FUNGI ASSOCIATED WITH BARLEY SEED IN KANSAS

by

LAUREL GRINNELL OUYE

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INTRODUCTION

Cereals occupy the largest acreage under field crops in the United States of America. Although barley ranks only fourth among cereals raised, it finds considerable use as a feed and as a source of malt. Barley is grown in all the temperate regions, mainly in the spring wheat areas, and is favored by cool, moist climates. Since these conditions are not typical of Kansas, barley ranks only fifth in importance among all crops grown here; however, the amount of barley produced yearly exceeds the combined production of vegetables and fruits. It is grown in every section of Kansas, its chief use being as a feed.

Some of the most destructive plant pathogens are carried over from year to year either within or on the surface of the seed. The diseases caused by these pathogens are commonly referred to as "seed-borne diseases". Among the various types of diseases that attack barley, many of them seed-borne, one group causes rotting of the seed and kills the seedlings. Such fungi are favored by low temperatures which retard seed germination and thus give the fungus ample time to attack. In the case of seedling blight, the fungus probably has invaded and destroyed the endosperm, starchy tissue of the seed, thereby robbing it of the food necessary to produce a healthy seedling. In addition, certain fungi are pathogenic to the seedlings themselves. Weather conditions such as those described prevail at times in various sections of Kansas and thus could result in a poor stand of barley.

A review of the literature showed no record of a study of the microflora associated with recently harvested barley seed. In view of similar work having been or now being done with other seeds at Kansas State College, it was felt that this investigation would add valuable information to the general store of such knowledge; and the information obtained would aid in

comparing it with that obtained from other seeds. The primary purpose of this investigation, therefore, was the isolation and identification of the fungi present in samples of barley seed obtained from various sections of the state of Kansas.

MATERIALS AND METHODS

Farmers from all over the state of Kansas send seed samples for a test of germination and weed seed analysis to the State Seed Laboratory at Topeka, Kansas. It was from these samples that the seed used in this investigation was obtained.

Seven different varieties of barley, three of winter barley and four of spring barley were used for the tests and were chosen on the basis of their popularity in Kansas. The varieties tested were Reno, Dicktoo and B-400, winter barley and Beecher, Custer, Flynn and Otis, spring barley. These seed samples were from the 1956 winter and spring barley crops. The number of samples tested for each variety is shown in Table 1.

Table 1. Barley samples in Kansas during 1956 selected for the investigation.

Variety	Number of Samples Winter	Variety	Number of Samples Spring
Reno	20	Beecher	23
Dicktoo	20	Custer	15
B-400	10	Flynn	6
		Otis	6
Total	50	Total	50

Since the number of samples was small, those used in this investigation were selected one from each county as far as possible. If an insufficient

number of samples resulted, two or at most, three samples were taken at random from those counties having more representative samples.

To determine the internal microflora of the seeds, slightly more than 100 seeds from each sample were placed between strainers and immersed for one minute in 70 percent alcohol. The strainers with seed were then transferred to a one percent solution of sodium hypochlorite for one minute. The cups and strainers used had been previously steam sterilized.

Treatment of seeds with 70 percent alcohol, as recommended by Riker and Riker (1936) was done in order to remove air bubbles from their surfaces and to act as a wetting agent for the seeds so that they could be more effectively surface sterilized by the sodium hypochlorite solution.

After the seeds had been sterilized they were plated individually by means of a pair of sterile forceps onto Petri dishes containing sterile potato dextrose agar medium which had been previously acidified with five percent lactic acid to an approximate pH of five. Ten seeds were placed on each plate and 100 seeds per sample were plated.

All plating was done in an inoculation chamber which had been previously sterilized by wiping all surfaces with a one part per thousand solution of mercuric bichloride and filling the chamber with live steam which upon settling pulled the fungus spores down into the mercuric bichloride and killed them. The Petri dishes of plated seeds were allowed to remain in this chamber at room temperature for three to four days after plating (or until the fungi had emerged and were mature enough for tentative identification) in order to reduce the danger of contamination by air-borne fungus spores.

After the fungi had emerged, records of the observations were made. The number of germinated and non-germinated seeds with the number of fungi emerging from each was noted, and the fungi were identified as far as possible.

All fungi except those positively identified as Alternaria tenuis (a common isolate which was readily recognized) were subcultured into test tubes and their origins recorded. The Petri dish cultures were kept as long as two weeks at room temperature after subculturing unless contamination had occurred since many of the fungi matured within that length of time and could be identified directly from the dishes at least to genus.

For many genera special media were used in order to conform to the media used in the monographs containing the keys for identification of the fungi to species. All fungi which failed to sporulate after two weeks on potato dextrose agar or other special media were placed on a water agar medium which very often induced sporulation. Those which did not respond to this treatment were placed on a water agar medium containing sterilized pieces of straw. Any fungus which failed to sporulate on both of these media and at the end of this investigation still had not produced any fruiting structures on potato dextrose agar in the test tubes was categorized as unknown.

EXPERIMENTAL RESULTS

A total of 5,000 seeds were plated for each type of barley. For winter barley 4,516 seeds germinated and 484 remained non-germinated. There were 2,518 germinated seeds from which fungi emerged and 213 non-germinated seeds. For spring barley 4,316 seeds germinated leaving 684 non-germinated seeds. Fungi emerged from 2,108 of the germinated seeds and 261 of the non-germinated seeds.

In order to have a more orderly presentation, the fungi listed on the following pages have been organized under the classes of fungi alphabetically in their respective mycological order and family. The following classes of fungi – Phycomycetes, Ascomycetes and Deuteromycetes – were among the fungi

isolated. No fungus belonging to the class Basidiomycetes was isolated from the barley seeds in this investigation.

Most of the following descriptions of orders, families and genera have been adapted from those of Swarup (1955). The descriptions of species are based entirely on the actual isolates even though in many cases only one isolate represented the species being described.

Phycomycetes

Fungi in this class typically have a coenocytic mycelium although there is a tendency toward the septate condition in the more advanced orders. Asexual reproduction varies from spores borne within a sporangium through naked conidia in many of the higher forms. Sexual reproduction always results in a thick walled resting spore which may result from the fusion of two motile zoospores or, on the other extreme, from the contact and fusion of two hyphal portions. Since the sexual stages are so similar, the asexual stage of reproduction is utilized mainly in the classification of this group of fungi.

Only one isolate represented this group.

Order – Mucorales

Members of this group produce zygospores by hyphal fusion. The asexual stage is typically a sporangium containing many sporangiospores although considerable variation is possible.

Family – Mucoraceae. Sporangia are all columellate and alike. Sporangial membrane is thin, fugaceous and breaks up to liberate the sporangiospores. Zygospores are rough.

Genus – Rhizopus Ehrenb. ex Corda. The members of this genus character-

istically produce spherical sporangia formed on stolons opposite the rhizoids. The mycelium is heterothallic, two strains being necessary for the production of zygospores.

Rhizopus oryzae Went & Gerlings. (PLATE I, Fig. 1). This isolate would not form zygospores with known strains of R. stolonifer (R. nigricans) and produced abundant growth at 37°C., a characteristic not applicable to R. stolonifer; therefore, it was assumed to be a different species. It was identified to species using the key presented by Gilman (1957) on the basis of spore size and development of rhizoids.

This isolate was from a germinated seed of the variety Reno.[1]

Ascomycetes

Fungi belonging to this class are commonly referred to as "Sac Fungi". The diagnostic characteristic of this class is the ascus which may be clavate, cylindrical, globose or pyriform, containing a definite number of spores, usually eight. Individual asci may be formed on the hyphae or cells, but generally they are grouped in ascocarps, or in locules in a stroma. The asexual stage of reproduction in the Ascomycetes is typified by the production of conidia. The classification of this group is based mostly on the characteristics of the perfect stage. Some of the criteria which have been used to differentiate between orders and families are: type and nature of the ascocarp wall, the shape, size and arrangement of asci in ascocarps and the presence or absence of paraphyses and periphyses (Alexopoulos, 1952).

Three orders were represented in the fungi that were isolated in this group.

1. Percentages of infected seeds were calculated for each species on the basis of total germinated or non-germinated seeds from which fungi had emerged. Any percentage less than 0.5 percent is not given in the text.

EXPLANATION OF PLATE I

Fig. 1. Rhizopus oryzae

Fig. 2. Chaetomidium sp.

Fig. 3. Chaetomidium sp.

Fig. 4. Eurotium amstelodami

Fig. 5. Eurotium sp. (Aspergillus mangini)

PLATE I

Order – Eurotiales

Members of this order usually produce small closed fruiting bodies called cleistothecia. Perithecia with ostioles also are found in some of the members. Fruiting bodies are produced on the mycelium without formation of stromata.

One family, three genera and four species were represented in this order.

Family – Eurotiaceae. The fruiting bodies of the members of this family have thin, dark, carbonaceous or brightly colored peridia. The distinctive feature of the group is the scattered arrangement of asci in the fruit body.

Genus – Chaetomidium (Zopf.) Sacc. Members of this genus produce ostiolate perithecia clothed with a few short unbranched hairs. The genus displays some features of both Thielavia and Chaetomium. Ascospores are dark brown and lemonshaped. The short unbranched hairs distinguish it from Thielavia. The scattered arrangement of asci within the cleistothecia places it in the family Eurotiaceae.

Chaetomidium spp. (PLATE I, Figs. 2 and 3). Colonies on potato dextrose agar (PDA) were creamy at first with considerable aerial mycelium. Cleistothecia began to form at the center of the culture and became purple to black with age. Asci were clavate, hyaline and deliquesed early. Ascospores were fairly large, dark brown and more or less lemonshaped. There was some variation among the isolates with regard to size of spores and cleistothecia and in cultural characteristics suggesting the presence of more than one species.

Table 2 shows the comparative analysis of seeds infected with this fungus.

Genus – Eurotium Link ex Fr. The position of this genus in the class of Ascomycetes was based on the production of cleistothecia in culture. Fungi producing only the imperfect stage of this genus have been classified with

Table 2. Comparative analysis of samples infected with Chaetomidium spp.

Winter			Spring		
Variety	G	NG	Variety	G	NG
Reno	0	0	Beecher	2	1
Dicktoo	0	0	Custer	4	0
B-400	1	0	Flynn	3	0
			Otis	5	0
Total	1	0	Total	14	1
% of Inf. Seed	0.04	0.00	% of Inf. Seed	0.66	0.38

the imperfect fungi under the Genus – Aspergillus and are dealt with later in this thesis. The name Aspergillus formerly included both the perfect and imperfect stages of this fungus.

