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ABSTRACT

THE MICROFLORA OF BARLEY KERNELS; THEIR ISOLATION, CHARACTERIZATION, ETIOLOGY, AND EFFECTS ON BARLEY, MALT, AND MALT PRODUCTS

by Evan Harold Pepper

The various methods used in the isolation of the microflora of barley were evaluated. Eighteen different nutrient media, eleven surface-disinfectants, and six disinfection-time periods were compared. The dilution-plate method was also evaluated by using several kernel-aggregate sizes in the preparation of the dilution material. A differential series of nutrient media is described for the isolation and/or estimation of microfloral populations on barley kernels. The series employs Cycloheximide for the suppression of fungal growth and Chloramphenicol for the inhibition of bacterial growth.

Barley kernels of several varieties, grown in various locations, were plated and their microfloral populations were assessed. Eighteen bacterial species have been isolated and identified from barley foliage and/or kernels. Eight of these species have not been previously reported from barley kernels. Two previously unreported fungal species have also been isolated from barley kernels. The location of the kernel microflora, as well as the form in which they exist, were also studied.

Field studies on the etiology of barley kernel

infection were carried out, using plating and histological procedures, and exposed slides and culture plates. While varietal differences in resistance were noted, the patterns of infection were similar. Bacterial infection occurred earlier than fungal infection; with the bacterial populations declining as the fungal populations increased above fifty percent. The organisms isolated from exposed slides and culture plates were similar to those infecting barley kernels. Significant increases in infection were made as the harvest period was prolonged. Single applications of five chemical compounds failed to reduce microfloral infection.

Barley kernels, infected by both fungi and bacteria, usually become discolored. The common field fungi cause more severe discoloration than do the kernel-infecting bacteria. Under certain conditions, barley kernels may support large populations of bacteria without being discolored. Several species of bacteria and fungi have been shown to be effective germination-inhibitors; while other common kernel microflora have no effect on barley germination. The culture filtrates of three fungal species were inhibitory to barley germination. The inhibitory principle of two culture filtrates was heat stable. Evidence is presented for the possible storage deterioration of barley by several bacterial species under laboratory conditions. Barley, infected with a large

bacterial population, proved to be unpalatable to swine when offered as feed.

Three varieties of bright barley were inoculated with several isolates of bacteria, fungi, and yeasts. These barleys were pilot malted in a pilot scale apparatus constructed for this purpose. Several malt defects were obtained with inoculated grain although the only consistently defective malts were obtained with isolates of known pathogenicity. The progress of microfloral population changes during malting was also studied. Several fungal and bacterial species were found capable of surviving the kilning process. Circumstantial evidence was presented for the consistent infection of green malt by bacteria carried in the humidified air of germinating equipment.

Disinfected kernels of barley have been shown to exert an inhibitory effect on a species of Bacillus. This effect varied with the barley variety. Chromatograms of bright and stained barley husk extracts indicated that the discoloration principle is a melanin-like pigment. This brown pigment found in barley husks is water-and alcohol-soluble. Other evidence is presented to show that these pigments are produced as kernel-microorganism interaction products. The steeping of disinfected barley kernels in an aqueous solution of L-tyrosine showed promise as a possible measure of microfloral content. Brown pigments

produced in this solution varied in intensity with the barley variety as well as with the microfloral population of the barley lot. Hypotheses are presented which assert that the melanin-like pigments are produced by the kernel as an inefficient resistance mechanism. This pigment production is enhanced by the presence of kernel microflora. It is also suggested that these polyphenolic materials may be of importance in the production of non-biological beer hazes. A further hypothesis states that the gas-stability of beer may be reduced by the use of malt prepared from barley which is severely infected by microflora. This speculation is based on information concerning the physiological potential of the kernel microflora.

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INTRODUCTION

Barley has traditionally been the raw material of choice in the preparation of malt. Suitable malting varieties, grown under favorable environmental and climatic conditions and meeting rigid quality standards, command a substantial premium if accepted by the maltster. In addition to meeting the official grade standards for barley (6), acceptable malting barley must possess certain other quality characteristics. These include: (1) strong and powerful germination, (2) a moisture content below 14 percent, (3) plump and mellow kernels, (4) absence of skinned and broken kernels, (5) a clean bright color (166).

