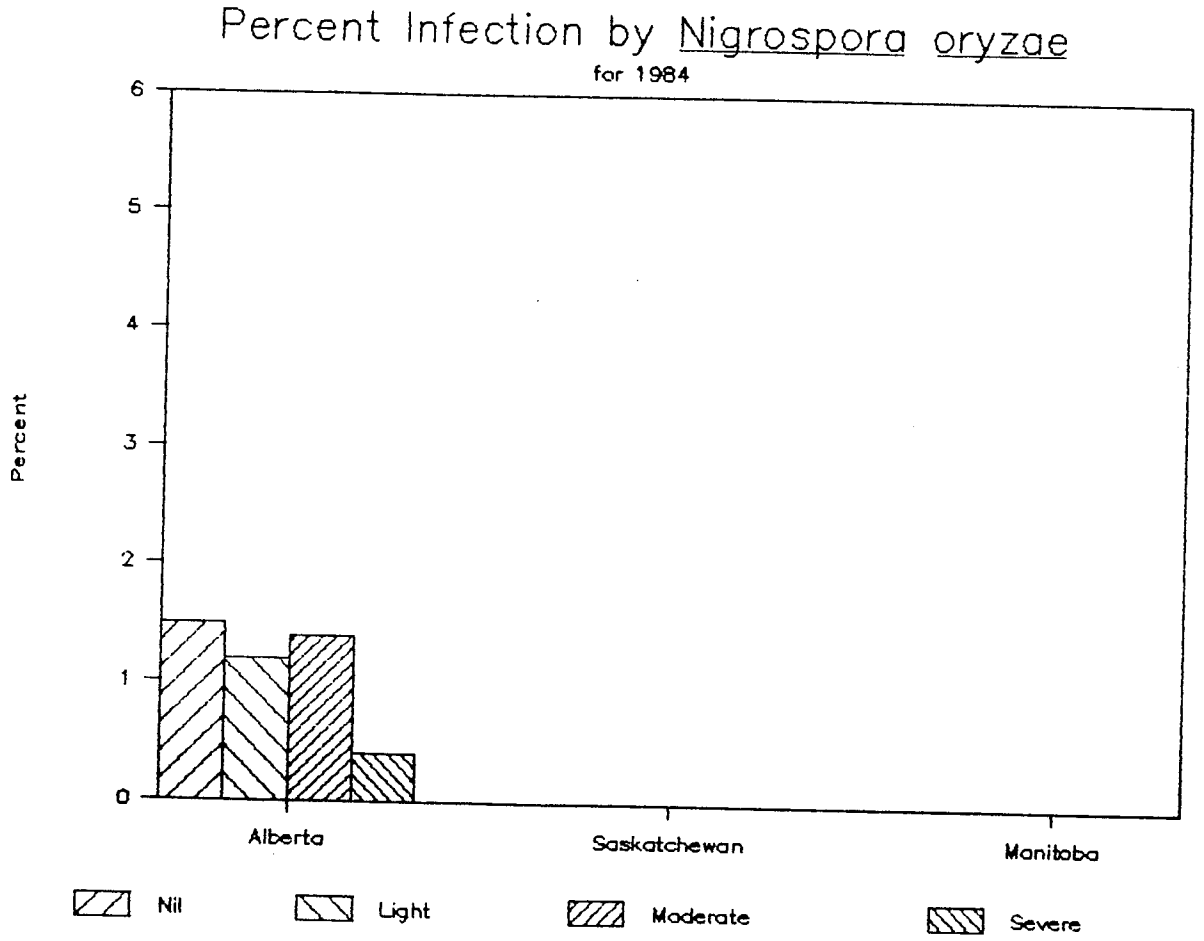


Figure 5C

Percent Infection by
Nigrospora oryzae
in Surface Sterilized Hard Red Spring Wheat Seed
Displaying Various Degrees of Mildew

1985 Number of samples examined

Degree of Mildew	Nil	Light	Moderate	Severe
Alberta	9	21	28	0
Saskatchewan	58	36	39	2
Manitoba	7	30	29	0

Percent *Nigrospora oryzae*

Alberta	1.11	0.86	0.43	0.00
Saskatchewan	2.90	3.31	4.57	2.00
Manitoba	3.14	3.33	3.83	0.00

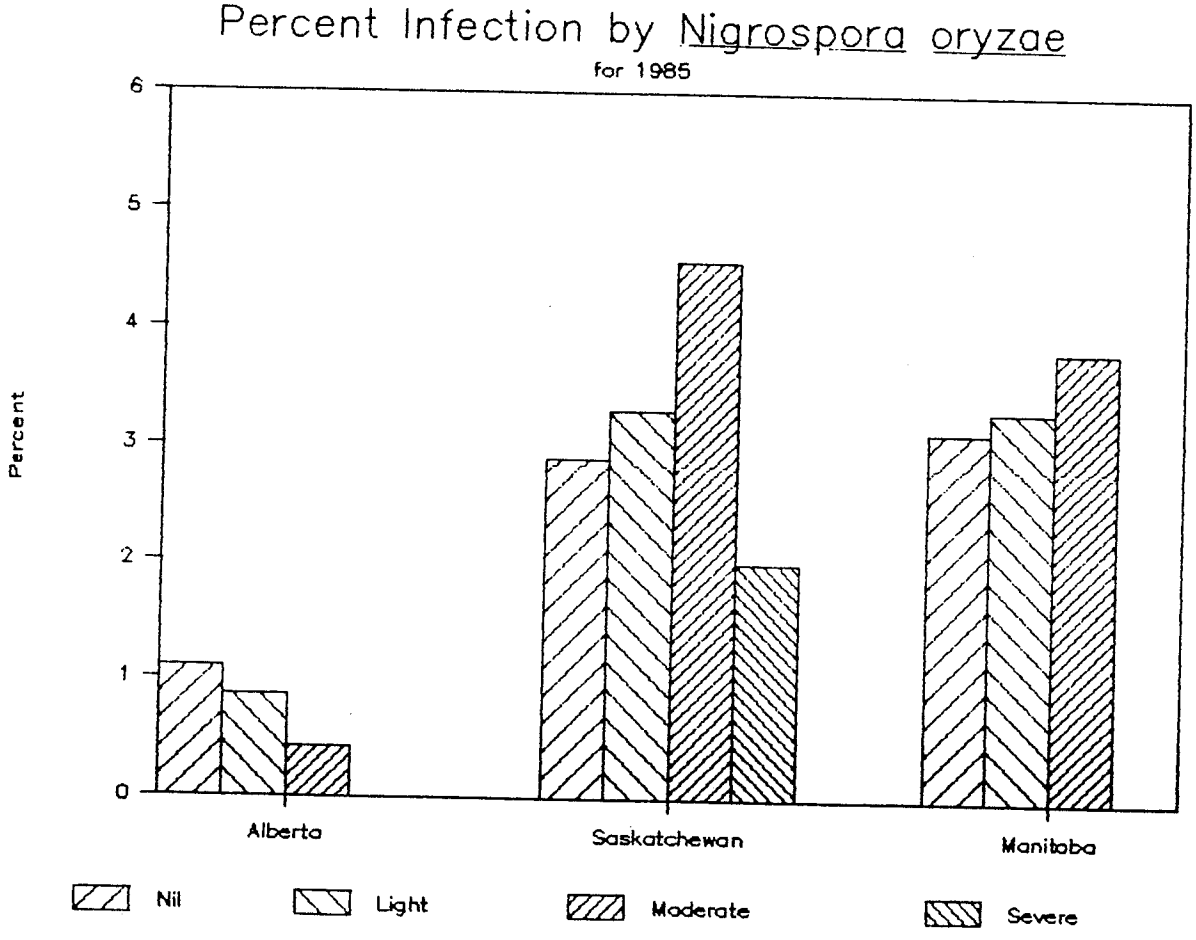


Figure 6A

Percent Infection by
Bacteria
in Surface Sterilized Hard Red Spring Wheat Seed
Displaying Various Degrees of Mildew

1983	Number of samples examined			
Degree of Mildew	Nil	Light	Moderate	Severe
Alberta	17	30	25	3
Saskatchewan		No data available		
Manitoba	20	11	23	4
Percent Bacteria				
Alberta	6.59	12.00	18.00	34.70
Saskatchewan	0.00	0.00	0.00	0.00
Manitoba	5.20	12.00	10.20	12.50

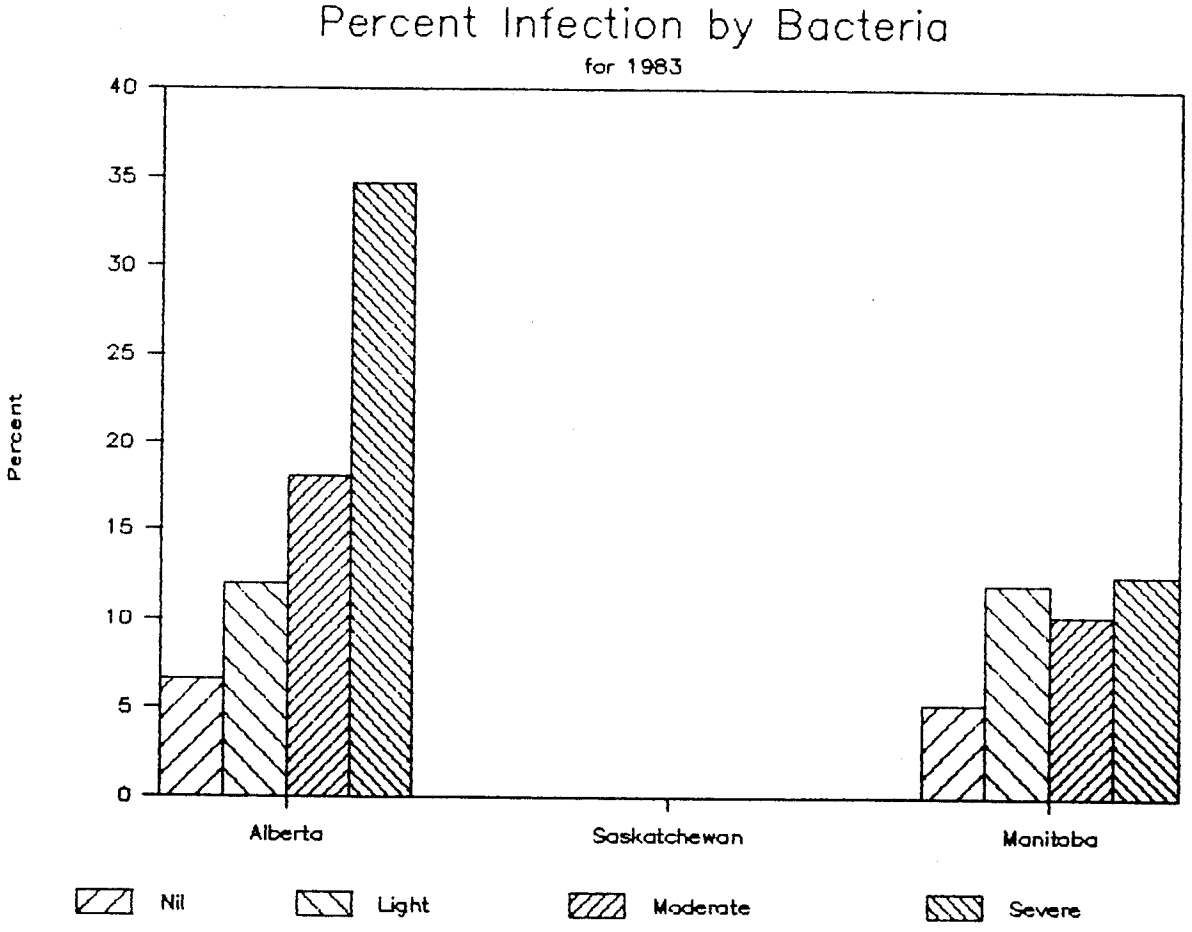


Figure 6B

Percent Infection by
Bacteria
in Surface Sterilized Hard Red Spring Wheat Seed
Displaying Various Degrees of Mildew