Two species of this genus were determined after growing the isolated fungi on Czapek's and Malt agars following the method of Thom and Raper (1945).

Eurotium amstelodami Mangin. (PLATE I, Fig. 4). Colonies on Czapek's agar were somewhat spreading and a dark greyish green in color. The bright yellow cleistothecia were very abundant and clustered in masses. Conidial heads were abundant at the colony center and profusely scattered over the entire surface of the colony tending to obscure the cleistothecia beneath. The reverse of the culture was persistantly yellow under the cleistothecial areas and more or less green under the conidial areas. Cleistothecia were globose to subglobose; ascospores were lenticular with a prominant V-shaped equatorial furrow and broad irregular ridges and with walls roughened over their entire surfaces. Conidia were finely spinulose, subglobose and variable in size.

A comparative analysis of seed samples infected with this species is shown in Table 3.

Table 3. Comparative analysis of samples infected with Eurotium amstelodami.

Winter				Spring		
Variety	G	NG		Variety	G	NG
Reno	0	0		Beecher	0	0
Dicktoo	0	0		Custer	0	1
B-400	4	4		Flynn	0	0
				Otis	0	0
Total	4	4		Total	0	1
% of Inf. Seed	0.16	1.88		% of Inf. Seed	0.00	0.38

Eurotium sp. ((Aspergillus mangini (Mangin) Thom & Raper)). (PLATE I, Fig. 5). Colonies on Czapek's agar were at first yellow gradually changing through shades of brown to deep maroon-brown with maturity. The yellow to orange, globose to subglobose cleistothecia matured first at the center of the colony and were abundantly borne in a close felt of red-encrusted hyphae at the agar surface. Conidial heads were few but scattered over the entire surface of the culture. The reverse was in shades of deep red-brown. Ascospores were lenticular, finely roughened in the equatorial area with a rather shallow V-shaped furrow and low rounded ridges. These spores were considerably larger than those of E. amstelodami. Conidia were large, dull green and elliptical to subglobose.

A comparative analysis of seed samples infected with this species is shown in Table 4.

Genus – Thielavia Zopf. This genus is characterized by globose dark cleistothecia which may be anchored to the substrate by hyphal strands but which are otherwise unadorned. Ascospores are dark brown, broadly ovoid with a pore at one end.

Thielavia terricola (Gilman & Abbott) Emmons. (Plate II, Fig. 1). Colonies on PDA were broadly spreading with white aerial mycelium. Cleistothecia

EXPLANATION OF PLATE II

Fig. 1. Thielavia terricula

Fig. 2. Chaetomium atrobruneum

Fig. 3. Chaetomium atrosporum

Fig. 4. Chaetomium aureum

Fig. 5. Chaetomium bostrychodes

PLATE II

Table 4. Comparative analysis of samples infected with Eurotium sp. (Aspergillus mangini)

Winter			Spring		
Variety	G	NG	Variety	G	NG
Reno	0	0	Beecher	0	0
Dicktoo	0	0	Custer	1	0
B-400	1	4	Flynn	0	0
			Otis	0	0
Total	1	4	Total	1	0
% of Inf. Seed	0.04	1.88	% of Inf. Seed	0.05	0.00

were brown to black and globose to oval forming first at the center of the culture. Ascospores were broadly elliptical and olivaceous with an apical pore. According to Gilman (1957) this fungus is of common occurrence in soil samples.

A comparative analysis of samples infected is shown in Table 5.

Table 5. Comparative analysis of samples infected with Thielavia terricola.

Winter			Spring		
Variety	G	NG	Variety	G	NG
Reno	6	0	Beecher	14	0
Dicktoo	2	0	Custer	10	0
B-400	2	3	Flynn	8	0
			Otis	3	0
Total	10	3	Total	35	0
% of Inf. Seed	0.40	1.41	% of Inf. Seed	1.66	0.00

Order – Sphaeriales

According to Alexopoulos (1952) this is the largest order of Ascomycetes. The characteristic features of Sphaeriales are the production of dark colored

more or less hard and carbonaceous, ostiolate perithecia, with or without the formation of stromata. The asci, which are usually clavate, are unitunicate and may be surrounded by paraphyses. There is a great variation in the form of ascospores. Most of the Sphaeriales have an asexual stage representing any of the chief divisions of the class Fungi Imperfecti.

Three families, four genera and 18 species are included among the fungi representing this group.

Family – Chaetomiaceae. Members of this family generally have long hairs, which may be straight, wavy, loosely coiled or dichotomously branched. Asci are usually clavate and are arranged in fasicles. Ascospores are of various shapes depending on the species and are discharged through the ostiole in the perithecium.

Genus – Chaetomium Kunze ex Fr. The present concept of this genus included species with subglobose to elongate, colored, superficial perithecia that are provided with an ostiole. The perithecial wall is membranous and is covered with variously modified hairs. The asci are thin walled and evanescent, usually clavate, sometimes cylindrical and typically eight spored. The ascospores are usually dark colored, typically lemonshaped, but variations in spore shape may range from globose to fusiform, triangular or square shape (Skolko and Groves, 1953).

The genus was first erected in 1817 by Kunze, based on Chaetomium globosum Kze. Various monographs of this genus have appeared since then and about 30 species are now retained in this genus. Skolko and Groves (1953) considered that shape and size of the ascospores provide a more practical basis for species separation than such variable characters as coloration of the substratum or medium upon which the fungus might be growing, the rhizoidal attachment of the perithecium and the incrustation of the hairs which seems

to be dependent of age and may dissolve in the mounting medium.

Members of this genus are for the most part saprophytic and are of particular economic importance as cellulose destroying fungi which grow on paper and fabrics causing considerable damage.

This genus was one of the most frequently isolated from barley seeds. Fourteen different species were isolated and identified from one year of samples. The keys given by Skolko and Groves (1948 and 1953) were followed in identification to the species level. Although for their keys, growth on malt agar was recommended, it was found that except for color, other major and more important characteristics were essentially the same when grown on PDA so that medium was used exclusively.

Chaetomium atrobruneum Ames. (PLATE II, Fig. 2). The colony representing this isolate on PDA was dark brown, the mycelium resembling a dense felt. Perithecia were dark brown, globose to subglobose and quite small. Terminal hairs were long, slender, light colored and smooth tapering somewhat. They were slightly wavy with occasional wide angle branching. Lateral hairs were similar but unbranched and had long collapsed tips. Ascospores were dark brown and ellipsoid. In contrast to most of the other species of Chaetomium isolated, this species required several months to mature.

This species was isolated only once from a non-germinated seed of variety Reno.

Chaetomium atrosporum Skolko & Groves. (PLATE II, Fig. 3). The colony on PDA was olive brown in color. Perithecia were dark brown and globose to subglobose. They were attached firmly to the substratum by dark-colored rhizoids. Terminal hairs were irregularly sinuous, long and untapered with a blunt tip, light colored and slightly roughened. Lateral hairs were similar. Ascospores were globose to subglobose to ovoid often flattened on

one side, dark brown and thick walled. The ascospores are the most distinctive feature of this species.

The single isolate was obtained from a germinated seed of variety Reno.

Chaetomium aureum Chivers. (PLATE II, Fig. 4). The colony on PDA was a dark grey-black and felt-like. Perithecia were small, globose to subglobose with a wide simple ostiole. Terminal hairs were arcuate from the base and incurved, regularly septate, smooth to finely roughened and colored brown in the middle, the incurved or once-recurved blunt tips being lighter. Lateral hairs were straight, septate and finely roughened. The dark colored ascospores were unevenly ellipsoid, often flattened on one side, and faintly apiculate at one or both ends with a germ pore at each end.

This species was isolated only once from a germinated seed of variety Flynn.

Chaetomium bostrychodes Zopf. (PLATE II, Fig. 5). Colonies on PDA were yellow to olive brown in color. Perithecia were globose to ovoid with light colored septate terminal hairs straight below and six to eight times spirally coiled above. Lateral hairs were straight, wide below, tapering above. Ascospores were oval to elliptical, faintly apiculate at both ends and light colored. This fungus was isolated twice from winter barley both from germinated seed, once each from varieties Reno and Dicktoo, and three times from spring barley, two from germinated seed and one from a non-germinated seed, all three isolates being from variety Beecher.

Chaetomium brasiliense Batista & Pontual. (PLATE III, Fig. 1). This colony on PDA was brown to grey brown. Perithecia were globose to ovoid, small and dark colored with terminal hairs five to six times spirally coiled above, dark in color and smooth. Lateral hairs were straight, lighter in color and finely roughened. Ascospores were broadly ovoid, light in color

EXPLANATION OF PLATE III

Fig. 1. Chaetomium brasiliense

Fig. 2. Chaetomium sp.

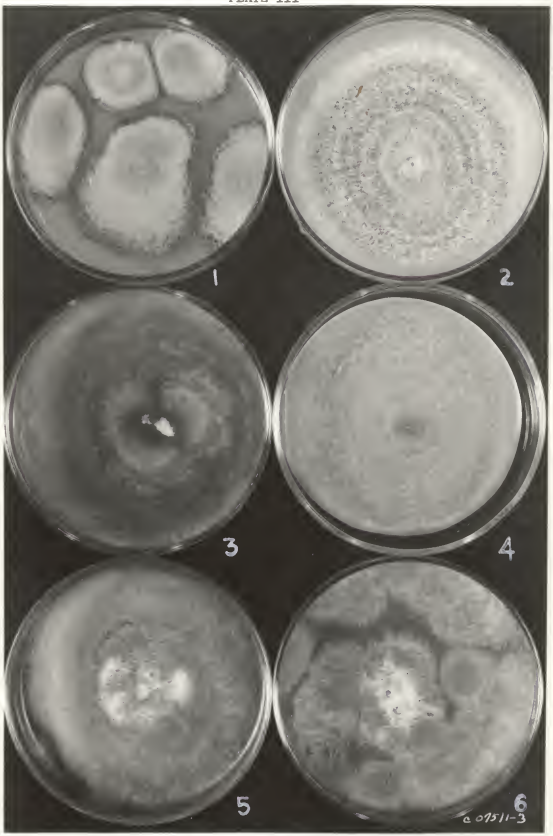
Fig. 3. Chaetomium dolichotrichum

Fig. 4. Chaetomium erectum

Fig. 5. Chaetomium funicolum

Fig. 6. Chaetomium globosum

PLATE III



and apiculate at one end.

This single isolate was obtained from a germinated seed of variety Otis.

Chaetomium sp. (PLATE III, Fig. 2). The colony on PDA was white, with abundant aerial mycelium. Perithecial bodies appeared as black specks all over the medium. Perithecia were dark colored and globose to ovoid. Terminal hairs were coiled up to five times, dark in color and smooth. Lateral hairs were straight, lighter in color and finely roughened. Ascospores were broadly ovoid, light colored and faintly apiculate at one end.

