THE UNIVERSITY OF MANITOBA

# ECOLOGICAL FACTORS AFFECTING THE VIABILITY AND MICROFLORA OF STORED CEREAL GRAINS, RAPESEED AND FABABEANS

by

Peter Leonard Sholberg

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ABSTRACT

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Several exploratory tests were made with wheat, barley, rapeseed and fababeans to determine the effect of moisture content, temperature and time on the microflora and germination of the stored seed. This was done by conditioning small samples of these crops to various moisture contents and storing the samples in different incubators for a suitable length of time. The seed was then assessed for microflora and germination.

Wheat, barley and rapeseed were surveyed as to the possible storage organisms that could grow on them after storage for 2 to 4 months. Any storage fungi on the grain at temperatures lower than 9°C were considered contaminants. At higher temperatures the Aspergillus species that appeared were A. glaucus (group) which preferred lower moisture content seed, and A. candidus, A. versicolor and A. flavus which preferred higher moisture content seed. Seeds stored at warm temperatures were infected by A. fumigatus and A. niger. Penicillium and Streptomyces occurred on seed at various

temperatures and moisture contents.

More extensive tests on barley stored for 27 weeks showed that A. glaucus (group) first, then A. candidus, and finally A. versicolor infected the seed. For Aspergillus species to appear the storage temperature had to be greater than 15.5°C. The rapidity of infection increased with increasing temperature and moisture content. An exception occurred on the 23.9 percent moisture content seed; of the Aspergillus species only A. versicolor appeared.

Fababeans inoculated with storage fungi decreased in viability when they were stored for 5 months at a moisture content of 18.4 percent and a temperature of 20°C. Of a limited number of Aspergillus species tested for pathogenicity on stored fababeans all were found capable of reducing the germination to zero. Fababeans inoculated with the maximum number of Aspergillus species that could infect them were found to be primarily infected by A. wentii, A. niger, A. clavatus, A. terreus, and A. candidus at the high moisture contents (18.4 - 23.8 percent) and by A. glaucus (group), A. ochraceus A. flavus, and A. versicolor at the lower moisture contents (13.2 - 18.4 percent).

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# 1 INTRODUCTION

## 1.1 Background

The annual over-all world loss of stored food grain due to damage or deterioration is estimated to be approximately 5 percent (Smith, 1969). For Canada with an average wheat crop of 20 million $m^3$ this would amount to an average loss of 1 million $m^3$ per year. Likely there would be correspondingly large losses in barley, oats, rapeseed, and flax.

Records of these staggering losses are difficult to find for the following reasons. Where rotting due to fungal growth is the cause of deterioration the grain is discarded by the farmer and no record of the loss is registered. If the grain is discovered before it is totally spoiled it is often used as feed. Further losses can occur to livestock, either because the use of spoiled grain is uneconomic, or it contains poisonous mycotoxins produced by the fungi in the grain. Mycotoxins are known to cause illness or death to animals (Scott, 1973).

Conditions of the abiotic environment of grain such as temperature and moisture content must be suitable for biotic agents to spoil grain. Favorable conditions are usually the result of one or more of the three following factors. Foremost, is a late harvest which often culminates in large amounts of tough and damp grain. Next, is in-

adequate storage where the farmer places surplus grain in piles on the ground or in structures unsuited for grain storage. This arises at times when large amounts of grain must be carried over from one year to the next. In a year of high carryover 66 percent of the grain elevator agents reported that farmers were placing some grain in open piles in their fields (Sinha, 1973). The third factor is long term storage under Western Canadian climatic conditions. Due to the variability of temperature, vapor pressure, barometric pressure, and the high insulation ability of stored grain a wide range of temperatures and moistures can exist in a bulk storage (H. A. H. Wallace, unpublished data). Given sufficient time these conditions will lead to pockets of high moisture grain which are susceptible to deterioration.

Biotic causes of grain deterioration are primarily molds, insects, and mites. Molds and mites are commonly associated with cool damp grain whereas insects prefer warm dry grain (Hyde and Burrell, 1973).

To avoid grain spoilage the crop must be harvested dry or failing this properly dried. Every attempt should be made to place harvested grain in storage under cool conditions such as in the early morning or late evening. Dried grain should be put into storage only after it has cooled or else it must be aerated. Once the grain is cool and thoroughly dry proper storage is required. Structures designed to keep abiotic conditions unfavorable to biotic agents of destruction are necessary.

By studying the effects of different conditions of temperature, moisture, and time on grain I endeavored to find out more information on these abiotic forces as they affected Manitoba grown grain. With this information I hoped to help Agricultural engineers in designing storage structures which would more effectively keep biotic agents of destruction inactive.

## 1.2 Objectives

The clarification of grain storage problems such as those which occur in designing a grain bin must be attacked from an ecological point of view (Sinha and Muir, 1973). The technique demands specific information on many variables in order to give realistic results. One of these variables is stored grain microflora.

I attempted to further research on grain storage structures by studying the grain microflora as it occurred on the seed. More specifically it was the purpose of this thesis to extend the knowledge on conditions of moisture, temperature and time which contribute to the fungal deterioration of some stored crops grown in Manitoba. With this objective in mind I attempted:

(A) To study the effect of temperature, moisture, time and crop on the microflora of stored grain.

(B) To identify the specific fungi involved in the deterioration of stored cereals and rapeseed grown in Manitoba.

(C) A special study to determine the factors affecting storage quality of fababeans involving temperature, moisture, time and species of fungi.

## 1.3 Delimitations

The delimitations of this study were essentially the duration of the experiments, the type of crops and the means of assessing seed quality. The data for this research project were obtained from various laboratory experiments lasting from 2 to 5 months conducted from June, 1973 to April, 1975. Crops such as wheat, barley, rapeseed and fababeans used in these experiments were chosen because they are commonly grown by Western Canadian farmers, or in the case of fababeans offer promise as a new crop. Seed quality was measured by ability to germinate.

## 1.4 Assumptions

Certain assumptions were necessary because of the nature of analysis. Most important are the following:

(A) All fungi that occur on or in a grain kernel will grow provided that the seed is placed on moist filter paper in a petri plate or it is cultured on the right type of media.

(B) Cross contamination does not normally occur between seeds in a petri dish.

(C) The sample taken for analysis is representative of the entire lot of grain.

(D) The moisture content of the experimental lots of grain stay relatively constant throughout the duration of the experiment unless decomposed by microorganisms.

(E) A limited amount of carbon dioxide produced in a sealed container does not affect the development of microorganisms.

## 1.5 Important Definitions

Field Fungi (Christensen, 1972): Fungi that invade seeds developing on the plants in the field, or after the seeds have matured and the plants are either still standing or are cut and swathed, awaiting threshing.

Storage Fungi (Christensen, 1973): Fungi that grow on products in storage.

Moisture Content: Moisture contents in this study are expressed on a percent wet basis, i.e., (100 x weight of water)/(Weight of water + weight of dry matter).

# 2. REVIEW OF LITERATURE

## 2.1 Introduction

Many researchers in laboratories around the world have already contributed information on the deterioration of stored grain. The following review of literature attempts to report their most important findings.

## 2.2 Abiotic Factors Affecting Grain Storage

2.2.1 Introduction. It is generally conceded that in the absence of insects and mites, microorganisms are the main cause of deterioration in stored grain (Christensen, 1974). Microorganisms in grain depend on specific abiotic conditions to survive and multiply. As stated by Semenuik (1954); the most important are temperature, moisture, and oxygen supply.

2.2.2 Temperature. Temperature directly affects both the grain in storage and the living organisms within the grain bulk. It does this by increasing the metabolic rate of both the seeds and microorganisms. This occurs because the rate of most chemical reactions doubles for a temperature increase of $10^{o}C$.

Microorganisms are often classed according to the temperatures at which they grow optimally. Psychrophiles develop best at around $20^{o}C$. Mesophiles thrive at $30^{o}C$

and thermophiles grow best at temperatures of about 50°C. Semeniuk (1954) has found psychrophiles, mesophiles and thermophiles in stored grain.

Temperature is usually not uniform throughout a bulk of grain because diurnal temperatures only act on grain up to 15 cm from the bin wall (Muir, 1970). Therefore, in farm granaries there will be large temperature differences between the periphery grain and the central grain both during the fall and spring. This may lead to damp pockets of grain near the top of the bin in the fall and near the floor in the spring (Hall, 1957).

The central grain mass of a simulated wooden grain storage structure 20 m in diameter would take at least 1251 d to cool from 25°C to 20°C at Winnipeg (Yaciuk, 1973). Therefore, if warm grain is put into a large bin such as a grain elevator most of it will stay warm, allowing microorganisms to survive and grow if moisture conditions are suitable.

2.2.3 Moisture. It is usually the low moisture within the grain bulk which prevents microorganisms from spoiling the grain because temperature, oxygen supply, and availability of food are sufficient for microbial growth. Since water is the limiting factor in a grain microorganism's life cycle, stored grain must be kept below a critical moisture content depending on the crop.

Moisture content only gauges the absolute amount of water present in a substrate. In considering storage potential of grain the equilibrium relative humidity is

more important than the moisture content because it measures the availability of water to microorganisms (Ayerst, 1969). Equilibrium relative humidity is the ratio of the vapor pressure of the water in the substrate to that of pure water at the same temperature and pressure expressed as a percentage.

Fungi that occur in stored grain have been classed by Panasenko (1944) into three groups depending on their relative humidity requirements. First are the exero-tolerant members which can develop in relative humidities ranging from 65 to 75 percent. In wheat this would correspond to moisture content between 13.5 and 16.0 percent (Pixton and Warburton, 1970). Second are the meso-hygrotolerant fungi which develop at a relative humidity of from 75 to 90 percent. For wheat this relative humidity would be in equilibrium with a moisture content of from 16 to 20 percent (Pixton and Warburton, 1970). Finally are the hygrotolerant organisms which grow at relative humidities of 90 percent and above. This grouping includes bacteria and actinomycetes as well as fungi (Semeniuk, 1954). These organisms would require a moisture content above 20 percent to live in wheat.

Grain samples representing various locations in a large bin when checked for moisture content usually show broad fluctuations. It is likely due to moisture migration. Muir (1973) states that moisture will move in a grain bulk of uniform moisture content if temperature

gradients exist in the bulk. As temperature gradients almost always exist in the case of large bins the grain will accumulate moisture in some locations and lose it in others. It is in the high moisture areas where the biotic agents among the seeds become active.

2.2.4 Oxygen. All living organisms which have evolved an efficient method for utilizing their food require oxygen. The cell uses oxygen from the atmosphere to accept hydrogen atoms which have provided energy and forms water as a waste product. This is the essence of aerobic respiration. The majority of organisms that live in stored grain, including the grain itself respire aerobically.

A difficulty experienced by scientists studying respiration in stored grain was whether the grain or the molds associated with the grain increased their respiration when the grain moisture content was raised. Oxley and Jones (1944) concluded that it was the molds growing in the pericarp of the grain kernel and not the grain itself which increased the respiration rate. Their conclusion was based on the following three facts:

(a) High respiration rates persisted even if the grain was moved to a lower temperature;

(b) wheat from which the embryo had been removed had a respiration rate nearly as high as undamaged grain; and

(c) the pericarp or bran of the wheat when removed by abrasion reduced the carbon dioxide output

to 5 percent of its previous value.

This important discovery of Oxley and Jones (1944) was supported by Hummel et al. (1954). When mold free wheat was compared to moldy wheat the former was found to have a much lower rate of respiration. It became evident that the respiration of wheat did not increase with increasing moisture content but that of the molds in the wheat did.

Molds that inhabit grain have a low oxygen requirement. Semenuik (1954) compiled data which shows that most fungi which live in stored grain have less than a 0.08 percent by volume demand for oxygen to grow optimally. Since air contains 22 percent oxygen and moves freely among the grain kernels in a grain pile molds would not likely be restricted by a lack of oxygen.

## 2.3 Biotic Factors Affecting Grain Storage

2.3.1 Introduction. There are several forms of life that may adversely affect stored grain. Some of these are man, birds, rodents, insects, mites, and microorganisms. In this study only microorganisms are considered. Microflora that are active in grain spoilage are fungi, bacteria, and actinomycetes.

2.3.2 Fungi. Fungi are the main cause of quality loss in stored grain. Papavizas and Christensen (1957) found that in all cases invasion of wheat by fungi preceded a decrease in germination and the decrease in germination preceded an increase in germ damage. Evidence that this

same sequence of events happens in other crops has been supported by various workers. Tuite and Christensen (1955) with barley, Qasem and Christensen (1960) with corn, Fields and King (1962) with peas and Dorworth and Christensen (1968) with soybeans all have demonstrated that the invasion by fungi preceded a decrease in germination.

Fungi are present among the grain kernels either as dormant spores or as living mycelia. Spores reside on the outside and within the grain kernel (Semeniuk, 1954). The mycelium is spread on the inner epidermis of the seed where it forms a network (Hyde and Galleymore, 1951). It often arises from spores or hyphae present on the outside of the grain. Fungal spores which may initiate decay in stored grain are ever present but occur in much larger concentrations around grain storage facilities (Tuite and Christensen, 1957).

Since fungal spores are ubiquitious, grain once threshed rapidly becomes exteriorly contaminated with these airborne propagules. If a survey of the interior fungi of grain is to be made surface-borne saprophytes must be removed for two reasons (Neergaard, 1973). First, the contaminants may hamper the growth of the actual spoilage fungi. Second, they may cause problems in recording fungi growing on seeds. For example, fast growing species of the "lower fungi," a heterogeneous group of primitive fungi, will over grow the culture plate making it impossible to recognize other fungi growing out of infected seeds. For

these reasons plant pathologists use surface disinfection.

Surface sterilization consists of washing the seeds in a fungitoxic solution long enough to kill the surface contaminants. Usually the seed is washed with sterile water after surface disinfection to remove all traces of the fungitoxin.

The most common surface disinfectants in grain storage research are mercuric chloride and sodium hypochlorite. A 0.1 percent solution of aqueous mercuric chloride in 95 percent alcohol was favored by Machachek et al. (1951) for their tests. Christensen and Kaufmann (1969), two of the foremost authorities on grain storage fungi, preferred the use of a 1 percent solution of sodium hypochlorite. This mild disinfectant is also recommended by the International Seed Testing Association (International Seed Testing Association, 1966).

Various techniques are used to detect fungi in and on grain kernels. Sometimes the grain is ground (Christensen and Kaufmann, 1969), but usually the whole kernels are placed on agar or filter paper moistened with sterile water (Neergaard, 1973). Generally a combination of agar types and filter paper are used. Potato dextrose agar is ideal for detection of field fungi such as Fusarium app. (Neergaard, 1973). Saprophytic fungi with relatively high moisture requirements are favored by Czapek-Dox agar while those with low moisture needs flourish on agar with a high osmotic potential. Bottomley et al. (1952)

showed that the Aspergillus glaucus group of fungi in corn kernels will grow best on malt agars containing high concentrations of sugar or salt.

A new procedure for monitoring living mycelium within grain kernels has recently been developed by Warnock (1973). It is an immunofluorescent method which depends on fluorescent dyes being attached to specific fungi in stored grain. As yet it is in the developmental stage and will require more research.

Fungi occurring in and on stored grain are of two types based on their ecology. Those that invade the crop in the field and those that become associated with the grain during storage. The fungi of stored grain are discussed under these two headings in the following paragraphs.

2.3.3 Field Fungi. Christensen (1973) states that the damage caused by field fungi is done by the time the grain is harvested because they require moisture contents in equilibrium with relative humidities above 95 percent. Grain is seldom stored at moistures this high since for wheat it would mean a moisture content over 24 percent. Field fungi eventually perish in warm dry stored grain. Lutey and Christensen (1963) showed that field fungi could be removed from barley by storing 14 percent moisture content seed at 30°C for 12 to 16 weeks.

The major field fungi are species of Alternaria,

Cladosporium, Drechslera and Fusarium (Wallace, 1973). The genera Alternaria, Cladosporium and Drechslera are members of the family Dematiaceae of the Fungi Imperfecti because of their dark colored spores. The genus Fusarium belongs to the family Moniliaceae which have hyaline spores.

Alternaria is the most commonly found fungal genus in freshly harvested grain. Machachek et al. (1951) detected it in 55 percent of the wheat and oats analyzed for fungi and in 66.5 percent of the barley examined. Christensen (1951) noticed that the mycelium of Alternaria spp. is often under the pericarp of high quality grain. This subepidermal mycelium likely increases during times of high humidity (Hyde, 1950). On fungal longevity studies in wheat seed Russell (1958) discovered that it took 7 years for Alternaria to completely disappear under Western Canadian climatic conditions.

Cladosporium spp. are common field fungi in Europe. They appear to be less numerous in Canada. Machachek et al. (1951) counted only a 0.3 percent infection in Canadian wheat. Flannigan (1970) recorded a 2.2 percent infection in Scotland. Cladosporium spp. manifested themselves on 20 percent of the seeds of Danish barley but were largely eliminated by surface sterilization (Jorgensen, 1969). The very humid climate of Northern Europe may be the reason for the higher infection.

Drechslera spp. often are pathogens of cereal crops causing various root, stem, and leaf disorders. They

can be spread by infected seeds. Wallace (1973) states that this parasitic mold occurs in about 5 to 10 percent of all wheat and oat seeds. Reproductive propagules of Drechslera have a long life span since viable spores were discovered in wheat after storage for 17 years (Russell, 1958).

Fusarium spp. are another group of fungi which are often pathogenic. Machechek et al. (1951) found a 0.65 percent infection in Canadian wheat while Flannigan (1970) recorded over 5 percent in Scottish wheat. Fusarium will increase in high moisture grain. Lund et al. (1971) showed that species of Fusarium vigorously developed in stored barley with a moisture content of 26 percent.

2.3.4 Storage Fungi. As previously mentioned, storage fungi differ from field fungi in that they are only associated with the grain after it is put into storage. Tuite and Christensen (1957) demonstrated that grain was not substantially invaded by storage fungi till the crop was threshed. Two other characteristics which distinguish storage fungi from field fungi are their high volume spore dessemination and lower pH requirements (Pelhate, 1968).

Christensen (1972) believes that the most important storage fungi are the following: Aspergillus restrictus Smith, A. glaucus (group), A. candidus Link ex Fr., A. ochraceus Wilhelm, A. versicolor (Vuill.) Tiraboschi, A. flavus Link ex Fr., and Penicillium spp. Other species of the genus Aspergillus that have been recorded on stored grain

are A. fumigatus Fresenius, A. niger van Tieghem, A. terreus Thom, A. clavatus group, A. flavipes (Bain. & Sartory) Thom & Church, A. ustus (Bain.) Thom & Church, and A. wentii Wehmer (Wallace, 1973). Although species of Aspergillus such as A. fumigatus, A niger, and A. terreus are not normally involved in starting deterioration of stored grain (Christensen, 1972), A. fumigatus is often found on spoiled grain (Wallace and Sinha, 1962).

A. restrictus and A. glaucus (group) have the lowest water requirements of all the storage fungi. The minimum relative humidity which permits growth of A. restrictus is 70 percent (Christensen, 1973). Conidia of A. glaucus may germinate at 70.3 percent relative humidity (Armolik and Dickson, 1956). Ascospores are often produced by A. glaucus (group) fungi which are released from yellow cleistotheca. The cleistotheca are an identifying feature of A. glaucus (group) fungi. These molds have an optimal temperature range of from 24 to 25°C with a minimum at 5°C and maximum at 37°C (Panasenko, 1967).

A. candidus, A. versicolor, and A. ochraceus have higher moisture requirements than the A. glaucus (group). They require minimum relative humidities of 76, 80, and 86 percent respectively before they will start to grow (Tsuruta, 1970). Their respective optimum temperatures are 25°C, 30°C, and 30°C (Panasenko, 1967 and Tsuruta, 1970). These figures indicate that A. versicolor and A. ochraceus will be in competition because they have the same optimum

temperature. A. candidus with its lower temperature and moisture requirements probably competes more directly with the A. glaucus group of fungi.

A. flavus requires the highest combination of moisture and heat for development of all the fungi from the genus Aspergillus which cause deterioration to stored grain. Spores of this fungus will only begin to germinate at a relative humidity of 85 percent (Christensen, 1973). It grows optimally at around 35°C and can tolerate a wide extreme of temperatures ranging from a minimum of 3°C to a maximum of 45°C (Panasenko, 1967).

The genus Penicillum contains a whole series of blue to green molds which live in grain. Mislivec and Tuite (1970) who isolated species of Penicillum from stored corn found that they grew on agar from -2 to 35°C and could germinate at a relative humidity of 81 percent. Most significant was their low optimal growth temperature range of 16 to 23°C. For this reason they are abundant in cool damp stored grain.

2.3.5 Bacteria. Bacteria do not seem to be of much importance in stored grain deterioration. Although they are extremely numerous on fresh grain, up to 99 percent of the microflora, their numbers decrease during storage to insignificant amounts (Semeniuk, 1954). The reason this decline occurs is probably because bacteria require higher moisture levels for growth than most molds (Semeniuk, 1954).