1984 Number of samples examined

Degree of Mildew	Nil	Light	Moderate	Severe
Alberta	47	33	33	11
Saskatchewan		No data available		
Manitoba		No data available		

Percent Bacteria

Alberta	3.40	15.90	18.30	16.90
Saskatchewan	0.00	0.00	0.00	0.00
Manitoba	0.00	0.00	0.00	0.00

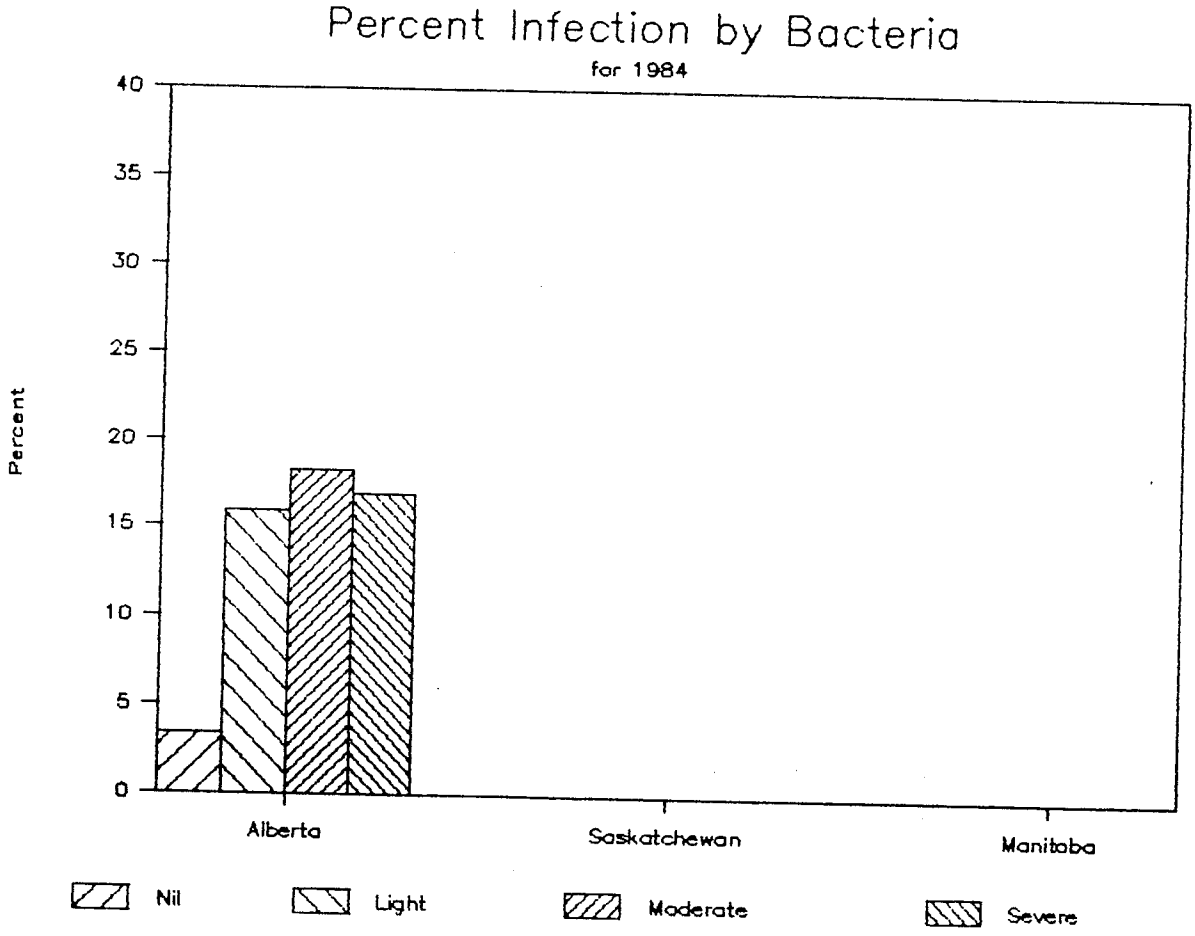


Figure 6C

Percent Infection by
Bacteria
in Surface Sterilized Hard Red Spring Wheat Seed
Displaying Various Degrees of Mildew

1985

Number of samples examined

Degree of Mildew	Nil	Light	Moderate	Severe
Alberta	9	21	28	0
Saskatchewan	58	36	39	2
Manitoba	7	30	29	0

Percent Bacteria

Alberta	5.30	26.70	36.40	0.00
Saskatchewan	4.80	13.90	21.30	25.00
Manitoba	7.90	23.20	30.10	0.00

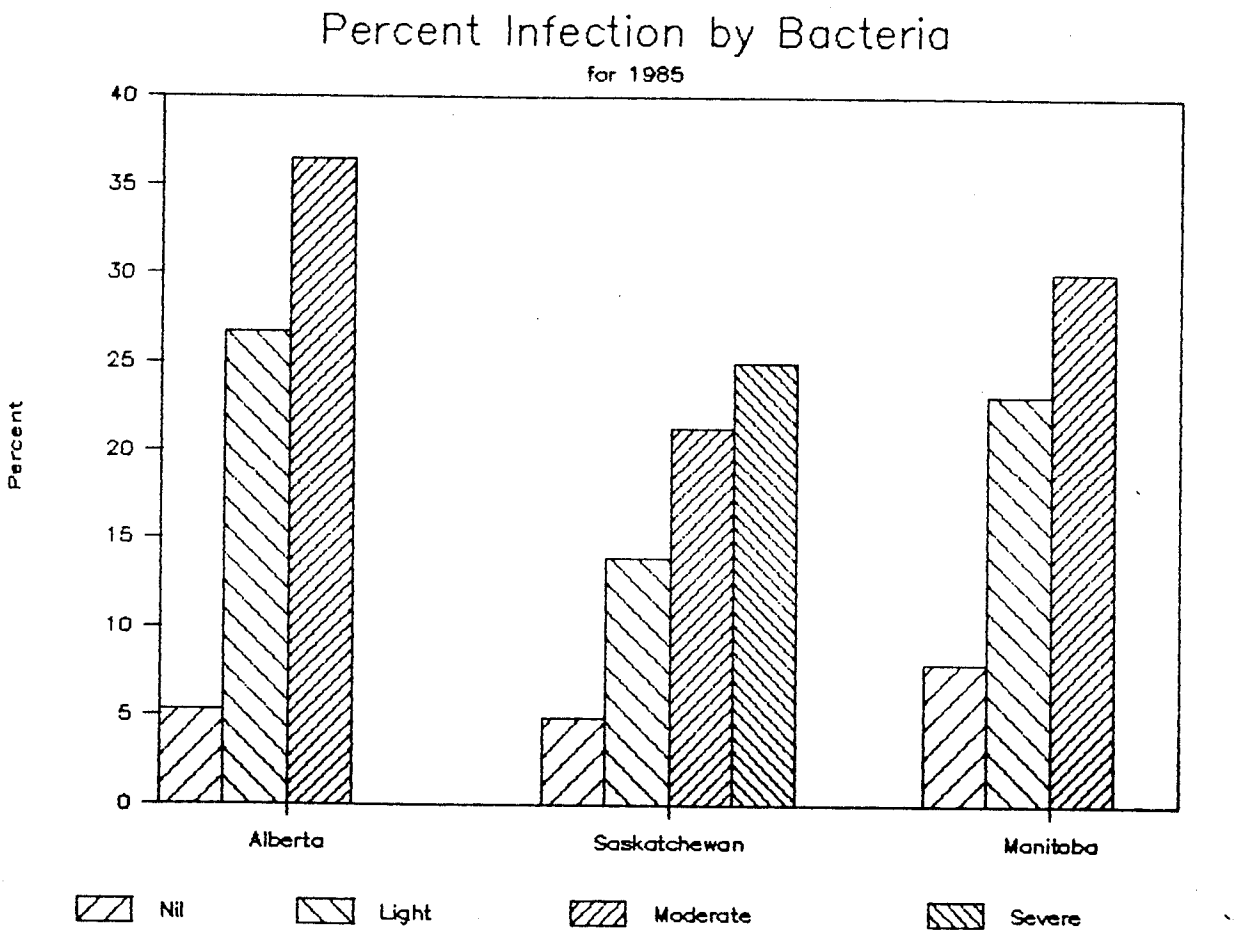


Figure 7A

A comparison between randomly selected seeds of samples graded as having moderate or severe mildew levels, and selected seeds which displayed mildew characteristics from the same samples.