It would appear that this species had features which would almost agree with C. brasiliense; however, there were two outstanding features which indicated that this might be a different species. According to Skolko and Groves (1953), C. brasiliense has a monostichus arrangement of ascospores in asci while this species had a clustered arrangement of ascospores within asci. The second difference was in size of perithecia, this species having much larger perithecia than those of C. brasiliense. Of minor importance was the fact that the apiculate condition of the spores was less obvious. Therefore, it was concluded that this was probably a new species.

This isolate was obtained from a germinated seed of variety Reno.

Chaetomium dolichotrichum Ames. (PLATE III, Fig. 3). Colonies on PDA were at first yellow turning almost black with perithecial development. Perithecia were dark colored, globose to subglobose and were produced in the center of the culture. This species produced both branched and unbranched terminal hairs at maturity. Lateral hairs were unbranched, straight and tapered to a fine tip. Terminal hairs varied from extremely long unbranched to once or several times regularly dichotomously branched. Often the branches were at right angles. They were regularly septate and dark brown with lighter tips. Ascospores were light brown in color and oval to ovoid in shape. The

branching of the terminal hairs was the most distinguishing feature of this species.

A comparative analysis of samples infected is shown in Table 6.

Table 6. Comparative analysis of samples infected with Chaetomium dolichotrichum.

Winter				Spring		
Variety	G	NG		Variety	G	NG
Reno	2	0		Beecher	16	2
Dicktoo	1	0		Custer	7	1
B-400	4	2		Flynn	6	1
				Otis	1	0
Total	7	2		Total	30	4
% of Inf. Seed	0.28	0.94		% of Inf. Seed	1.42	1.53

Chaetomium erectum Skolko & Groves. (PLATE III, Fig. 4). Colonies on PDA were dark yellowish green with light margins. Perithecia were dark, ovoid and with numerous rhizoids. Lateral hairs were not numerous, relatively short, septate, colored below becoming hyaline above and with collapsed tips. Terminal hairs were entirely branched. Branching was not frequent, however, but dichotomous. Hairs were dark and smooth below becoming hyaline and somewhat roughened above. Internodes were short, the hairs forming an erect, relatively short, stout cluster at the top of the perithecium. Ascospores were dark, unequally oval to elliptical and acute at both ends.

This species was isolated two times both from germinated seeds of variety Dicktoo.

Chaetomium funicolum Cooke. (PLATE III, Fig. 5). Colonies on PDA were at first grey becoming greenish. Perithecia were dark and subglobose. There were two types of terminal hairs, (1) unbranched straight hairs which

projected slightly above the branched hairs (2) dichotomously branched hairs which were dark in color and had a roughened appearance. This species closely resembled C. dolichotrichum but could be distinguished by the lack of extremely long unbranched hairs and right angle branching. Ascospores were light colored and ovoid to elliptical.

This fungus was isolated eight times from germinated seed. Winter barley included two isolates from variety Reno and one from variety Dicktoo. The six isolates from spring barley were two each from varieties Beecher, Custer and Flynn.

Chaetomium globosum Kunze. (PLATE III, Fig. 6). Colonies on PDA were of two general types, (1) perithecia appeared clothed in a dense mass of grey hairs becoming darker with age (2) perithecia clothed with dark olive green hairs. Perithecia in both cases were dark, and globose to subglobose. Terminal hairs were light colored and undulate for their entire length. Ascospores were dark colored and lemonshaped.

Table 7 shows the comparative analysis of infected samples.

Table 7. Comparative analysis of samples infected with Chaetomium globosum.

Winter			Spring		
Variety	G	NG	Variety	G	NG
Reno	2	0	Beecher	5	1
Dicktoo	6	1	Custer	5	0
B-400	45	1	Flynn	1	0
			Otis	3	0
Total	53	2	Total	14	1
% of Inf. Seed	2.10	0.94	% of Inf. Seed	0.66	0.38

Chaetomium indicum Corda. (PLATE IV, Fig. 1). Colonies on PDA were greyish-yellow at first becoming almost black in color with age. Perithecia

EXPLANATION OF PLATE IV

Fig. 1. Chaetomium indicum

Fig. 2. Chaetomium olivaceum

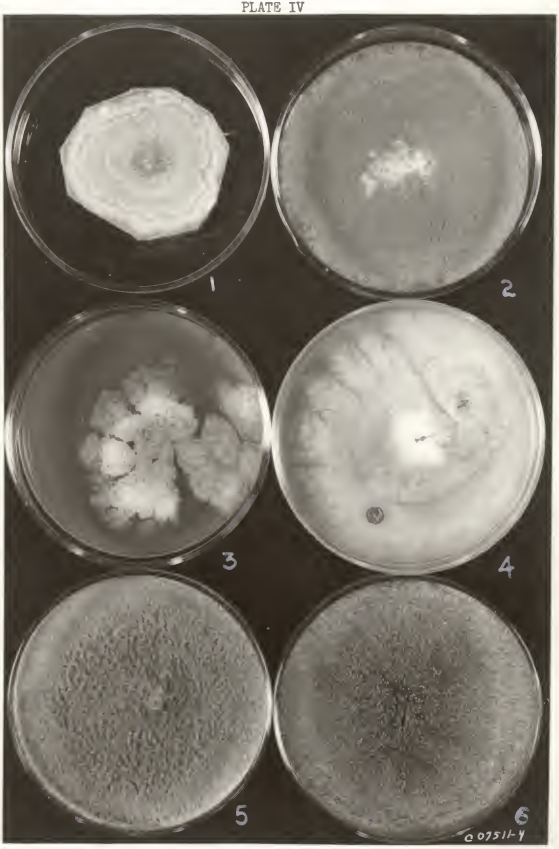
Fig. 3. Chaetomium reflexum

Fig. 4. Chaetomium spirale

Fig. 5. Sordaria fimicola

Fig. 6. Sordaria humana

PLATE IV



were dark olive green to black and subglobose. Lateral hairs were straight, dark colored and quite short. Terminal hairs were repeatedly dichotomously branched and formed a very close network of branched hairs. They were dark colored and slightly roughened at the tips. The profuse branching and lack of any unbranched terminal hairs distinguished this species from both C. dolichotrichum and C. funicolum.

This species was isolated only once from winter barley – a germinated seed of variety Reno, and only four times from spring barley – all four isolates being from germinated seeds of variety Beecher.

Chaetomium olivaceum Cooke & Ellis. (PLATE IV, Fig. 2). Colonies on PDA were grey, very closely resembling the grey colony type of C. globosum. Perithecia were dark colored and globose. Terminal hairs were dark colored and undulate. Ascospores were dark colored and lemonshaped. This species also closely resembled C. globosum in microscopic features; however, it could be distinguished by its larger perithecia, wider terminal hairs and larger ascospores.

A comparative analysis of samples infected with this species is shown in Table 8.

Table 8. Comparative analysis of samples infected with Chaetomium olivaceum.

Winter				Spring		
Variety	G	NG		Variety	G	NG
Reno	0	0		Beecher	10	3
Dicktoo	1	0		Custer	5	0
B-400	2	0		Flynn	1	0
				Otis	0	0
Total	3	0		Total	16	3
% of Inf. Seed	0.12	0.00		% of Inf. Seed	0.76	1.15

Chaetomium reflexum Skolko & Groves. (PLATE IV, Fig. 3). Colonies on PDA were at first light grey in color becoming darker with the formation of perithecia. Perithecia were black, ovoid to subglobose, small and forming a closely arranged almost continuous layer. Lateral hairs were few, unbranched, quite short and only slightly colored with rounded tips. Terminal hairs were short, stout, arcuate from the base with short, stout, strongly recurved dichotomous branches. The tips of these hairs were blunt, dark colored and slightly roughened. Ascospores were light olive brown, oval and slightly flattened on one side, rounded to slightly acute on the ends.

This species was isolated two times from winter barley, one isolate being from a germinated seed of variety Dicktoo, the other from a non-germinated seed of variety B-400; and once from spring barley from a germinated seed of variety Flynn.

Chaetomium spirale Zopf. (PLATE IV, Fig. 4). Colonies on PDA were greyish-yellow in color becoming darker with age. Perithecia were dark, medium sized and globose to ovate. Terminal hairs were dark colored and uniformly spirally coiled eight or more times. Lateral hairs were straight and dark with a rounded tip. Ascospores were dark colored, ovoid and faintly apiculate at both ends. The spores were much larger than those of most Chaetomium species. The terminal hairs of this species tended to mass together when mounted for microscopic examination thus frequently making it difficult to count more than eight spirals. However, on the basis of the large spores, if the hairs were spirally coiled and massed together, the isolate was placed in this species.

This species was isolated seven times but only from germinated spring barley. Six of the isolates were from variety Beecher the other being from variety Otis.

Family – Sordariaceae. Members of this family produce perithecia that are superficial, black, somewhat beaked, membranous and almost glabrous.

Genus – Sordaria Ces. & deNot. According to Cain and Groves (1948) some of the species such as S. fimicola (Rob.) Ces. & deNot. which are usually found on dung frequently occur on other substrata. It is, therefore, not surprising to find a few species belonging to this genus among a large number of seed samples. Members of this genus have mostly a coprophilous habitat.

Sordaria fimicola (Rob.) Ces. & deNot. (PLATE IV, Fig. 5). Colonies on PDA were dark, fast spreading with aerial grey mycelium. Perithecial bodies soon appeared as black dots scattered mostly at the margin and center of the medium. Perithecia were ovoid to pyriform and black in color. Asci were hyaline, long and cylindrical. Ascospores were black, ellipsoid and rounded at both ends – arranged in rows in the asci.

A comparative analysis of the samples infected with this species is given in Table 9.

Table 9. Comparative analysis of samples infected with Sordaria fimicola.

Winter				Spring		
Variety	G	NG	Variety	G	NG
Reno	8	0	Beecher	13	5
Dicktoo	7	1	Custer	9	2
B-400	5	0	Flynn	2	2
			Otis	0	0
Total	20	1	Total	24	9
% of Inf. Seed	0.79	0.47	% of Inf. Seed	1.14	3.45

Sordaria humana (Fuckel) Winter. (PLATE IV, Fig. 6). Colonies on PDA were very similar to S. fimicola except that the aerial mycelium was

somewhat more sparse and perithecia were not so abundant. This species was also similar microscopically to S. fimicola except that the ascospores were broadly ovoid and therefore considerably wider than for the former species.