Grain storage bacteria have unique temperature requirements. They will increase in moist wheat stored at 37°C but will decline or stay the same in numbers in moist wheat at 20°C or 50°C (Mirzoieva, 1939).

Generally, conditions which favor bacteria are not common in stored grain with the exception of heated grain which undergoes putrefaction by bacteria.

2.3.6 Actinomycetes. Relatively little is known about Actinomycetes in stored grain. Wallace (1973) found that some samples of nonsurface disinfected grain when placed on moist filter paper and incubated at room temperature yielded a high percentage of Streptomyces spp. Streptomyces are filamentous actinomycetes that form white, greenish, or greyish tufts on the surface of weathered or heated seed; they are rare on surface sterilized seed (Machachek et al. 1951). These microorganisms grow well at 16 to 21°C on grain in the laboratory and their presence usually indicates a history of exposure of the grain to a warm, damp condition for a brief period (Sinha and Wallace, 1964).

## 2.4 Interaction of Abiotic and Biotic Factors in Stored Grain

2.4.1 Introduction. The main consideration in this study are the effects of the living and nonliving environment of grain on storage fungi. First the physical environment is discussed which is then followed by the biological

environment.

2.4.2 Storage Fungi and the Physical Environment.

The temperature of stored grain determines the type of fungi that it will support. Most storage fungi will not germinate below 5°C with the exception of Penicillium spp. They will grow at temperatures below 0°C (Meslivec and Tuite, 1970).

The temperature at which a fungus grows best on grain is affected by the moisture content of the substrate, and the relative humidity of the air surrounding the fungus. Moisture content of the fungal substrate can determine if a particular mold can develop. Storage fungi are not likely to grow in grain with a moisture content in equilibrium with a relative humidity less than 70 percent. In wheat this corresponds to a moisture content of approximately 14 percent at 25°C (Pixton and Warburton, 1971). However, wheat with a moisture content less than 14 percent will quickly spoil if it is exposed to high humidity air because it will absorb the moisture from the air. Very serious damage can occur in less than a month to all grain stored at 90 percent relative humidity or higher (Robertson et al., 1939). Pixton and Warburton (1968) found that wheat will reach 90 percent of its total moisture change in less than 14 days when it is conditioned by high relative humidity air.

Since both moisture content and temperature affect stored grain many experiments have been conducted by manipulating these two variables. After a specific length of time

the grain is assessed for quality loss. The three principal methods of assessing the quality are by measuring the increase in fat acidity, the loss in germination and the number and kinds of fungi present.

An experiment of this type was conducted by Dorworth and Christensen (1968). They took soybeans and conditioned six lots to moisture contents ranging from 12.1 to 18.3 percent. These were further subdivided into samples and placed at temperatures ranging from 15 to 30°C. From this experiment they discovered that with increasing temperature, moisture content, and time, seeds yielding A. glaucus (group) increased. Penicillium spp. only multiplied in seeds with a moisture content over 18.3 percent. Germination in all cases decreased after invasion by storage fungi.

More grain storage experiments have been done with barley than soybeans. Lund et al. (1971) reported that barley stored at 20°C was invaded by Aspergillus spp. if the moisture content was below 18 percent, with Penicillium spp. at higher moisture contents and Fusarium spp. became prevalent at a moisture content of 26 percent. Work with lower moisture content barley shows that at 13.8 to 14.2 percent it is slowly invaded by A. restrictus. At moisture contents of 15 to 19 percent the barley is occupied by members of the A. glaucus group along with A. candidus and Penicillium spp. (Tuite and Christensen, 1955). The two barley experiments mentioned, were primarly designed to find the effects of changing moisture contents on the

quality of barley at a fixed temperature.

Grain storage studies have been done on wheat and corn also. In all cases at any one moisture content in equilibrium with a relative humidity greater than 70 percent, germination decreased, and fungi increased with increasing temperature and time. In wheat, storage fungi rapidly developed at a moisture content of 15 percent and a temperature of 40°C (Wyllie and Christensen, 1958). Experiments with corn were more intensive. If the temperature was above 10°C corn stored at 12 to 14 percent moisture content was attacked by A. glaucus (group), at 14 to 16 percent by A. candidus and at 16 to 18 percent by A. flavus (Qasem and Christensen, 1958). It became apparent that as long as the temperature was above 10°C and the moisture content above 14 percent, damage eventually befell the grain.

These previously mentioned experiments depend on a natural inoculum of storage fungi within the grain. Because of the uncertainty that all the storage fungi will be present in sufficient numbers to cause spoilage, researchers in stored grain have artificially infected grain on occasion. Another reason artificial inoculation has been used is to prove that it is a particular fungus or group of fungi causing the quality loss in stored grain and not the physical environment itself. In this way specific fungi have been implicated as primary storage decay organisms.

Preinoculated seed was used by Fields and King (1962) on peas. Idaho peas were obtained which prior to the

experiment were thought to contain no storage fungi. Samples of the peas were then inoculated with a single storage mold and incubated at different temperatures and relative humidities. This was repeated for each of five common Aspergillus storage molds. A. flavus was the most pathogenic species at 85 percent relative humidity and 30°C with A. candidus, and A. amstelodami (Mang.) Thom and Church second. A. restrictus and A. ruber Thom and Church were less noxious under these conditions. An increase in the moisture content of the seed or in the storage temperature, increased the rate of fungus invasion and decreased germination.

This experiment and others like it lead one to ask the question: would mixtures of storage fungi be more harmful than a single pathogenic species alone? Papavizas and Christensen (1960) supplied the answer by inoculating almost sterile wheat with single as well as mixtures of storage fungi. They found no difference in loss of germination or germ damage caused by single species or mixtures.

Moisture and temperature relationships of storage fungi may be used to advantage in predicting the safe storage life of a grain bulk. It was discovered that the moisture content which permits invasion by storage fungi is a function of both time and temperature, and that as the temperature is decreased the moisture content may be increased without danger of deterioration (Papavizas and

Christensen, 1958). Grain with high moisture contents must be kept at low temperatures to ensure safe storage.

Storage fungi require oxygen in order to survive. Peterson et al. (1956) found that decreasing the oxygen concentration in the absence of carbon dioxide progressively retarded mold growth and reduced losses in grain viability. Muir et al. (1973) put this fact to practical use. They stored wheat containing 21.6 percent moisture in air-tight containers at 20 to 30°C. The grain in the air-tight containers kept its viability for about 5 weeks whereas that in unsealed containers began to lose its viability almost immediately.

Gases such as carbon dioxide or nitrogen can be used to produce a mold suppressive atmosphere. Carbon dioxide concentrations over 18.6 percent markedly reduce mold growth, grain respiration, and development of fat acidity (Peterson et al., 1956). Storage in nitrogen will prevent mold growth at all moisture contents (Glass et al., 1959). Suppressive atmospheres may be used in future practical grain storage applications.

With the accumulation of experimental data, grain storage studies have offered many clues on how storage fungi may be controlled by manipulating the physical environment. The result has been improved drying techniques for all types of grain and new storage methods utilizing refrigeration, air-tight storage, and suppressive atmosphere.

2.4.3 Storage Fungi and The Biological Environment. Besides being acted on by the physical environment of a grain bulk, storage fungi are affected by their biological surroundings. Factors important in the biological environment are the grain variety and condition, the presence of other fungi, and carriers such as insects and mites.

It was recognized long ago that grain containing cracked or broken kernels was more of a storage risk than undamaged grain. A. Hurd (1921) showed that a break in the seed coat allowed saprophytic fungi to invade seeds. Once this has occurred all that is needed to start deterioration are physical conditions favorable to the fungi.

There is some evidence that seed variety can influence the amount of fungal invasion. Moreno-Martinez and Christensen (1971) noted that the variety of corn used in storage tests may determine the amount of damage by storage fungi. If this characteristic proved to be genetic, it would be possible to breed for resistance to storage fungi. To find such resistance and to incorporate it into otherwise desirable crops has not yet been attempted.

Storage fungi may be destroyed by other fungi in stored grain. For example, damp grain heating is due to a whole series of fungi, each increasing the temperature till the preceding species is destroyed. Sinha and Wallace (1965) have traced this ecological succession. Although species overlap, the general sequence of events starts with Penicillium spp. and ends with Streptomyces spp.

with Aspergillus spp. and Absidia in the middle. These organisms raise the temperature from -5 to 64°C in a matter of two weeks.

Insects and mites affect the microflora of stored grain. Insects initiate hot spots in grain which allows various storage molds to flourish. Sinha and Wallace (1966) state that insects do this by increasing the moisture content and temperature of the grain. Insects and mites damage grain and spread spores of storage fungi. Agrawal, et al. (1957) on studies of the relationship between grain storage fungi and the granary weevil have presented evidence that this pest spreads the spores of A. glaucus (group) throughout the grain bin. Mites carry spores on their bodies and in their digestive tract and feces (Griffiths et al., 1959). Without biological agents aiding storage fungi by increasing the grain temperature and moisture, weakening the kernels, and spreading their spores, storage fungi would be less of a problem.

## 2.5 Summary of Literature Review

Several types of microorganisms live in stored grain. They may be divided into fungi, actinomycetes, and bacteria.

The microorganisms which are chiefly responsible for grain losing its quality are fungi. Stored grain fungi are further divided into two classes based on their ecology and referred to as field fungi and storage fungi. Field fungi do not damage the stored grain after it is harvested.

On the other hand, storage fungi are the main cause of deterioration in grain free of insects. Most of them belong to the genera Penicillium or Aspergillus.

Grain storage fungi rely upon specific conditions of moisture, temperature, and oxygen supply to multiply. Storage molds follow a natural succession in stored grain depending on the changing moisture and temperature conditions within the bin. Generally Penicillium spp. are found at low temperatures and high moistures and Aspergillus spp. develop at average temperatures and moistures. Most storage fungi require a temperature higher than 10°C and a moisture content greater than that in equilibrium with a relative humidity of 70 percent. Furthermore, storage molds will grow at temperatures below their optimum growth temperature as the seed moisture content rises beyond that which is in equilibrium with a relative humidity of 70 percent.

Storage fungi are influenced by crop, variety and conditions of seed as well as insects and mites. Damaged seeds are quickly invaded by storage molds. Insects and mites help to spread storage fungi throughout the grain bulk by increasing moisture and temperature, damaging seed, and by dispersing spores.

# 3. METHOD AND MATERIALS

## 3.1 Introduction

In order to determine the conditions under which microorganisms will attack stored grain certain standard procedures have been developed. The methods used in this study have in the most part been taken from Christensen and Kaufmann (1969), Wallace and Sinha (1962), and Machacek *et al.* (1951).

## 3.2 Source and Type of Seed

Various crops (Table 3.1) were used all of which had high germination and were Manitoba grown.

TABLE 3.1

CROPS USED AND PERTINENT BACKGROUND INFORMATION

| Crop | Variety | Year Harvested | Percent Germination |
|---|---|---|---|
| New Wheat | Neepawa | 1973 | 98 ± 2 |
| Old Wheat | Selkirk | 1968 | 98 ± 2 |
| Barley | Conquest | 1973 | 98 ± 2 |
| Rapeseed | Zephyr | 1973 | 98 ± 2 |
| Fababeans | Diana | 1973 | 98 ± 2 |

Seed not used immediately in the tests was usually kept in a $0^{o}C$ cold room.

## 3.3 Preparation and Incubation of Samples

In all cases where barley, wheat and rapeseed had to be conditioned to different moisture contents the following procedure was observed. From 100 to 1,000 g of grain were put into flasks or mason jars large enough to permit thorough shaking of the seed. To this seed appropriate amounts of sterile water were added depending on the desired moisture content of the sample. Sterile water is distilled water autoclaved at 120°C for 15 min. The containers were sealed and shaken occasionally over a period of from 24 to 72 h at room temperature (20 - 25°C). The small amount of growth which possibly occurred at this time was assumed to be unimportant. After this period of moisture equilibration these large samples were tested for moisture content. They were then divided up into smaller samples and incubated at different temperatures. For some tests moisture determinations were made on the grain stored at different temperatures after a few weeks of storage.

The small samples were put into 14.5 cm by 2 cm diameter test tubes, capped with metal tops and sealed with vinyl tape. Where more than one test tube of seed was required of the same moisture content, they were put into a mason jar which was then sealed. Erlenmyer flasks (150 ml) were also used in one test to hold grain samples. These were tightly sealed with corks.

Fababeans were treated in a slightly different manner because no data existed on the time required for moisture

equilibration and because of the large seed size. From preliminary tests it was found that fababeans require more time to absorb water than small grains. Therefore the fababeans were left to equilibrate 4 d at 5°C before moisture content was determined. The low temperature was used to keep fungal growth in check. Medicine bottles (200 ml) had to be used for fababean samples in one test because of the large size of the seeds. After incubation of the fababeans in medicine bottles the caps were removed from the bottles every day for the first week in order to prevent the atmosphere in the bottles from going anaerobic. After this initial period only the bottles at temperatures over 15°C were opened every third or fourth d for the next 2 wk. Opening the bottles for sampling was thought to provide sufficient oxygen once this 3 wk period was concluded.

For the various tests reported in this thesis many different incubators were used. The range of temperatures were 2 ± 1, 9 ± 1, 15.5 ± 1, 17 ± 1, 20 ± 2, 21 ± 2, 25 ± 2, 33 ± 2, 37 ± 2, 40 ± 2 and 47 ± 2°C. The 33 ± 2°C incubator was fitted with a recording thermograph and its relative humidity was controlled at 70 percent. To test the effects of actual weather conditions on grain some samples were placed in an unheated shed at Winnipeg from September 18, 1973 to January 15, 1974. During this time the highest temperature recorded was 23.3°C and the lowest was -41.7°C.

The duration of the experiments ranged from 2 to 5 months. Depending on the test, seed was taken from the

samples at regular intervals to assess it for germination and microorganisms.

## 3.4 Moisture Content

When the accuracy of the seed moisture content measurement was not critical (± 1 percent) a Halross Model 919 moisture meter was used. For precise moisture determination the single stage oven method (Hart and Neustadt, 1957) in conjunction with a Sartorius Model scale accurate to 0.00001 g was used.

For fababeans an oven method had to be developed since I was unable to find any standard procedure in the literature. After preliminary tests comparing ground seed, a number of seeds and a single seed for oven moisture determinations it was decided that the single seed was the most reliable. Hence, four replicates of one seed were heated at 130 ± 2°C for 40 h.

## 3.5 Inoculation of Fababeans

In this study only the fababeans were preinoculated with storage fungi because little was known about the effects of storage fungi on this particular crop. The fungi used for inoculation had been previously found on stored fababeans from Manitoba (Platford, Wallace, and Bernier, 1974). The fungi were deposited on the seeds by placing agar disks of sporulating molds into a plastic bag containing 4.2 kg of seed and then mixing thoroughly. The seed was equilibrated with sterile water, moisture determinations made, subsampled

and incubated.

The following fungi were inoculated on the fababeans: Alternaria alternata (Fr.) Keissler, Cladosporium cladosporioides (Fres.) de Vries, Aspergillus amstelodami (Mang.) Thom & Church, A. candidus Link ex Fr., A. clavatus Desm., A. flavus Link ex Fr., A. fumigatus Fres. A. nidulans (Eidam) Wint., A. niger van Tigehem, A. ochraceus Wilhelm, A. repens (Corda) Sacc., A. sejunctus Bain & Sartory (= A. ruber Thom & Church), A. terreus Thom, A. versicolor (Vuill.) Tiraboschi, and A. wentii Wehmer.

In a similar experiment only one type of fungus was used at a time. First the seeds were sterilized with a 0.6 percent solution of sodium hypochlorite to remove the fungi on the surface of the seed. The seeds were then conditioned to different moisture contents. Spore suspensions of Aspergillus fumigatus, A. nidulans, A. repens, A. versicolor and A. wentii were made. A 0.2 ml spore suspension of one fungus was inoculated on the seed of a particular sample. A sample inoculated with 0.2 ml of water was used to determine the moisture content of the incubated seed. The remaining samples were incubated at different temperatures.

## 3.6 Germination of Seed

Germination of the seed was established by counting the number of seeds showing shoots after 7 d of incubation at room temperature. In one instance the plates were incubated at 33 and 40$^{o}$C and placed in plastic bags to retain moisture.

For barley, wheat and rapeseed 25 seeds were placed around the periphery of a 9 cm diameter No. 3 Whatman filter paper disk. The filter paper was contained in a petri plate, both having been sterilized for 3.5 h at 175°C. To provide sufficient moisture for germination but not to drench the seed, 4 ml of sterile water was used to saturate the filter paper.

Once again some preliminary testing was necessary for fababeans. It was found that they require more water to germinate than other smaller seeds. Therefore two filter papers saturated with 7 ml of sterile water were used. Because of the large seed size only five seeds a plate, dispersed evenly over the filter paper, were possible. For fababeans germination was also determined on Czapek agar.

## 3.7 Determination of Natural Inoculum

The natural microflora of the seeds were determined by examining the kernels on filter paper after they had been counted for germination. Wallace (1962) states that the filter paper method is the most suitable because it simulates the micro-environment in which fungi usually grow under natural conditions. First, identification with the aid of a stereo microscope at magnifications of 50X to 100X was made. In some cases final identifications were made later at a higher magnification. If this was not possible fungi were identified only after incubation on Potato Dextrose, Czapek or Malt Salt agar slants.

In the identification of microorganisms they are

usually referred to by their generic names. The following genera were represented by a single species: Alternaria alternata, Cladosporium cladosporoides, Drechslera sorokiniana (Sacc.) Subram & Join (= Helminthosporium sativum Pammel & al.) Rhizopus arrhizus Fischer, Mucor pusillus Lindt, Nigrospora oryzae (Berk & Br.) Petch, Paecylomyces varioti Bain, Tricothecium roseum Link, Arthrinium phaesospermum (Corda) M. B. Ellis and Epicoccum purpurascens Ehrenb. ex Schlecht. The commonest species in each genus were: Fusarium: F. poae (Peck) Wollenw. Absidia: A. corymbifera (Cohn) Sacc. & Trott., A. glauca Hagem. Penicillium: P. cyclopium Westling, P. funiculosum Thom, P. chrysogenum Thom, P. viridicatum Thom.

Since certain species of Aspergillus are not always well defined, they are sometimes referred to as "groups." This is particularly true of the A. glaucus group. In these experiments the A. glaucus group consisted of A. amstelodami, A. repens, and A. sejunctus.

Actinomycetes recognized in these tests belonged mainly to the genus Streptomyces. S. griseus (Krainsky) Waxmann and Henrici is the most common species found on grain (Wallace, 1973).

The bacteria were usually of two types. Yellow cultures resembling Bacterium herbicola aureum Duggeli (James, 1955) and white cultures resembling the unnamed species of Pseudomonas referred to by James et al. (1946). Yeasts were not identified.

Malt Salt and Czapek agar were used in the detection of microorganisms on fababeans. Malt Salt agar is made by adding 100 g of sodium chloride to 1 liter of regular Malt agar. It is used to indicate storage fungi such as the Aspergillus glaucus (group) which grow best on media of high osmotic pressure. Papavizas and Christensen (1957) stated that Malt Salt media was superior to any other tested for the isolation of various storage fungi. However, Malt Salt agar does not allow seed germination.

Czapek agar is an all purpose media and will reveal most fungi that are present on or in seeds. It has the added advantage of allowing seeds to germinate.

About 18 ml of media were placed in 9.5 cm diameter by 1.5 cm deep petri plates to a depth of 0.5 to 0.75 cm. Five kernels of fababeans were placed on each of these agars. After incubating at room temperature for 7 d the plates were counted for germination and read for fungi.

## 3.8 Determination of Internal Inoculum

In order to find out what type of microflora has actually penetrated the seed coat surface, sterilization of the pericarp is necessary. In this study for most cases a 0.6 percent aquaeous solution of sodium hypochlorite was adequate. By immersing the seeds in this fungicide for 2 min most of the exterior fungi were killed. The seeds were then rinsed twice in sterile water to remove any traces of the sterilant.

Unfortunately, the sodium hypochlorite was not as effective as desired on the fababeans. Therefore in some tests a mercuric chloride solution was used. It consisted of one part of 95 percent ethyl alcohol in three parts of a 0.1 percent aqueous solution of mercuric chloride. Seed was immersed in this solution for 3 min followed by two rinses in sterile water.

Following disinfection the seeds were plated asceptically on filter paper or agar, incubated usually at room temperature and analyzed after 7 d in the same way as described above.

# 4. ANALYSIS OF RESULTS

## 4.1 Introduction

There was a wide range in significance of the results because these experiments were of an exploratory nature. The following tests yielded the most information:

## 4.2 A General Survey of the Microflora on or in Grain

4.2.1 Freshly Harvested Wheat. The seed was divided into seven lots and moisture content was adjusted to give a range of 13.0 to 39.9 percent. Each lot was subdivided into nine samples each of which was stored at temperatures ranging from 2 to 47°C. After storage for 2 to 4 months the seed was plated on filter paper moistened with 4 ml of water. Results were recorded after incubation for 7 d at room temperature. Tables 4.1 and 4.2 contain the essence of these results.