Alberta

Fungi	Code	Random	Selected
Alternaria spp.	A	46.60	45.20
Cladosporium spp.	C	18.10	30.50
D. sorokiniana	D	0.43	0.29
Epicoccum nigrum	E	12.10	12.60

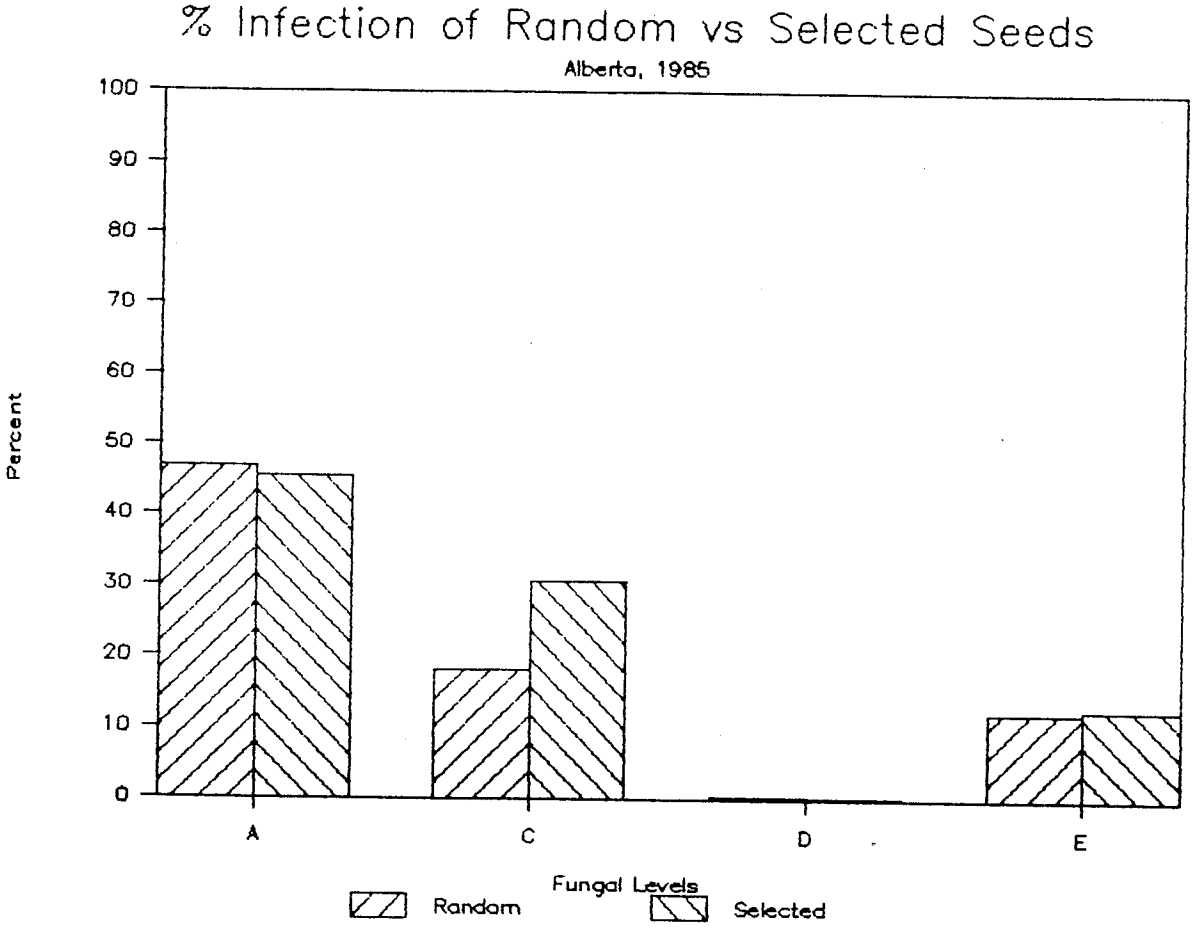


Figure 7B

A comparison between randomly selected seeds of samples graded as having moderate or severe mildew levels, and selected seeds which displayed mildew characteristics from the same samples.

Saskatchewan

Fungi	Code	Random	Selected
Alternaria spp.	A	91.40	93.60
Cladosporium spp.	C	14.30	25.10
D. sorokiniana	D	4.57	3.43
Epicoccum nigrum	E	7.21	10.14

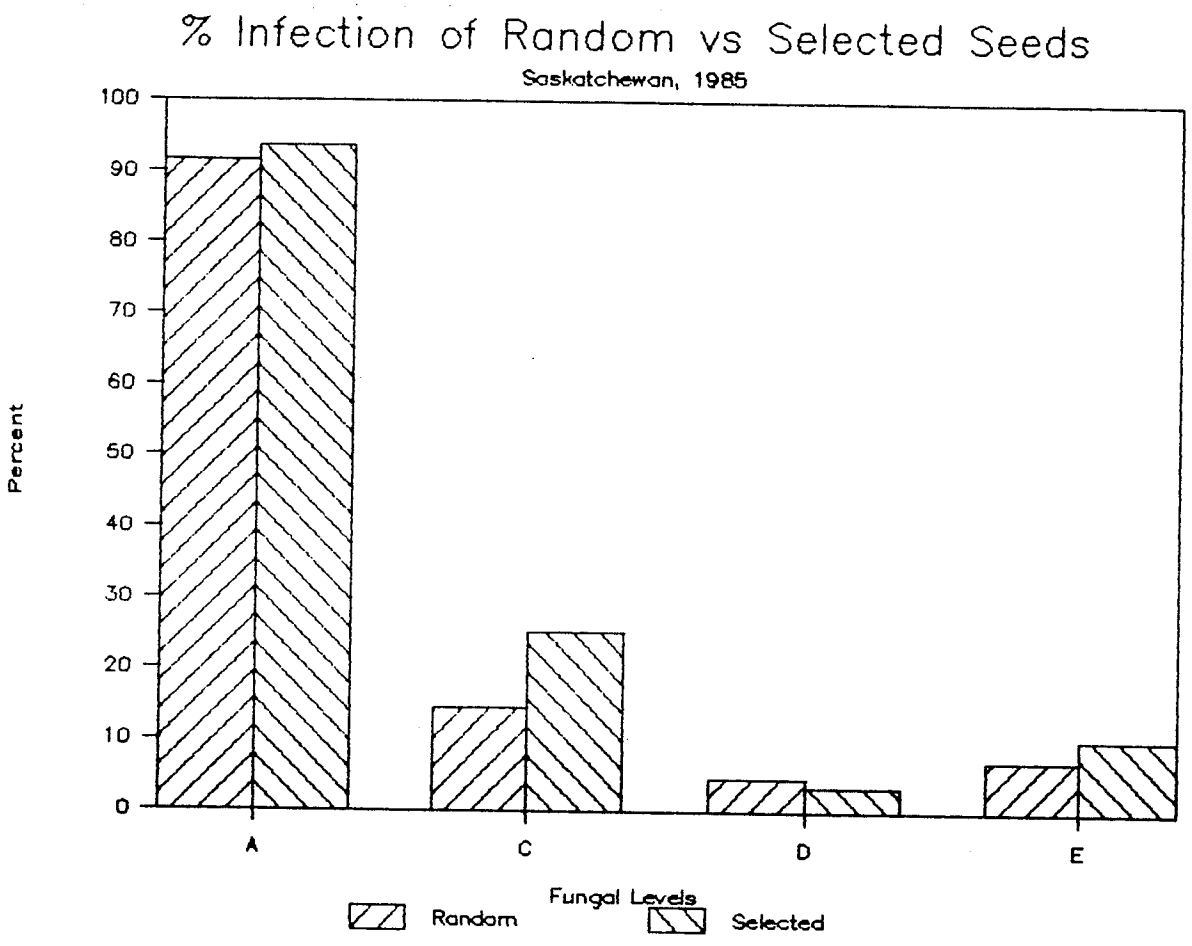
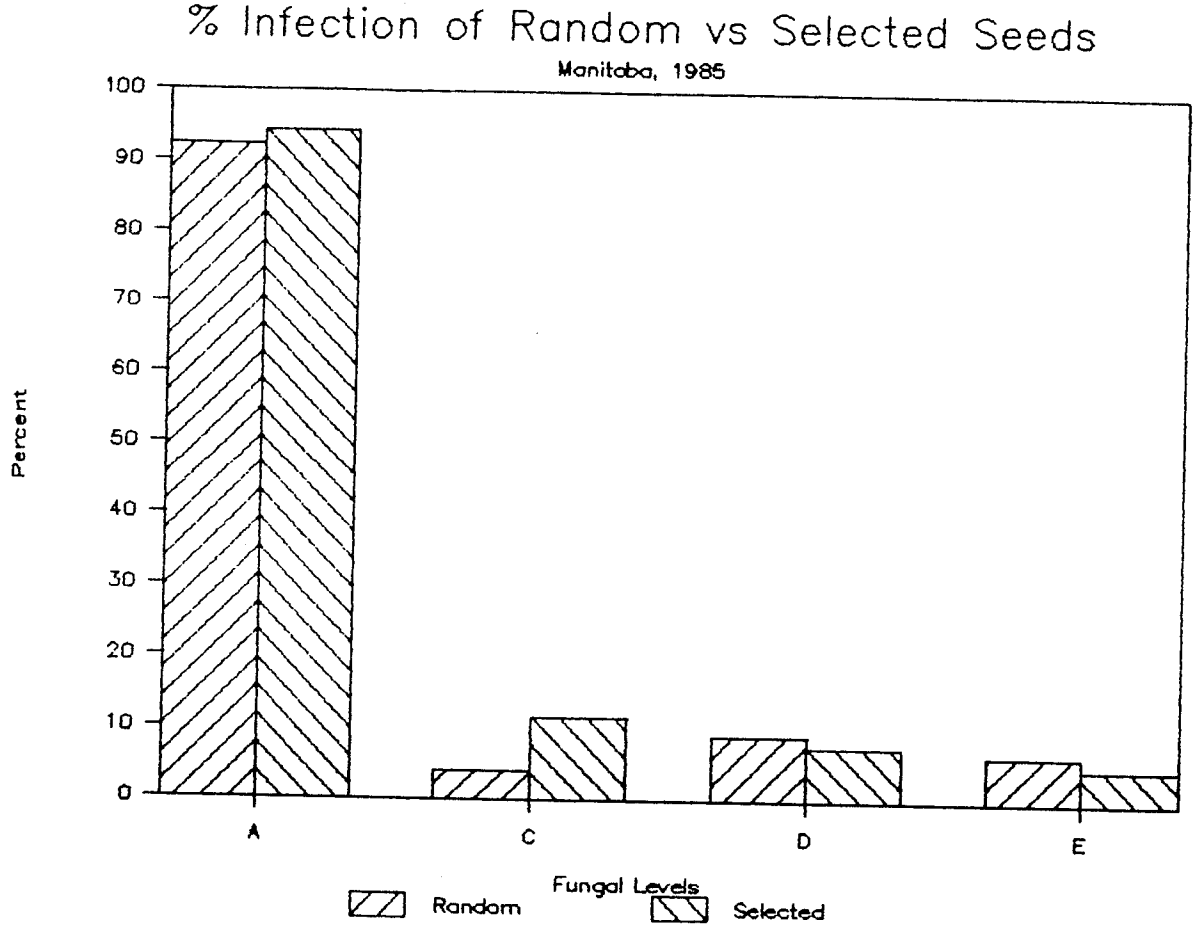


Figure 7C

A comparison between randomly selected seeds of samples graded as having moderate or severe mildew levels, and selected seeds which displayed mildew characteristics from the same samples.