S. humana was isolated only once from winter barley from a germinated seed of variety B-400 and once from spring barley from a germinated seed of variety Beecher.

Family – Xylariaceae. Members of this family have perithecia with a firm wall and a simple osticle. Perithecia are produced sometimes superficially on the substratum, sometimes on a felty mass of mycelium or in a stroma.

Genus – Rosellinia de Not. Members of this genus have nearly spherical perithecia each with a small ostiolar papilla. They are formed externally on the post with their bases sunk in a more or less well developed subiculum.

Rosellinia limoniispora Ell. & Ev. (PLATE V, Fig. 1). Colonies on PDA were white with black fruiting bodies sparsely arranged on the mycelial mat. Perithecia were large and almost spherical. Ascospores were black and lemonshaped.

This fungus was isolated three times from winter barley – once from a non-germinated seed and once from a germinated seed of variety Reno and once from a germinated seed of variety Dicktoo. It was also isolated once from spring barley from a germinated seed of variety Beecher.

Genus – Xylaria Hill ex Grey. Perithecia are produced in stromata which are upright, simple or branched. The stromata are at first pale and produce conidia. Later they turn black and the perithecia are formed.

Xylaria spp. (PLATE V, Figs. 2 and 3). Although no perithecia were ever formed in culture during this investigation, these isolates were classified as Xylaria on the basis of their morphology which resembled the

EXPLANATION OF PLATE V

Fig. 1. Rosellinia limoniispora

Fig. 2. Xylaria sp.

Fig. 3. Xylaria sp.

Fig. 4. Sporormia australis

Fig. 5. Sporormia minima

PLATE V

known forms of Xylaria species. In some cases very small hyaline conidia or spermatia were observed but mostly the stromata were sterile. Two general types were observed in culture on PDA (1) the stromata were rather short and stout with a suggestion of branching at the tip (Fig. 2). Spermatia or conidia were usually associated with this type. (2) stromata were long and narrow tapering to a fine point. Branching, when it occurred, was from the base of the stroma (Fig. 1).

Table 10 shows the comparative analysis of samples infected with these species.

Table 10. Comparative analysis of samples infected with Xylaria spp.

Winter				Spring		
Variety	G	NG		Variety	G	NG
Reno	3	0		Beecher	2	1
Dicktoo	0	0		Custer	2	0
B-400	1	1		Flynn	0	0
				Otis	0	0
Total	4	1		Total	4	1
% of Inf. Seed	0.16	0.47		% of Inf. Seed	0.19	0.38

Order – Pseudosphaeriales

This order includes those fungi which produce asci and ascospores in a pseudothecium, the cavity of which has been dissolved out so that the spores and asci are free. No paraphyses or periphyses are ever present. The asci are typically bitunicate.

A single family and genus with two species represent this order among the fungi isolated during this investigation.

Family – Sporormiacese. The distinguishing characteristics of this family

are the unilocular pseudothecium and the presence of bitunicate asci. Ascospores are dark colored and readily fall apart into part-spores with germ pores or germ slits.

Genus – Sporormia deNot. Members of this genus have dark brown to black pseudothecia which are usually immersed in the substratum. The spores are transversely three to many septate, dark brown and are surrounded by a hyaline sheath.

Sporormia australis Speg. (PLATE V, Fig. 4). Colonies on PDA were dark grey in color and produced pseudothecia all over the medium. Pseudothecia were somewhat globose and dark. Ascospores in asci were present in two to three series and were cylindrical with tapering ends. They were dark brown, transversely septate and the septate segments were easily separated.

This species was isolated only from winter barley variety B-400. A total of three isolates were obtained – one from a germinated seed and two from non-germinated seeds giving percentages of 0.04 and 0.94 respectively.

Sporormia minima Auersw. (PLATE V, Fig. 5). Colonies on PDA were very similar to the above species but differed in having smaller pseudothecia and ascospores.

Two isolates were obtained both from germinated seeds of the same sample of variety Reno.

Deuteromycetes (Fungi Imperfecti)

This class comprises fungi which have septate mycelium and reproduce only by means of asexual spores.

The Deuteromycetes may be conidial stages of Ascomycetes, or more rarely, Basidiomycetes, whose sexual stages have not been discovered or no longer exist.

The classification of this group is purely an artificial system and has been formed as a matter of convenience which enables workers to identify the conidial stages of many known Ascomycetes and other groups.

Three form orders are included among the fungi isolated representing this group.

Form Order – Sphaeropsidales

This order is characterized by production of conidia inside flask-shaped stromatic structures called pycnidia. These are with or without ostioles or other openings and are separate or joined by stromatic tissue. The pycnidiospores or conidia may be of various sizes and shapes and may be hyaline or colored.

A single family, two genera and at least two species are represented in this order by the fungi isolated.

Form Family – Sphaeropsidaceae. Members of this family have dark colored pycnidia which are leathery to carbonous and usually have an opening.

Form Genus – Phoma Sacc. Members of this genus have hyaline single celled spores.

Phoma spp. (PLATE VI, Figs. 1, 2 and 3). Colonies on PDA varied from light grey to dark grey with usually abundant mycelium. Some of the isolates sporulated very easily, but some had to be put on straw agar before they sporulated. Differences in isolates besides colony appearance were in size of pycnidia and spores. Since the identification of this genus to species involves its appearance on its host, no further identification was attempted.

Three isolates were obtained, all from germinated seeds of variety Reno.

Form Genus – Sphaeronema Fr. Members of this genus have submerged pycnidia with very long beaks protruding above the substrate.

EXPLANATION OF PLATE VI

Fig. 1. Phoma sp.

Fig. 2. Phoma sp.

Fig. 3. Phoma sp.

Fig. 4. Sphaeronema sp.

Fig. 5. Colletotrichum graminicolum

PLATE VI

Sphaeronema sp. (PLATE VI, Fig. 4). This colony on PDA was pale at first becoming black with age. At maturity small black dots could be seen over the surface of the medium. The entire base of the pycnidium was submerged in the medium with only the long beak projecting above. Spores were fairly large, oblong with rounded ends and hyaline. The identification of this fungus to species is based on host and so was not attempted for this study.

The single isolate representing this genus came from a germinated seed of variety B-400.

Form Order – Melanconiales

The distinguishing feature of this order is the production of conidia in an erumpent mycelial structure called an acervulus. The mycelium is within the host or substratum. Conidia are hyaline or dark and are generally enveloped in a slimy mass.

Form Family – Melanconiaceae. The order has only one family which represents all of the genera in this order.

A single genus and species within this group was isolated.

Form Genus – Colletotrichum Corda. The chief characteristic feature of this genus is the production of a well developed acervulus which has black setae-like structures. Conidia are sickle shaped or cylindrical.

Colletotrichum graminicolum (Ces.) Wils. (PLATE VI, Fig. 5). The colony on PDA was greyish white with abundant mycelial formation which gave the appearance of a felt-like cover over the surface of the medium. The acervuli were produced under the mycelium. Acervuli were dark brown to black with long black setae. Conidia were falcate, sickle-shaped and borne on very short conidiophores.

This isolate was obtained from a germinated seed of variety Dicktoo.

Form Order – Moniliales

Members of this order produce conidia on conidiophores which have a wide distribution over the hyphae, or are united into groups or massed in other ways. No pycnidia or acervuli are formed. Conidia are hyaline or dark colored and may be of various shapes.

Three families, 16 genera and 44 species were included in this order.

Form Family – Dematiaceae. The genera included here have dark colored mycelium and conidiophores. Conidia are usually dark colored but may be light colored. The conidia are formed on the ends of short conidiophores not distinguishable from other branches of the mycelium or are terminal or lateral on distinct branched or unbranched septate conidiophores.

Form Genus – Alternaria Nees ex Wallr. Conidiophores are dark, simple, typically bearing a simple or branched chain of conidia. Conidia are dark with both cross and longitudinal septa. They are obclavate to elliptical or ovoid frequently borne in acropetal chains.

Alternaria tenuis auct. sensu str. Neergaard. (PLATE VII, Fig. 1). Colonies on PDA were of varying shades of light grey to almost black, occasionally with pink mycelium. Some of the isolates readily produced conidia in culture, others did not and had to be grown on water or straw agar to induce sporulation. Conidiophores were long, dark colored and septate. Conidia were dark colored, obclavate and muriform. The conidia often had a long beak and were borne in acropetal chains consisting of eight or more conidia. This was the most commonly isolated genus from all varieties tested (Table 11).

Form Genus – Cladosporium Link ex Fr. Members of this group have dark conidiophores which are branched near the upper or middle portion and are

EXPLANATION OF PLATE VII

Fig. 1. Alternaria tenuis

Fig. 2. Cladosporium cladosporioides

Fig. 3. Cladosporium herbarum

Fig. 4. Cladosporium macrocarpum

Fig. 5. Curvularia geniculata

PLATE VII

Table 11. Comparative analysis of samples infected with Alternaria tenuis.

Winter				Spring		
Variety	G	NG		Variety	G	NG
Reno	1025	53		Beecher	854	109
Dicktoo	968	87		Custer	631	58
B-400	270	26		Flynn	159	10
				Otis	143	9
Total	2263	166		Total	1787	186
% of Inf. Seed	89.87	77.34		% of Inf. Seed	84.77	71.26

scattered singly or in clustered condition. Conidia are light to dark colored, variable in size and shape and may be one to eight celled.

Cladosporium cladosporioides (Fres.) deVries. (PLATE VII, Fig. 2). Isolates on PDA produced restricted, felted colonies, greyish green to olive with a somewhat powdery appearance. Conidiophores and conidia were generally abundant. Conidia were variable – globose, elliptic, fusoid – one to two celled, smooth to slightly warted and more or less dark colored.

This species was isolated three times from germinated seed of winter barley, twice from variety Reno and once from variety Dicktoo. Seven isolates were obtained from spring barley, six from germinated seed as follows – three from Beecher, two from Flynn and one from Otis – and one from a non-germinated seed of variety Otis.

Cladosporium herbarum Link ex Fr. (PLATE VII, Fig. 3). Colonies on PDA were very similar to those of C. cladosporioides but were much darker in color. Conidia varied as above but were somewhat larger, darker and more warted.

This species was isolated three times from winter barley, once each from germinated seeds of Reno and Dicktoo and once from a non-germinated seed of variety B-400. Two isolates were obtained from spring barley, one each from

germinated seeds of varieties Flynn and Otis.

Cladosporium macrocarpum Preuss. (PLATE VII, Fig. 4). Colonies on PDA were quite restricted but with more aerial mycelium than either C. cladosporioides or C. herbarum. The colonies showed more grey color than those of the other two species. Chlamydospores were more or less abundant. Conidia were somewhat larger than those of C. herbarum, very dark, one to two celled, plump, elliptic and echinulate.