Malting is a carefully controlled process whereby the barley is: (1) cleaned and sized, (2) steeped in water until the moisture content reaches 43 to 47 percent, (3) germinated under exacting temperature and humidity conditions, (4) dried on kilns to stop germination, preserve enzymatic activity, and to reduce moisture content to four percent or less, (5) cleaned and stored (326).

The major malting barley producing areas in the United States are the North Central states, the Pacific Coast states, and certain irrigated lands west of the Rocky Mountains (235). Barleys grown in the North Central

states are subject to kernel staining when moderate to heavy rainfall occurs during the final stages of ripening and harvest. This staining condition is frequently termed "weathering" and may be caused by a variety of factors including: (1) moisture, (2) genetic composition of the barley variety, (3) microflora and microfauna. Barleys grown in the arid regions of the west, under irrigation, are usually free of kernel stain and are bright in appearance. The "bright" western barleys while possessing desirable color may, however, be subject to other undesirable features such as steeliness of the kernel and high percentages of skinned and broken kernels.

The annual consumption of barley in the production of malt for food and beverage purposes is in excess of 100,000,000 bushels (235). Even if "brightness" alone were the only quality criterion employed in the marketing of malting barley, the western areas could never produce an adequate supply of the grain (236). Hence, the barley-buyer and the maltster must rely on the barley supplied by the North Central states, and in an unseasonably moist year the barley may be heavily stained. As a direct result of the climatic conditions generally existing in the Great Lakes region and the concommitant "weathering" of grain crops, barley culture has been pushed westward to the drier areas found in the Red River valley. This westward shift in barley culture has been largely due to the reluctance of growers to plant malting barley which might prove

unsalable because of kernel staining (236, 337, 377).

While several factors play a part in the staining of grain kernels, microflora, and especially fungi, are most influential in the weathering process. If "weathered" barley truly represents a decrease in malting quality, criteria other than aesthetic appeal must be formulated in order to properly and scientifically evaluate a specific lot of barley. A search for such criteria must, at least in part, begin with an investigation of the weathering process; how, when, and why it occurs. With this assumption as a basis for the overall investigation of kernel staining and its effect on malting quality, it is possible to subdivide the problem into a number of other related investigations. Most previous studies of barley microflora have overlooked, or have deliberately excluded, the bacteria and yeasts commonly found associated with the barley kernel. Consequently the isolation and identification of these organisms, as well as the filamentous fungi, has been undertaken in this study. In order to both qualitatively and quantitatively distinguish between these various microorganisms, it was necessary to carry out extensive methodological studies on the determination of seed-borne microflora. The techniques developed in this phase of the program were additionally employed in the study of the weathering process. The time and mode of infection by staining organisms was studied, using histological techniques. Barley inoculated with pure cultures of bacteria

and fungi were pilot-malted and analyzed in order to determine what effect these organisms might have on the quality of malt. Finally, the physiology of staining and its effect on both malt and malt products were examined with respect to both the barley kernel and the staining microflora.

CHAPTER I

THE ISOLATION OF MICROFLORA

"In some sorts of seeds the goodness cannot be known by the eye". . . Jethro Tull

LITERATURE REVIEW

"Seed-borne disease" is in actuality a misnomer. As Groves and Skolko have pointed out, the disease is not seed-borne; the bacteria and fungi which cause the disease are (150). Seed-microflora may exist in a number of forms with respect to the seed. Dorogin has listed five forms of microfloral contamination: (1) the admixture of sclerotia or spore masses with the seed, (2) the mummification of the seed by fungal stromata or by internal spores, (3) the presence of mycelium in definite parts or organs within the seed, (4) fructifications [and mycelial fragments and bacterial cells] on the surface of seeds, (5) spores [on the surface of and within seeds] (103). The seed-borne viruses must be appended to such a classification. Chen has also categorized the manner in which seed-borne organisms exist (64). The way in which seed-flora occur determines which method will be employed for the isolation and/or the identification of the organism. Some general methods of seed analysis, based on the form in which the organisms occur, have been listed by Neergaard (284), Doyer (106), and Dorogin (103). These methods fall into

the following broad categories:

1. Visual methods
   a. macro
   b. micro
2. Washing methods and the use of the centrifuge
3. Assorted histological and staining techniques
4. Soil and blotter tests and the use of moist chambers
5. Various disinfection and plating methods