Table 4.3 shows the temperature and moisture content of the stored Neepawa wheat at which specific fungi were detected. The extent of infection under these conditions is also indicated.

TABLE 4.1

MEAN EFFECT OF MOISTURE CONTENT ON GERMINATION AND GROWTH OF MICROFLORA OF NINE LOTS OF NEEPAWA WHEAT STORED AT DIFFERENT TEMPERATURES (2 - 47°C)

| | | Percent of Seed Infected by | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Percent Moisture Content | Percent Germination | *Alternaria* | *Cephalosporium* | *Tricothecium* | *Fusarium* | *A. glaucus* (group) | *A. versicolor* | *A. fumigatus* | *Penicillium* | *Streptomyces* | Bacteria |
| 13.0 | 88 | 50 | 0 | 0 | 0 | 10 | 0 | 0 | 1 | 0 | 0 |
| 16.9 | 63 | 51 | 0 | 0 | 0 | 11 | 1 | 0 | 3 | 6 | 0 |
| 19.8 | 54 | 52 | 0 | 0 | 0 | 3 | 15 | 0 | 18 | 40 | 0 |
| 23.8 | 38 | 36 | 0 | 22 | 16 | 0 | 0 | 18 | 2 | 48 | 0 |
| 27.4 | 7 | 19 | 26 | 24 | 0 | 0 | 3 | 25 | 0 | 65 | 17 |
| 32.9 | 28 | 19 | 34 | 17 | 4 | 0 | 0 | 37 | 0 | 43 | 0 |
| 39.9 | 30 | 14 | 23 | 10 | 28 | 0 | 0 | 28 | 0 | 36 | 1 |

TABLE 4.2

MEAN EFFECT OF TEMPERATURE ON GERMINATION AND GROWTH OF MICROFLORA OF SEVEN LOTS OF NEEPAWA WHEAT STORED AT DIFFERENT MOISTURE CONTENTS (13.0 - 39.9 Percent)

| | | Percent of Seed Infected by | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Temperature °C | Percent Germination | *Alternaria* | *Cephaloporium* | *Tricothecium* | *Fusarium* | *A. glaucus* (group) | *A. versicolor* | *A. fumigatus* | *Penicillium* | *Streptomyces* | Bacteria |
| 2 | 61 | 92 | 20 | 0 | 2 | 0 | 0 | 0 | 2 | 16 | 0 |
| 9 | 75 | 67 | 15 | 13 | 1 | 0 | 0 | 0 | 2 | 36 | 1 |
| 17 | 81 | 63 | 1 | 9 | 2 | 0 | 0 | 0 | 7 | 40 | 0 |
| 21 | 60 | 33 | 12 | 20 | 13 | 1 | 0 | 0 | 4 | 48 | 4 |
| [1]CL | 66 | 49 | 17 | 13 | 9 | 0 | 0 | 0 | 0 | 24 | 1 |
| 25 | 47 | 15 | 8 | 5 | 13 | 5 | 4 | 11 | 7 | 39 | 8 |
| 33 | 25 | 5 | 3 | 3 | 14 | 25 | 14 | 7 | 1 | 50 | 0 |
| 40 | 11 | 1 | 2 | 12 | 6 | 4 | 0 | 47 | 4 | 10 | 0 |
| 47 | 11 | 2 | 0 | 3 | 0 | 0 | 1 | 53 | 6 | 1 | 1 |

[1]CL - temperatures found in an unheated shed in Winnipeg, Manitoba (-41.7 to 23.3°C).

TABLE 4.3

MOISTURE AND TEMPERATURE RANGE OF IMPORTANT MICROFLORA ON NEEPAWA WHEAT

| Microflora | Storage Temperature °C | Percent Moisture Content | Approximate Percentage of Seed Infected ± 10 |
|---|---|---|---|
| Alternaria | 2 - 25 | 13.0-19.8 | 50 |
| Cephalosporium | 2 - 21 | 27.9-39.9 | 30 |
| Penicillium | 2 - 47 | 13.0-23.8 | 10 |
| Tricothecium | 9 - 21 | 23.8-39.9 | 20 |
| Streptomyces | 9 - 33 | 19.8-39.9 | 55 |
| Fusarium | 21 - 33 | 39.9 | 20 |
| A. glaucus | 25 - 33 | 13.0-16.9 | 15 |
| A. versicolor | 33 | 19.8 | 15 |
| A. fumigatus | 40 - 47 | 23.8 - 39.9 | 45 |

4.2.2 Five-Year-Old Wheat. In this test seed was divided into nine lots and moisture content was adjusted to give a range of 10.2 to 42.8 percent, and each lot was further subdivided into nine samples each of which was stored at temperatures ranging from 2 to 47°C. After 2 to 4 months storage, seed was placed on moist filter paper and incubated at room temperature for 7 d before analysis. Tables 4.4 and 4.5 contain the essence of this analysis.

Table 4.6 shows the temperature and moisture content of stored Selkirk wheat at which specific fungi were detected. The extent of infection under these conditions is also indicated.

TABLE 4.4

MEAN EFFECT OF MOISTURE CONTENT ON GERMINATION AND GROWTH OF MICROFLORA OF NINE LOTS OF 5-YEAR-OLD SELKIRK WHEAT STORED AT DIFFERENT TEMPERATURES (2 - 47°C)

| Percent Moisture Content | Percent Germination | Percent Seed Infected By | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cepholosporium | Tricothecium | Arthrinium | Mucor | A. glaucus (group) | A. candidus | A. versicolor | A. niger | A. fumipatus | Penicillium | Streptomyces | Bacteria |
| 10.2 | 91 | 0 | 0 | 0 | 0 | 32 | 0 | 0 | 0 | 6 | 17 | 0 | 0 |
| 12.0 | 73 | 0 | 1 | 0 | 0 | 37 | 0 | 0 | 0 | 12 | 23 | 0 | 1 |
| 14.7 | 61 | 0 | 0 | 0 | 0 | 29 | 0 | 0 | 0 | 14 | 22 | 0 | 0 |
| 17.9 | 40 | 0 | 0 | 1 | 0 | 37 | 19 | 1 | 1 | 12 | 10 | 0 | 1 |
| 20.8 | 36 | 0 | 0 | 0 | 5 | 0 | 17 | 6 | 0 | 13 | 65 | 11 | 0 |
| 25.7 | 8 | 11 | 0 | 22 | 16 | 0 | 8 | 0 | 0 | 31 | 27 | 15 | 0 |
| 31.5 | 11 | 5 | 13 | 0 | 20 | 0 | 0 | 1 | 3 | 36 | 19 | 22 | 2 |
| 37.4 | 4 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 3 | 32 | 20 | 0 | 9 |
| 42.8 | 0 | 0 | 0 | 2 | 6 | 2 | 0 | 0 | 22 | 27 | 21 | 2 | 1 |

TABLE 4.5

MEAN EFFECT OF TEMPERATURE ON GERMINATION AND GROWTH OF MICROFLORA OF NINE LOTS OF 5-YEAR-OLD SELKIRK WHEAT STORED AT DIFFERENT MOISTURE CONTENTS (10.2 - 42.8 percent)

| | | Percent Seed Infected By | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Temperature °C | Percent Germination | Cephalosporium | Tricothecium | Arthrinium | Mucor | A. glaucus (group) | A. candidus | A. versicolor | A. niger | A. fumigatus | Penicillium | Strephomyces | Bacteria |
| 2 | 65 | 0 | 0 | 2 | 6 | 0 | 0 | 0 | 0 | 7 | 41 | 0 | 1 |
| 9 | 47 | 0 | 0 | 8 | 24 | 13 | 0 | 0 | 3 | 6 | 51 | 0 | 0 |
| 17 | 42 | 0 | 0 | 8 | 8 | 15 | 9 | 1 | 14 | 8 | 55 | 0 | 0 |
| 21 | 41 | 0 | 8 | 11 | 13 | 30 | 7 | 4 | 8 | 25 | 11 | 0 | 1 |
| [1]CL | 49 | 0 | 0 | 14 | 6 | 12 | 0 | 1 | 5 | 24 | 27 | 0 | 4 |
| 25 | 38 | 16 | 5 | 1 | 0 | 39 | 12 | 0 | 7 | 10 | 5 | 11 | 2 |
| 33 | 23 | 0 | 1 | 0 | 1 | 27 | 20 | 1 | 11 | 32 | 1 | 7 | 0 |
| 40 | 8 | 0 | 2 | 0 | 0 | 1 | 3 | 0 | 5 | 43 | 8 | 24 | 5 |
| 47 | 11 | 0 | 0 | 0 | 2 | 0 | 3 | 0 | 6 | 30 | 18 | 7 | 1 |

[1]CL - Temperatures found in an unheated shed in Winnipeg, Manitoba (-41.7 to 23.3°C).

TABLE 4.6

MOISTURE AND TEMPERATURE RANGE OF IMPORTANT MICROFLORA ON 5-YEAR-OLD SELKIRK WHEAT

| Microflora | Storage Temperature °C | Percent Moisture Content | Approximate Percentage of Seed Infected ± 10 |
|---|---|---|---|
| Mucor | 2 - 21 | 20.8-42.8 | 15 |
| Penicillium | 2 - 17 | 20.8 | 50 |
| A. glaucus | 9 - 33 | 10.2-17.9 | 25 |
| A. candidus | 17 - 47 | 17.9-25.7 | 10 |
| A. niger | 17 - 47 | 42.8 | 15 |
| A. fumigatus | 21 - 47 | 25.7-42.8 | 30 |
| Streptomyces | 25 - 47 | 20.8-31.5 | 15 |

4.2.3 Freshly Harvested Barley. The seed was divided into nine lots and moisture content was adjusted to give a range of 13.1 to 44.2 percent. Each lot was subdivided into nine samples each of which was stored for 2 to 4 months at temperatures ranging from 2 to 47°C. The seed was plated on filter paper moistened with 4 ml of water and examined after 7 d at room temperature. Tables 4.7 and 4.8 contain the essence of this examination.

Table 4.9 shows the temperature and moisture content of stored Conquest barley at which specific fungi were detected. The extent of infection under these conditions is also indicated.

TABLE 4.7

MEAN EFFECT OF MOISTURE CONTENT ON GERMINATION AND GROWTH OF MICROFLORA OF NINE LOTS OF CONQUEST BARLEY STORED AT DIFFERENT TEMPERATURES (2 - 47°C)

| | | Percent Seed Infected By | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Percent Moisture Content | Percent Germination | *Alternaria* | *Drechslera* | *Cephalosporium* | *Paecilomyces* | *Fusarium* | *A. glaucus* (group) | *A. versicolor* | *A. flavus* | *A. niger* | *A. fumigatus* | *Penicillium* | *Streptomyces* | Bacteria |
| 13.1 | 76 | 61 | 7 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 3 | 0 | 0 |
| 14.9 | 73 | 53 | 8 | 0 | 1 | 0 | 12 | 1 | 1 | 0 | 4 | 3 | 0 | 1 |
| 17.3 | 62 | 52 | 13 | 0 | 0 | 0 | 9 | 0 | 2 | 1 | 1 | 8 | 0 | 0 |
| 19.4 | 59 | 58 | 8 | 0 | 0 | 4 | 6 | 0 | 1 | 1 | 2 | 26 | 12 | 0 |
| 23.2 | 48 | 40 | 4 | 2 | 9 | 12 | 0 | 2 | 1 | 2 | 12 | 8 | 36 | 9 |
| 29.1 | 11 | 21 | 1 | 4 | 0 | 33 | 0 | 4 | 5 | 1 | 3 | 12 | 20 | 0 |
| 34.9 | 16 | 14 | 3 | 12 | 3 | 46 | 0 | 3 | 35 | 9 | 2 | 5 | 11 | 3 |
| 39.3 | 19 | 12 | 0 | 11 | 0 | 37 | 0 | 0 | 6 | 3 | 16 | 2 | 1 | 0 |
| 44.2 | 0 | 7 | 0 | 1 | 0 | 37 | 0 | 0 | 0 | 7 | 23 | 14 | 2 | 0 |

TABLE 4.8

MEAN EFFECT OF TEMPERATURE ON GERMINATION AND GROWTH OF MICROFLORA OF NINE LOTS OF CONQUEST BARLEY STORED AT DIFFERENT MOISTURE CONTENTS (13.1 - 44.2 Percent)

| Temperature °C | Percent Germination | Percent Seed Infected By | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Alternaria | Drechslera | Cephalosporium | Paecilomyces | Fusarium | A. glaucus (group) | A. versicolor | A. flavus | A. niger | A. fumigatus | Penicillium | Streptomyces | Bacteria |
| 2 | 74 | 90 | 10 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | 52 | 55 | 7 | 9 | 3 | 36 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 0 |
| 17 | 61 | 52 | 11 | 0 | 0 | 26 | 0 | 0 | 0 | 0 | 0 | 16 | 20 | 0 |
| 21 | 61 | 50 | 9 | 6 | 0 | 21 | 1 | 0 | 4 | 2 | 16 | 2 | 19 | 0 |
| [1]CL | 51 | 59 | 4 | 10 | 0 | 23 | 0 | 0 | 0 | 1 | 9 | 0 | 2 | 0 |
| 25 | 40 | 13 | 4 | 3 | 0 | 37 | 3 | 0 | 9 | 2 | 1 | 15 | 16 | 3 |
| 33 | 30 | 1 | 0 | 0 | 9 | 12 | 20 | 0 | 10 | 3 | 2 | 18 | 16 | 13 |
| 40 | 0 | 1 | 0 | 0 | 1 | 2 | 2 | 7 | 14 | 11 | 22 | 18 | 1 | 1 |
| 47 | 0 | 2 | 0 | 0 | 0 | 3 | 2 | 4 | 15 | 6 | 12 | 12 | 7 | 2 |

[1]CL - Temperatures found in an unheated shed in Winnipeg, Manitoba (-41.7 to 23.3°C).

TABLE 4.9

MOISTURE AND TEMPERATURE RANGE OF IMPORTANT MICROFLORA ON CONQUEST BARLEY

| Microflora | Storage Temperature °C | Percent Moisture Content | Approximate Percentage of Seed Infected ± 10 |
|---|---|---|---|
| Alternaria | 2 - 21 | 13.1-23.2 | 50 |
| Fusarium | 9 - 25 | 29.1-44.2 | 30 |
| Streptomyces | 17 - 33 | 19.4-34.9 | 15 |
| Penicillium | 17 - 47 | 17.3-44.2 | 15 |
| A. glaucus (group) | 33 | 14.9-19.4 | 15 |
| A. flavus | 25 - 47 | 29.1-39.3 | 15 |
| A. fumigatus | 21 - 47 | 23.2-44.2 | 15 |

4.2.4 Freshly Harvested Rapeseed. The seed was divided into eight lots and moisture content was adjusted to give a range of 6.1 to 43.4 percent. Each lot was subdivided into nine samples each of which was stored for 2 to 4 months at temperatures ranging from 2 to 47°C. Seeds were plated on filter paper moistened with 4 ml of water and incubated for 7 d at room temperature before analysis. The essence of the analysis is contained in Tables 4.10 and 4.11.

Table 4.12 shows the temperature and moisture content of stored Zephyr rapeseed at which specific fungi were detected. The extent of infection under these conditions is also indicated.

TABLE 4.10

MEAN EFFECT OF MOISTURE CONTENT ON GERMINATION AND GROWTH OF MICROFLORA OF NINE LOTS OF ZEPHYR RAPESEED STORED AT DIFFERENT TEMPERATURES (2 - 47°C)

| | | Percent Seed Infected By | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Percent Moisture Content | Percent Germination | *Alternaria* | *Cladosporium* | *Arthrinium* | *Fusarium* | *A. glaucus* (group) | *A. versicolor* | *A. flavus* | *A. niger* | *A. fumigatus* | *Penicillium* |
| 6.1 | 97 | 2 | 38 | 0 | 0 | 4 | 0 | 0 | 0 | 11 | 16 |
| 7.8 | 78 | 1 | 12 | 0 | 0 | 7 | 0 | 1 | 0 | 9 | 34 |
| 10.4 | 67 | 2 | 8 | 0 | 0 | 15 | 0 | 0 | 0 | 12 | 31 |
| 13.2 | 64 | 5 | 15 | 0 | 0 | 2 | 6 | 0 | 6 | 4 | 47 |
| 17.6 | 59 | 6 | 17 | 0 | 0 | 0 | 24 | 0 | 8 | 11 | 56 |
| 22.4 | 35 | 10 | 17 | 13 | 6 | 0 | 8 | 1 | 5 | 14 | 28 |
| 29.3 | 21 | 2 | 4 | 0 | 16 | 0 | 1 | 6 | 10 | 16 | 19 |
| 43.4 | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 8 | 21 | 31 |

TABLE 4.11

MEAN EFFECT OF TEMPERATURE ON GERMINATION AND GROWTH OF MICROFLORA OF EIGHT LOTS OF ZEPHYR RAPESEED STORED AT DIFFERENT MOISTURE CONTENTS (6.1 - 43.4 Percent)

| Temperature °C | Percent Germination | Percent Seed Infected By | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Alternaria | Cladosporium | Arthrinium | Fusarium | A. glaucus (group) | A. versicolor | A. flavus | A. niger | A. fumigatus | Penicillium |
| 2 | 98 | 0 | 52 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14 |
| 9 | 88 | 8 | 15 | 10 | 6 | 0 | 0 | 6 | 0 | 0 | 60 |
| 17 | 60 | 2 | 4 | 0 | 3 | 0 | 14 | 1 | 0 | 2 | 81 |
| 21 | 54 | 5 | 7 | 0 | 15 | 3 | 1 | 0 | 1 | 15 | 35 |
| [1]CL | 65 | 7 | 35 | 2 | 10 | 0 | 0 | 6 | 3 | 9 | 40 |
| 25 | 66 | 2 | 4 | 1 | 0 | 6 | 21 | 0 | 11 | 25 | 22 |
| 33 | 23 | 2 | 1 | 0 | 0 | 17 | 4 | 8 | 18 | 20 | 8 |
| 40 | 11 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 8 | 12 | 4 |
| 47 | 12 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 15 | 28 | 8 |

[1]CL - Temperatures found in an unheated shed in Winnipeg, Manitoba (-41.7 - 23.3°C).

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TABLE 4.12

MOISTURE AND TEMPERATURE RANGE OF IMPORTANT MICROFLORA ON ZEPHYR RAPESEED

| Microflora | Storage Temperature °C | Percent Moisture Content | Approximate Percentage of Infected Seed ± 10 |
|---|---|---|---|
| Cladosporium | 2 - 9 | 6.1-22.4 | 20 |
| Arthrinium | 9 | 22.4 | 10 |
| Fusarium | 9 - 21 | 22.4-29.3 | 15 |
| Penicillium | 9 - 17 | 13.2-17.6 | 50 |
| A. glaucus (group) | 21 - 33 | 6.1-13.2 | 10 |
| A. versicolor | 17 - 25 | 13.2-22.4 | 15 |
| A. fumigatus | 21 - 47 | 6.1-43.4 | 15 |
| A. niger | 25 - 47 | 13.2-43.4 | 15 |

## 4.3 Effect of Temperature, Moisture and Time on The Deterioration of Stored Barley

4.3.1 Introduction. The effect of different moisture contents and temperatures on stored barley germination were studied over a period of 27 wk. Six lots of barley were conditioned to moisture contents from 13.8 to 23.9 percent. These were subdivided into six samples and incubated at temperatures from 2 to 33°C. The seeds were periodically plated on moist filter paper over 27 wk and counted for germination.

4.3.2 Effect of Moisture Content and Time on Barley Germination. Germination of barley kernels declined with increasing moisture content and time (Fig. 4-1). At high moisture contents germination began to decrease in less than 6 wk while at the medium moisture contents it took about 11 wk. At 15.5 percent moisture content germination did not decline for 15 wk. At 13.8 percent moisture content the seed did not decline in germination.

4.3.3 Effect of Temperature and Time on Barley Germination. Germination also decreased with increasing temperature and time. In 27 wk there was no loss in germination at 2 or 9°C and only a slight loss at 15.5 and 20°C (Fig. 4-2). The seed viability quickly degenerated at 25 and 33°C. At 33°C a loss in germination occurred almost immediately.