This species was isolated only from germinated spring barley, one isolate each from varieties Beecher and Otis.

Form Genus – Curvularia Boedijn. This genus is typically characterized by dark ellipsoid or cylindrical, curved or bent, three or four septate spores in which one of the central cells is distinctly larger and darker than the terminal cells.

Curvularia geniculata (Tracy & Earle) Boed. (PLATE VII, Fig. 5). Colonies on PDA were dark brown to black and spreading with a somewhat powdery appearance at the center. Conidiophores were dark and unbranched bearing conidia in a dense cluster at the tip. Conidia were more or less boat shaped and quite strongly curved with four septa.

A comparative analysis of infected samples is given in Table 12.

Form Genus – Helminthosporium Link ex Fr. Members of this genus have light to dark mycelium in culture. Conidiophores are short or long, simple or branched bearing conidia on tips. Conidia are usually dark with more than three cells and have varying shapes and sizes.

Helminthosporium halodes Drechsl. var. tritici Mitra. (PLATE VIII, Fig. 1). Colonies on PDA were dark grey with abundant spreading mycelium. Conidiophores were dark and septate. Conidia were brown to olivaceous, usually straight, broad in the middle and tapering at both ends. The hilum protruded

Table 12. Comparative analysis of samples infected with Curvularia geniculata.

Winter			Spring		
Variety	G	NG	Variety	G	NG
Reno	3	0	Beecher	7	5
Dicktoo	5	1	Custer	2	0
B-400	0	0	Flynn	0	0
			Otis	1	0
Total	8	1	Total	10	5
% of Inf. Seed	0.32	0.47	% of Inf. Seed	0.47	1.92

rather prominently. The two polar septa were much darker than the center ones. The variety tritici was erected chiefly on the basis of spore size, spores of H. halodes being longer and narrower than those of the variety.

This fungus was isolated twice from winter barley, once each from germinated seeds of varieties Reno and B-400; and twice from spring barley, once from a non-germinated seed of variety Beecher and once from a germinated seed of variety Custer.

Helminthosporium sorokinianum Sacc. & Sorokin (H. sativum Pam., King & Bakke). (PLATE VIII, Fig. 2). Colonies on PDA were greyish white to almost black, fluffy, with abundant mycelium and often with sterile stromatic projections. Conidia were dark olivaceous in color and straight or curved. If curved, they either tapered evenly toward each end or were irregularly bent. They were three to nine septate and quite large.

Table 13 gives a comparative analysis of infected samples.

Helminthosporium spiciferum (Bain) Nicot. (PLATE VIII, Fig. 3). Colonies on PDA were dark grey, spreading with abundant aerial mycelium. Conidia were brown, three septate, short cylindric or ovoid and borne in clusters at the tips of the conidiophores (Table 14).

EXPLANATION OF PLATE VIII

Fig. 1. Helminthosporium halodes var. tritici

Fig. 2. Helminthosporium sorokinianum (H. sativum)

Fig. 3. Helminthosporium spiciferum

Fig. 4. Nigrospora oryzae

Fig. 5. Nigrospora sphaerica

Fig. 6. Papularia sphaerosperma

PLATE VIII

Table 13. Comparative analysis of samples infected with Helminthosporium sorokinianum (H. sativum).

Winter			Spring		
Variety	G	NG	Variety	G	NG
Reno	7	2	Beecher	7	2
Dicktoo	11	2	Custer	1	0
B-400	3	0	Flynn	0	0
			Otis	0	0
Total	21	4	Total	8	2
% of Inf. Seed	0.83	1.88	% of Inf. Seed	0.38	0.77

Table 14. Comparative analysis of samples infected with Helminthosporium spiciferum.

Winter			Spring		
Variety	G	NG	Variety	G	NG
Reno	6	0	Beecher	4	2
Dicktoo	7	1	Custer	3	2
B-400	4	0	Flynn	2	0
			Otis	0	0
Total	17	1	Total	9	4
% of Inf. Seed	0.67	0.47	% of Inf. Seed	0.43	1.53

Form Genus – Nigrospora Zimm. This genus has an extensive hyaline mycelium with dark, somewhat inflated short conidiophores. Conidia are black and one celled.

Nigrospora oryzae (Berk. & Br.) Petch. (PLATE VIII, Fig. 4). Colonies on PDA were light grey with a faint tinge of violet. Conidiophores were simple, unbranched and ended in a flat vesicle-type structure. Conidia were dark colored, one celled and globose.

This species was isolated 11 times from winter barley, 10 times from germinated seed as follows – Reno –three, Dicktoo –five and B-400 –two – and

once from a non-germinated seed of variety Dicktoo. Only three isolates were obtained from spring barley all of them coming from germinated seed, one each from varieties Custer, Flynn and Otis.

Nigrospora sphaerica (Sacc.) Mason. (PLATE VIII, Fig. 5). Colonies on PDA were more felt-like than those of N. oryzae and were a mottled cream and light greyish-tan in color. Microscopic features were similar to those described for N. oryzae except that the conidia were noticeably larger in diameter.

This species was isolated only from germinated seed of winter barley, once each from varieties Reno and B-400.

Form Genus - Papularia Fr. Members of this genus have hyaline to brown hyphae with lenticular conidia which are black and have a characteristic hyaline rim around the periphery.

Papularia sphaerosperma (Pers.) von Höhnel. (PLATE VIII, Fig. 6). This species produced colonies on PDA which were spreading, floccose and wooly, at first white becoming pink and dotted with dark brown to black masses of spores. Conidiophores were prostrate and hyaline. Conidia were borne at the apex in an irregular head and were smooth and black with a hyaline rim around the periphery.

This fungus was isolated only from germinated seed, once each from varieties Dicktoo and Beecher.

Form Genus - Stemphylium Wallr. Conidiophores are dark, mostly simple, short to long, bearing a single terminal conidium. Conidia are dark colored with cross and longitudinal septa, globose to broadly ellipsoid, smooth or echinulate.

Stemphylium botryosum Wallr. The colony on PDA was brown to black in color, floccose and spreading. Conidiophores were brown and swollen at

the apex just below the attachment of the spores. Conidia were oblong, dark brown, verrucose with transverse, longitudinal and oblique septa and were borne singly at the tips of conidiophores.

This isolate was from a germinated seed of variety Reno.

Form Family – Moniliaceae. Hyphae and conidia are hyaline or brightly colored. The conidiophores are not grouped in sporodochia or synnemata. According to Bessey (1950) the classification of this family is based on number of cells in the conidia, whether or not these are borne on special conidiophores or are merely modified portions of the vegetative mycelium; and the number of conidia at the apex of the conidiophore and their arrangement, whether in chains or formed in a head.

Six genera and 14 species representing this family were isolated from barley seeds.

Form Genus – Aspergillus Mich. ex Fr. Conidiophores are upright, terminating in a globose to elliptical swelling, bearing phialides at the apex or radiating from the entire surface. Conidia are one celled and spherical.

Two species producing cleistothecia have already been described under Ascomycetes.

Aspergillus alliaceus Thom & Church. (PLATE IX, Fig. 1). Colonies on Czapek's agar were rapidly and broadly spreading with loosely floccose aerial sterile mycelium bearing scattered yellowish heads among abundant black sclerotia. The reverse was uncolored. Conidial heads were dull yellow to ochraceous. Conidia were elliptical to globose and yellowish.

This species was isolated only from spring barley seed, twice from non-germinated seeds of variety Beecher and once from a germinated seed of variety Flynn. Percentages of infected seed were 0.05 and 0.77 respectively.

Aspergillus flavus Link. (PLATE IX, Fig. 2). Colonies on Czapek's

EXPLANATION OF PLATE IX

Fig. 1. Aspergillus alliaceus

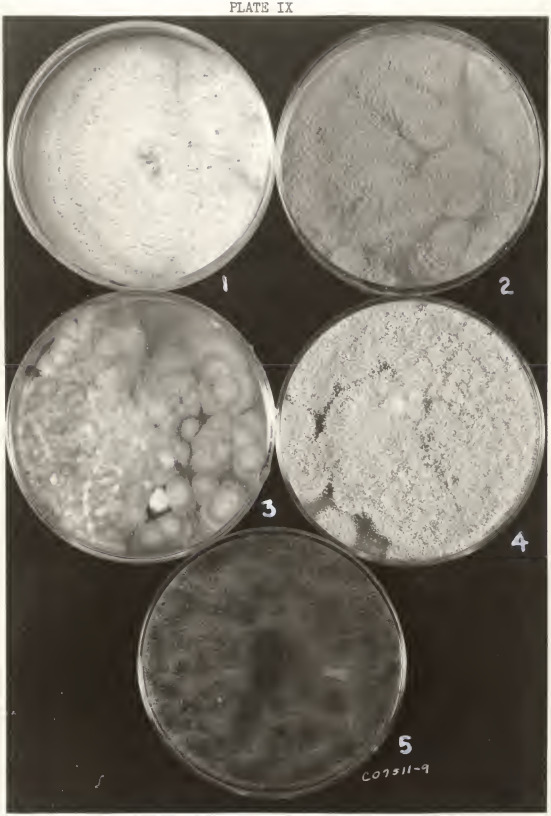
Fig. 2. Aspergillus flavus

Fig. 3. Aspergillus fumigatus

Fig. 4. Aspergillus malleus

Fig. 5. Aspergillus niger

PLATE IX



agar spread rapidly but with scanty mycelium. Conidial heads ranged in color through various shades of yellow-green. The reverse was at first yellow becoming brown with age. There was a considerable range in size of conidial heads. Conidia were pyriform to almost globose, nearly colorless to definitely yellow-green, variously marked with pits and echinulations and were quite variable in size.

This fungus was isolated three times from spring barley, twice from variety Beecher, once each from a germinated and a non-germinated seed, and once from a germinated seed of variety Flynn.

Aspergillus fumigatus Fres. (PLATE IX, Fig. 3). The colony on Czapek's agar spread broadly over the substratum, was rather velvety, white at first becoming greenish. The reverse showed some yellow becoming reddish with age. Conidiophores were short and somewhat green. Conidia were dark green in mass, echinulate and globose.

This single isolate was from a germinated seed of variety Dicktoo.

Aspergillus melleus Yukawa. (PLATE IX, Fig. 4). Colonies on Czapek's agar were rapidly growing and spreading forming an aerial felt of sterile hyphae and conidiophores and producing sclerotia in a shade of yellow orange to pinkish. The reverse was reddish to brown. Conidiophores were quite long with yellow pitted walls. Conidial heads varied considerably in size and were in shades of yellow. Conidia were in long chains, almost colorless when mounted, very thin walled, smooth and slightly elliptical.