Manitoba

Fungi	Code	Random	Selected
Alternaria spp.	A	92.20	94.10
Cladosporium spp.	C	4.00	11.60
D. sorokiniana	D	9.00	7.60
Epicoccum nigrum	E	6.50	4.80



DISCUSSION

1.) Fungal Isolation Techniques

a) Surface Sterilization

The relevance of the criticisms of Harmon and Pfleger (1974) and Sauer and Burroughs (1986) to the use of NaOC1 treatment in the present study is somewhat reduced when it is considered that mildew is a pre-, not post-harvest phenomenon. However, what applied for the Aspergilli and Penicillia, may also apply in different degrees for other fungi. Standardizing the conditions and comparing results directly, however, makes it possible to detect patterns and trends without necessarily being certain that one's results are exactly and only formed by the organisms growing on the seed.

b) Media for Isolation of Field Fungi

Which medium was to be used, and its method of use was determined by four factors: i) agar-plate tests detect a greater range of saprophytes than a blotter test (Flannigan, 1969)

ii) the period of plant development when the mildew discoloration occurs corresponds to when field fungi (as defined by Christensen and kaufmann, 1965) are predominant,

iii) many descriptions of these field fungi are based on cultures grown on potato dextrose agar (PDA), a medium on which many thrive and

iv) a preliminary test showed PDA supported good growth and, when combined with UV light treatment, induced good sporulation by the developing fungi.

The use of broad spectrum media such as PDA was preferred over selective media since no specific target organism was the object of this study, but rather a group of organisms known as seed-borne field fungi.

c) Isolation of Storage Fungi

A primary distinction between field and storage fungi is the latter's ability to grow under higher osmotic pressure. Since the latter's ecological niche is the storage environment, and the discoloration of the seed occurred before storage, it was assumed these fungi were not involved in the production of mildewed kernels. However, it is useful to assess the levels of storage fungi as they may be affected in some way by the conditions which produce the mildewed kernels. Most obviously, wet field conditions could result in damp grain being stored with a consequent increase in the levels and variety of storage fungi. The detection of the xerophilic storage fungi was achieved using the SFP method, as it was simple and apparently superior to other methods (Mills et al., 1978).

d) Temperature and Moisture Conditions

As the dominant type of seed-borne field fungi are mesothermotolerant Deuteromycetes (Panasenko, 1967), a temperature in the mid-twenty degree Celsius range seemed most appropriate for fungal isolations. A fluctuating temperature and light regime was incorporated into the incubation conditions, as it is well recognized that

such a regime is the most effective way to induce sporulation in many fungi (Houston and Oswald, 1946; Snyder and Hansen, 1947; Lilly, 1951; Hawker, 1958; Cochrane, 1958; Aragaki, 1961; Lukens, 1966; Leach, 1962, 1967).

In the present experiment, conditions of moisture suitable for the growth of field fungi were achieved by using 3-day old potato dextrose agar plates. By using 3-day old PDA plates, the inhibitory effect of high moisture reported by de Tempe and Limonard (1966) was avoided.

2.) Fungi

Alternaria spp.

Alternaria spp.are normally present as saprophytes and are frequently the causal agent of other seed discolorations such as blackpoint (Brentzel, 1944; Machacek and Greaney, 1938). They have also been reported to be toxic to animals (Joffe, 1965) possibly due to production of mycotoxins such as alternariol, alternariol methyl ether, altenuene, tenuazonic acid and altertoxin I (Meronuck et al., 1972; Harvan and Pero, 1976; Bruce et al., 1984; Stinson, 1985; Wei and Swartz, 1985). Machacek et al. (1951) reported Alternaria spp. to be less common on wheat seed grown in Alberta than Manitoba, which is in agreement with the present findings where the difference was found to be significant at the 0.01 level (Table 2). They also grew rapidly on the PDA media, and this likely resulted in the inhibition of slower growing, less aggressive fungi (Boyer, 1955).

In the field, Alternaria spp. normally invade the seed while it is still green, becoming dormant in the outer layers of the seed when the grain matures. Wet harvest conditions allow the fungus to develop further. Although this continued growth, along with new infections, does not appear to affect the germinability of the seed (Machacek and Greaney, 1938; Brentzel, 1944; Hanson and Christensen, 1953), they are likely responsible for the production of many of the mildewed kernels in a typical sample. The presence of the mildewed kernels was found to positively affect the levels of Alternaria spp. in 1983 and 1984 (Table 2). The lack of significance in 1985 may be due to three factors. First, the small number of Manitoba and Alberta samples with no mildew and secondly the high levels of Alternaria spp. in both mildewed and non-mildewed samples. The latter was likely due to the wet growing conditions which resulted in more kernels being infected during growth of the plant, but which did not show the mildew discoloration associated with growth after crop maturity. The third factor was the extra competition from other fungi on the mildewed seeds. The combination of these three factors may account for the lack of significance of the effect of mildew on Alternaria spp. in 1985. Certainly the abundance of Alternaria spp. and their recognized ability to discolor grain must place them as primary agents of the mildew discoloration of wheat.

Cladosporium spp.

Cladosporium spp. commonly occur on dead and dying plant substrates (Domsch et al., 1980) and are a well-known cause of wood staining (Hedgcock, 1906). Growth of these fungi can also cause grain discoloration. Baker et al. (1958) found high levels of Cladosporium spp. on British wheat in 1956 after a cold, wet harvest. They noticed superficial growth on the brush end of the kernels, which sometimes discolored the bran of the seed and resulted in poor flour color after milling. In all likelihood, this wheat would have been graded mildewed under the Canadian grain grading guide-lines. In the present study, Cladosporium spp. were normally associated with the brush end of the seed, and dark fungal structures which Baker et al. (1958) called sclerotia were sometimes observed forming streaks or circular areas of dark discoloration just beneath the epidermis of the seed (plate 2, 4). Cladosporium spp. were among the dominant mycofloral components in growing areas where cool, wet weather at harvest is common (Alberta). However, it is possible these fungi, as well as others, were detected less often in Manitoba because of the greater abundance of Alternaria spp. masking their presence on the grain. The levels of Cladosporium spp. were significantly affected by the area in which the seed was grown, and in 1984 and 1985, were related to the presence of mildew (Table 2). No interaction between area and presence of mildew was apparent, indicating that these factors operated independently, both being significant in their own right. The non-significant effect of mildew in 1983 is hard to understand in light of

PLATE 4

Cladosporium spp. Growing on the Brush End of Wheat Seed

the results of 1984 and 1985. The answer may lie in the growing conditions of 1983 or other factors as yet unassessed.

The reported of the presence of Cladosporium spp. at the brush end of discolored wheat after wet harvests, the observation that Cladosporium spp. were usually associated with the brush end of wheat in this study, and the fact that they were significantly more common on the discolored grain of all three provinces (Table 9) support the conclusion that Cladosporium spp. are major factors in the production of mildewed kernels.

Epicoccum nigrum

Machacek et al.(1951) recorded only one species, E. purpurascens Ehrenberg, on cereal grains in Canada. This name is considered a synonym of E. nigrum Link ex Link by Schol-Schwarz and others (1959). It has been reported as both a parasite (Ito and Iwadare, 1934) and a saprophyte (Schol-Schwarz, 1959; Siu, 1951) and is frequently found on dead plant parts. Campbell (1956) reported it as an internal parasite of spores and mycelium of Drechslera sorokiniana, reducing their pathogenic capability.

This fungus was unlikely to be hidden by the growth of other fungi as it produced a distinctive discoloration in the agar and also appeared aggressive in its development on the media. The significantly higher levels of seed infection by this fungus in the mildewed samples (Table 2) indicates it may have contributed to the discoloration of the wheat seeds, especially in Alberta where it was most

common. However, reports of seed discoloration by E. nigrum mention a reddish discoloration combined with visible mycelium and sprouting of the seed (Tekauz et al., 1986).

Drechslera sorokiniana

Commonly referred to as Helminthosporium sativum Pammel, King and Bakke, D. sorokiniana is a well-known pathogen of cereals, causing crown and root rot of wheat in the prairies, as well as head blight, smudge, and black point of seed (Martens et al., 1984). There are also recent reports it can produce a number of mycotoxins such as sterigmatocystin (Maes and Steyn, 1984).

Machacek et al.(1951) isolated D. sorokiniana from wheat seed more frequently in Manitoba (3.6%) than Saskatchewan (0.78%) or Alberta (0.32%), and Wallace (1964) reported an average of 0.3% of the seeds of durum wheat from Alberta and 3.6% of the durum wheat seeds from Manitoba were infected by D. sorokiniana. In the present study, the difference in levels of this fungus between Manitoba, Saskatchewan and Alberta were found to be significant at the 0.01 level of significance (Table 2).

Its relative obscurity in Alberta would preclude it from having a major effect on the production of mildewed kernels in that province, but its ability to discolor grain where it is more abundant cannot be overlooked. It was more frequent in the discolored Manitoba samples, where it was consistently more common than Cladosporium spp. (Table 1). It is unlikely that growth of Alternaria

spp. would have prevented D. sorokiniana from expressing its presence on the seed or agar, as the latter organism is very aggressive and usually inhibited the development of other fungi, including Alternaria spp.

Nigrospora oryzae

Nigrospora oryzae can be either a parasite or saprophyte (Barnett and Hunter, 1972), and has been reported by Wilson et al. (1986) to produce an antibiotic. Machacek et al. (1951) found Nigrospora spp. were "particularly prevalent in Manitoba when excessively wet weather delayed threshing of grain". They identified only one species, N. sphaerica (Sacc.) Mason. Some question remains as to what species of Nigrospora have been identified on cereal seed in Canada since confusion between these two species is not uncommon. For example, Starratt and Loschiavo (1974) misidentified N. oryzae as N. sphaerica. Conners (1967) lists all recorded pathogenic species of fungi found on plants in Canada. N. sphaerica was recorded on wheat, oats and barley, and N. oryzae on several grasses and other crops, including corn, but not on wheat, oats or barley. Another report of a Nigrospora sp. on cereal seed in Canada occurred when Wallace et al. (1976) identified N. oryzae as being present on 12% of the freshly harvested wheat seed in Manitoba in 1976. It may be that growth under different conditions could result in a different spore size, the measurement of which is a key factor in distinguishing between these two species of Nigrospora. Hawker (1958) demonstrated that UV and

visible radiation, temperature and nutrition may affect morphology and size of spores, as well as the gross morphology of colonies.

In agreement with Wallace et al. (1976), only Nigrospora oryzae was identified in this study. Many isolates were examined for spore size, and all were in the range typical of N. oryzae. A culture sent to the BRI was identified as N. oryzae. Cultures of N. sphaerica (confirmed by BRI) obtained from Ontario corn and wheat seed and incubated under identical conditions, produced spores which were definitely larger in size.