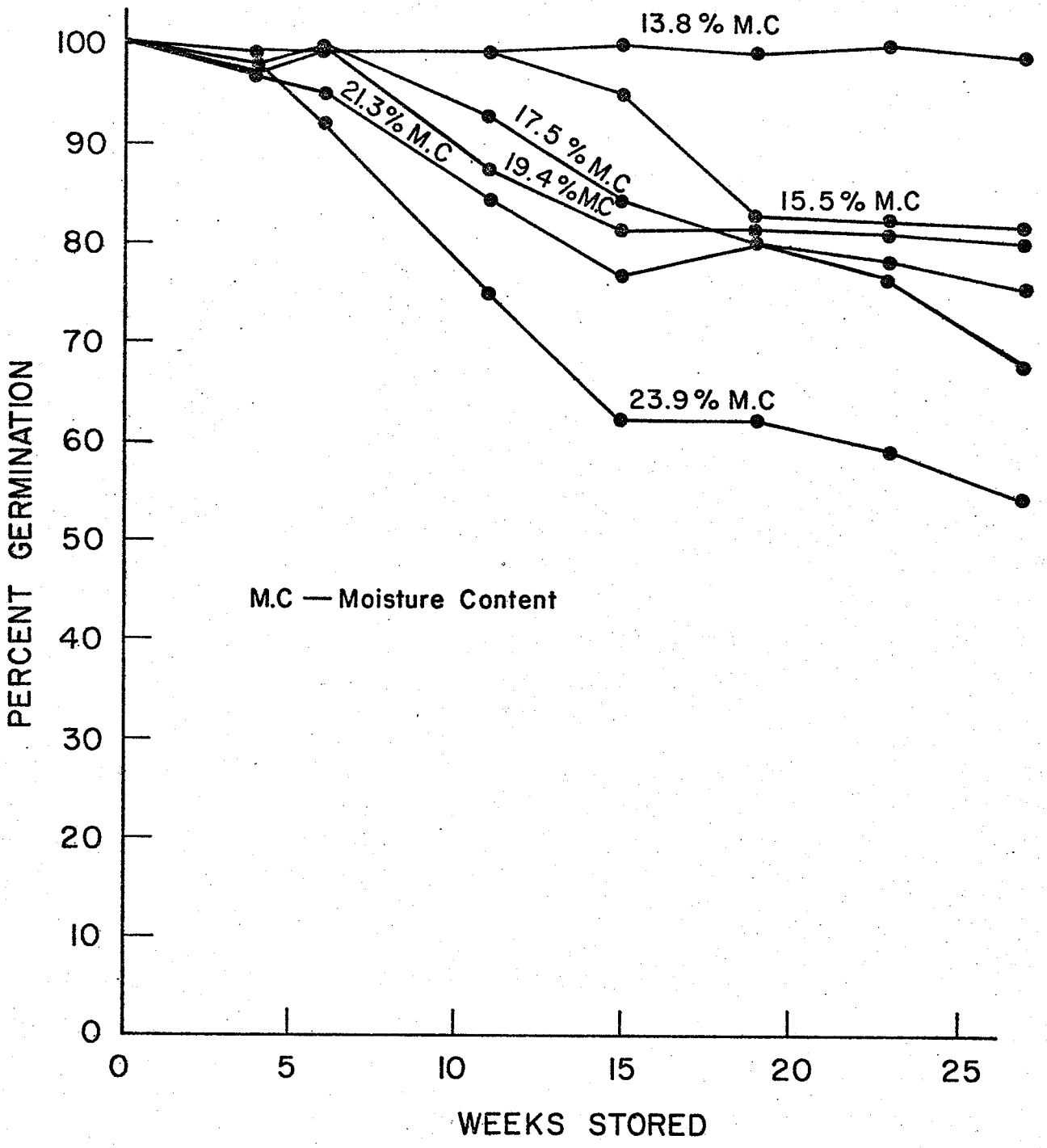


**Fig. 4.1** Effect of moisture and time on mean barley germination of seed stored at 2 to 33°C.

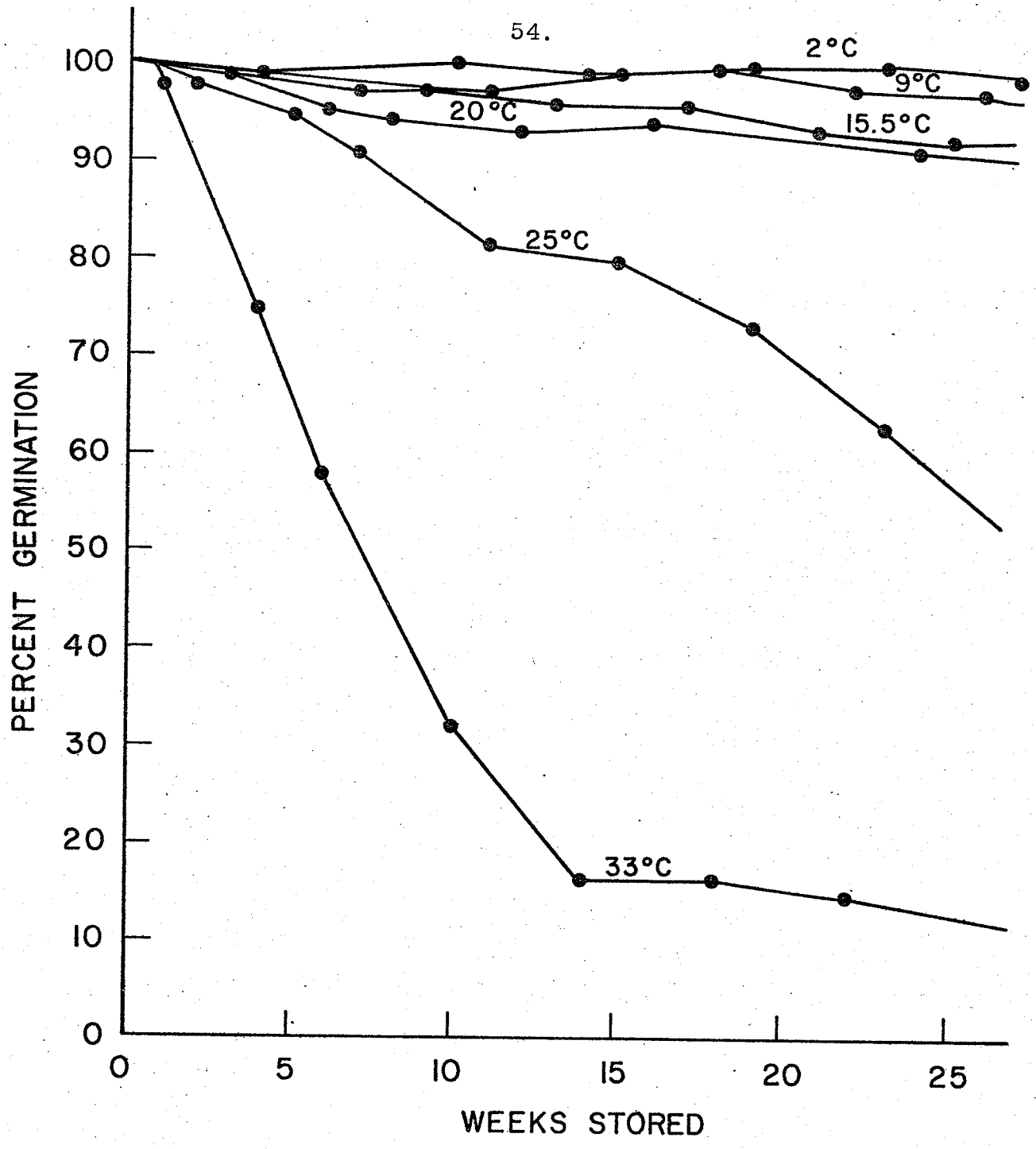


Fig. 4.2 Effect of temperature and time on mean barley germination of seed stored at moisture contents of 13.8 to 23.9 percent.

4.3.4 Effect of Moisture Content, Temperature and Time on Barley Germination. When both temperature and moisture content were analyzed together an important relation became apparent. Table 4.13 shows that seed stored at high temperatures will deteriorate at lower moisture contents.

TABLE 4.13

EFFECT OF TEMPERATURE, MOISTURE CONTENT AND TIME ON STORED BARLEY GERMINATION OVER 27 WEEKS

| Seed Storage Temperature °C | [1]M.C. at which Germination Decreased at Least 5% | Time (wk) For Germination To Drop at 23.9% M.C. | Percent Germination at 23.9% M.C. After 27 wk |
|---|---|---|---|
| 9 | 21.3 - 23.9 | 22 | 94 ± 2 |
| 15.5 | 21.3 - 23.9 | 3 | 69 ± 2 |
| 20 | 19.4 - 23.9 | 6 | 64 ± 2 |
| 25 | 17.5 - 23.9 | 1 | 0 |
| 33 | 15.5 - 23.9 | 1 | 0 |

[1]Moisture Content.

## 4.4 Effect of Moisture, Temperature and Time on the Most Common Microflora of Stored Barley

4.4.1 Introduction. The effect of different moisture contents and temperatures on stored barley microflora were studied over a period of 27 wk. As mentioned in the previous section six lots of barley were conditioned to different moisture contents (13.8 to 23.9 percent) and subdivided into six samples stored at different temperatures (2 to 33°C).

The seeds were periodically incubated on moist filter paper and examined after 7 d at room temperature for microflora.

4.4.2 Effect of Moisture Content and Time on Common Microflora of Barley. Even when the barley was stored at a moisture content of 13.8 percent the microflora changed considerably after 27 wk of storage (Table 4.14). For example, the percentage of Alternaria infection declined from 99 to 66 percent.

TABLE 4.14

THE EFFECT OF TIME (4 - 27 WEEKS) ON THE MEAN PERCENTAGE OF MICROFLORA OCCURRING ON STORED BARLEY OF 13.8 PERCENT MOISTURE CONTENT (MEAN OF SIX TEMPERATURES)

| | Storage Time (Weeks) | | | | | | |
|---|---|---|---|---|---|---|---|
| Microflora | 4[1] | 6[2] | 11 | 15 | 19 | 23 | 27 |
| Alternaria | 99 | 91 | 81 | 75 | 71 | 67 | 66 |
| Cladosporium | 4 | 1 | 4 | 5 | 3 | 3 | 2 |
| Drechslera | 14 | 22 | 12 | 16 | 15 | 13 | 14 |
| A. glaucus (group) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A. candidus | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A. versicolor | 0 | 0 | 12 | 0 | 1 | 0 | 4 |
| Penicillium | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Streptomyces | 3 | 47 | 18 | 32 | 14 | 3 | 22 |

[1]Includes only temperatures 2, 9, 15.5, and 20°C.

[2]Includes only temperatures 20, 25 and 33°C.

At a moisture content of 15.5 percent an important change in the microflora of the stored barley seed took place (Table 4.15). Aspergillus glaucus (group) appeared after 11 wk.

TABLE 4.15

THE EFFECT OF TIME (4 - 27 WEEKS) ON THE MEAN PERCENTAGE OF MICROFLORA OCCURRING ON STORED BARLEY OF 15.5 PERCENT MOISTURE CONTENT (MEAN OF SIX TEMPERATURES)

| | Storage Time (Weeks) | | | | | | |
|---|---|---|---|---|---|---|---|
| Microflora | 4[1] | 6[2] | 11 | 15 | 19 | 23 | 27 |
| Alternaria | 97 | 92 | 71 | 72 | 61 | 55 | 52 |
| Cladosporium | 6 | 3 | 4 | 7 | 4 | 5 | 2 |
| Drechslera | 15 | 10 | 11 | 15 | 11 | 8 | 11 |
| A. glaucus (group) | 0 | 0 | 1 | 7 | 12 | 13 | 16 |
| A. candidus | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A. versicolor | 0 | 0 | 13 | 0 | 0 | 0 | 6 |
| Penicillium | 0 | 0 | 0 | 0 | 2 | 0 | 1 |
| Streptomyces | 1 | 37 | 20 | 25 | 12 | 1 | 20 |

[1]Includes only temperatures 2, 9, 15.5 and 20°C.

[2]Includes only temperatures 20, 25 and 33°C.

At the 17.5 percent moisture content *Aspergillus versicolor* and *A. candidus* appeared after 11 and 15 wk, respectively (Table 4.16).

TABLE 4.16

THE EFFECT OF TIME (4 - 27 WEEKS) ON THE MEAN PERCENTAGE OF MICROFLORA OCCURRING ON STORED BARLEY OF 17.5 PERCENT MOISTURE CONTENT (MEAN OF SIX TEMPERATURES)

| | Storage Time (Weeks) | | | | | | |
|---|---|---|---|---|---|---|---|
| Microflora | 4[1] | 6[2] | 11 | 15 | 19 | 23 | 27 |
| *Alternaria* | 97 | 95 | 86 | 75 | 70 | 65 | 62 |
| *Cladosporium* | 10 | 1 | 1 | 4 | 4 | 5 | 2 |
| *Drechslera* | 18 | 11 | 8 | 13 | 9 | 8 | 9 |
| *A. glaucus* (group) | 0 | 0 | 15 | 33 | 26 | 29 | 23 |
| *A. candidus* | 0 | 0 | 0 | 1 | 3 | 11 | 13 |
| *A. versicolor* | 0 | 0 | 7 | 0 | 0 | 6 | 17 |
| *Penicillium* | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| *Streptomyces* | 2 | 23 | 30 | 20 | 7 | 2 | 10 |

[1]Includes only temperatures 2, 9, 15.5 and 20°C.

[2]Includes only temperatures 20, 25 and 33°C.

At the 19.4 percent moisture content the appearance of storage fungi occurred in the following sequence: *Aspergillus glaucus* (group) (6 wk), *A. candidus* and *Penicillium* (11 wk), and *A. versicolor* which became the dominant fungus after 19 wk (Table 4.17).

TABLE 4.17

THE EFFECT OF TIME (4 - 27 WEEKS) ON THE MEAN PERCENTAGE OF MICROFLORA OCCURING ON STORED BARLEY OF 19.4 PERCENT MOISTURE CONTENT (MEAN OF SIX TEMPERATURES)

| | Storage Time (Weeks) | | | | | | |
|---|---|---|---|---|---|---|---|
| Microflora | 4[1] | 6[2] | 11 | 15 | 19 | 23 | 27 |
| Alternaria | 96 | 96 | 78 | 79 | 76 | 76 | 73 |
| Cladosporium | 8 | 2 | 1 | 3 | 3 | 2 | 1 |
| Drechslera | 18 | 14 | 12 | 16 | 12 | 11 | 8 |
| A. glaucus (group) | 0 | 12 | 13 | 11 | 5 | 1 | 1 |
| A. candidus | 0 | 1 | 13 | 15 | 14 | 16 | 2 |
| A. versicolor | 0 | 0 | 1 | 1 | 32 | 27 | 39 |
| Penicillium | 0 | 1 | 12 | 13 | 13 | 8 | 14 |
| Streptomyces | 1 | 15 | 14 | 23 | 0 | 3 | 7 |

[1]Includes only temperatures 2, 9, 15.5 and 20°C.

[2]Includes only temperatures 20, 25 and 33°C.

At the 21.3 percent moisture content Aspergillus candidus and A. glaucus (group) reached their peak infection after only 11 wk while it took A. versicolor 19 wk (Table 4.18).

On the 23.9 percent moisture content barley only the Aspergillus versicolor infection increased of the Aspergillus species (Table 4.19).

TABLE 4.18

THE EFFECT OF TIME (4 - 27 WEEKS) ON THE MEAN PERCENTAGE OF MICROFLORA OCCURING ON STORED BARLEY OF 21.3 PERCENT MOISTURE CONTENT (MEAN OF SIX TEMPERATURES)

| | Storage Time (Weeks) | | | | | | |
|---|---|---|---|---|---|---|---|
| Microflora | 4[1] | 6[2] | 11 | 15 | 19 | 23 | 27 |
| Alternaria | 98 | 96 | 73 | 78 | 72 | 71 | 72 |
| Cladosporium | 10 | 5 | 0 | 6 | 4 | 4 | 2 |
| Drechslera | 13 | 11 | 8 | 8 | 9 | 7 | 7 |
| A. glaucus (group) | 0 | 5 | 17 | 5 | 17 | 12 | 12 |
| A. candidus | 0 | 0 | 16 | 8 | 10 | 10 | 1 |
| A. versicolor | 0 | 0 | 3 | 7 | 23 | 16 | 20 |
| Penicillium | 1 | 8 | 5 | 17 | 14 | 34 | 35 |
| Streptomyces | 3 | 88 | 54 | 72 | 40 | 23 | 55 |

[1]Includes only temperatures 2, 9, 15.5 and 20°C.

[2]Includes only temperatures 20, 25 and 33°C.

TABLE 4.19

THE EFFECT OF TIME (4 - 27 WEEKS) ON THE MEAN PERCENTAGE OF MICROFLORA OCCURRING ON STORED BARLEY OF 23.9 PERCENT MOISTURE CONTENT (MEAN OF SIX TEMPERATURES)

| | Storage Time (Weeks) | | | | | | |
|---|---|---|---|---|---|---|---|
| Microflora | 4[1] | 6[2] | 11 | 15 | 19 | 23 | 27 |
| Alternaria | 98 | 95 | 75 | 61 | 62 | 52 | 40 |
| Cladosporium | 9 | 3 | 0 | 8 | 6 | 9 | 5 |
| Drechslera | 9 | 7 | 5 | 6 | 4 | 3 | 3 |
| A. glaucus (group) | 0 | 1 | 0 | 3 | 0 | 0 | 0 |
| A. candidus | 0 | 0 | 0 | 0 | 0 | 6 | 1 |
| A. versicolor | 0 | 1 | 10 | 13 | 37 | 25 | 25 |
| Penicillium | 0 | 0 | 3 | 22 | 3 | 19 | 12 |
| Streptomyces | 25 | 98 | 67 | 65 | 70 | 66 | 55 |

[1]Includes only temperatures 2, 9, 15.5 and 20°C.

[2]Includes only temperatures 20, 25 and 33°C.

4.4.3 Effect of Temperature and Time on Common Microflora of Barley. After analyzing these selected micro-organisms of stored barley seed at 2, 9, 15.5, 20, 25 and 33°C I found that most of the change took place at 25 and 33°C. The data on the microflora of seed stored at 15.5, 25 and 33°C are given to show the contrast between the low, medium and high temperatures (Tables 4.20, 4.21 and 4.22).

TABLE 4.20

THE EFFECT OF TIME (3 - 25 WEEKS) ON THE MEAN PERCENTAGE OF MICROFLORA OCCURRING ON BARLEY STORED AT 15.5°C (MEAN OF SIX MOISTURE CONTENTS)

| | Storage Time (Weeks) | | | | | | |
|---|---|---|---|---|---|---|---|
| Microflora | 3 | 7 | 9 | 13 | 17 | 21 | 25 |
| Alternaria | 99 | 96 | 94 | 93 | 85 | 79 | 72 |
| Cladosporium | 7 | 5 | 1 | 8 | 1 | 3 | 1 |
| Drechslera | 15 | 14 | 12 | 15 | 11 | 11 | 12 |
| A. versicolor | 0 | 0 | 0 | 10 | 5 | 7 | 22 |
| A. candidus | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A. glaucus | 0 | 2 | 1 | 1 | 0 | 1 | 5 |
| Penicillium | 0 | 2 | 2 | 4 | 4 | 7 | 5 |
| Streptomyces | 15 | 21 | 38 | 59 | 23 | 22 | 41 |

At 25°C the occurrence of Aspergillus versicolor and Penicillium on seed increased with time and Streptomyces, Alternaria and Drechslera declined with time (Table 4.21). Aspergillus candidus and A. glaucus (group) remained relatively constant.

TABLE 4.21

THE EFFECT OF TIME (2 - 23 WEEKS) ON THE MEAN PERCENTAGE OF MICROFLORA OCCURRING ON BARLEY STORED AT 25°C (MEAN OF SIX MOISTURE CONTENTS)

| | Storage Time (Weeks) | | | | | | |
|---|---|---|---|---|---|---|---|
| Microflora | 2 | 5 | 7 | 11 | 15 | 19 | 23 |
| Alternaria | 94 | 88 | 77 | 63 | 47 | 41 | 33 |
| Cladosporium | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Drechslera | 16 | 11 | 9 | 11 | 6 | 4 | 3 |
| A. glaucus (group) | 2 | 15 | 17 | 20 | 11 | 13 | 9 |
| A. candidus | 0 | 1 | 4 | 3 | 3 | 7 | 5 |
| A. versicolor | 0 | 0 | 4 | 2 | 33 | 37 | 35 |
| Penicillium | 2 | 6 | 3 | 20 | 11 | 27 | 29 |
| Streptomyces | 53 | 75 | 66 | 21 | 23 | 18 | 14 |

At 33°C the Alternaria infection of the seed disappeared after 10 wk (Table 4.22). At this high temperature there was a tendency for Aspergillus versicolor and A. candidus to be inconsistent with time. Contrastingly, the A. glaucus (group) infection rate was consistent after 10 wk storage.