This fungus was isolated only from spring barley, all four isolates being from non-germinated seeds of variety Beecher giving an infection percentage of 1.53.

Aspergillus niger van Tieghem. (PLATE IX, Fig. 5). Colonies on Czapek's agar had very little aerial mycelium. Black conidial heads appeared

immediately covering the entire surface of the medium. Conidia were globose and hyaline with a considerable range in size.

A total of three isolations were made all from non-germinated seeds. Two isolates were from variety Beecher, the other from variety Reno.

Aspergillus restrictus G. Smith. (PLATE X, Fig. 1). Colonies on Czapek's agar were quite restricted and velvety and had a dark dull green color which turned grey or brownish-grey with age. The reverse showed some shade of green. The heads formed long, compact, slender columns with rough, spinulose, elliptical or somewhat pyriform, greenish-brown conidia.

This species was isolated five times from winter barley, all five isolates being from variety B-400 and only one of these from a germinated seed. Percentages of infection for germinated and non-germinated seeds were 0.04 and 1.88 respectively.

Aspergillus terreus Thom. (PLATE X, Fig. 2). Colonies on Czapek's agar were spreading and somewhat velvety with a tendency toward floccosity in central colony areas. Conidial heads were long columnar with conidial chains compacted together, of uniform diameter throughout the entire length and giving a cinnamon shade to the colony. An amber exudate was produced. Conidia were globose to slightly elliptical.

This fungus was isolated twice from spring barley only, one isolate being from a non-germinated seed of variety Beecher and the other from a germinated seed of variety Flynn.

Form Genus – Gonatobotrys Corda. Conidiophores are simple, erect, slightly swollen at the point of attachment of the conidia. Conidia are oval to rounded and borne in a whorl-like fashion.

Gonatobotrys simplex Cda. (PLATE X, Fig. 3). Colony appearance on PDA was white with sparse aerial mycelium and abundant conidial heads.

EXPLANATION OF PLATE X

Fig. 1. Aspergillus restrictus (white overgrowth is a contaminant)

Fig. 2. Aspergillus terreus

Fig. 3. Gonatobotrys simplex

Fig. 4. Penicillium thomii

Fig. 5. Penicillium variabile

PLATE X

Conidiophores were long and erumpent and were slightly swollen at the points of conidial attachment. Conidia were sessile, oval to rounded and were borne in whorls of eight to ten.

This fungus was not obtained in pure culture but was associated with Alternaria tenuis.

The single isolate was obtained from a germinated seed of variety Custer.

Form Genus – Penicillium Link ex Fr. Conidiophores arise from the mycelium singly and are branched near the apex to form a brush-like conidia-bearing structure. Conidia are hyaline or bright colored in mass, one celled and mostly spherical.

Penicillium thomii Maire. (PLATE X, Fig. 4). This colony on Czapek's agar grew and spread quite rapidly. The mycelium was a white to pale blue-green in color sporulating throughout. Hard rounded to oblong pinkish sclerotia were produced abundantly. The reverse was in pale yellow to pinkish brown shades. Penicilli were strictly monoverticillate bearing conidial chains in loose columns. Conidia were elliptical to subglobose and smooth walled.

This fungus was isolated just once from a non-germinated seed of variety Dicktoo.

Penicillium variabile Sopp. (PLATE X, Fig. 5). This colony on Czapek's agar spread rapidly and had a velvety appearance. Conidial structures were produced chiefly in central areas. Colors ranged from white through yellow and various shades of green. The reverse was in yellow to orange-brown shades. Penicilli were typically biverticillately symmetrical; sterigmata were lanceolate and tapered. Conidia were strongly elliptical with ends more or less pointed, smooth to irregularly roughened and varying considerably in size.

This single isolate was from a germinated seed of variety Beecher.

Form Genus – Sporotrichum Link ex Fr. Conidiophores are hyaline, usually irregularly branched with spore bearing portion near the apex. Conidia are hyaline, one-celled, globose or ovoid and attached apically and laterally.

Sporotrichum roseolum Oudemans & Beijerinck. (PLATE XI, Fig. 1). The colony on PDA was widely spreading, pale rose in color. Hyphae were prostrate, irregularly branched, rarely septate and very delicate with erect simple or branched laterals which served as conidiophores. Conidia were globose to ovoid and hyaline – in mass, pale rose.

This isolate was obtained from a germinated seed of variety Beecher.

Form Genus – Trichoderma Pers. ex Fr. This genus is characterized by creeping septate, sterile hyphae which form a flat, firm turf. Conidiophores are erect, arising from short, branched side-branches, branching usually being opposite. The branches taper toward the tips and bear clusters of small globose, bright colored or hyaline conidia.

Trichoderma viride (Tode) Harz. (PLATE XI, Fig. 2). Colonies on PDA were broadly spreading, at first white, becoming various yellow-green to dark green shades with age. Conidiophores arose as branches of aerial mycelium, occasionally in whorls. Conidia were globose to ovate and smooth.

This species was isolated three times from the same sample of spring barley. Two of the isolates were from germinated seed and the other from a non-germinated seed of variety Custer.

Form Genus – Verticillium Nees ex Wallr. Conidiophores arise from sterile creeping branched hyphae which are hyaline. Branches of conidiophores are arranged in whorls bearing various shaped deciduous conidia singly at their tips.

EXPLANATION OF PLATE XI

Fig. 1. Sporotrichum roseolum

Fig. 2. Trichoderma viride

Fig. 3. Verticillium puniceum

Fig. 4. Verticillium sp.

Fig. 5. Epicoccum purpurascens

PLATE XI

Verticillium puniceum Cooke & Ellis. (PLATE XI, Fig. 3). The colony on PDA was rose colored and spreading widely. Conidiophores were verticillately branched bearing small, elliptic, one celled, hyaline conidia.

This single isolate was obtained from a germinated seed of variety Otis.

Verticillium sp. (PLATE XI, Fig. 4). This colony on PDA was spreading with floccose white aerial mycelium. Branching was as for V. puniceum. Conidia were more or less globose, hyaline and rather small. None of the species given in the key by Gilman (1957) matched the characteristics of this isolate thus it was impossible to identify it beyond the genus level.

The isolate was from a germinated seed of variety Beecher.

Form Family – Tuberculariaceae. The conidia are borne on short or rarely long conidiophores arising from a fungal tissue called a sporodochium which varies in color and consistancy and is superficial.

Form Genus – Epicoccum Link ex Wallr. Sporodochia are dark, more or less cushion-shaped and are variable in size. Conidiophores are compact or loose and dark colored. Conidia are dark colored and globose with one to several cells.

Epicoccum purpurascens Ehrenberg. (PLATE XI, Fig. 5). Colonies on PDA were bright yellow-orange mottled with brown with a distinct reddish cast. Sporodochia were black-brown and globose united into a long turf of elongate purplish spots. Conidia were globose, at first yellow, then brown, reticulate, verrucose and with a tapering hyaline stripe at the base.

This fungus was isolated only from germinated seed of winter barley, once each from varieties Dicktoo and B-400.

Form Genus – Fusarium Link ex Fr. This genus is characterized by the presence of fusoid, curved, septate macroconidia in slimy masses (sporodochia) on branched conidiophores. Microconidia are also commonly produced. The

mycelium and spores are hyaline or bright colored. A composite key based on those of Wollenweber and Reinking (1935), Snyder and Hansen (1940) and Gordon (1952) and compiled by Dr. Clark T. Rogerson of Kansas State College was used for species identification.

Sixteen species were determined for this genus.

Fusarium acuminatum Ell. & Ev. (PLATE XII, Fig. 1). Colonies on potato sucrose agar (PSA) were spreading with rather flocculant aerial mycelium and were carmine red to pink in color with dark bluish-black areas denoting sclerotia. Microconidia were present but not abundantly. Macroconidia were characterized by a whip-like extension of the upper cell. Chlamydospores were fairly abundant.

Two isolates were obtained from spring barley; one from a germinated seed of variety Beecher and the other from a non-germinated seed of variety Flynn.

Fusarium arthrosporioides Sherb. (PLATE XII, Fig. 2). The colony on PSA was a rosy-purple with the conidial area being mostly orange-red. The mycelium was spreading and somewhat felt-like. Both micro- and macroconidia were found.

This isolate was obtained from a germinated seed of variety Flynn.

Fusarium avenaceum (Fr.) Sacc. (PLATE XII, Fig. 3). The colony on PSA was pale rose with dark sclerotia and orange-pink areas denoting spore masses. Only macroconidia were observed.

This isolate came from a germinated seed of variety Dicktoo.

Fusarium equiseti (Cda.) Sacc. (PLATE XII, Fig. 4). Colonies on PSA were spreading with abundant floccose mycelium. Color ranged from pinkish-yellow to golden brown with darker brown areas denoting sclerotia and sporodochia. This species is similar to F. acuminatum microscopically and

EXPLANATION OF PLATE XII

Fig. 1. Fusarium acuminatum

Fig. 2. Fusarium arthrosporioides

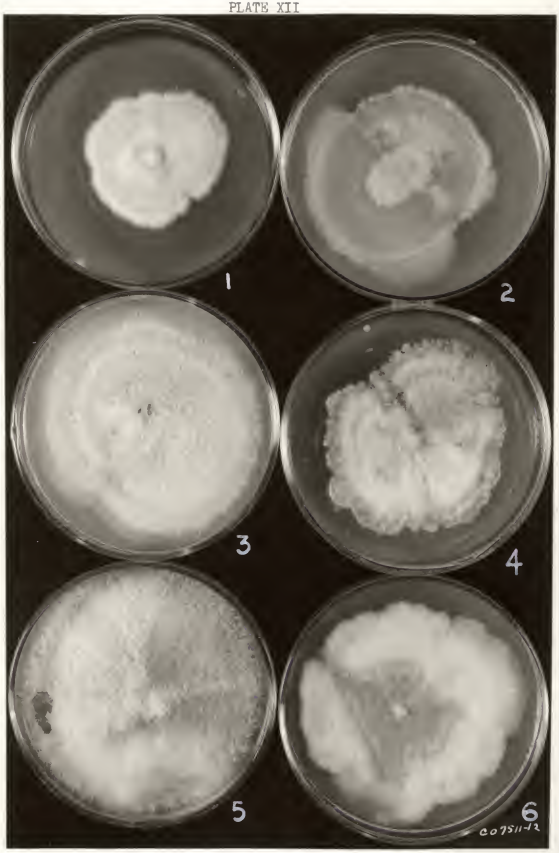
Fig. 3. Fusarium avenaceum

Fig. 4. Fusarium equiseti

Fig. 5. Fusarium expansum

Fig. 6. Fusarium heterosporum

PLATE XII



is often considered to be the yellow counterpart of it.