Barley grains are examined visually for several quality characteristics which may be affected by the presence of microflora. The intensity and location of the hull color is frequently indicative of the occurence of certain organisms. Smudged kernels may indicate the presence of field organisms. Black-pointed kernels may harbor several bacterial and fungal species, including species of Pseudomonas and Helminthosporium. Shrunken, whitened kernels often with a pinkish discoloration are characteristic of scabbed grain, a condition caused by Fusarium spp. (144, 226, 333). Skinned, broken, and harvest damaged barley kernels are also suspect because of the ease with which storage organisms attack the exposed embryo (140, 188, 226). The removal of the hulls from barley grains, using a barley pearler, is another valuable diagnostic procedure. Heat-damaged barley characteristically possesses a dark-red, pearled kernel which is the result of heat generated by microflora in storage (226). Microscopic examination of whole barley kernels may reveal other

signs of [illegible] invasion, in the form of fructifications, mycelial masses, etc. (69, 103).

Surface contamination of seed by microorganisms can be determined by the washing method and the use of the centrifuge. The washing method is carried out by either placing the sample of grain under a stream of water or by shaking the seed in a container of sterile water. The washings are plated on suitable nutrient media after which the number of viable microflora are determined from the developing colonies (144, 195). Populations can also be assessed, without plating, by the use of a hemacytometer or counting chamber. A variation of the washing method is used in which the kernels are placed in water, washed with agitation, and the washings centrifuged to concentrate the cells, spores, and hyphal fragments which have been removed from the grain [illegible]. These methods are of greatest value in the isolation and identification of the externally-borne smuts.

Histological and other staining techniques are of great value in assessing the location and amount of microflora present in seeds, but these methods provide little or no information about the identity of the microflora involved (416). One notable exception to this statement is, of course, the mycelium of loose-smut, which is located in the tissues of the barley embryo, chiefly in the scutellum. Methods for the detection of this mycelium are constantly being modified and developed (278). Simmonds

has reviewed the techniques employed in the detection of this parasite and has outlined two methods which give reasonably satisfactory results (357). The whole embryo method employs a dehydration and clearing process with no staining or sectioning required. The sectioned embryo method necessitates embedding, sectioning, and staining of the germ. A procedure, similar to the whole embryo method, has been described for use with sick wheat and corn grains by Christensen and Qasem (71). Hyde and Galleymore have described a simple and rapid method for demonstrating the presence of fungal mycelium in the outer tissues of cereal grains (191). A similar procedure, involving the use of aniline blue to stain the inner pericarp layers of grain was presented by Oxley and Jones (295). A method for the determination of Fusarium-damaged grain has been reported by Gadd and Kjaer (121) which employs the staining of longitudinally-sectioned kernels with a selenite and indigo-carmine dual stain. Christensen has developed a method in which histological and plating techniques are combined (68). This procedure does provide information on species identification as well as location of the mycelium. The seeds are washed under running tap water with the addition of a small amount of detergent, and strips of pericarp tissue are removed and transferred aseptically to agar blocks held on coverslips. The cover slips are placed on van Tieghem cells in petri plates, and the plated tissue is examined at intervals for several

days. The living and dead mycelium is then tabulated by observing the formation of new hyphae and sporulation by the fungus. Other workers have suggested that surface-disinfected pericarp strips be plated on agar plates (191), or that the disinfected paleae and lemmas as well as the caryopsis be plated and population counts made separately (398). The latter method has the additional advantage in that it provides some information as to the location of the seed-flora.

Seed microflora are determined, in many cases, in the course of routine germination tests. These tests are ordinarily conducted in seed-laboratories, using moist blotters or paper toweling containing the germinating seeds. The seed-borne organisms are subsequently identified as they grow out from the germinating seedling (246, 306, 307, 311). While providing a limited amount of information about the attendant microflora, the method is inadequate for the determination of bacteria and many slow-growing or minute fungi. Various soil tests have been employed in seed-testing work. These tests employ various combinations of sterile and non-sterile soil, sand, vermiculite, and brick-dust, as well as treated and un-treated seed. These methods are of great value when dealing with organisms which preferentially attack seedlings or older plants (144, 246, 248, 265, 306, 307).