TABLE 4.22

THE EFFECT OF TIME (1 - 22 WEEKS) ON THE MEAN PERCENTAGE OF MICROFLORA OCCURRING ON BARLEY STORED AT 33°C (MEAN OF SIX MOISTURE CONTENTS)

| | Storage Time (Weeks) | | | | | | |
|---|---|---|---|---|---|---|---|
| Microflora | 1 | 4 | 6 | 10 | 14 | 18 | 22 |
| Alternaria | 92 | 40 | 15 | 3 | 0 | 0 | 0 |
| Cladosporium | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| Drechslera | 14 | 4 | 1 | 0 | 0 | 0 | 0 |
| A. glaucus | 7 | 21 | 9 | 30 | 45 | 38 | 33 |
| A. candidus | 1 | 27 | 25 | 20 | 24 | 36 | 12 |
| A. versicolor | 0 | 15 | 36 | 0 | 28 | 8 | 14 |
| Penicillium | 2 | 12 | 15 | 18 | 6 | 6 | 0 |
| Streptomyces | 70 | 57 | 36 | 35 | 11 | 10 | 25 |

## 4.5 The Effects of Certain Special Conditions on the Microflora of Stored Barley at Different Moisture Contents and Temperatures

4.5.1 Introduction. For these tests seed had been divided into six lots and conditioned to moisture contents from 10.9 to 28.8 percent and stored at 33°C from October 23, 1974 to January 30, 1975. Similarly six lots of seed had been conditioned to moisture contents from 11.0 to 35.0 percent and stored at 40°C for the same length of time.

4.5.2 Barley Incubated at High Temperatures After Being Plated on Moist Filter Paper. Normally the petri plates containing seeds are incubated at room temperature, but under these conditions the thermophilic fungi may not appear. Therefore, I decided to find what effect incubation at high temperatures would have on the microflora of barley. The petri plates had to be enclosed in plastic bags to retain their mositure over 7 d since the temperatures of incubation were 33 and 40°C. The controls at room temperature were also placed in plastic bags.

Aspergillus fumigatus was common on seed incubated at 33°C, but rare on seed that was incubated at room temperature (Table 4.23). Streptomyces and bacteria were favored by the high incubation temperature to a lesser degree.

Aspergillus fumigatus and Streptomyces were most common on seeds that had been incubated on petri plates at 40°C (Table 4.24). Other fungi grew but appeared to be repressed by the high temperature.

TABLE 4.23

THE EFFECT OF INCUBATION OF STORED BARLEY AT 33°C SHOWING PERCENTAGE OF MICROFLORA AND GERMINATION (SEED STORED FOR 3 MONTHS AT 33°C)

| Description | Temperature of Incubation | Percent Moisture Content of Seed | | | | | |
|---|---|---|---|---|---|---|---|
| | | 10.9 | 13.4 | 16.6 | 21.2 | 25.2 | 28.8 |
| Germination | 33 | 84 | 16 | 0 | 0 | 0 | 0 |
| Germination | [1]R.T. | 100 | 88 | 0 | 0 | 0 | 0 |
| *A. glaucus* (group) | 33 | 0 | 0 | 0 | 0 | 40 | 0 |
| *A. glaucus* (group) | R.T. | 8 | 0 | 8 | 0 | 0 | 0 |
| *A. candidus* | 33 | 0 | 0 | 92 | 0 | 0 | 0 |
| *A. candidus* | R.T. | 0 | 0 | 88 | 0 | 12 | 0 |
| *A. flavus* | 33 | 4 | 16 | 0 | 0 | 68 | 84 |
| *A. flavus* | R.T. | 12 | 0 | 0 | 24 | 84 | 64 |
| *A. fumigatus* | 33 | 56 | 48 | 32 | 96 | 50 | 68 |
| *A. fumigatus* | R.T. | 0 | 0 | 0 | 4 | 8 | 0 |
| *A. nidulans* | 33 | 0 | 0 | 0 | 0 | 84 | 0 |
| *A. nidulans* | R.T. | 0 | 0 | 4 | 0 | 100 | 0 |
| Streptomyces | 33 | 88 | 100 | 100 | 96 | 8 | 92 |
| Streptomyces | R.T. | 72 | 48 | 96 | 72 | 4 | 48 |
| Bacteria | 33 | 0 | 12 | 20 | 8 | 0 | 0 |
| Bacteria | R.T. | 0 | 0 | 0 | 16 | 0 | 0 |

[1]R.T. - Room Temperature.

TABLE 4.24

THE EFFECT OF INCUBATION OF STORED BARLEY AT 40°C SHOWING PERCENTAGE OF MICROFLORA AND GERMINATION (SEED STORED FOR 3 MONTHS AT 40°C)

| Description | Temperature of Incubation | Percent Moisture Content of Seed | | | | | |
|---|---|---|---|---|---|---|---|
| | | 11.0 | 17.0 | 21.9 | 25.8 | 31.9 | 35.0 |
| Germination | 40 | 0 | 0 | 0 | 0 | 0 | 0 |
| Germination | [1]R.T. | 100 | 0 | 0 | 0 | 0 | 0 |
| A. candidus | 40 | 0 | 0 | 0 | 0 | 0 | 0 |
| A. candidus | R.T. | 0 | 24 | 100 | 0 | 0 | 0 |
| A. terreus | 40 | 0 | 0 | 100 | 0 | 0 | 0 |
| A. terreus | R.T. | 0 | 0 | 96 | 0 | 0 | 0 |
| A. fumigatus | 40 | 64 | 0 | 0 | 100 | 92 | 64 |
| A. fumigatus | R.T. | 0 | 0 | 0 | 100 | 0 | 40 |
| A. nidulans | 40 | 48 | 4 | 4 | 0 | 64 | 0 |
| A. nidulans | R.T. | 28 | 56 | 4 | 4 | 100 | 40 |
| Paecilomyces | 40 | 0 | 0 | 0 | 0 | 0 | 0 |
| Paecilomyces | R.T. | 0 | 0 | 0 | 88 | 0 | 0 |
| Streptomyces | 40 | 96 | 80 | 80 | 48 | 20 | 100 |
| Streptomyces | R.T. | 0 | 0 | 40 | 4 | 0 | 92 |
| Bacteria | 40 | 0 | 24 | 0 | 0 | 0 | 0 |
| Bacteria | R.T. | 0 | 16 | 0 | 0 | 0 | 0 |

[1]R.T. - Room Temperature.

4.5.3 Effect of Ensuring Humid Atmospheres on Plated Barley Seeds. The moist filter paper used in petri dishes tends to dry out. Since this could have some effect on what microflora is detected on the seed an experiment was designed to keep the atmosphere around the barley humid by placing the petri plates in plastic bags.

Little change occurred between seeds incubated in petri dishes in bags compared with those exposed to the relatively dry atmosphere of the room. Only the results for the seed stored at 33°C are reproduced here to show that no significant change took place (Table 4.25).

4.5.4 Effect of Surface Sterilization on Seed with 0.6 Percent Sodium Hypochlorite Before Plating on Moist Filter Paper. Since there was some question about the effect of surface sterilization on barley seed I analyzed both surface sterilized and none surface sterilized seed for microflora and germination.

An important result of surface sterilization at 33°C was to remove all traces of Streptomyces (Table 4.26). For other microflora the number of infected seeds were increased or decreased when the seed was surface sterilized.

The main effect of surface sterilization on the seed stored for 3 months at 40°C was to remove infection by Aspergillus nidulans and A. terreus (Table 4.27). At 17.0 percent moisture content surface sterilized seed gave zero percent germination but only 8 percent were infected by fungi and for the none surface sterilized seed 64 percent was infected.

TABLE 4.25

THE EFFECT ON SEED MICROFLORA AND GERMINATION BY PLACING PETRI PLATES IN POLYETHYLENE BAGS AT ROOM TEMPERATURE

| Description | Type of Enclosure | Percent Moisture Content of Seed | | | | | |
|---|---|---|---|---|---|---|---|
| | | 10.9 | 13.4 | 16.6 | 21.2 | 25.2 | 28.8 |
| Germination | [1]B | 100 | 88 | 0 | 0 | 0 | 0 |
| Germination | [2]UB | 100 | 96 | 0 | 0 | 0 | 0 |
| *A. glaucus* (group) | B | 8 | 0 | 8 | 0 | 0 | 0 |
| *A. glaucus* (group) | UB | 0 | 0 | 44 | 0 | 0 | 0 |
| *A. candidus* | B | 0 | 0 | 88 | 0 | 12 | 0 |
| *A. candidus* | UB | 0 | 0 | 40 | 16 | 8 | 0 |
| *A. flavus* | B | 12 | 0 | 0 | 24 | 84 | 64 |
| *A. flavus* | UB | 0 | 12 | 0 | 8 | 52 | 68 |
| *A. fumigatus* | B | 0 | 0 | 0 | 4 | 8 | 0 |
| *A. fumigatus* | UB | 0 | 0 | 0 | 12 | 20 | 8 |
| *A. nidulans* | B | 0 | 0 | 4 | 0 | 100 | 0 |
| *A. nidulans* | UB | 0 | 0 | 0 | 0 | 88 | 4 |
| *Penicillium* | B | 0 | 0 | 8 | 0 | 0 | 0 |
| *Penicillium* | UB | 0 | 0 | 8 | 0 | 0 | 0 |
| *Streptomyces* | B | 72 | 48 | 96 | 72 | 4 | 48 |
| *Streptomyces* | UB | 72 | 80 | 92 | 80 | 44 | 56 |
| Bacteria | B | 0 | 0 | 0 | 16 | 0 | 0 |
| Bacteria | UB | 0 | 0 | 0 | 0 | 0 | 0 |

[1]B - Bagged.
[2]UB - Unbagged.

TABLE 4.26

COMPARISON OF SURFACE STERILIZED AND NONE SURFACE STERILIZED SEED FOR GERMINATION AND MICROFLORA ON BARLEY STORED FOR 3 MONTHS AT 33°C

| Description | Seed Condition | Percent Moisture Content of the Seed | | | | | |
|---|---|---|---|---|---|---|---|
| | | 10.9 | 13.4 | 16.6 | 21.2 | 25.2 | 28.8 |
| Germination | [1]SS | 100 | 84 | 0 | 0 | 0 | 0 |
| Germination | [2]NSS | 100 | 96 | 0 | 0 | 0 | 0 |
| *A. glaucus* (group) | SS | 0 | 0 | 72 | 4 | 0 | 0 |
| *A. glaucus* (group) | NSS | 0 | 0 | 44 | 0 | 0 | 0 |
| *A. candidus* | SS | 0 | 0 | 12 | 0 | 4 | 0 |
| *A. candidus* | NSS | 0 | 0 | 40 | 16 | 8 | 0 |
| *A. flavus* | SS | 0 | 0 | 0 | 0 | 92 | 12 |
| *A. flavus* | NSS | 0 | 12 | 0 | 8 | 52 | 68 |
| *A. fumigatus* | SS | 0 | 0 | 0 | 0 | 16 | 0 |
| *A. fumigatus* | NSS | 0 | 0 | 0 | 12 | 20 | 8 |
| *A. nidulans* | SS | 0 | 0 | 0 | 0 | 24 | 4 |
| *A. nidulans* | NSS | 0 | 0 | 0 | 0 | 88 | 4 |
| *Penicillium* | SS | 0 | 0 | 0 | 0 | 0 | 0 |
| *Penicillium* | NSS | 0 | 0 | 8 | 0 | 0 | 0 |
| *Streptomyces* | SS | 0 | 0 | 0 | 0 | 0 | 0 |
| *Streptomyces* | NSS | 72 | 80 | 92 | 80 | 44 | 56 |

[1]SS - Surface Sterilized.

[2]NSS - Nonsurface Sterilized.

TABLE 4.27

COMPARISON OF SURFACE STERILIZED AND NONE SURFACE STERILIZED SEED FOR GERMINATION AND MICROFLORA ON BARLEY STORED FOR 3 MONTHS AT 40°C

| Description | Seed Condition | Percent Moisture Content of Seed 11.0 | 17.0 | 21.0 | 25.8 | 31.9 | 35.0 |
|---|---|---|---|---|---|---|---|
| Germination | [1]SS | 100 | 0 | 0 | 0 | 0 | 0 |
| Germination | [2]NSS | 100 | 8 | 0 | 0 | 0 | 0 |
| Alternaria | SS | 12 | 0 | 0 | 0 | 0 | 0 |
| Alternaria | NSS | 4 | 0 | 0 | 0 | 0 | 0 |
| A. glaucus (group) | SS | 0 | 8 | 12 | 0 | 0 | 0 |
| A. glaucus (group) | NSS | 0 | 0 | 0 | 0 | 0 | 0 |
| A. candidus | SS | 0 | 0 | 24 | 0 | 0 | 0 |
| A. candidus | NSS | 0 | 0 | 100 | 0 | 0 | 0 |
| A. terreus | SS | 0 | 0 | 0 | 0 | 0 | 0 |
| A. terreus | NSS | 0 | 0 | 80 | 0 | 0 | 0 |
| A. fumigatus | SS | 0 | 0 | 0 | 4 | 80 | 0 |
| A. fumigatus | NSS | 0 | 0 | 0 | 100 | 4 | 0 |
| A. nidulans | SS | 0 | 0 | 0 | 0 | 12 | 0 |
| A. nidulans | NSS | 4 | 64 | 4 | 8 | 100 | 32 |
| Paecilomyces | SS | 0 | 0 | 0 | 80 | 0 | 0 |
| Paecilomyces | NSS | 0 | 0 | 0 | 72 | 0 | 0 |
| Streptomyces | SS | 0 | 0 | 0 | 0 | 0 | 32 |
| Streptomyces | NSS | 0 | 0 | 0 | 0 | 8 | 100 |

[1]SS - Surface Sterilized.

[2]NSS - Nonsurface Sterilized.

Generally surface sterilization lowered the percentage of occurrence of the other fungi.

## 4.6 The Effect of Specific Aspergilli on Fababeans

### 4.6.1 Comparison of Media for Detecting Aspergilli on Fababeans.

Fababeans were surface sterilized with 0.6 percent sodium hypochlorite, divided into four lots and conditioned to moisture contents from 15.4 to 23.1 percent, subdivided and stored at 17, 25 and 37$^{o}$C. Before storage the samples had been inoculated with 0.2 ml of spore suspension from various fungi, one sample for each type of fungi. After 2 months the seeds were analyzed for germination and microflora on moist filter paper, Czapek and Malt Salt agars.

The moist filter paper was compared with the two agars to determine under which condition the fungi grew most suitably from the seed. The seed had been inoculated with either *Aspergillus fumigatus*, *A. nidulans*, *A. repens*, *A. versicolor* or *A. wentii*.

Generally, Czapek agar produced the most colonies of fungi and it had the advantage over the Malt Salt agar in that it allowed the fababeans to germinate (Table 4.28). Malt Salt agar was only superior to Czapek agar in the case of *Aspergillus repens*.

TABLE 4.28

GROWTH MEDIUM EFFECTS ON THE PERCENTAGE OF FUNGI OF STORED FABABEANS INOCULATED WITH SEVERAL SPECIES OF ASPERGILLUS (SEED STORED FOR 2 MONTHS AT 25°C AND AT MOISTURE CONTENTS FROM 15.4 TO 23.1 PERCENT)

| Seed Inoculated With | Aspergillus spp. Found | Percent on Malt Salt Agar | Percent on Czapek Agar | Percent on Moist Filter Paper |
|---|---|---|---|---|
| Water | Various Fungi | 12 | 21 | 5 |
| *A. repens* | *A. repens* | 38 | 14 | 12 |
| *A. versicolor* | *A. versicolor* | 30 | 33 | 29 |
| *A. wentii* | *A. wentii* | 15 | 16 | 14 |
| *A. nidulans* | *A. nidulans* | 8 | 20 | 8 |
| *A. fumigatus* | *A. fumigatus* | 5 | 11 | 0 |
| Mean | | 18 | 19 | 11 |

4.6.2 The Effect of Specific Aspergilli on the Germination of Fababeans. The effect of specific fungi on germination was only analyzed for fababeans plated on Czapek agar after surface sterilization with sodium hypochlorite.

At 17°C little change occurred in the viability of the fababeans at moisture contents lower than 20 percent (Table 4.29). Germination decreased at moisture contents above 20 percent. Since the non-inoculated seed was also reduced in germination conclusions are difficult. It is possible that *Aspergillus repens* had some deleterious effect. It would appear that the 2 month storage period at this temperature was too short.

TABLE 4.29

THE EFFECT OF SOME SPECIES OF ASPERGILLUS ON THE GERMINATION OF FABABEANS THAT HAD BEEN INOCULATED AND STORED FOR 2 MONTHS AT 17°C

| Seed Inoculated With | Percent Moisture Content | Percent Germination | Percent Seed Contaminated With | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | A. wentii | A. nidulans | A. repens | A. versicolor | A. fumigatus | A. sejunctus |
| A. wentii | 15.4 | 100 | 100 | | | | | |
| A. wentii | 18.4 | 80 | 100 | | | | | |
| A. wentii | 20.1 | 100 | 100 | | | | | |
| A. wentii | 23.1 | 80 | 100 | | | | | |
| A. nidulans | 15.4 | 100 | | 100 | | | | |
| A. nidulans | 18.3 | 100 | | 60 | | | | |
| A. nidulans | 20.1 | 40 | | 100 | 80 | | | |
| A. nidulans | 23.1 | 100 | | 100 | | 60 | | |
| A. repens | 15.4 | 100 | | | 60 | | | |
| A. repens | 18.4 | 20 | | | 40 | | | |
| A. repens | 20.1 | 60 | | | 100 | | | |
| A. repens | 23.1 | | | | 100 | | | |
| A. versicolor | 15.4 | 100 | | | | 80 | | |
| A. versicolor | 18.4 | 80 | | | | 100 | | |
| A. versicolor | 20.1 | 100 | | | | 100 | | |
| A. versicolor | 23.1 | 40 | | | 20 | 100 | | |
| A. fumigatus | 15.4 | 100 | | | | | 100 | |
| A. fumigatus | 18.4 | 100 | | 20 | | | 80 | |
| A. fumigatus | 20.1 | 20 | | | | | 40 | 80 |
| A. fumigatus | 23.1 | 100 | | | | 100 | | |
| Water | 15.4 | 100 | | | | | | |
| Water | 18.4 | 80 | | | | | | |
| Water | 20.1 | 60 | | | | | | |
| Water | 23.1 | 40 | | | | | | |

At 25°C some reduction in germination can occur at all moisture contents. Seed inoculated with Aspergillus wentii or A. versicolor were reduced in germination. A. candidus and A. versicolor appeared on seed inoculated with A. repens and A. fumigatus making it difficult to determine the cause of lowered germination (Table 4.30).

The fungus inoculum usually died on seed stored at 37°C having a moisture content of 15.4 or 18.4 percent (Table 4.31). In contrast, all species survived and reduced germination to zero on seed of higher moisture contents. The same effect occurred on seed that had been only inoculated with water.

All the Aspergillus species appear to reduce germination to zero when given a requisite moisture, temperature and time but conclusions are complicated by the failure of seed at high moisture content to germinate.

TABLE 4.30

THE EFFECT OF SOME SPECIES OF ASPERGILLUS ON THE GERMINATION OF FABABEANS THAT HAD BEEN INOCULATED AND STORED FOR 2 MONTHS AT 25°C

| Seed Inoculated With | Percent Moisture Content | Percent Germination | Percent Seed Contaminated With | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | A. wentii | A. nidulans | A. repens | A. versicolor | A. fumigatus | A. sejunctus | A. candidus |
| A. wentii | 15.4 | 100 | | | | | | | |
| A. wentii | 18.4 | 100 | 100 | | | | | | |
| A. wentii | 20.1 | 80 | 100 | | | | | | |
| A. wentii | 23.1 | 40 | 100 | | | | | | |
| A. nidulans | 15.4 | 100 | | 100 | | | | | |
| A. nidulans | 18.4 | 100 | | 100 | | | | | |
| A. nidulans | 20.1 | 80 | | 100 | | | | | |
| A. nidulans | 23.1 | 80 | | 100 | | 100 | | | |
| A. repens | 15.4 | 40 | | | 40 | | | | |
| A. repens | 18.4 | 100 | | | 100 | | | | |
| A. repens | 20.1 | 80 | | | 100 | | | | |
| A. repens | 23.1 | 20 | | | 20 | 40 | | | |
| A. versicolor | 15.4 | 60 | | | | 60 | | | |
| A. versicolor | 18.4 | 60 | | | | 100 | | | |
| A. versicolor | 20.1 | 40 | | | | 100 | | | |
| A. versicolor | 23.1 | 20 | | | | 100 | | | |
| A. fumigatus | 15.4 | 60 | | | | | 100 | | |
| A. fumigatus | 18.4 | 80 | | | | | 80 | | |
| A. fumigatus | 20.1 | 100 | | | | 40 | 60 | | |
| A. fumigatus | 23.1 | 20 | | | | 60 | | | 80 |
| Water | 15.4 | 80 | | | | | | 20 | |
| Water | 18.4 | 100 | | | | | | | |
| Water | 20.1 | 100 | | | | | | | |
| Water | 23.1 | 60 | | | | 100 | | | 20 |

TABLE 4.31

THE EFFECT OF SOME SPECIES OF ASPERGILLUS ON THE GERMINATION OF FABABEANS THAT HAD BEEN INOCULATED AND STORED FOR 2 MONTHS AT 37°C

| Seed Inoculated With | Percent Moisture Content | Percent Germination | Percent Seed Contaminated With | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | A. wentii | A. nidulans | A. repens | A. versicolor | A. fumigatus | A. sejunctus | A. candidus | A. niger |
| A. wentii | 15.4 | 80 | | | | | | | | 40 |
| A. wentii | 18.4 | 40 | | | 20 | | | | | |
| A. wentii | 20.1 | | 100 | | | | | | | |
| A. wentii | 23.1 | | 100 | | | | | | | |
| A. nidulans | 15.4 | 80 | | 20 | | | | | | |
| A. nidulans | 18.4 | 20 | | 40 | 20 | | | | | |
| A. nidulans | 20.1 | | | 100 | | | | | | |
| A. nidulans | 23.1 | | | 100 | | | | | | |
| A. repens | 15.4 | 100 | | | | | | | | |
| A. repens | 18.4 | 100 | | | | | | | | 60 |
| A. repens | 20.1 | | | | 100 | | | | | 20 |
| A. repens | 23.1 | | | | 20 | | | | 100 | |
| A. versicolor | 15.4 | 80 | | | | | | | | |
| A. versicolor | 18.4 | 80 | | | | 20 | | | | |
| A. versicolor | 20.1 | | | | | 100 | | | | 20 |
| A. versicolor | 23.1 | | | | | 100 | | | | |
| A. fumigatus | 15.4 | 80 | | | | | 20 | | | |
| A. fumigatus | 18.4 | 80 | | | | | | | | |
| A. fumigatus | 20.1 | | | | 40 | 20 | | 40 | | |
| A. fumigatus | 23.1 | | | | | | 100 | | | |
| Water | 15.4 | 100 | | | | | | | | |
| Water | 18.4 | 60 | | | | | | | | 20 |
| Water | 20.1 | | | | 100 | | | | | 40 |
| Water | 23.1 | | | | | | | | | |

## 4.7 The Effect of Temperature, Moisture and Time on the Germination of Fababeans Stored for 5 Months

4.7.1 Introduction. Fababeans were inoculated with a mixture of Aspergillus species known to occur on fababeans. The seed was then divided into six lots and conditioned to moisture contents from 13.2 to 23.8 percent. These were subdivided and stored for 5 months at temperatures ranging from 2 to 33°C.