The comparative analysis of samples infected is shown in Table 15.

Table 15. Comparative analysis of samples infected with Fusarium equiseti.

Winter				Spring		
Variety	G	NG		Variety	G	NG
Reno	0	0		Beecher	2	1
Dicktoo	0	0		Custer	3	1
B-400	1	0		Flynn	0	0
				Otis	0	0
Total	1	0		Total	5	2
% of Inf. Seed	0.04	0.00		% of Inf. Seed	0.24	0.77

Fusarium expansum Schlecht. (PLATE XII, Fig. 5). The colony on PSA was spreading with rather sparse aerial mycelium. The mycelium was orange in color developing a purple tint with age. Only macroconidia were observed.

This isolate came from a non-germinated seed of variety Beecher.

Fusarium heterosporum Nees. (PLATE XII, Fig. 6). Colonies on PSA were a golden-yellowish-brown in color with abundant floccose, spreading mycelium. Chlamydospores and macroconidia were abundant.

This species was isolated twice each from winter and spring barley, once each from germinated and non-germinated seed of variety Dicktoo and twice from germinated seed of variety Beecher.

Fusarium lateritium Nees emend. Snyder & Hansen. (PLATE XIII, Fig. 1). Colonies on PSA were pallid to pale salmon in color; spreading with abundant aerial mycelium. Only macroconidia were present.

This fungus was isolated only from germinated seed once each from varieties Reno, Dicktoo, Beecher and Custer.

EXPLANATION OF PLATE XIII

Fig. 1. Fusarium lateritium

Fig. 2. Fusarium moniliforme

Fig. 3. Fusarium nivale

Fig. 4. Fusarium oxysporum

Fig. 5. Fusarium reticulatum

Fig. 6. Fusarium sambucinum

PLATE XIII

Fusarium moniliforme Sheld. emend. Snyder & Hansen. (PLATE XIII, Fig. 2). Colonies on PSA ranged in color from almost white with a violet tinge to pale salmon or rose. Mycelium was generally scanty. This species was characterized by having long chains of microconidia which often broke up to form "false heads". Macroconidia were present but usually not in abundance.

Table 16 shows the comparative analysis of infected samples.

Table 16. Comparative analysis of samples infected with Fusarium moniliforme.

Winter			Spring		
Variety	G	NG	Variety	G	NG
Reno	1	0	Beecher	13	11
Dicktoo	2	0	Custer	2	0
B-400	0	0	Flynn	2	2
			Otis	10	0
Total	3	0	Total	27	13
% of Inf. Seed	0.12	0.00	% of Inf. Seed	1.28	4.98

Fusarium nivale (Fr.) Ces. emend. Snyder & Hansen. (PLATE XIII, Fig. 3). Colonies on PSA were spreading with abundant floccose aerial mycelium which ranged from golden-yellow to brown in color. Only macroconidia were present and these were extremely small in comparison to those of the other species isolated. F. nivale is parasitic on grasses.

Three isolates were obtained from germinated seed. Two of these were from variety Dicktoo and the other from variety Beecher.

Fusarium oxysporum Schlecht. emend. Snyder & Hansen. (PLATE XIII, Fig. 4). Colonies on PSA were carmine to rose with a spreading, somewhat felt-like aerial mycelium. The culture had a distinct aromatic odor. Both micro- and macroconidia were present and chlamydospores were produced abundantly.

This species was isolated once from a germinated seed of variety Reno and once from a germinated seed of variety Beecher.

Fusarium reticulatum Montagne . (PLATE XIII, Fig. 5). The colony on PSA was spreading with abundant floccose aerial mycelium which ranged from creamy rose to carmine. Sclerotia were almost black. Macroconidia and chlamydospores were present.

This single isolate came from a non-germinated seed of variety Flynn.

Fusarium sambucinum Fuckel. (PLATE XIII, Fig. 6). The colony on PSA was spreading with a rather compact felt-like mycelium ranging in color from yellowish-brown to brown. Only macroconidia were observed.

The isolate was from a germinated seed of variety Beecher.

Fusarium sambucinum Fuckel var. caeruleum Wr. (PLATE XIV, Fig. 1). This colony on PSA was similar to F. sambucinum except that the mycelium ranged from pale rose through carmine and purple in color and was dotted with dark bluish-black sclerotia.

The isolate was from a germinated seed of variety Beecher.

Fusarium semitectum Berk. & Rav. (PLATE XIV, Fig. 2). Colonies on PSA were spreading with considerable floccose-granular mycelium of a pale salmon color. Micro-, macro- and chlamydospores were abundant.

This fungus was isolated twice from non-germinated seeds of variety Dicktoo and twice from spring barley - once from a germinated seed of Custer and once from a non-germinated seed of Flynn.

Fusarium solani (Mart.) App. & Wr. emend. Snyder & Hansen. (PLATE XIV, Fig. 3). This colony on PSA was characterized by a spreading, rather scanty mycelium in shades of cream to butter yellow becoming a deep yellow brown with age. Micro- and macroconidia as well as chlamydospores were observed.

The isolate was from a germinated seed of variety Dicktoo.

EXPLANATION OF PLATE XIV

Fig. 1. Fusarium sambucinum var. caeruleum

Fig. 2. Fusarium semitectum

Fig. 3. Fusarium solani

Fig. 4. Fusarium tricinctum

Fig. 5. Myrothecium verrucaria

PLATE XIV

Fusarium tricinctum (Cda.) Sacc. (PLATE XIV, Fig. 4). The colony on PSA was a pale rose in color and spreading but with scanty mycelium. Slimy orange masses of spores dotted the mycelium. Both micro- and macroconidia were present.

This isolate was from a germinated seed of variety Reno.

Form Genus – Myrothecium Tode ex Fr. This genus is characterized by the presence of sporodochia in shallow cups, with or without a pseudoparenchymatous base, covered with a viscid spore-mass at first green becoming jet black with a white margin of flocculant contorted hyphae with or without setae. Conidiophores are hyaline or olivaceus, once or twice branched, crowned with a whorl of phialides bearing small cylindrical or elliptical conidia which are subhyaline to pale olive green with a truncate base.

Myrothecium verrucaria (Alb. & Schw.) Ditwar ex Fr. (PLATE XIV, Fig. 5). Colonies on PDA were at first white becoming nearly black with the development of spores. The spore mass was at first green becoming jet black, viscid and globose or conical, finally becoming dry, dull black and flattened. Conidia were elliptical with a truncate base, smooth, continuous and subhyaline to olive green.

Two isolates of this species were obtained both from germinated seed, one isolate each from varieties Dicktoo and Custer.

Unknowns

This group included all those fungi which failed to sporulate even after special media were employed to induce sporulation (Table 17).

Table 17. Comparative analysis of samples infected with fungi classified as unknowns.

Winter			Spring		
Variety	G	NG	Variety	G	NG
Reno	9	0	Beecher	27	7
Dicktoo	17	2	Custer	19	1
B-400	17	6	Flynn	11	1
			Otis	5	2
Total	43	8	Total	62	11
% of Inf. Seed	1.71	3.75	% of Inf. Seed	2.94	4.21

SUMMARY OF RESULTS

The total fungi isolated during this experiment are shown in Table 18 listed by the varieties of barley tested. The percentages of infection were

Table 18. Total fungi isolated from seven varieties of 1956 barley seed.

Winter			Spring		
Variety	G	NG	Variety	G	NG
Reno	1098	58	Beecher	1009	163
Dicktoo	1052	100	Custer	714	67
B-400	368	55	Flynn	207	19
			Otis	178	12
Total	2518	213	Total	2108	261
% of Inf. Seed	55.76	45.01	% of Inf. Seed	48.84	38.16

calculated on the basis of total germinated and non-germinated seed of winter and spring barley (see page 4).

As a matter of convenience, the state of Kansas has been divided into nine sections of approximately equal size, corresponding to the agricultural

districts of the state. These districts are shown in PLATE XV and include northwest (NW), north central (NC), northeast (NE), west central (WC), central (C), east central (EC), southwest (SW), south central (SC) and southeast (SE).

Table 19 shows the number of samples selected at random from those available for each section of the state. It will be noted that more samples

Table 19. Number of 1956 barley samples from the sections of the state of Kansas.

	NW	NC	NE	WC	C	EC	SW	SC	SE	Total
Winter	4	2	1	1	6	13	2	10	11	50
Spring	5	6	3	0	20	4	5	7	0	50

were available from the east central, south central and southeast sections for winter barley whereas the central section was most frequently represented for spring barley.

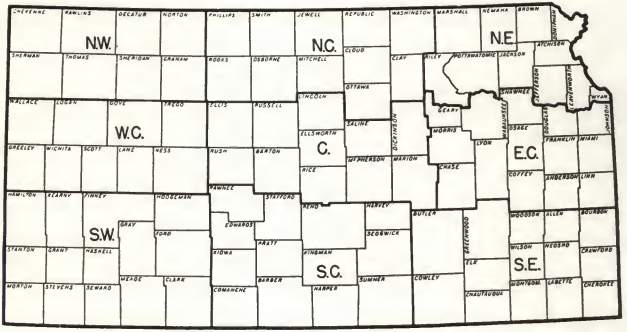
Table 20 shows the distribution of isolated fungi in the state by sections. As would be expected, in general, those fungi isolated most frequently were found most frequently in the sections of the state contributing most of the seed samples.

A comparison by percentage of those genera of fungi isolated most frequently is given in Table 21. There are some differences between the isolates from winter and spring barley; however, the chief differences are between germinated and non-germinated seed and these differences being similar for winter and spring barley, the two have been averaged for general comparison.

EXPLANATION OF PLATE XV

Map of the state of Kansas showing the nine agricultural districts.

PLATE XV



KANSAS CROP-REPORTING DISTRICTS

Table 20. Distribution in the state of total fungi isolated from barley seed.