Since it was relatively common in Manitoba and Saskatchewan, it may have had a small effect on kernel appearance. Although there have been no reports of this fungus discoloring seed, it must still be considered as a potential factor, especially when it is found at high levels in individual samples, e.g. a 1983 Manitoba sample where 22% of the seeds were infected by N. oryzae. Generally, however, its importance is minor as its overall frequency on seed never exceeded 5.5% nor was its' isolation frequency significantly affected by the presence of mildew (Table 2).

Fusarium spp.

None of the 11 Fusarium spp. isolated could be considered as important factors in the production of mildewed kernels. Their importance, however, may lie in their effect on other parameters of grain quality such as palatability, toxicity, and germination.

Fusarium avenaceum

F. avenaceum was the most commonly isolated species due to its high frequency in Alberta samples, but it was noticeably less common in the other two provinces. This result is similar to that of Gordon (1952) who found F. avenaceum to be the second most frequently isolated Fusarium spp. next to F. poae, from wheat, oat and barley kernels in Canada. However he rarely isolated it from soil in Manitoba cereal plots (Gordon, 1954) or the soil of other provinces (Gordon, 1956). Fusarium avenaceum has been associated with diseases such as fusarium head blight of cereals (Atanasoff, 1920; Martens et al., 1984; Chelkowski et al., 1985) root rot of cereals (Atanasoff, 1920; Booth, 1971), seedling blight (Atanasoff, 1920; Booth and Waterston, 1964), and as a saprophyte (Domsch et al., 1980). Booth (1971) reported it to be more frequent in cold wet areas than warm dry ones. This may be one reason F. avenaceum was the dominant Fusarium spp. from the northern Alberta samples in all three sampling years. Gordon (1944) isolated F. avenaceum from 0.01% of common wheat seeds in Manitoba between 1937-1942, but from none of the root rot samples collected in North and South Dakota in 1940 (Gordon and Sprague, 1941).

There are no reports that F. avenaceum is a mycotoxin producer in field infections. As it was so dominant in Alberta, this implies that mildewed HRS wheat from Alberta would be at a low risk of fusariotoxins.

Fusarium acuminatum

Fusarium acuminatum is cosmopolitan, occurring as a soil saprophyte and a secondary invader associated with foot, root and stem rots (Marasas et al., 1984). Gordon (1944) found F. acuminatum to be the most frequently isolated Fusarium species from common wheat seed in Manitoba, and the third most common in Canada (Gordon, 1952) between 1937 and 1942; however, it was still not commonly seed borne. Gordon and Sprague (1941) recovered F. scirpi var. acuminatum more frequently from diseased root tissue of Triticum aestivum (Link) than on any other of the 55 host plant species and varieties examined. Only F. equiseti and F. oxysporum were isolated more often from root rot diseased tissue of common wheat in north and south Dakota.

The relative abundance of this species during this study agrees with the results obtained by Gordon (1944). Its apparent lack of toxin production in field infections is a reassuring sign to producers and the grain industry, as it is one of the more common Fusarium species.

Fusarium poae

Gordon found F. poae to be the most commonly isolated Fusarium sp. from cereal seed in Canada. It was more common in eastern than western Canada, and the second most common species on farm samples of wheat in Manitoba (Gordon, 1944, 1952). It was rarely isolated from diseased roots of wheat in North and South Dakota, or soil in Canada (Gordon, 1954, 1956). However, from the description of

F. sporotrichioides given by Gordon (1952) it is obvious that his F. sporotrichioides was actually F. tricinctum (Corda) Sacc.. Consequently, one must assume his F. poae also included F. sporotrichioides. Fusarium poae is reported to cause head blight of wheat (Martens et al, 1984), although Stack and McMullen (1985) found it did not blight the heads of spring wheat.

F. poae was found more often in Manitoba and at higher levels in the mildewed samples. Its frequent presence in Manitoba is similar to results obtained by Gordon (1944), although Gordon's definition of F. poae must also include F. sporotrichioides. However, since F. sporotrichioides was also common in Manitoba, Gordon's observations of the distribution of these species combined, are still in agreement with the present findings.

There are reports of in vitro toxin production by isolates of F. poae recovered from Manitoba wheat and corn (Scott et al., 1980). If members of this species have a similar capacity to produce toxins during natural infections, then presumably Manitoba wheat may be at a greater risk than Alberta or Saskatchewan grown wheat.

Fusarium sporotrichioides

Overall the third most frequently isolated Fusarium sp., it was isolated almost exclusively from Manitoba, where in 1985 it was the most commonly isolated Fusarium spp. In a study of 1985 Manitoba wheat samples containing tombstone kernels, F. sporotrichioides was second in frequency to F. graminearum, and comprised

17.8% of the Fusaria isolated (Abramson et al., 1986). The detection of diacetoxyscirpenol (DAS), HT-2, and T-2 toxin as well as deoxynivalenol (DON) in these naturally infected samples supports in vitro experiments where F. sporotrichioides has, among other toxins, produced HT-2 (Scott et al., 1980), T-2 (Neish et al, 1982) and DAS (Marasas et al., 1984). Canadian isolates of F. graminearum have, in vitro, produced DAS (Miller et al., 1983), and is a well-known producer of DON in the field (Sutton, 1982).

The dominance of F. sporotrichioides in Manitoba wheat, and its association with mycotoxin contaminated grain, implies Manitoba wheat to be of greater concern of mycotoxin contamination than Saskatchewan or Alberta wheat.

Fusarium equiseti

Fusarium equiseti is a cosmopolitan saprophyte as well as reportedly a casual agent of stem, root and crown rots of cereals (Booth, 1971; Domsch et al., 1980; Maas and Kotze, 1986). Johnston and Greaney (1942) however found F. equiseti to be non-pathogenic to wheat roots. Gordon and Sprague (1941), Gordon (1944, 1952, 1954, 1956) frequently isolated this species from soil but less frequently from seed.

Although Canadian isolates of F. equiseti have produced zearalenone in culture (Neish et al., 1982), there are no reports of its association with mycotoxins in field samples. Its abundance in Saskatchewan samples would therefore presumably not pose a mycotoxin threat.

Fusarium graminearum

A well-known pathogen of a number of cereals, F. graminearum can cause pre- and post-emergence blight, root rot, foot rot, crown rot, head blight and culm decay. F. graminearum is much more common in eastern than western Canada. Gordon rarely isolated F. graminearum from cereal seed in western Canada (Gordon, 1944, 1952) or soil (Gordon, 1956), or root-rotted Gramineae in North and South Dakota (Gordon, 1941). However, it was economically important in Ontario in 1980 when cool, wet weather was cited as a primary factor in infection of wheat by F. graminearum (Neish and Cohen, 1981; Trenholm et al., 1983) and subsequently in the production of the mycotoxin deoxynivalenol (DON). Reports of F. graminearum in western Canada are rare. But in 1984 Clear and Abramson (1986) analyzed a sample of hard red spring wheat and one of amber durum wheat, both from south eastern Manitoba, and found high levels of F. graminearum and DON present in the samples. It was considered that a corn-wheat rotation and rains at anthesis favored the development of the disease. A subsequent survey, in 1985, of samples of Manitoba wheat seed with fusarium head blight kernels found that F. graminearum was the predominant species (Abramson et al, 1986).

In this study, F. graminearum was isolated only from samples originating from southeast Manitoba in 1985. Therefore, the three main species of toxigenic Fusaria isolated, F. graminearum, F. sporotrichioides and F. poae, were all found mainly in Manitoba samples. Thus, Manitoba grown wheat is at a greater risk of having

fusariotoxins present than wheat from Saskatchewan or Alberta, where the main Fusarium species are not considered to be of concern in the production of toxins in the field. It has been the author's experience that this indeed is the situation. In four years of examining producer deliveries of cereal grains for the presence of fungi and toxins, no samples west of Manitoba have been found to contain the fusariotoxins DON, DAS, HT-2, T-2, or zearalenone (unpublished data). All except the latter toxin have been found in Manitoba wheats.

Other Fusarium spp.

Of the other Fusarium spp. isolated, only F. culmorum can be considered a toxin producer in the field. However, it is so uncommon it does not warrant concern. Gordon (1944, 1952) and Gordon and Sprague (1941) also rarely isolated this species from wheat seed or root rot collections in the prairies.

F. oxysporum's importance is as a disease agent. Gordon and Sprague (1941) isolated it from 43.73% of root rot collections of cereals and grasses collected in North and South Dakota, common and durum wheats being the most frequent hosts. However, Gordon (1944, 1952) seldom isolated it from cereal seed, and it was seldom seed-borne in the present study.

The other three Fusarium spp. isolated, F. sambucinum, F. semitectum and F. subglutinans are rarely reported from wheat seed (Booth, 1971).

Septoria nodorum

S. nodorum is the causal agent of glume blotch of wheat. Reportedly more severe in eastern Canada, it can be economically important in the prairies during prolonged periods of rainfall (Martens et al., 1984). The organism is seed-borne, but also survives well on crop residues (Sutton and Waterston, 1966), which are the sources of primary inoculum. The seed-borne inoculum has been shown to adversely affect seedling development (Machacek, 1945). Shipton et al. (1971) mention that accurate assessment of seed infection by S. nodorum is hindered by more rapidly growing fungi which suppress the slower growing S. nodorum. Ponchet (1960) reported the success of seed infection by S. nodorum was reduced by competition from saprophytic fungi. The last two points may explain the higher observed level of S. nodorum in the 1983 and 1984 non-mildewed samples.

Its effect on the visual appearance of the seed may be more in the aspect of reducing the size or plumpness of kernels due to parasitic action, than to any form of discoloration. Possibly the reduction of kernel size by a heavy infection of this organism would in fact reduce the amount of discoloration, this by virture of a smaller seed being less exposed to organisms growing on the wheat head. This was suggested by Machacek and Greaney (1938) for black-point of wheat.

Drechslera tritici-repentis

Drechslera tritici-repentis is the causal agent of tan spot on wheat, and of red smudge on durum wheat seed. Clear and Cooke (1985) examined 4 samples of hard red spring wheat grown on the prairies in 1985 and reported that 73% of the seeds described as being mostly plump and purplish mottled were infected by this organism. Nonetheless, seed transmission is not considered an important mode of dissemination of this pathogen.

A drought which occurred over much of the prairies in 1984 may be an important factor in the levels detected in 1985. Vanterpool (Wallace, 1964) suggested the high incidence of this fungus in 1962 may have been due to the 1961 trash cover being carried over to 1962. Due to the drought in 1961, other straw rotting organisms had been unable to decompose the substrate upon which D. tritici-repentis was able to complete its life cycle. Rees (1981) states the main cause of increased yellow spot disease in the northestern wheat growing areas of Australia is the trend toward retention of stubble for soil conservation purposes, a trend that is increasing in the prairie provinces. This increased crop residue results in increased inoculum survival.