4.7.2. The Effect of Temperature, Moisture and Time on the Germination of Fababeans Stored for 5 Months. Fababeans stored for 2 wk and 5 months were compared for rate of germination by plating the seeds on moist filter paper. Surface sterilization was assumed to have no effect on their germination.

Germination after 5 months storage decreased as the moisture content increased (Fig. 4-3). Lower germination after 5 months was most pronounced on seed with a moisture content greater than 16.7 percent.

The effect of temperatures over 9°C was to cause a correspondingly greater loss in germination with increasing temperature (Fig. 4-4). After 5 months storage the loss in germination was considerably greater than after 2 wk storage at all temperatures over 9°C.

Germination of fababeans decreased most sharply at moisture contents over 16.9 percent and at temperatures over 15.5°C.

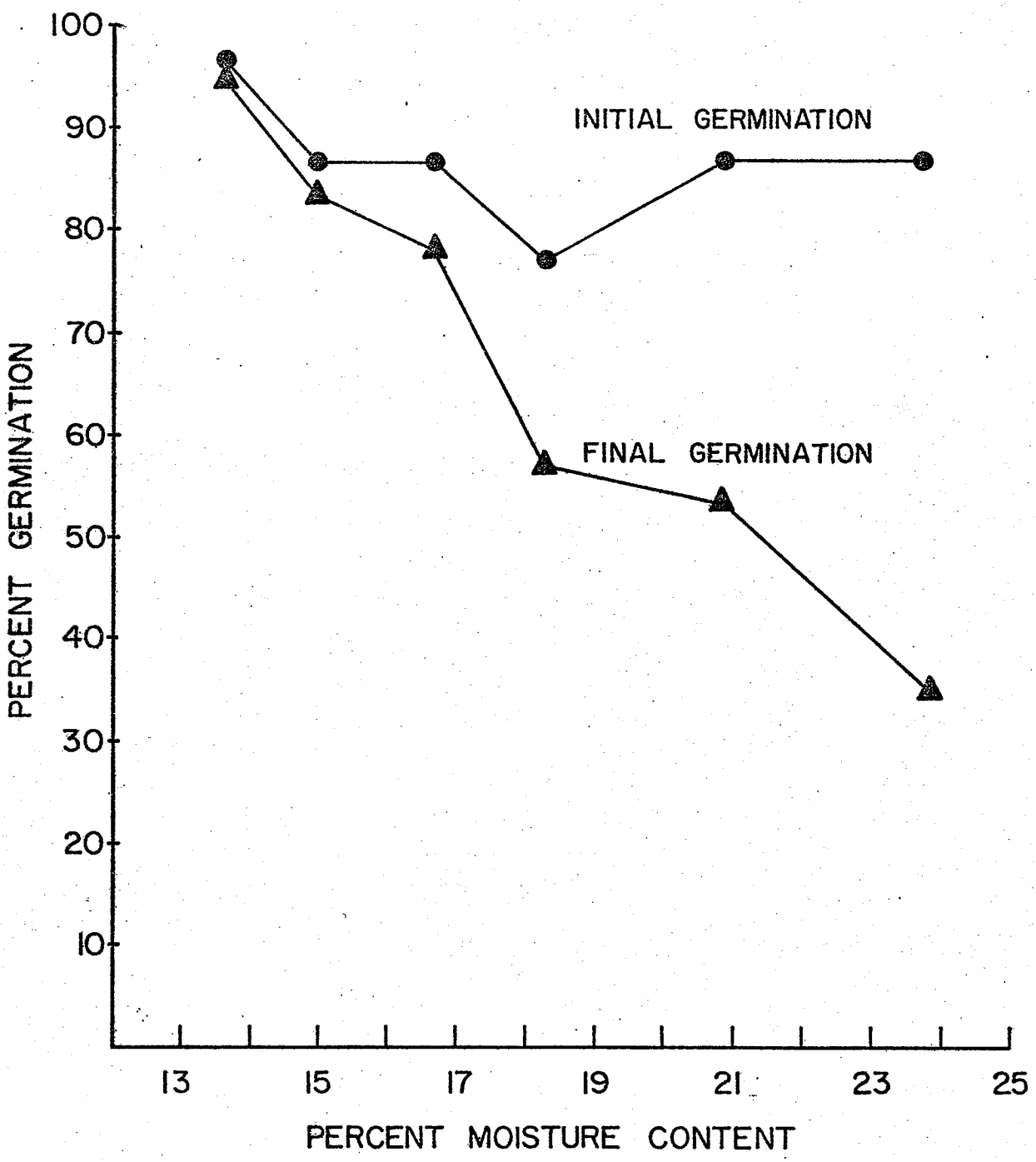


**Fig. 4-3** Mean percentage germination of fababeans after 5 months storage at various moisture contents (seed stored at 2 to 33°C).

INITIAL GERMINATION - Recorded after 2 wk storage.

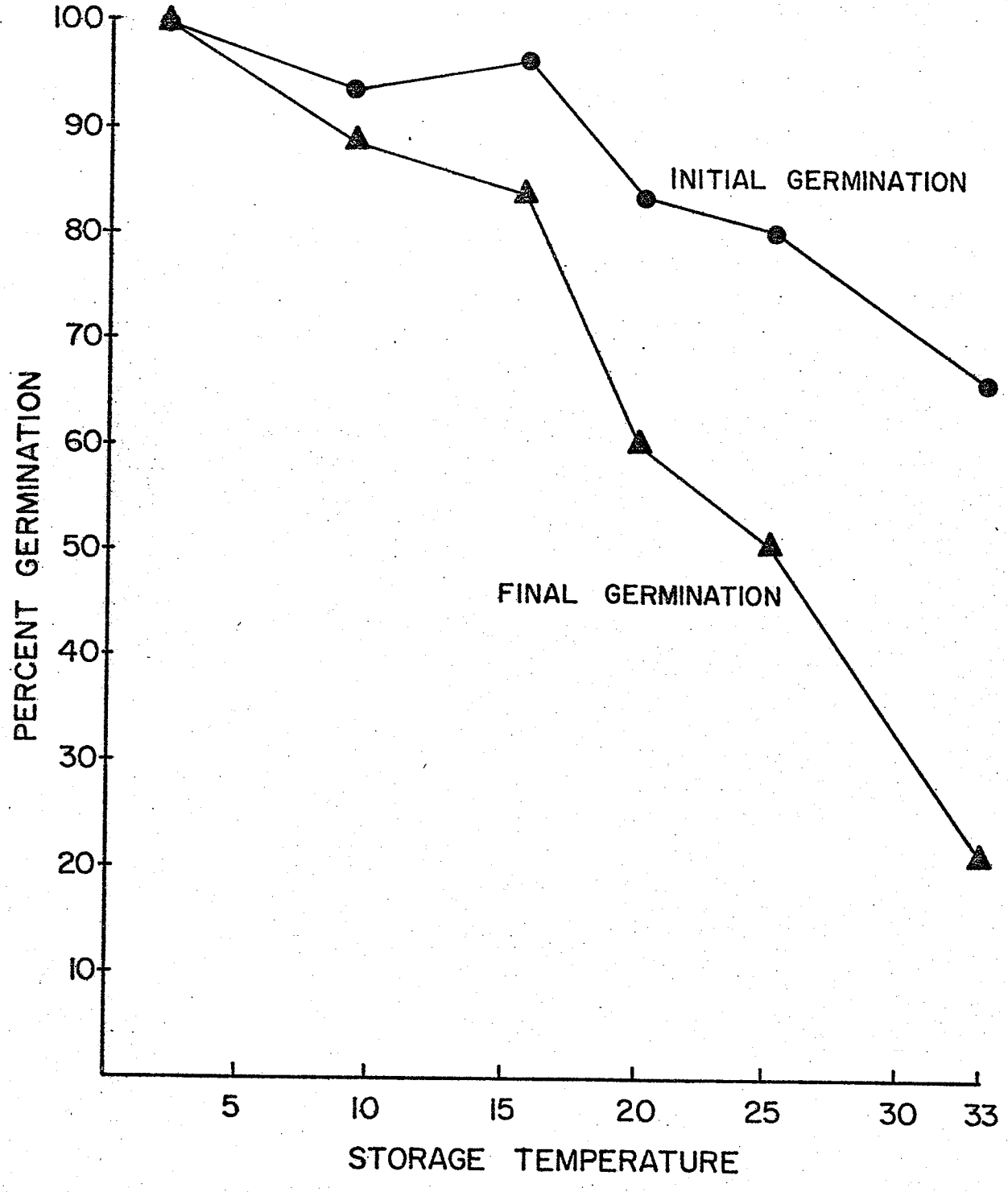


**Fig. 4-4** Mean percentage germination of fababeans after 5 months storage at various temperatures (seed stored at 13.2 to 23.8 percent moisture content).

INITIAL GERMINATION - Recorded after 2 wk storage.

## 4.8 The Effect of Moisture Content, Temperature and Time on the Dominant Microflora of Fababeans Stored for 5 Months.

4.8.1 Introduction. As previously described the fababeans were inoculated with several species of Aspergillus, conditioned to various moisture contents and stored at different temperatures. After allowing 5 months for the inoculated and natural microflora of the fababeans to interact the seeds were studied on both Czapek and Malt Salt agars after surface sterilization with mercuric chloride.

4.8.2 Effect of Moisture Content and Temperature on the Dominant Microflora of Fababeans Plated on Czapek Agar. Aspergillus flavus, A. niger, A. clavatus and A. wentii predominated on seed plated on Czapek agar.

The optimum moisture content for infection by A. niger and A. wentii were 20.9 percent and for A. clavatus, 23.8 percent (Fig. 4-5). A. flavus was not affected by moisture content.

Aspergillus wentii, A. niger, and A. flavus infected seed plated on Czapek agar equally well from 9 to 20°C. (Fig. 4-6). For A. clavatus the optimum was 20 to 25°C. A. niger was found on many seeds incubated at 33°C.

4.8.3 Effect of Moisture Content and Temperature on the Dominant Microflora of Fababeans Plated on Malt Salt Agar. Aspergillus terreus, A. glaucus (group), A. ochraceus, A. versicolor and A. candidus predominated on seed plated on

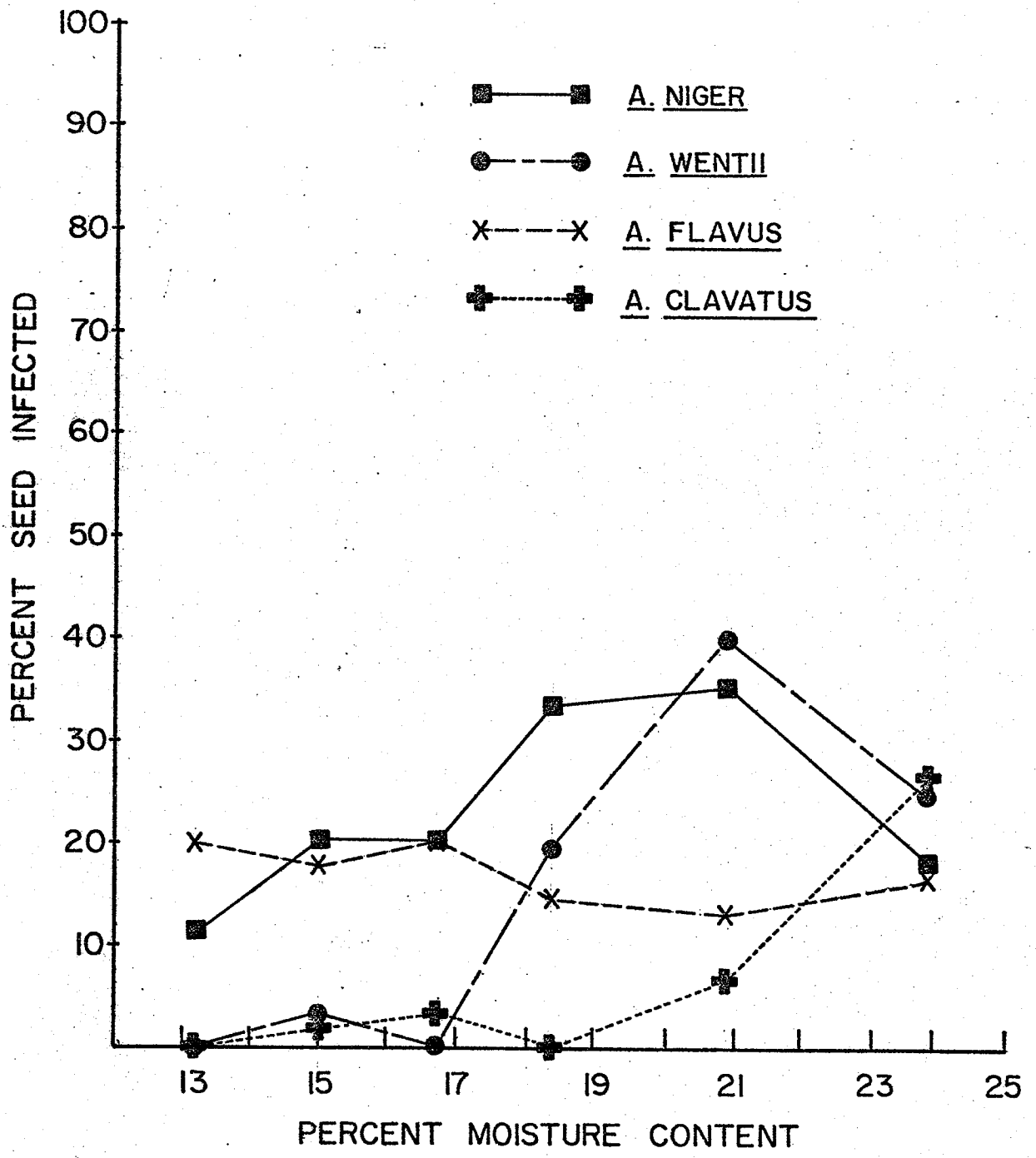


**Fig. 4-5** Effect of moisture content on the mean percentage of various species of Aspergillus of surface sterilized fababeans (seed plated on Czapek agar after storage for 5 months at 2 to 33°C).

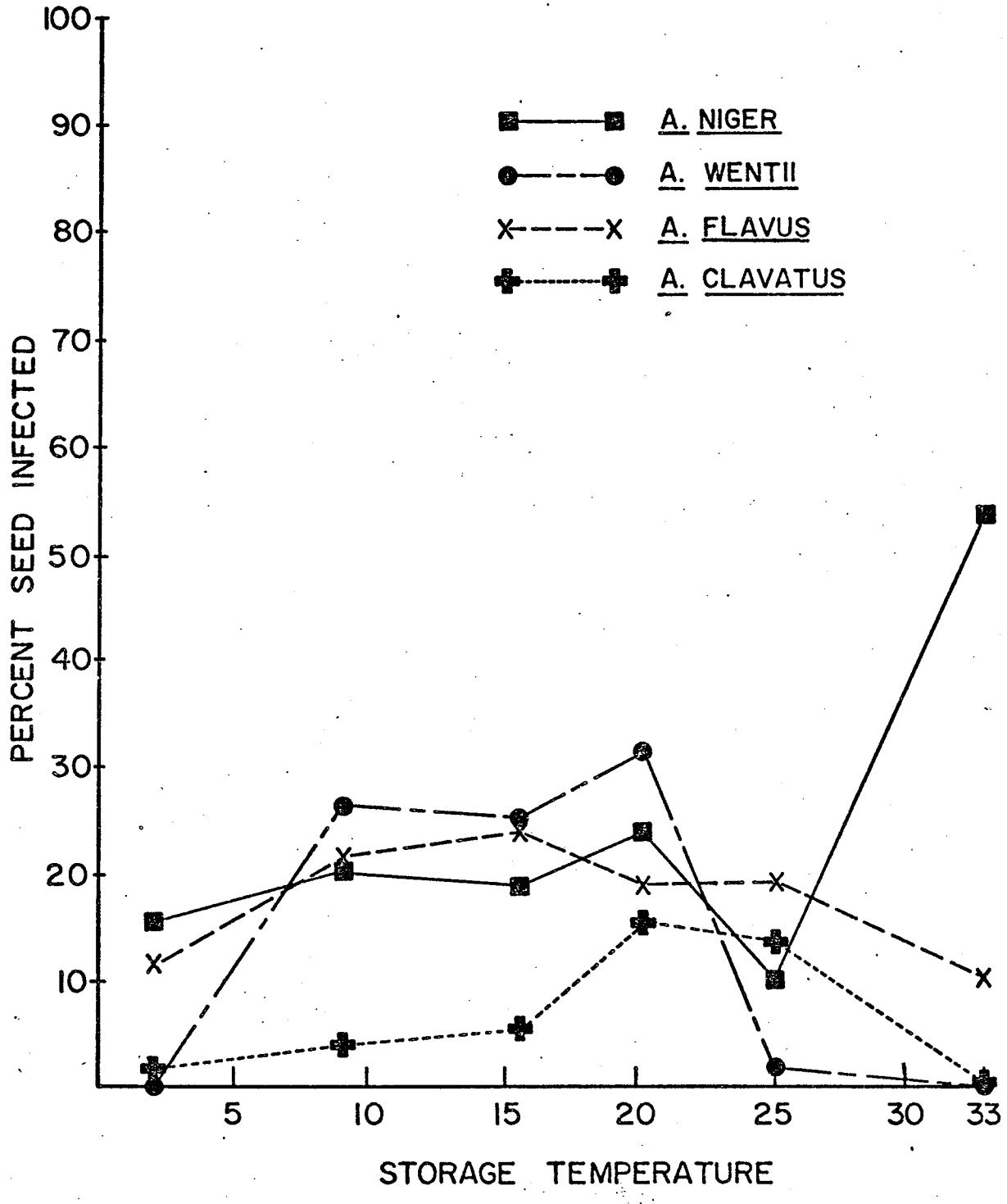


Fig. 4-6 Effect of temperature on the mean percentage of various species of Aspergillus of surface sterilized fababeans (seed plated on Czapek agar after storage for 5 months at 13.2 to 23.8 percent moisture content).

Malt Salt media.

Fababeans showed high infections by the A. glaucus (group), A. ochraceus and A. versicolor at the 16.7 and 20.9 percent moisture contents (Fig. 4-7). A. terreus and A. candidus infected seed with moisture contents greater than 16.7 percent.

When infection by storage temperature was studied on fababeans plated on Malt Salt agar it was found that the A. glaucus (group) had an optimum range of 9 to 20°C, and for A. ochraceus the optimum was 15.5°C, for A. candidus 20°C, and A. terreus and A. versicolor 33°C (Fig. 4-8).