Class	Fungi	+	Tot. Isol.	Section of the state NW	NC	NE	WC	C	EC	SW	SC	SE
Phycomycetes	Rhizopus oryzae	W	1					1				
		S	0									
Ascomycetes	Chaetomidium spp.	W	1									1
		S	15	1	1			6		1	6	
	Eurotium	W	8					1				7
	amstelodami	S	1						1			
	E. mangini	W	5									5
		S	1					1				
	Thielavia	W	13	1				5	2	2	3	
	terricola	S	35	1	2			22		1	9	
	Chaetomium	W	1							1		
	atrobruneum	S	0									
	C. atrosporum	W	1							1		
		S	0									
	C. aureum	W	0									
		S	1					1				
	C. bostrychodes	W	2					1			1	
		S	4		1	2		1				
	C. brasiliense	W	0									
		S	1	1								
	C. sp.	W	1								1	
		S	0									
	C. dolichotrichum	W	9	1				4			3	1
		S	34		3			25		1	5	
	C. erectum	W	2	2								
		S	0									
	C. funicolum	W	3	1	1			1				
		S	6	1				5				
	C. globosum	W	55		1	1	1		6		1	45
		S	15		4	2		9				
	C. indicum	W	1								1	
		S	4		1			3				
	C. olivaceum	W	3						1		1	1
		S	19		2	3		8	1	1	4	
	C. reflexum	W	2					1		1		
		S	1							1		
	C. spirale	W	0									
		S	7			3		3			1	
	Sordaria fimicola	W	21	1	2			1	6		4	7
		S	33		4	4		8	4	12	1	
	S. humana	W	1									1
		S	1		1							

+ Winter barley = W, Spring barley = S.

Table 20 (con't.).

Class	Fungi	+	Tot. Isol.	Section of the state NW	NC	NE	WC	C	EC	SW	SC	SE
Ascomycetes (con't.)	Rosellinia	W	3	1				1		1		
	limoniispora	S	1		1							
	Xylaria spp.	W	5		1			1		1		2
		S	5			1		3			1	
	Sporormia	W	3								1	2
	australis	S	0									
	S. minima	W	2									2
		S	0									
Deuteromycetes	Phoma spp.	W	3						1		1	1
		S	0									
	Sphaeronema sp.	W	1									1
		S	0									
	Colletotrichum	W	1			1						
	graminicolum	S	0									
	Alternaria tenuis	W	2429	134	105	26	22	219	929	41	467	486
		S	1973	125	288	203		821	281	72	183	
	Cladosporium	W	3					1		1	1	
	cladosporioides	S	7					2		5		
	C. herbarum	W	3					1			1	1
		S	2							2		
	C. macrocarpum	W	0									
		S	2					1		1		
	Curvularia	W	9						7		2	
	geniculata	S	15		1	1		11	1		1	
	Helminthosporium	W	2						1			1
	halodes var.	S	2		2							
	tritici											
	H. sorokinianum	W	25						14	1	6	4
	(H. sativum)	S	10	1	1	3		2		1	2	
	H. spiciferum	W	18						9	1	5	3
		S	13		1	3		5		1	3	
	Nigrospora oryzae	W	11				1	2	5	1	2	
		S	3	1				1			1	
	N. sphaerica	W	2						1			1
		S	0									
	Papularia	W	1							1		
	sphaerosperma	S	1								1	
	Stemphylium	W	1									1
	botryosum	S	0									
	Aspergillus	W	0									
	alliaceus	S	3			2		1				
	A. flavus	W	0									
		S	3					2			1	
	A. fumigatus	W	1							1		
		S	0									

Table 20. (con't.).

Class	Fungi	+	Tot. Isol	Section of the state NW	NC	NE	WC	C	EC	SW	SC	SE
Deuteromycetes (con't.)	Aspergillus melleus	W	0									
		S	4			4						
	A. niger	W	1						1			
		S	2			2						
	A. restrictus	W	5									5
		S	0									
	A. terreus	W	0									
		S	2			1				1		
	Gonatobotrys simplex	W	0									
		S	1						1			
	Penicillium thomii	W	1							1		
		S	0									
	P. variabile	W	1							1		
		S	0									
	Sporotrichum roseolum	W	0									
		S	1					1				
	Trichoderma viride	W	0									
		S	3			3						
	Verticillium puniceum	W	0									
		S	1								1	
	V. sp.	W	0									
		S	1							1		
	Epicoccum purpurescens	W	2								2	
		S	0									
	Fusarium acuminatum	W	0									
		S	2		1			1				
	F. arthro-sporioides	W	0									
		S	1					1				
	F. avenaceum	W	1						1			
		S	0									
	F. equiseti	W	1								1	
		S	7		2	2			1		2	
	F. expansum	W	0									
		S	1							1		
	F. heterosporum	W	2		1					1		
		S	2			1		1				
	F. lateritium	W	2	1							1	
		S	2					1	1			
	F. moniliforme	W	3							3		
		S	40	2	3			7		24	4	
	F. nivale	W	2	1							1	
		S	1					1				
	F. oxysporum	W	1								1	
		S	1								1	

Table 20 (concl.).

Class	Fungi	+	Tot. Isol	Section of the state NW	NC	NE	WC	C	EC	SW	SC	SE
Deuteromycetes (Concl.)	Fusarium reticulatum	W	0									
		S	1							1		
	F. sambucinum	W	0									
		S	1					1				
	F. sambucinum Var. caeruleum	W	0									
		S	1								1	
	F. semitectum	W	2						1		1	
		S	2						1	1		
	F. solani	W	1						1			
		S	0									
	F. tricinctum	W	1								1	
		S	0									
	Myrothecium verrucaria	W	1			1						
		S	1		1							
Unknowns		W	51	3	1	2	5	8	5	2	6	18
		S	73	6	10	6		29	3	5	14	
Totals		W	2731	146	112	31	29	248	992	61	516	596
		S	2369	139	330	246		984	295	133	242	

Table 21. Comparison of the genera of fungi isolated most frequently from barley seed.

Genus	Winter G	Winter NG	Spring G	Spring NG	Averages G	Averages NG
	percent		percent		percent	
Alternaria	89.87	77.34	84.77	71.26	87.32	74.30
Chaetomium	2.94	2.82	3.93	3.44	3.44	3.13
Fusarium	.52	1.41	2.05	7.27	1.29	4.34
Aspergillus plus Eurotium	.28	6.11	.24	4.21	.26	5.16
Helminthosporium	1.58	2.35	.86	2.68	1.22	2.52
Sordaria	.83	.47	1.19	3.45	1.01	1.96
Curvularia	.32	.47	.47	1.92	.40	1.20
Thielavia	.40	1.41	1.66	0.00	1.03	.71
Totals	96.74	92.38	95.17	94.23	95.97	93.32

DISCUSSION

Although only 100 samples of barley were tested during the course of this study, a wide range of fungus species were isolated.

It is interesting to note that winter barley had a higher percentage of infection than did spring barley (Table 18). This is true for both germinated and non-germinated seed. Climatic conditions at the time and the inoculum available probably played a role in creating these differences.

Unfortunately, there were not enough samples of barley available to select an equal number from each section of the state (Table 19). Because of this no attempt was made to correlate or compare the number of isolates from the various sections of the state. The information in Table 20 has been included only as a summary of the species isolated and in case these data should be utilized at a later date. Table 21, however, shows some rather striking differences between the percentage of infection of germinated and non-germinated seed by the various genera of fungi. These differences are particularly evident with those genera which include species pathogenic on barley. It was noted that these genera were isolated most frequently from non-germinated seed. It is possible that the pathogenicity played a role in lowering the germination of these seed samples infected thus creating the higher percentage obtained from non-germinated seed.

The high percentage of Alternaria isolated from both germinated and non-germinated seed was to be expected since freshly harvested seed was used exclusively in this investigation. Beardmore (1955) and Swarup (1955), working on freshly harvested oat and sorghum seeds respectively, both reported Alternaria as comprising the majority of the fungus isolates. Furthermore, it has been shown by Semeniuk (1954) that the fungi isolated from seed which

has undergone long periods of storage are chiefly members of the genus Aspergillus, Alternaria being isolated rarely. Stored seed also tends to have a lower germination percentage than fresh seed. Certain Aspergillus species do produce pathological conditions on seed or young seedlings and could conceivably be one of the agents involved in lowering the germination percentage of stored seed.

Chaetomium, Sordaria and Thielavia are fungi commonly found in the soil and as saprophytes on a wide range of living and dead vegetative matter. Therefore, their high frequency of isolation is not unexpected and should be about equal from both germinated and non-germinated seed. The variation between spring and winter barley probably was due to different climatic conditions.

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FUNGI ASSOCIATED WITH BARLEY SEED IN KANSAS

by

LAUREL GRINNELL OUYE

B. S., Allegheny College, 1954

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Although only fifth among crops produced in Kansas, barley finds considerable use as a feed and thus is important as a cereal crop in the state. Some of the most severe diseases which attack barley are carried over from year to year by fungi which lodge within or on the surfaces of the seeds. These are referred to as "seed-borne diseases". Little information was available on the microflora associated with recently harvested barley seed, therefore it was considered a worthwhile project for investigation. The primary purpose of this work was the isolation and identification of the fungi associated with barley seed from a one year sample.

Seven different varieties of barley, Reno, Dicktoo and B-400, winter barley, and Beecher, Custer, Flynn and Otis, spring barley, were selected at random on the basis of their popularity in Kansas. The small number of samples available made it impossible to select an equal number of samples from each of the nine agricultural sections of the state. A total of 100 samples were selected at random from the available samples from the 1956 winter and spring barley crop. They were obtained from the State Seed Laboratory at Topeka, Kansas.

One hundred seeds of each sample were surface sterilized for one minute in 70 percent ethyl alcohol and then with one percent sodium hypochlorite solution for one minute. They were then plated out on previously acidified sterile potato dextrose agar. Records were kept of the observations of the fungi growing out of the seeds.

Fungi were isolated from the three classes of fungi—Phycomycetes, Ascomycetes and Deuteromycetes (Fungi Imperfecti). The total number of fungi isolated in these groups was 5,100 with 4,626 being from germinated seed and 474 from non-germinated seed. Seven orders, 11 families, 28 genera and at least 72 species were identified from the fungi isolated.

It was found that nine of the genera represented included 96 percent of the fungi from germinated seeds and 93 percent of the fungi from non-germinated seeds which were isolated in this investigation. These were in order of abundance, Alternaria, Chaetomium, Fusarium, Aspergillus plus Eurotium, Helminthosporium, Sordaria, Curvularia and Thielavia. Alternaria alone made up 87 percent of the fungi from germinated seeds and 74 percent of the fungi isolated from non-germinated seeds.

The high frequency of isolation for Alternaria was expected since recently harvested seed was employed in this investigation. Various workers have substantiated this finding. Furthermore, work on stored seed has shown that the number of Alternaria isolations decreases markedly with storage while the incidence of Aspergillus and Eurotium increases.

There seems to be a tendency for those fungi which are pathogenic on barley to be isolated more frequently from non-germinated seed.