In addition to the red to purplish discoloration of the seed, D. tritici-repentis often produced dark circular areas of fungal material beneath the pericarp after incubation. However, this discoloration was rarely seen beforehand and thus would not have had any impact on the assessment of the grade.

D. tritici-repentis on the seed is unlikely a factor in the formation of mildewed wheat. However, its presence does indicate areas where the tan-spot disease is now present, and perhaps the relative abundance of the disease in those areas.

Other Fungi

The overall scarcity of the other fungi isolated from seed would suggest that their importance in the production of mildewed kernels is at best slight. However, some may be present in appreciable levels in individual samples, and here they may indeed have an effect. With fungi such as A. pullulans, which are known to cause discoloration on seeds, this would be especially true.

The significance of some of these fungi may be as indicators of the potential presence of mycotoxins. For example, high levels of Trichothecium roseum should alert one to the possibility of the presence of the trichothecene mycotoxins T-2 and Trichothecin (Beuchat, 1978; Ishii et al, 1986). Many of the storage fungi have also been involved in mycotoxin production. Members of the Aspergillus versicolor group species are capable of producing sterigmatocystin (Mills and Abramson, 1986) and Aspergillus flavus group species, aflatoxin (Lancaster et al., 1961). Penicillium species produce a wide range of toxins, most notable in Canada is Ochratoxin A (Abramson et al., 1982). The higher levels of storage fungi in the Alberta and mildewed samples presumably indicates a greater risk that these toxins might be present. However, the levels of the potentially toxigenic species were so low as to pose no danger at present.

The occurrence of some fungi may be indicators of growing conditions in particular geographical areas. For example, Arthrinium spp. are reportedly present as saprophytes on dead plant material, mainly Gramineae, and are found primarily in swampy habitats and water logged soils (Domsch, 1980). Thus when recorded, such fungi may merely reflect adverse conditions which existed on specific farms.

3.) Bacteria

The considerably higher levels of bacteria in the mildewed samples was probably due in large part to the irregularity in the seed surface of the more discolored kernels, thus allowing the bacteria to penetrate beneath the seed coat and/or to escape the surface sterilant. The non-mildewed samples were usually a high quality seed graded No. 1 or No. 2 CWRS and were relatively free of surface imperfections. It is also likely that wet harvest conditions associated with the production of mildewed kernels would have stimulated the development of bacteria.

4.) Germination

Most of the decreased viability is more likely attributable more to the direct effects of the wet weather conditions on the seed than to the action of parasitic fungi. Sprouting was common in years when mildew was also a major degrading factor (Anonymous, 1979), and increased levels of alpha-amylase, an indication of sprouting (Dexter and Matsuo, 1982), were found in mildewed samples of wheat.

The samples collected in the spring (Manitoba and Alberta 1983, Alberta 1985) would also have been affected by storage fungi reducing their viability as higher levels of these organisms were found in those samples. However, the levels were not particularily high overall, so the results of their actions on the seeds was likely limited.

5.) Geographic Distribution of Fungi

The location the samples originated from, as defined by province, significantly influenced the frequency with which Alternaria spp., Cladosporium spp., E. nigrum, D. sorokiniana, N. oryzae, and S. nodorum were isolated (Table 2). Machacek et al (1951) also found D. sorokiniana, Alternaria spp. and Nigrospora spp. to have a lower frequency west of Manitoba. However, the present study also revealed that Cladosporium spp., Epicoccum nigrum, Septoria nodorum and Ulocladium spp. are more common on seed west of Manitoba, reaching a peak in samples from Alberta. Wallace and Sinha (1981) reported Cladosporium spp. and Epicoccum spp. to be more common on Alberta grown barley seed than Saskatchewan or Manitoba grown seed. Drechslera tritici-repentis mirrored the distribution pattern of D. sorokiniana, Nigrospora oryzae and Alternaria spp. by decreasing in frequency west of Manitoba. Two Fusarium spp. had definite distribution patterns, F. avenaceum being primarily from Alberta seed and F. sporotrichioides almost exclusively from Manitoba. Aureobasidium pullulans and Botrytis cinerea were not frequently isolated, but when they were

it was usually from Alberta grown wheat seed. The elucidation of distribution patterns for other fungi would require the analysis of many more samples as they were isolated from the seed at very low frequencies.

GENERAL DISCUSSION

1) Discoloration of Mildewed Kernels

The cause of the greyish discoloration of the brush end, to which the grading term "mildew" refers, is not clear. The wet weather conditions under which mildewed kernels are produced allows the development of a number of saprophytic and parasitic fungi to begin or continue. Representatives of the two main genera (*Alternaria*, *Cladosporium*) found on the mildewed samples, plus *Epicoccum nigrum* and *D. sorokiniana*, have all been reported to be involved in discolorations of seeds, but only species of *Alternaria* and *Cladosporium* were frequent in mildewed kernels from all three provinces.

Alternaria spp. and *D. sorokiniana* (*H. sativum*) have been shown to cause the blackpoint and smudge diseases of wheat, which are dark discolorations of the germ end of the seed (blackpoint), that may also spread further toward the brush end, especially along the crease (smudge) (Machacek and Greaney, 1938; Brentzel, 1944; Hanson and Christensen, 1953). These infections are thought to occur when the head is still green, before the tissues mature. In contrast, infection of the brush end of English wheat by *Cladosporium* spp. (Baker *et al.*, 1958) occurred after the tissues were mature, and was considered to be due to exceptionally wet, cold weather delaying harvest. During this time, *Cladosporium* spp. that were growing on the glumes infected the exposed brush end of the seed, darkening the brush. In the present study, *Cladosporium* spp. were normally associated with the brush end of the seed, and were the only fungi which had a significant in

crease in frequency when seeds displaying a grey brush end were compared with the original heterogenous samples from which they had been selected. Their occurrence was also positively related to the presence of mildew a relationship which was statistically significant in 1984 and 1985.

Species of the genus Alternaria were also very likely involved in the production of mildewed kernels, as they were the most frequently isolated entities during this study. They have also been shown to be involved in other types of seed discoloration, and had a positive relationship with the presence of mildew in 2 of the 3 years studied. It would seem plausible, therefore, that the chief organisms involved in producing mildew symptoms on wheat are species of Alternaria and Cladosporium. However, the relative distribution of each varied with the area in which the grain was grown. Other fungi may also be involved, but their frequency on seed is usually so low they would not be a major factor.

Approximately 80% of the time mildewed kernels appear bleached. A sample with kernels still vitreous (not bleached) and with mildew on the brush end would always be termed lightly mildewed (R. Bevilacqua, personal communication). Simmonds (1968) noted that the testa and outer layers were little changed from normal in bleached wheat seeds, but the endosperm was distinctly altered. The main cause of the appearance of the bleached kernels was the change in endosperm color to starchy, which appeared opaque when viewed with transmitted light. Simmonds compared kernels bleached by rainfall in the field with those

bleached by soaking the seeds in the laboratory from 1/4 to 24 hours before drying and found them to be similar.

One possible scenario is that prolonged wet weather after the crops have reached maturity results in bleaching of the kernels as well as stimulating the further growth of Alternaria spp. and Cladosporium spp. at the exposed brush end of the wheat seed. This fungal growth, due perhaps to the production of exoenzymes or other substances, discolors the brush end a grey color.

2) Possible Effects of this Study on the Grading System

Results obtained in this study have shed some light on the fungi associated with mildewed HRS wheat kernels, but while the fungi themselves produce the discoloration, it is primarily the wet harvest weather which affects the actual end-use of the grain by inducing the germination process. The fungus induced discoloration and the bleaching of the kernels resulting from wet weather serve the grain inspectors as visual indicators of damp harvest conditions. Therefore, degrading a grain sample on the basis of the amount and severity of mildew is realistic since there is a direct relationship between the presence of mildewed kernels and wet harvest weather. This is not to say the discoloration itself is not a factor affecting quality, for the appearance of a sample is considered important. However, the Alternaria spp. and Cladosporium spp. which appear to cause the discoloration are not known to affect the wheat kernel or its end use beyond the appearance.

At present the principle of degrading grain due to mildewed kernels does not require modification as it is soundly based on actual quality reduction. The main fungi isolated (Alternaria spp. and Cladosporium spp.) are not presently viewed as a health risk. If future research implicates them or their products as a health problem, it could affect the grading system in the same fashion as did the association of fusarium head blight and tombstone kernels with DON (vomitoxin).

CONCLUSION

The main fungi associated with mildewed kernels of hard red spring wheat are Alternaria spp. and Cladosporium spp. Members of these genera are extremely common in grain fields and have been implicated in various other discolorations of wheat seed. Growth of Alternaria and Cladosporium species on the wheat head normally halts as the crop matures. However, wet harvest conditions stimulate further fungal growth on the wheat head, discoloring the exposed brush end of the seed. Frequently the wet weather also alters the endosperm to a starchy appearance, resulting in what is described as bleaching of the kernels. The combination of the fungal discolored brush end and starchy endosperm results in the typical 'mildew' appearance.

Generally, the main fungi isolated from mildewed and non-mildewed seeds were the same in all three provinces. However, the relative frequency of these fungi was dependent on the samples' geographic origin and the moisture conditions at the time of harvest. Alternaria spp., D. sorokiniana, D. tritici-repentis, N. oryzae and F. sporotrichioides were more frequently isolated from samples originating from the south-eastern prairies. Cladosporium spp., E. nigrum, S. nodorum, Ulocladium spp. and F. avenaceum were more common in samples originating from the north-west prairies.

Wet harvest conditions, as determined by the presence of mildewed kernels in a sample, resulted in increased levels of almost all microorganisms. However, Cladosporium spp. were the only fungi to show a

marked increase in frequency when just the mildewed kernels were compared with the heterogenous mixture from which they had been removed.

The degrading of a sample due to the presence of mildew is a sound method of visually assessing the quality of the sample. The wet conditions which result in the mildewed condition do affect the end-use of the grain, while the discoloration reduces the aesthetic appeal of the grain and its end-products.

There is, however, a problem with the actual term of mildew. Mildew can have quite different meanings to a plant pathologist and a grain inspector. A more descriptive and definitive term would be desirable. Blackpoint is an excellent example of a term which describes a visual discoloration. Perhaps the term grey-brush would serve as a suitable replacement for the term mildew in the grading system.

FUNGI ISOLATED AND AUTHOR CITATIONS

Acremoniella atra(Corda) Sacc.

Alternaria spp. Nees ex Fries; Nees

Arthrinium spp. Kunze ex. Fr.

Aspergillus candidus group species Link ex Link

flavus Link ex Gray

glaucus Link ex Gray

nidulans (Eidam) Winter

versicolor (Vuill.) Tiraboschi

Aureobasidium pullulans (De Bary) Arnaud

Botrytis cinerea Pers. ex. Pers; Persoon

Cephalosporium spp. Corda

Chaetomium spp. Kunze ex Fr.