4.8.4 Ecological Niche of the Predominant Microflora of Stored Fababeans. On fababeans each species of Aspergillus tended to have a niche at which it was best suited to exploit its environment (Table 4.32). For example, the optimum temperature for A. ochraceus and A. flavus on stored fababeans was 15.5°C and the optimum moisture contents were 20.7 and 16.7 percent, respectively. A. glaucus (group) and A. wentii were competitive at 20°C and 20.9 percent moisture content, A. candidus and A. clavatus had an optimum at 20°C and the optimum moisture contents were 18.4 and 23.8 percent respectively. At 20°C A. glaucus (group) and A. wentii infected most seeds. The optimum temperature for A. terreus, A. versicolor and A. niger was 33°C and the optimum moisture contents were 23.8, 16.7 and 20.9 percent respectively. Although A. nidulans and A. fumigatus were also used they failed to develop.

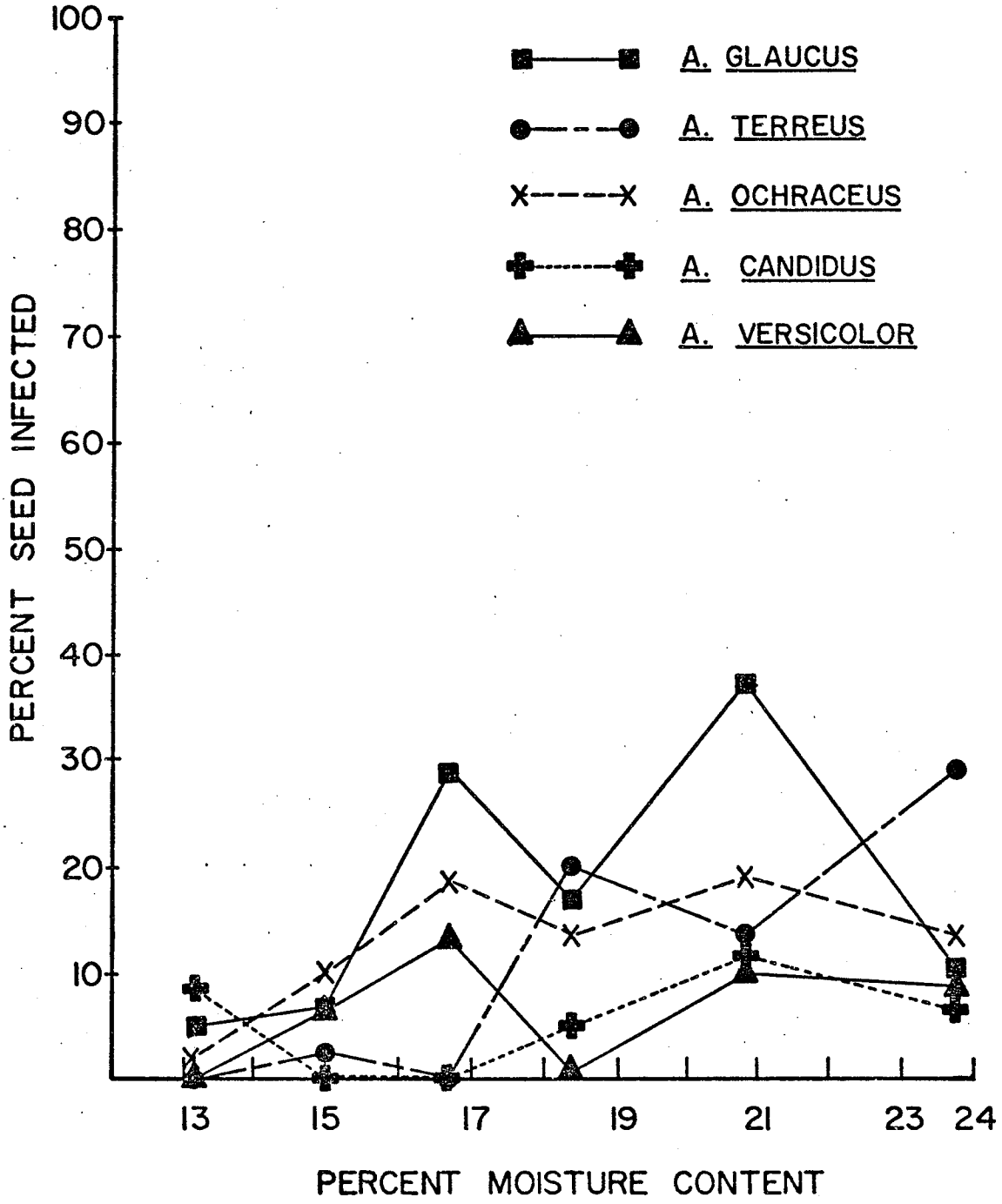


Fig. 4-7 Effect of moisture content on the mean percentage of various species of Aspergillus of surface sterilized fababeans (seed plated on Malt Salt agar after storage for 5 months at 2 to 33°C).

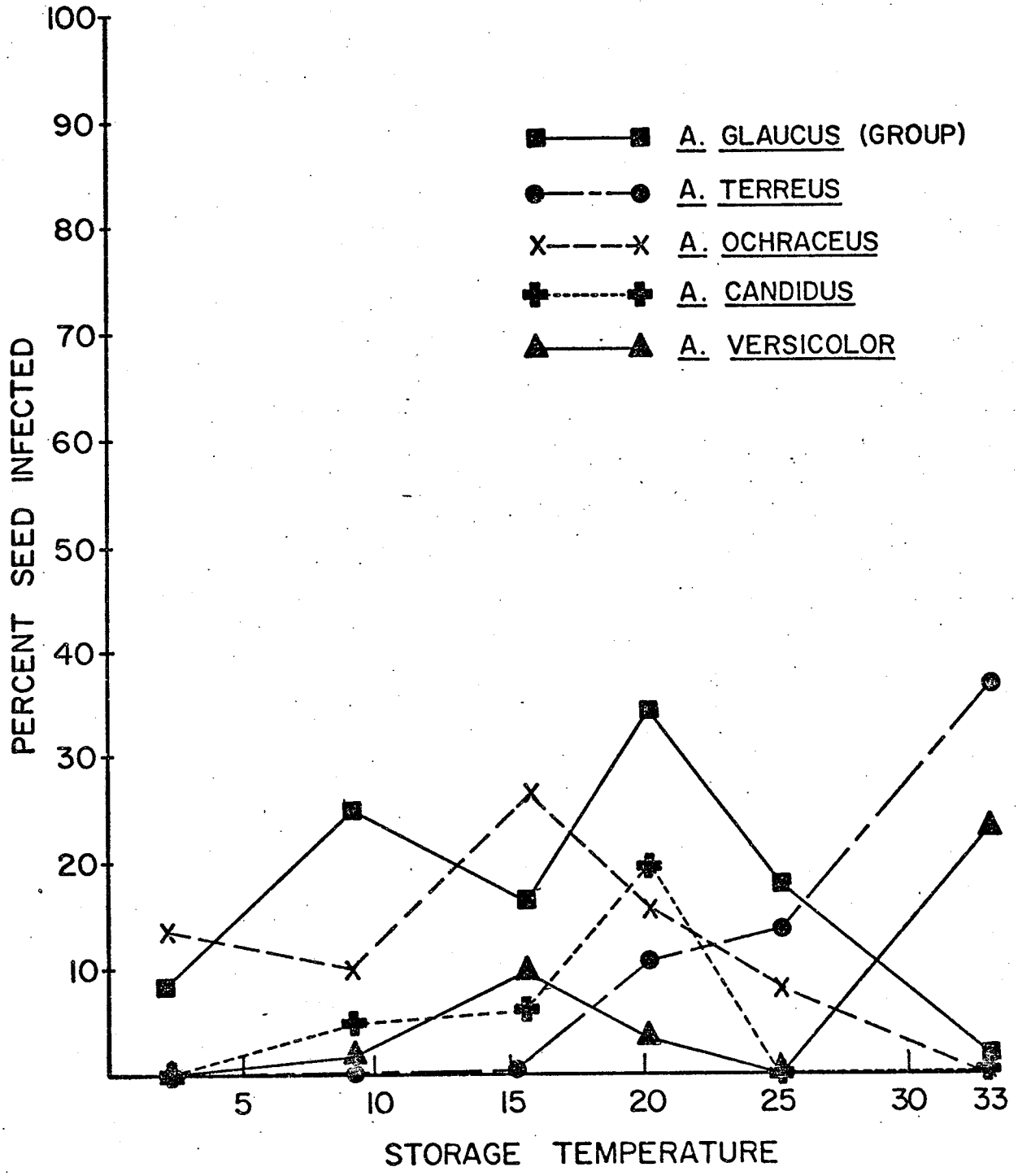


Fig. 4-8 Effect of temperature on the mean percentage of various species of Aspergillus of surface sterilized fababeans (seed plated on Malt Salt agar after storage for 5 months at 13.2 to 23.8 percent moisture content).

TABLE 4.32

OPTIMUM TEMPERATURES AND MOISTURE CONTENTS OF FABABEANS FOR MAXIMUM INFECTION BY INTERACTING SPECIES OF ASPERGILLUS

| Species of Aspergillus | Medium | Optimum Temperature °C | Optimum Moisture Content % | Optimum Percent Infection ± 10 |
|---|---|---|---|---|
| A. ochraceus | [1]MS | 15.5 | 20.9 | 20 |
| A. glaucus | MS | 20 | 20.9 | 30 |
| A. candidus | MS | 20 | 18.4 | 15 |
| A. terreus | MS | 33 | 23.8 | 30 |
| A. versicolor | MS | 33 | 16.7 | 15 |
| A. flavus | [2]CZ | 15.5 | 16.7 | 25 |
| A. wentii | CZ | 20 | 20.9 | 35 |
| A. clavatus | CZ | 20 | 23.8 | 20 |
| A. niger | CZ | 33 | 20.9 | 45 |

[1]MS - Malt Salt agar.

[2]CZ - Czapek agar.

## 4.9 Summary of the Results

4.9.1 Introduction. In all cases during these tests both germination and Alternaria decreased with increasing moisture content, temperature, time and storage fungi. The amount of seed spoilage was directly related to increasing moisture content, temperature and time.

4.9.2 Some Effects of Petri Plate Incubation Temperature and Surface Sterilization on the Microflora of Barley Plated on Filter Paper. Incubation of plated seed at room temperature of grain that had been stored at 33 or 40°C for 3 months produced the greatest variety of fungi but retarded the occurrence of Aspergillus fumigatus, Streptomyces and bacteria. These were more abundant on seed incubated in

petri plates at 33°C. On plates incubated at 40°C only Streptomyces and Aspergillus fumigatus appeared. The greatest effect of surface sterilization on grain microflora was to lower the numbers and kinds of fungi. It increased the number of seeds infected with Aspergillus glaucus (group) however. Surface sterilization removed nearly all traces of Streptomyces, Aspergillus nidulans, A. fumigatus and Penicillium from the seed. There is also evidence that surface sterilization may be selective, and sometimes too severe eliminating nearly all fungi from dead seed.

4.9.3 Comparison of Wheat, Barley and Rapeseed Microflora. Freshly harvested rapeseed, barley, wheat and 5 year old wheat differ in the predominant microorganisms found on them after storage for at least 2 months at 2 to 47°C and moisture contents up to 40 percent (Fig. 4-9). For example, freshly harvested rapeseed had a different microflora than freshly harvested wheat and barley.

Freshly harvested wheat and barley both had a high amount of field fungi on them. When Aspergillus glaucus (group) was present it was restricted to seed stored at 25 to 33°C and having moisture contents below 20 percent. Streptomyces was common on both crops. The chief difference between the two crops was the high rate of infection by Fusarium on barley as compared to the higher rate of infection by Cephalosporium and Paecilomyces on new wheat.

Freshly harvested rapeseed differed from the barley

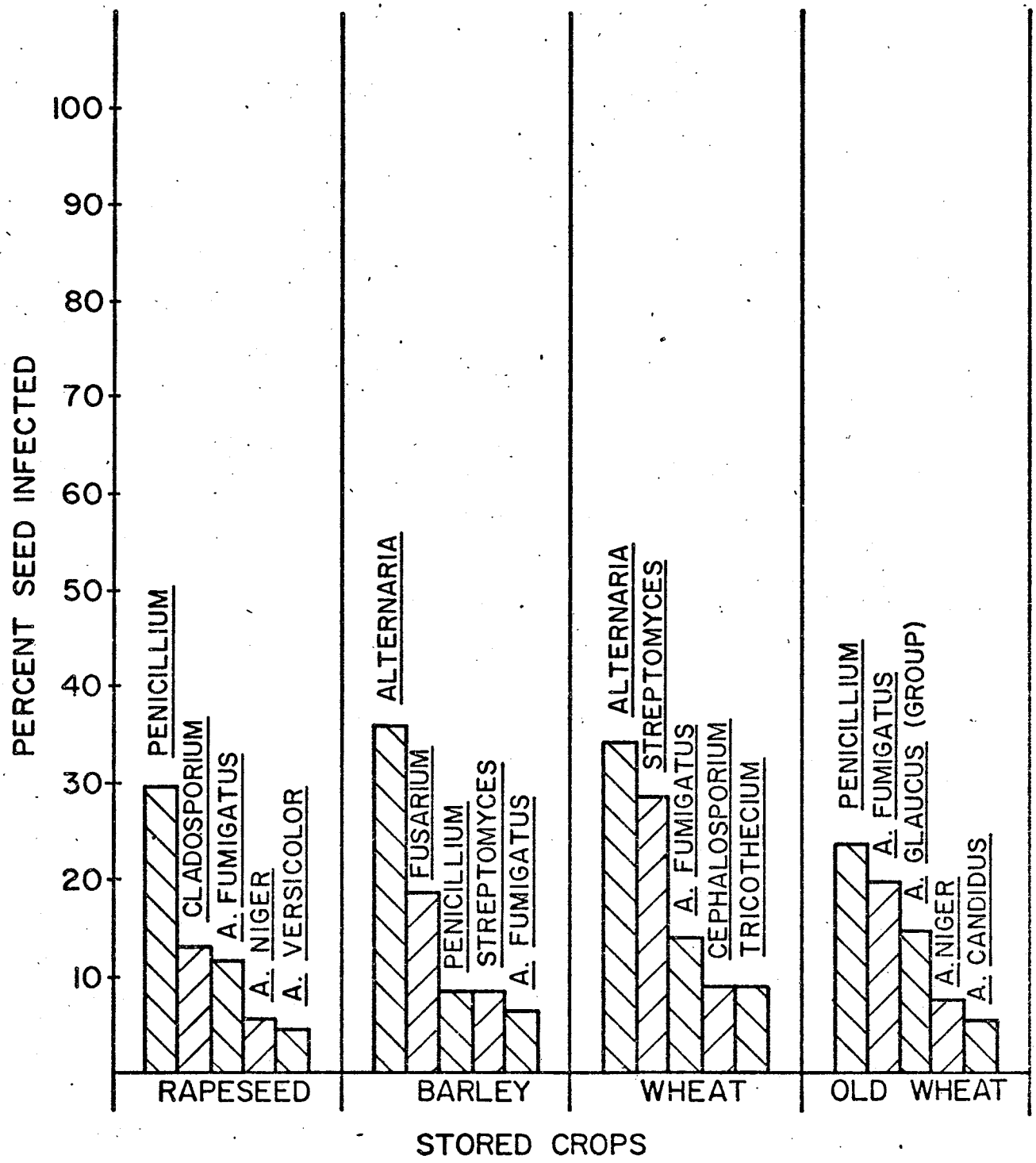


Fig. 4-9 Comparison of the predominant microflora of four crops after storage for 2 to 4 months at different temperatures and moisture contents.

and wheat. The predominant field fungus occurring on it was Cladosporium rather than Alternaria. Rapeseed had higher levels of infection by Penicillium and Aspergillus fumigatus than new wheat or barley. It was also infected by A. glaucus (group) at low moisture contents, A. versicolor at medium moisture contents and A. flavus at high moisture contents. No Streptomyces was found on rapeseed.

Old wheat differed from the new grain in that it had a low percentage infection of field fungi. Aspergillus glaucus (group) infection was higher than on any of the other grains and occurred over a wide temperature range. The seed was infected by A. candidus at moisture contents from 18 to 26 percent. The wheat was also heavily infected with Penicillium and Aspergillus fumigatus. There was less Streptomyces on the old wheat as compared to the fresh wheat.

4.9.4 Relationship Between Loss in Germination and Type of Fungi in Barley. Barley stored at temperatures lower than 9°C and moisture contents ranging from 13.8 to 23.9 percent did not deteriorate. Seed stored at 13.8 percent moisture content did not decrease in germination over 27 wk at any temperature from 2 to 33°C.

Where barley deterioration occurred there was an accompanying increase in storage fungi (Table 4.33). Aspergillus glaucus (group) and A. candidus appear to be the cause of the loss in germination to grain stored at moisture

contents below 21 percent. A. versicolor may be the cause of the loss in germination at higher moisture contents. These fungi are active at temperatures from 15.5 to 33°C.

TABLE 4.33

MEAN EFFECT OF STORAGE FUNGI ON THE GERMINATION OF BARLEY STORED AT DIFFERENT MOISTURE CONTENTS FOR 23 WEEKS (MEAN OF TEMPERATURES FROM 15.5 TO 33°C)

| Time in Weeks | Percent Moisture Content | Percent Germination | Percent Infection By | | |
|---|---|---|---|---|---|
| | | | A. glaucus | A. candidus | A. versicolor |
| 6 | 13.8 | 99 | | | |
| 15 | 13.8 | 100 | | | |
| 23 | 13.8 | 98 | | | |
| 6 | 15.5 | 99 | | | |
| 15 | 15.5 | 94 | 7 | | |
| 23 | 15.5 | 83 | 13 | | |
| 6 | 17.5 | 100 | | | |
| 15 | 17.5 | 84 | 33 | 1 | |
| 23 | 17.5 | 68 | 29 | 11 | 6 |
| 6 | 19.4 | 99 | 12 | 1 | |
| 15 | 19.4 | 81 | 11 | 15 | 1 |
| 23 | 19.4 | 82 | 1 | 16 | 27 |
| 6 | 21.3 | 95 | 5 | | |
| 15 | 21.3 | 77 | 5 | 8 | 7 |
| 23 | 21.3 | 78 | 12 | 10 | 16 |
| 6 | 23.9 | 98 | 1 | | 1 |
| 15 | 23.9 | 62 | 3 | | 13 |
| 23 | 23.9 | 59 | | 6 | 25 |

Seed stored at 33°C and having a moisture content greater than 15.5 percent partially lost its viability in less than a month. At this temperature Aspergillus glaucus (group) and A. candidus infection rapidly increased. At 33°C A. glaucus (group) infected over 45 percent of the seed after

14 wk of storage. Together A. candidus and A. glaucus (group) infected over 70 percent of the seed. The germination at this point was reduced to around 20 percent.

The effect of A. versicolor is not clear. Where it appears there is not always a corresponding reduction in germination.

4.9.5 Effects of Fababean Microflora on Seed Germination. First it was confirmed that fababean microflora is best revealed by Czapek or Malt Salt agar.

Fababeans inoculated with pathogenic species of Aspergillus decreased in germination providing the temperature and moisture content were favorable to the Aspergilli. It was established that fababeans with a moisture content of 18.4 percent and stored at a temperature of 20°C eventually lost their viability. This loss in viability was attributed to various species of Aspergillus which were found on surface sterilized fababeans after 5 months storage. A. wentii, A. niger, A. clavatus, A. terreus and A. candidus were found primarily on high moisture content seed and A. glaucus (group), A. ochraceus, A. flavus and A. versicolor were predominant on lower moisture content seed.

A. flavus, A. wentii, A. glaucus (group) and A. ochraceus occurred at temperatures below 15.5°C, while at temperatures over 15.5°C these and other fungi were involved in fababean spoilage.

4.9.6 Effect of Competition on the Microflora of Fababeans. Competition among the various species of Aspergillus which was allowed to develop on stored seed for 5 months led to the exploitation by individual species of seed stored at specific temperatures and moisture contents. A. ochraceus and A. flavus infected many seeds stored at 15.5°C with A. ochraceus preferring the higher moisture content seed. At 20°C A. flaucus (group), A. candidus, A. wentii and A. clavatus were prominent in exploiting seed with moisture contents of from 18.4 to 23.8 percent. A. terreus, A. niger and A. versicolor were found primarily on seed stored at 33°C. A. versicolor was able to grow on seed with a lower moisture content than A. terreus and A. niger.

# 5. DISCUSSION

## 5.1 Introduction

This study has endeavoured to add knowledge to the existing information on microflora of Manitoba grain crops as affected by temperature, moisture content and time. Wheat, rapeseed and barley were exposed to a range of temperatures and moistures to determine the effect on development of microorganisms. The effect of temperature and moisture on fababeans inoculated with storage fungi was also determined.

In this chapter the results of these tests are discussed in relation to the findings of others. Furthermore, practical implications arising from this study are examined and some recommendations for further research are put forward.

## 5.2 Interpretation of Findings from Storage Tests

First, I would like to discuss certain methods and assumptions used by others which this writer feels are not justified. Many workers surface sterilize grain before it is examined for microflora (Christensen and Kaufmann, 1969). I found that surface sterilization with 0.6 percent sodium hypochlorite may be selective and sometimes too severe. It removes all traces of Streptomyces and most Aspergillus

_fumagatus_ and _A. nidulans_ from the seed and appears to increase the amount of the _A. glaucus_ (group). Sometimes surface sterilization removes all fungi from dead seed giving no clue as to why the seed died.