Perhaps the most valuable diagnostic procedure in the determination of seed-microorganisms is the agar-

plating method. In general terms, the technique consists in the placing of seed, either with or without surface-disinfection, on a suitable nutrient medium and observing the microflora that grow out on the surface of the medium and/or the infected seed. Frequently, investigators employ deep agar and submerge the seeds in the plating process to prevent the seed from lifting itself above the agar surface during germination. This method is sometimes known as the "New Zealand" technique (288). Agar-plating has been in use in phytopathological work for at least 50 years. Some of the earlier papers dealing with the method are those of Bolley (47), Selby and Manns (343), and Chen (64). The so-called "Ulster" method was developed to measure the seed-borne organisms of flax-seed. In this procedure, the flax-seed are transferred without surface-disinfection or washing to malt agar with sterile forceps. This technique has been discussed in papers by Muskett (280), Muskett and Malone (281), and Noble (288). More frequently the plated seeds are disinfected prior to plating in order to remove post-harvest surface contaminants. This procedure, termed the "Ottawa" method by Noble (288), employs numerous combinations of disinfecting materials and nutrient media. The choice of disinfectant and growth medium is dictated in each case by the information desired by the operator. Porter has indicated that, in spite of its usefulness, the

disadvantages of the plating method are two-fold: (1) the method is time-consuming and permits the examination of only a small seed population and (2) incorrect population counts may be obtained because of the selective destruction of certain species (307). Simmonds and Mead evaluated several methods used in the examination of diseased wheat seed and concluded that the plating method was inferior to direct examination of the seed and the use of sterile sand plantings and the moist chamber (356). Several workers have discussed the selective nature of the various disinfection, plating, and isolation techniques, as well as the importance of using proper culture media (70, 150, 162, 245, 247, 254, 364, 398).

Although washing methods and the use of chemical compounds generally have been employed in the surface-disinfection of seeds, gaseous sterilants have been recommended and evaluated by several workers for the determination of microorganisms or for the production of sterile plants (376, 400, 401). The use of gaseous sterilization has also been proposed for the preparation of culture media composed of heat-labile biological materials (162). Evaluation of a number of seed-disinfection materials have been made by Porter (307, 308) and deZeeuw (94). Wilson compiled a list of seed-sterilizing methods which had been previously used to remove microflora and/or to produce sterile plants (419). He found that the existing methods were unsatisfactory and devised a technique

which employed calcium hypochlorite as the active agent. Levine and Schoenlien prepared a comprehensive listing of various types of culture media which is of great value in the proper selection of suitable plating media (231). Some commonly used media, disinfectants, and techniques are included in the manual prepared by Riker and Riker (322). In an effort to determine the most satisfactory culture medium for use in the isolation of cereal storage fungi, a number of various media were tested and evaluated by Christensen (67), Christensen and Tuite (72), and Bottomley and his co-workers (51). In each case, malt-salt agar proved to be the most satisfactory isolation medium for species of Aspergillus.

While the whole-kernel plating method has been valuable in the determination of relative percentages of microorganisms present on and within cereal grains, it does not provide an absolute population figure. Consequently, several investigators have suggested that the seed be crushed or ground and dilution plates be prepared from a suspension of the 'flour' (50, 51, 52, 67, 70, 367, 398).

A serious problem in the plating of seeds and other plant tissues has been the suppression of certain organisms by seed flora and the masking of developing colonies by other, faster-growing microorganisms. Only partially satisfactory methods for the selective inhibition of certain types of organisms were available until

recently. Bacterial colonies were usually inhibited by the use of acidified media; the suppression of the filamentous fungi was not as easily accomplished. The advent of antibiotics has made it possible to selectively suppress one class of organisms to the advantage of another (109). Martin has described an anti-bacterial medium containing streptomycin and rosebengal which has proved useful in the isolation of soil-fungi (254). Cycloheximide (Actidione) was incorporated into culture media in a study by Vaughn and his co-workers (403). They demonstrated that the growth of ten phytopathogenic fungi was strongly inhibited at a concentration of 100 p.p.m. in media. Phillips and Hanel obtained effective control of twelve species of fungi with the addition of Actidione to solid culture media at a rate of 0.1 mg. of the compound to one ml. of the media (302). Actidione was employed by Green and Gray in a selective medium for use in the enumeration of contaminating bacteria in brewery yeast (146, 147). This medium has received wide acceptance and is now available commercially in de-hydrated form (Difco W-L differential medium). The same compound has also been employed in plant pathological practice by Jeffers (198) as a fungal-contaminant suppressant, and by Schneider (339) as a selective medium for Graphium ulmi Schw. which apparently is tolerant of the compound. Streptomycin was also incorporated in this selective medium to suppress bacterial contamination.