Cladosporium spp. Link ex Fr; Link

herbarum (Pers.) Link ex. Gray

cladosporioides (Fresen) de Fries

Phoma spp. Sacc.

Colletotrichum spp. Corda

Curvularia geniculata (Tracy and Earle) Boedijn

Drechslera avenacea (Curtis ex Cooke) Shoemaker

bicolor (Mitra) Subram. and Jain

biseptata (Sacc. and Roum.) Richardson and Fraser

catenaria (Drechs.) Ito

graminea (Rab. ex Schlecht.) Shoem.

phlei (Graham) Shoem.

sorokiniana (Sacc.) Subram. and Jain

teres (Sacc.) Shoem.

tritici-repentis (Died.) Shoem.

tuberosa (Atk.) Shoem.

victoriae (Meehan and Murphy) Subram. and Jain

Epicoccum nigrum Link ex Link

Fusarium acuminatum Ell. and Everh.

avenaceum (Fr.) Sacc.

culmorum (W.G. Smith) Sacc.

equiseti (Cda.) Sacc.

graminearum Schwabe

oxysporum Schlecht. emend. Snyder and Hansen

poae (Peck) Wollenw.

sambucinum Fuckel

semitectum Berk. and Rav.

sporotrichioides Sherb.

subglutinans (Wollenw. and Reinking) Nelson, Toussoun and Marasas.

Gonatobotrys spp. Corda

Microdochium bolleyi (Sprague) de Hoog and Hermanides - Nijhof

Mucor spp. Mich. ex. St.-Am.

Nigrospora oryzae Hudson

Papulospora spp. Preuss

Penicillium spp. Link ex Fr.

Phaeoramularia spp. Muntanola

Phycomyces spp. Kunze

Pithomyces spp. Berk. and Br.

Rhizopus spp. Ehrenb.

Scopulariopsis spp. Bain

Septoria nodorum (Berk.) Berk. in Berk. and Br.

Sordaria fimicola (Rob.) Ces. and de Not.

Stemphylium spp. Wallroth

Trichoderma spp. Pers. ex. Fr.

Trichothecium roseum (Pers.) Link ex Gray

Ulocladium spp. Preuss

atrum Preuss

Verticillium spp. Nees ex Link ; Nees

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TABLE 1

PERCENTAGE INFECTION BY SEED-BORNE MICROORGANISMS IN SURFACE STERILIZED HARD RED SPRING WHEAT SEED DISPLAYING VARIOUS DEGREES OF MILDEW

	ORDER OF FREQUENCY	MANITOBA * 1983				MANITOBA 1985				SASKATCHEWAN 1985				ALBERTA 1983				ALBERTA 1984				ALBERTA 1985			
		NIL	LIGHT	MOD.	SEV.	NIL	LIGHT	MOD.	SEV.	NIL	LIGHT	MOD.	SEV.	NIL	LIGHT	MOD.	SEV.	NIL	LIGHT	MOD.	SEV.	NIL	LIGHT	MOD.	SEV.
No. Samples Tested		20	11	23	4	7	30	29	---	58	36	39	2	17	30	25	3	47	32	33	11	9	21	28	---
Moisture Content		11.7	12.3	11.9	12.4	14.3	13.5	14.1	---	13.3	14.4	14.8	15.5	13.4	13.5	13.4	13.9	12.6	13.3	13.5	14.1	13.9	13.5	13.6	---
% Germination (normal)		93.4	88.6	81.4	77.5	71.7	87.4	72.0	---	89.6	85.7	76.4	35.5	89.0	83.9	79.3	64.0	92.1	75.6	62.6	62.9	88.1	58.6	49.0	---
Alternaria	1	70.2	84.4	80.9	79.5	95.7	92.1	92.8	---	66.4	89.0	92.1	95.5	24.7	40.3	43.4	37.3	42.6	60.0	77.8	66.9	70.2	55.0	49.3	---
Arthrinium	13	---	---	---	---	.4	1.2	.8	---	.2	.2	.2	---	1.1	.4	.6	---	t	.3	---	---	---	.86	.82	---
Aspergillus candidus		---	---	---	---	---	---	---	---	---	---	---	---	---	.1	.1	---	---	---	---	---	---	---	---	---
flavus		---	---	---	---	---	---	---	---	t	---	---	---	---	.1	---	---	---	---	---	---	---	---	.1	---
glaucus		.5	.2	1.4	5.5	.3	.1	t	---	.4	.5	.3	---	2.1	2.0	5.9	---	.3	.8	.5	.2	2.1	3.5	2.9	---
nidulans		---	---	---	---	---	---	---	---	---	---	---	---	.1	---	---	---	---	---	---	---	---	---	---	---
versicolor		---	---	---	---	---	---	---	---	.1	---	---	---	---	---	---	---	---	---	---	---	---	t	---	---
Total Aspergillus	8	.5	.2	1.4	5.5	.3	.1	t	---	.5	.5	.3	---	2.2	2.2	6.0	---	.3	.8	.5	.2	2.1	3.5	3.0	---
Aureobasidium pullulans	11	---	---	---	---	---	.1	.1	---	.6	.6	.3	---	---	.6	.6	2.7	---	---	---	.6	.1	1.8	1.2	---
Bacteria		5.2	12.0	10.2	12.5	7.9	23.2	30.1	---	4.8	13.9	21.3	25.0	6.4	11.7	18.0	34.7	3.4	15.9	18.3	16.9	5.3	26.7	36.4	---

t < 0.1%

* Nil - No mildew symptoms
LIGHT - Lightly mildewed
MOD. - Moderately mildewed
SEV. - Severely mildewed

TABLE 1 (Cont'd)

PERCENTAGE INFECTION BY SEED-BORNE MICROORGANISMS IN SURFACE STERILIZED HARD RED SPRING WHEAT SEED DISPLAYING VARIOUS DEGREES OF MILDEW

	ORDER OF FREQUENCY	MANITOBA 1983				MANITOBA 1985				SASKATCHEWAN 1985				ALBERTA 1983				ALBERTA 1984				ALBERTA 1985			
		NIL	LIGHT	MOD.	SEV.	NIL	LIGHT	MOD.	SEV.	NIL	LIGHT	MOD.	SEV.	NIL	LIGHT	MOD.	SEV.	NIL	LIGHT	MOD.	SEV.	NIL	LIGHT	MOD.	SEV.
Botrytis cinerea	12	.1	---	---	---	---	---	t	---	---	.1	t	---	.5	.5	.2	---	.5	1.5	1.1	2.2	---	.2	.7	---
Cephalosporium	19	---	---	---	.5	---	.1	.1	---	---	---	t	---	---	---	---	---	---	---	---	---	---	t	---	---
Chaetomium	23	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	.2	t	---
Cladosporium	2	1.9	2.2	1.7	1.5	2.7	3.1	3.8	---	11.2	15.3	10.9	4.0	18.6	14.0	10.5	8.7	17.7	28.1	23.8	39.6	7.4	13.1	17.4	---
Colletotrichum	25	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	.1	---	---	---	---	---
Curvularia	17	---	.2	.1	---	.3	.2	.1	---	.1	.1	.3	---	---	---	---	---	---	---	---	---	---	---	---	---
Drechslera sorokiniana	}	5.1	7.8	14.6	10.5	6.3	5.8	8.2	---	1.1	2.4	4.4	6.0	1.2	1.0	2.0	1.3	.7	.4	.4	.7	.4	.3	.5	---
D. tritici-repentis	} 4	---	---	---	---	1.9	4.3	3.3	---	.9	1.0	.8	---	---	---	---	---	.1	.1	.2	---	.3	.2	.3	---
Drechslera spp.	}	.3	.2	.1	---	1.3	1.7	.8	---	.7	1.5	.9	1.0	.4	.9	.9	---	2.0	1.8	2.2	2.9	.9	1.6	1.1	---
Epicoccum nigrum	3	1.0	3.3	2.1	1.5	3.6	5.6	6.6	---	4.4	9.7	7.1	3.0	4.7	9.1	12.6	4.0	2.8	15.3	18.9	22.2	11.3	14.4	11.9	---

t $<$0.1%

TABLE 1 (cont'd)

PERCENTAGE INFECTION BY SEED-BORNE MICROORGANISMS IN SURFACE STERILIZED HARD RED SPRING WHEAT SEED DISPLAYING VARIOUS DEGREES OF MILDEW

	ORDER OF FRE-QUENCY	MANITOBA 1983				MANITOBA 1985				SASKATCHEWAN 1985				ALBERTA 1983				ALBERTA 1984				ALBERTA 1985			
		NIL	LIGHT	MOD.	SEV.	NIL	LIGHT	MOD.	SEV.	NIL	LIGHT	MOD.	SEV.	NIL	LIGHT	MOD.	SEV.	NIL	LIGHT	MOD.	SEV.	NIL	LIGHT	MOD.	SEV.
Fusarium	}																								
acuminatum	}	.1	.4	.2	---	.1	.4	.7	---	.1	.6	1.4	3.0	.1	.9	.6	---	---	.1	.5	---	.4	.1	.1	---
avenaceum	}	---	---	.7	---	---	.1	.4	---	.1	.2	t	---	1.2	1.1	1.4	.7	.3	1.2	2.1	2.2	---	.3	.4	---
culmorum	}	---	---	---	---	---	.1	---	---	t	---	.1	---	---	---	---	---	---	---	.1	---	---	---	---	---
equiseti	}	---	---	---	---	.1	.5	1.0	---	.2	1.1	1.6	1.0	---	---	.1	---	---	.1	.1	---	---	---	t	---
graminearum	}	---	---	---	---	---	.2	.6	---	---	---	---	---	--	---	---	---	---	---	---	---	---	---	---	---
oxysporum	} 6	---	---	.3	---	---	---	---	---	---	---	t	---	---	---	---	---	---	---	---	---	---	---	---	---
poae	}	.4	.9	.2	---	---	.4	.3	---	---	.1	.1	---	.2	.1	.2	---	.1	.2	.6	.2	---	.1	.1	---
sambucinum	}	---	---	---	---	---	---	---	---	---	t	---	---	---	---	---	---	---	---	---	---	---	.1	---	---
semitectum	}	---	---	---	---	---	---	t	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
sporot-	}																								
richioides	}	.1	.6	.7	.5	.9	.8	1.5	---	t	.1	.2	---	.1	.2	.1	---	---	---	.2	---	---	---	---	---
subglutinans	}	---	---	---	---	---	t	---	---	--	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Fusarium spp.	}																								
(not ident.)	}	.1	.4	.7	2.0	---	t	.2	---	.1	.2	.3	---	.5	.1	.9	---	t	.1	.5	.9	.1	.1	t	---
Gonatobotrys	15	.1	---	.2	---	---	t	.5	---	t	---	.3	1.0	---	---	---	---	---	.1	.1	.4	---	---	.1	---
Microdochium bolleyi	26	---	---	---	---	---	.1	---	---	---	---	t	---	---	---	---	---	---	---	---	---	---	t	---	---
Mucor	22	---	---	---	---	---	t	t	---	t	t	---	---	---	---	---	---	t	---	---	---	.1	---	.2	---
Nigrospora oryzae	5	4.8	5.5	3.6	3.0	3.1	3.3	3.8	---	2.9	3.3	4.6	2.0	.6	.5	.2	---	1.5	1.2	1.4	.4	1.1	.9	.4	---
Papulospora	29	---	---	---	---	---	---	---	---	---	---	t	---	---	---	---	---	---	---	---	---	---	---	---	---
Penicillium	9	.2	---	.9	3.5	.1	.5	.1	---	.5	.3	.2	---	1.1	1.7	1.4	---	.2	.2	.9	.2	.1	.4	1.1	---

t < 0.1%

TABLE 1 (Cont'd)