An assumption often made is that at room temperature all the fungi on or in a seed can be induced to grow. Although the greatest range of species of microorganisms will appear on seeds at room temperature some thermophililic fungi may not grow. Therefore deteriorated seed plated for fungal analysis should be incubated at room temperature and 40°C if the full range of microorganisms are to be observed.

Various studies on freshly harvested (Machacek et al., 1950) as well as stored grain microflora (Christensen and Kaufmann, 1969) have been conducted. Studies on fresh grain did not consider what happened to the grain once it was stored whereas those on stored grain usually examined only a narrow range of temperatures and moisture contents of a particular crop. This study shows which storage fungi can develop on specific crops in Manitoba over a wide range of temperatures and moisture contents when the seed is stored for 2 to 5 months.

Plating seed on filter paper or various other media and incubation at room temperature will only produce fungi that will grow under these conditions. For this reason tests on the microflora of relatively dry grain stored for several months at 2°C will always produce the field fungi which infected the grain before it was harvested. Storage fungi

that occur on these seeds when plated at room temperature are almost certainly contaminants because most storage fungi cannot grow at 2°C (Panasenko, 1967).

True infection by storage fungi has only occurred when a corresponding decrease in the viability of the seed has occurred (Table 5.1). Research scientists in stored grain have proven that storage fungi cause the seed to lose its viability by infecting the seed (Christensen, 1972). For example, the occurrence of Penicillium on rapeseed at 2°C had no effect on germination and hence was due to contaminated seed being used. On the other hand, the low germination of seed stored at 33°C is proof that such seed was infected by storage fungi.

Of the species of storage fungi listed in Table 5.1 Aspergillus glaucus (group), A. candidus, A. versicolor, and A. flavus have been implicated in grain deterioration of commercial bulks. A. fumigatus and A. niger are less involved in the initiation of grain spoilage (Christensen, 1972). Little work has been done on the effects of Penicillium and Streptomyces on stored grain.

For control of storage fungi it is important to know the conditions of temperature and moisture content at which they develop on various crops. Figures 5.1 and 5.2 graphically illustrate the temperature and moisture content range of various storage fungi found on crops stored in Manitoba. It is emphasized that the optimum conditions of temperature and moisture content shown below for storage fungi have come

from actually observing these microorganisms on grain and not from growing them in culture as reported by Panasenko (1967).

TABLE 5.1

PERCENTAGE GERMINATION AND MICROFLORA OF WHEAT, BARLEY AND RAPESEED STORED FOR 2 TO 4 MONTHS AT 2° AND 33° C (MEAN OF DRY TO WET MOISTURE CONTENTS)

| Crop | Germination | Alternaria | Drechslera | Cladosporium | Cephalosporium | Tricotherium | Arthrinium | Paecilomyces | Mucor | Fusarium | Aspergillus glaucus (group) | A. versicolor | A. candidus | A. flavus | A. fumigatus | A. niger | Penicillium | Streptomyces | Bacteria |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Old Wheat at 2°C | 65 | | | | | | 2 | | 6 | | | | | | 7 | | 41 | | 1 |
| Old Wheat at 33°C | 23 | | | | | 1 | | | | | 27 | | 1 | 20 | 32 | 11 | 1 | 7 | |
| Fresh Wheat at 2°C | 61 | 92 | | | 20 | | | | | 2 | | | | | | | 2 | 16 | |
| Fresh Wheat at 33°C | 25 | 5 | | | 3 | 3 | | | | 14 | 25 | | 14 | | 7 | | 1 | 50 | |
| Barley at 2°C | 74 | 90 | 10 | | | | | | | 12 | | | | | | | | | |
| Barley at 33°C | 30 | 1 | | | | | | 9 | | 12 | 20 | | | 10 | 2 | 3 | 18 | 16 | 13 |
| Rapeseed at 2°C | 98 | | | 52 | | | | | | | | | | | | | 14 | | |
| Rapeseed at 33°C | 23 | 2 | | 1 | | | | | | | 17 | | 4 | | 8 | 20 | 18 | 8 | |

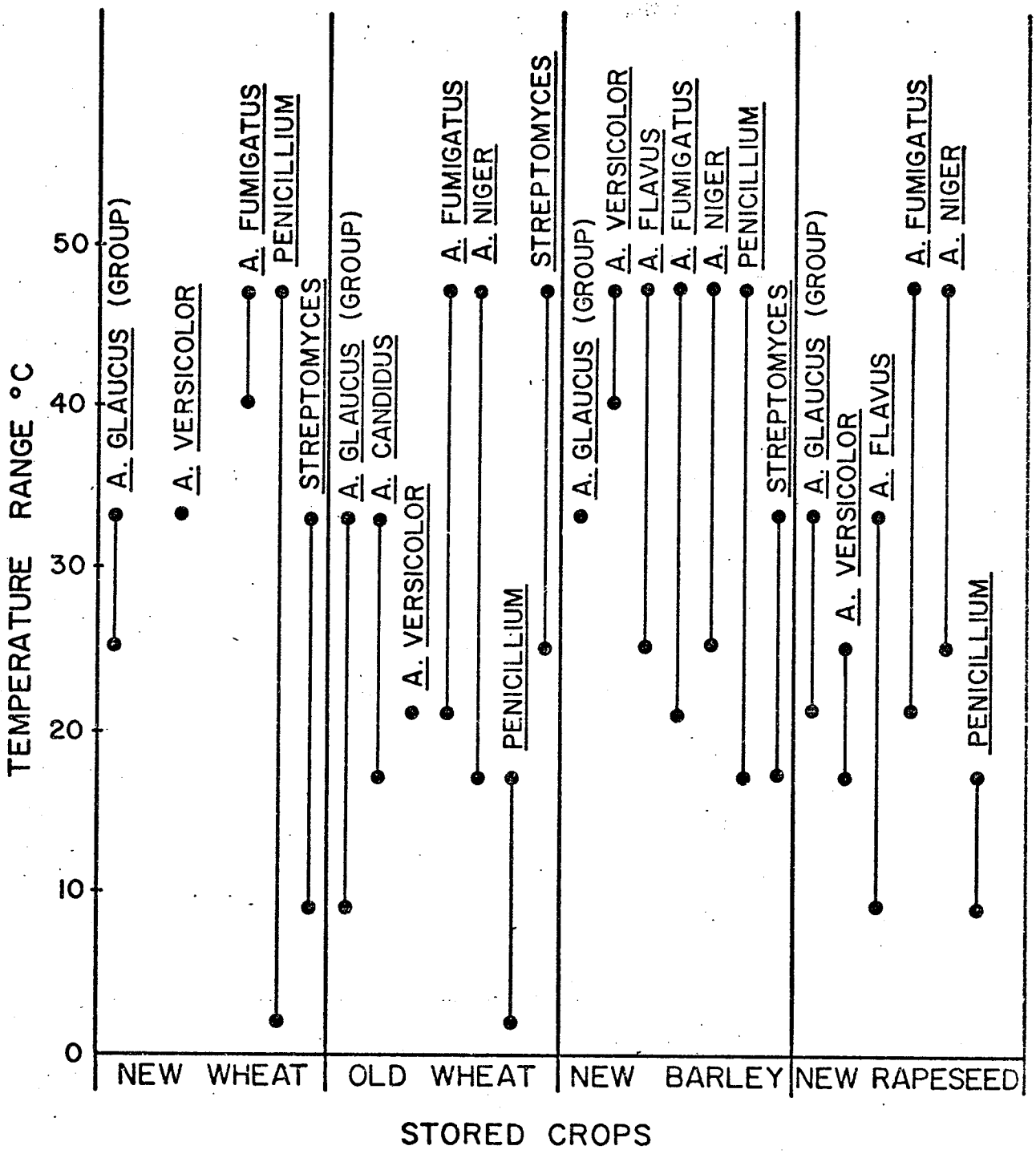


Fig. 5-1 Optimum temperature range for storage fungi when found on wheat, barley and rapeseed.

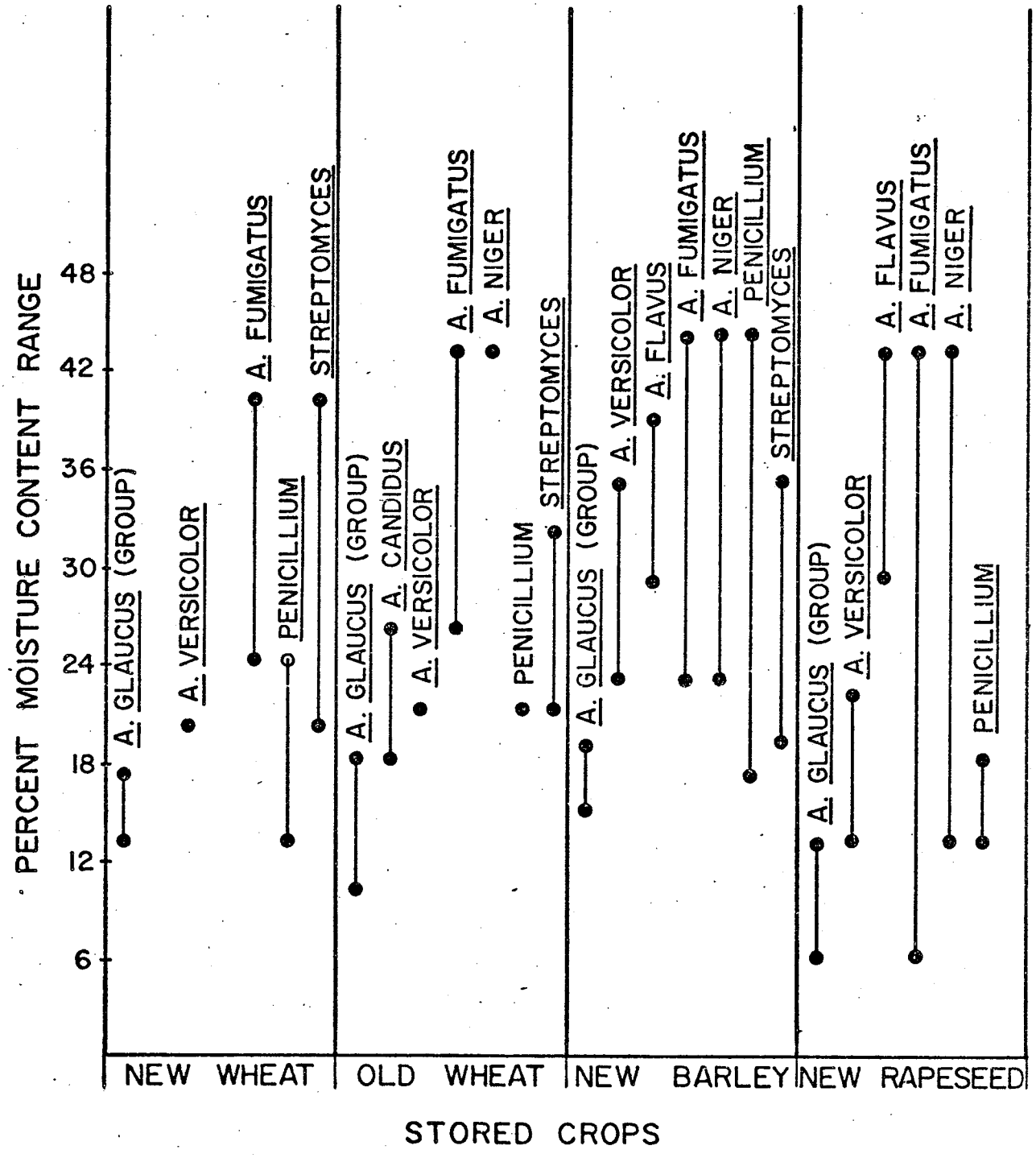


**Fig. 5-2** Optimum moisture content range for storage fungi when found on wheat, barley and rapeseed.

In previously mentioned tests no attempt was made to determine the effect of time on seed infection by storage fungi. Therefore, some tests were made with barley to find out the effect time had on infection by storage fungi and germination.

Barley was only infected by storage fungi in less than 27 wk when the storage temperature was raised higher than 9°C and the moisture content was greater than 13.8 percent. Seed viability decreased proportionally faster as the temperature or the seed moisture content were increased.

Generally the first storage fungi to appear were Aspergillus glaucus (group) followed by A. candidus and finally A. versicolor. It was confirmed that barley infected by Aspergillus glaucus (group) and A. candidus soon decreased in germination (Tuite and Christensen, 1955). An exception to this sequence occurred on the highest moisture content barley (23.8%). In this case an increase in Streptomyces preceded an initial small loss in germination which was then followed by an increase in A. versicolor which corresponded with a much larger decrease in germination.

In grain storage literature it is commonly stated that the A. glaucus group initiates deterioration in stored grain (Christensen and Kaufmann, 1969) but at and above 20 percent moisture content A. versicolor or other storage micro-organisms initiates the deterioration.

At the start of this study the microflora of faba-beans was relatively unknown. There was no literature on

their microflora although studies on soybeans (Dorworth and Christensen, 1968) could partially apply to these very large seeds.

In order to determine which storage fungi attack fababeans they were innoculated with Aspergillus species that had been previously isolated from seeds of this crop. This made it necessary to surface sterilize them before any assessment could be made about the microorganisms occurring in them after 5 months storage at various temperatures and moisture contents. Aspergillus species that infect fababeans stored at temperatures over 15.5°C and moisture contents greater than 15.0 percent were A. glaucus (group), A. ochraceus, A. flavus, and A. versicolor; and at moisture contents greater than 18.4 percent A. wentii, A. niger, A. clavatus, A. terreus, and A. candidus.

The fababeans were infected by a greater variety of Aspergilli species than any of the other crops tested. This could have been due to the preinoculation of the fababeans with a large number of Aspergilli species. However, it is worth noting that only fababeans were infected to any large extent by A. wentii, A. clavatus, A. terreus and A. ochraceus. This could indicate that fababeans are susceptible to a wider range of storage microorganisms than either cereals or oilseeds.

Fababeans decreased in viability similar to other crops with respect to moisture and temperature. Seeds with moisture contents of 23.8 percent lost their viability when

the temperature of storage was over 15.5°C, and seeds with a lower moisture content stored at 33°C had zero germination also after 5 months storage. As with other crops a combination of high moisture content and temperature cause deterioration of fababeans in storage.

## 5.3 Practical Implications of the Thesis

These tests provided at least two aspects of practical value. First, they added some information on how to safeguard the quality of Manitoba stored grain and second, they provided a technique for assessing fababeans for microflora and germination.

From the study it was learned that in a 2 month period the safe moisture contents for freshly harvested rapeseed, wheat and barley stored under 25°C were 6.1, 16.9 and 17.3 percent respectively. Fababeans could safely be stored at 13.2 percent moisture content for 5 months at a temperature of 33°C. If the storage temperature was lower the fababeans could safely be stored at higher moisture contents. This was also found to be the case for barley. The tests also showed that wheat stored for several years is more susceptible to deterioration by a wider range of storage fungi than freshly harvested grain so is therefore more of a storage risk.

Barley could be stored without loss in germination at 2°C with a moisture content of 23.9 percent for at least 27 wk. At 33°C grain with the same moisture content decreased

in viability almost immediately. Therefore the cooler the grain is put into storage, then the longer it will remain in good condition. The cool conditions of Manitoba that prevail in the fall should be used to full advantage in preserving the grain quality.

At moisture contents under 20 percent the Aspergillus glaucus (group) initiated deterioration. Knowing this fact allows one to take measures to safeguard the stored grain from these microorganisms. Control by chemicals, or deleterious temperatures and moisture contents may be used to inhibit these pests.

The second practical aspect of this study was the development of a method to assess fababeans for germination and microflora. Fababeans must be plated on both Malt Salt and Czapek agars to properly determine their microflora. They must still be plated on moist filter paper to discover their viability because Czapek agar does not provide enough moisture for proper fababean germination (H. A. H. Wallace, Unpublished Data). Tests such as these offer agricultural engineers a method of scientifically following the quality of the stored product and hence enabling them to design superior storage structures.

## 5.4 Recommendations For Further Research

1. The effect that seed size and shape has on convection currents and diffusion of moisture in a grain bin.

2. The effect of broken seeds within the grain bulk on the storability of fababeans.
3. Tests on grain stored at temperatures below $15^{o}C$ lasting more than 1 year are required.
4. Most stored grain research has been based on deterioration of relatively dry grain. It is erroneously assumed that damp grain is never stored. Therefore research is required on storage of damp grain. This research is important because spoiled grain is usually fed to livestock and may be infected with fungi producing mycotoxins.
5. Another ramification of damp grain which deserves attention is the occurrence of thermophilic fungi in "heated" grain. These fungi are involved in allergies and lung diseases which affect both man and livestock.
6. Studies should be undertaken to discover the succession of fungi occurring on seeds in a grain bin as the moisture content and temperature increase.
7. Alternaria and storage fungi can be used as indicators of biological changes occurring in grain storage bulks because rising temperature and moisture content decrease Alternaria infection and increase storage fungi occurrence before seed germination is reduced. Determining the effectiveness of different types of storage structures by

monitoring the presence or absence of Alternaria and storage fungi is therefore recommended.

## 6. SUMMARY

The object of the thesis was to find out more information on the conditions under which grain stored by Manitoba farmers deteriorates and to identify the fungi which brings about this deterioration. Wheat, barley, rapeseed and fababeans were studied.

The general method followed was to take small samples of seed and condition them to various moisture contents. These samples were then stored in a wide array of incubators for from 2 to 6 months. At regular time intervals samples of the stored grain were removed and placed on moist filter paper incubated at room temperature for 7 days. The seeds were then counted for germination and examined by microscope for fungi. In the case of fababeans they were also plated on Malt Salt and Czapek agars. The seed was only surface sterilized where contamination was known to exist on the seed or to study its effect. It was found that surface sterilization with 0.6 percent sodium hypochlorite may be selective or too severe.

Seed incubated at room temperature did not always produce a true picture of the seed microflora. Incubation temperatures of 40°C or higher are needed to induce thermophilic fungi to grow on deteriorated seed.

Wheat, barley, and rapeseed were surveyed to determine what types of fungi could possibly grow on them in storage by conditioning them to moisture contents producing dry to wet seed and storing them at temperatures from 2 to 47°C. In all cases storage fungi infected the seeds at temperatures over 9°C after storing them for 2 to 4 months. Any storage fungi on the seed at temperatures lower than 9°C were contaminants. The storage fungi that appeared with respect to increasing moisture content were; Aspergillus glaucus (group), A. candidus, A. versicolor, and A. flavus. Seed stored at warm temperatures were infected by A. fumigatus and A. niger. Penicillium and Streptomyces occurred under various conditions probably due to the fact that each genera consisted of several species.

More extensive tests with barley also studied the effect of time. Invariably Aspergillus glaucus (group) first infected the grain, then within a month a loss in germination occurred. After the grain became infected with A. glaucus (group) it was infected by A. candidus and then A. versicolor. This sequence of infection with time occurred on grain stored over 15.5°C at a moisture content of 13.0 to 21.3 percent. There was an exception to this pattern when the seed was stored at a moisture content of 23.9 percent. Only A. versicolor was prominent of the three storage fungi so it was concluded that at high moisture contents this fungus or various other fungi with high moisture requirements infect the grain. Furthermore, the higher the temperature and moisture

content, then the quicker the seed lost its viability.

Fababeans were studied to find out which storage fungi would infect them. They were inoculated with many species of Aspergillus and stored for 5 months at various temperatures (2 to 33°C) and moisture contents (13.2 to 23.8%). After plating them on filter paper it was found that they had decreased in viability in all cases when their moisture content was over 16.7 percent and they had been stored at temperatures greater than 15.5°C. Fababeans surface sterilized with 0.1 percent mercuric chloride were plated on Czapek and Malt Salt agars to determine their microflora. These mediums had previously been found to be the best for assessing microflora on fababeans. Combining the results from both mediums it was found that A. wentii, A. niger, A. clavatus, A. terreus, and A. candidus primarily infected high moisture content seed (18.4 - 23.8 percent), and A. glaucus (group), A. ochraceus, A. flavus, and A. versicolor were predominant on lower moisture content seed (13.2 - 18.4 percent). In a test with a limited amount of Aspergillus species all were found to be capable of reducing the germination of infected fababeans to zero if conditions of moisture and temperature were satisfactory.

The study has supplied some practical information that could be used by farmers. For example, grain should be stored at the coolest possible temperature because this greatly increases storage life. In addition newly harvested grain is less likely to undergo deterioration than grain that has been stored for a number of years.

This study has two important recommendations to offer the agricultural community. First, more research should be conducted on damp grain because of health hazards that may be involved in its use. Second, the disappearance of Alternaria and appearance of storage fungi in stored grain should be used as a measure of the usefulness of different types and designs of storage structures.

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