PERCENTAGE INFECTION BY SEED-BORNE MICROORGANISMS IN SURFACE STERILIZED HARD RED SPRING WHEAT SEED DISPLAYING VARIOUS DEGREES OF MILDEW

	ORDER OF FRE-QUENCY	MANITOBA 1983				MANITOBA 1985				SASKATCHEWAN 1985				ALBERTA 1983				ALBERTA 1984				ALBERTA 1985			
		NIL	LIGHT	MOD.	SEV.	NIL	LIGHT	MOD.	SEV.	NIL	LIGHT	MOD.	SEV.	NIL	LIGHT	MOD.	SEV.	NIL	LIGHT	MOD.	SEV.	NIL	LIGHT	MOD.	SEV.
Phaeoramularia	10	---	---	.1	---	---	.1	.1	---	.9	.5	.7	4.5	---	---	---	---	t	.5	.4	---	4.0	.2	.3	---
Phoma	16	---	---	---	---	---	.1	.1	---	.1	.1	.2	---	---	---	---	---	.1	---	---	.2	---	.5	.6	---
Phycomyces	24	---	.2	---	---	---	---	t	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Pithomyces	27	---	---	---	---	---	---	.1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Rhizopus	18	.1	.2	.1	---	---	---	---	---	t	---	---	---	---	.2	.2	---	t	---	---		---	---	.1	---
Scopulariopsis	30	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	t	---
Septoria nodorum	7	.3	1.3	1.3	---	.3	.2	.1	---	.7	.5	.3	---	6.1	5.0	2.8	3.3	4.1	1.9	1.4	1.8	.6	1.2	1.5	---
Sordaria fimicola	31	---	---	---	---	---	---	---	---	---	---	t	---	---	---	---	---	---	---	---	---	---	---	t	---
Stemphylium	20	---	---	---	---	---	t	.2	---	t	.1	.1	---	---	---	---	---	---	---	---	---	---	t	---	---
Trichothecium roseum	21	---	---	---	---	---	---	---	---	.3	---	---	---	---	---	t	---	---	---	---	---	---	---	.1	---
Ulocladium	14	---	---	.1	---	---	.1	t	---	.4	.2	.1	.5	.1	.1	.1	---	---	.6	.6	.2	2.1	.6	1.2	---
Verticillium	28	---	---	.1	---	---	---	t	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

t < 0.1%

TABLE 2: SUMMARY OF ANALYSIS OF VARIANCE

	ALTERNARIA SPP.			CLADOSPORIUM SPP.		
	1983	1984	1985	1983	1984	1985
MAIN EFFECTS						
PROVINCE	**	—	**	**	—	**
MILDEW	**	**	NS	NS	**	*
INTERACTION EFFECT						
PROVINCE X MILDEW	NS	—	**	NS	—	NS

	DRECHSLERA SOROKINIANA			NIGROSPORA ORYZAE		
	1983	1984	1985	1983	1984	1985
MAIN EFFECTS						
PROVINCE	**	—	**	**	—	**
MILDEW	**	NS	NS	NS	NS	NS
INTERACTION EFFECT						
PROVINCE X MILDEW	**	—	*	NS	—	NS

	EPICOCCUM NIGRUM			SEPTORIA NODORUM		
	1983	1984	1985	1983	1984	1985
MAIN EFFECTS						
PROVINCE	**	—	**	**	—	*
MILDEW	**	**	**	NS	**	NS
INTERACTION EFFECT						
PROVINCE X MILDEW	NS	—	NS	NS	—	*

** SIGNIFICANT AT $\alpha = .01$

* SIGNIFICANT AT $\alpha = .05$, BUT NOT SIGNIFICANT AT $\alpha = .01$

NS NOT SIGNIFICANT AT $\alpha = .05$

— NOT APPLICABLE

TABLE 3:

MEAN PERCENTAGE OF KERNELS INFECTED WITH ALTERNARIA SPP.

A) 1985

PROVINCE	MILDEW ASSESSMENT: MILDEW	MILDEW ASSESSMENT: NO MILDEW	
MANITOBA	92.41	95.71	92.76
SASKATCHEWAN	90.69	66.38	80.24
ALBERTA	51.73	70.22	54.60
	80.92	69.62	

B) 1984

PROVINCE	MILDEW ASSESSMENT: MILDEW	MILDEW ASSESSMENT: NO MILDEW	
ALBERTA	68.71	42.60	58.73
	68.71	42.60	

C) 1983

PROVINCE	MILDEW ASSESSMENT: MILDEW	MILDEW ASSESSMENT: NO MILDEW	
MANITOBA	81.74	70.20	77.76
ALBERTA	41.48	24.71	37.68
	57.42	49.30	

TABLE 4:

MEAN PERCENTAGE OF KERNELS INFECTED WITH *CLADOSPORIUM* SPP.

A) 1985

PROVINCE	MILDEW ASSESSMENT: MILDEW	MILDEW ASSESSMENT: NO MILDEW	
MANITOBA	3.47	2.71	3.39
SASKATCHEWAN	12.81	11.19	12.11
ALBERTA	15.57	7.44	14.31
	10.56	9.93	

B) 1984

PROVINCE	MILDEW ASSESSMENT: MILDEW	MILDEW ASSESSMENT: NO MILDEW	
ALBERTA	27.89	17.70	24.00
	27.89	17.70	

C) 1983

PROVINCE	MILDEW ASSESSMENT: MILDEW	MILDEW ASSESSMENT: NO MILDEW	
MANITOBA	1.84	1.90	1.86
ALBERTA	12.21	18.59	13.65
	8.10	9.57	

TABLE 5:

MEAN PERCENTAGE OF KERNELS INFECTED WITH *EPICOCCUM NIGRUM*

A) 1985

PROVINCE	MILDEW ASSESSMENT: MILDEW	MILDEW ASSESSMENT: NO MILDEW	
MANITOBA	6.07	3.57	5.80
SASKATCHEWAN	8.19	4.38	6.56
ALBERTA	12.96	11.33	12.71
	8.78	5.15	

B) 1984

PROVINCE	MILDEW ASSESSMENT: MILDEW	MILDEW ASSESSMENT: NO MILDEW	
ALBERTA	17.84	2.81	12.10
	17.84	2.81	

C) 1983

PROVINCE	MILDEW ASSESSMENT: MILDEW	MILDEW ASSESSMENT: NO MILDEW	
MANITOBA	2.37	1.00	1.90
ALBERTA	10.34	4.71	9.07
	7.19	2.70	

TABLE 6:

MEAN PERCENTAGE OF KERNELS INFECTED WITH *DRECHSLERA SOROKINIANA*

A) 1985

PROVINCE	MILDEW ASSESSMENT		
	MILDEW	NO MILDEW	
MANITOBA	6.98	6.29	6.91
SASKATCHEWAN	3.51	1.10	2.47
ALBERTA	0.43	0.44	0.43
	3.80	1.51	

B) 1984

PROVINCE	MILDEW ASSESSMENT		
	MILDEW	NO MILDEW	
ALBERTA	0.45	0.68	0.54
	0.45	0.68	

C) 1983

PROVINCE	MILDEW ASSESSMENT		
	MILDEW	NO MILDEW	
MANITOBA	12.21	5.10	9.76
ALBERTA	1.48	1.18	1.41
	5.73	3.30	

TABLE 7:

MEAN PERCENTAGE OF KERNELS INFECTED WITH *NIGROSPORA ORYZAE*

A) 1985

PROVINCE	MILDEW ASSESSMENT: MILDEW	NO MILDEW	
MANITOBA	3.58	3.14	3.53
SASKATCHEWAN	3.81	2.90	3.41
ALBERTA	0.61	1.11	0.69
	2.89	2.70	

B) 1984

PROVINCE	MILDEW ASSESSMENT: MILDEW	NO MILDEW	
ALBERTA	1.18	1.49	1.30
	1.18	1.49	

C) 1983

PROVINCE	MILDEW ASSESSMENT: MILDEW	NO MILDEW	
MANITOBA	4.05	4.80	4.31
ALBERTA	0.31	0.59	0.37
	1.79	2.86	

TABLE 8:

MEAN PERCENTAGE OF KERNELS INFECTED WITH *SEPTORIA NODORUM*

A) 1985

PROVINCE	MILDEW ASSESSMENT: MILDEW	MILDEW ASSESSMENT: NO MILDEW	
MANITOBA	0.15	0.29	0.17
SASKATCHEWAN	0.38	0.66	0.50
ALBERTA	1.57	0.56	1.41
	0.62	0.61	

B) 1984

PROVINCE	MILDEW ASSESSMENT: MILDEW	MILDEW ASSESSMENT: NO MILDEW	
ALBERTA	1.71	4.13	2.63
	1.71	4.13	

C) 1983

PROVINCE	MILDEW ASSESSMENT: MILDEW	MILDEW ASSESSMENT: NO MILDEW	
MANITOBA	1.16	0.30	0.86
ALBERTA	3.97	6.12	4.45
	2.85	2.97	

TABLE 9:

STATISTICAL COMPARISON OF THE INFECTION LEVELS OF THE MAIN FUNGI ISOLATED FROM SEEDS DISPLAYING THE MILDEW DISCOLORATION AND THE HETEROGENOUS MIXTURE OF SEEDS FROM WHICH THEY WERE SELECTED

	FUNGI			
PROVINCE	ALTERNARIA SPP.	CLADOSPORIUM SPP.	EPICOCCUM NIGRUM	DRECHSLERA SOROKINIANA
MANITOBA	NS	* *	NS	NS
SASKATCHEWAN	NS	* *	NS	NS
ALBERTA	NS	* *	NS	NS

** - Infection of the selected discolored seeds is significantly greater than the infection of the heterogenous mixture of seeds at $\alpha = 0.01$

NS - Not significant at $\alpha = 0.01$