Other techniques have been developed to evaluate the seed-borne organisms of barley and other seeds. Skolko has described a technique for determining the presence of certain bacterial pathogens of bean seed by inoculating suspensions of the comminuted seed with bacteriophages specific for the pathogens (359). An increase in the number of phage particles determined by plaque formation is presumptive evidence that the bacterial-host cells are present in the seed sample. Such a method might be employed with certain of the bacterial pathogens of barley since phages specific for Xanthomonas translucens f.spp.hordei, secalis, and hordei-avenae have been isolated from barley and oat kernels (380). Danckwortt has reported that barley seed infected with Gibberella saubinetii (Mont.) Sacc. displays a green discoloration at the tips of the seed when exposed to ultra-violet irradiation (87). Simmonds has designed an apparatus to isolate fungi from the seeds of cereals which employs an aseptic-washing principle (354). This device was constructed in order to avoid damage to the seed-tissues and retardation of fungal growth as a result of chemical-disinfection injury. The plating of large numbers of clover seeds was facilitated by the use of a disinfecting apparatus, aseptic seed-drier, and vacuum counters (220).

The methods outlined above were not generally suited to the determination of obligate parasites. Consequently the seed-borne viruses of barley must be determined in a

different fashion. McKinney has outlined methods for detecting these pathogens in developing barley seedlings (260).

Several authors have reviewed the many papers dealing with the isolation and identification of seed-borne organisms (85, 247, 263, 309). Comparisons of seed-testing results, using samples of the same seed-lot, have been made by various investigators (7, 267). In general, agreement between participating laboratories was consistent although certain testing procedures yielded greater uniformity than others. Some of the isolation and identification procedures, which have been successfully used with barley microflora, are tabulated in Appendix A.

MATERIALS AND METHODS

The methods used in any given laboratory for the determination of seed-microflora have largely been devised to meet the needs of the research program carried on in that laboratory. It must be emphasized that no one method is wholly satisfactory for the determination of all microflora in either a qualitative or a quantitative sense. The purpose of this study was to evaluate the various methods employed in barley-microflora research in order to determine the best method for assessing the microbiological population of stained barley kernels. The effectiveness and selectivity of various nutrient media, surface-disinfectants, disinfection-times, and plating techniques were investigated as well as some preliminary work on the location of microflora on and within stained kernels.

Two barley lots were employed throughout the study: a moderately-stained lot of Hannchen barley grown at East Lansing in 1959, and a sample of Hudson feed barley of extremely poor quality grown in Michigan in 1958.

Eighteen types of nutrient culture media were used in the study (Table 1). Eleven surface-disinfecting treatments were also employed as well as a non-disinfected check (Table 2). Various periods of disinfection ranging from zero minutes to ten minutes were employed in conjunction with two surface-disinfection methods.

In the evaluation of the various types of media the standard surface-disinfecting procedure of the author was employed. This method is carried out as follows: One hundred kernels are counted out at random. The kernels are placed ten at a time in a Gooch crucible and immersed in a finger-bowl containing the surface-disinfectant. The disinfecting material is a mixture of one part 70% ethyl alcohol to two parts of commercial laundry bleach (5.25% available chlorine). The kernels remain submerged for a period of one minute with agitation of the container to prevent the kernels from floating on the surface. At the termination of the disinfecting period the crucible is removed from the finger-bowl, and the kernels are transferred aseptically to suitable culture media with a pair of sterile forceps. The kernels are placed uniformly in the petri plates, ten kernels to a plate, and ten plates to a series. The plates are incubated at room temperature (ca. 25°C.)

Table 1.-Culture media employed in the study of influences on barley microflora exerted by the type of plating-media employed.

Name of medium	Source of medium	pH of medium	Ref.
Potato-dextrose agar (PDA)	Difco	5.6	322
Acidified PDA (lactic acid)	do	4.0	322
PDA plus actidione (100 p.p.m.)	do [a]	5.6	---
PDA plus chloromycetin (50 p.p.m.)	do [b]	5.6	---
Water agar	self	5.6	322
Barley agar	do	5.3	300
Malt-extract agar	Difco	4.7	82
Malt-rootlet agar	self [c]	5.0	---
Malt-salt agar (10% NaCl)	do	3.8	70
Lima-bean agar	Difco	5.6	82
V-8 agar	self	7.2	82
Prune agar	do	3.8	322
Cornmeal agar	do	6.0	322
Nutrient agar	do	7.4	82
Nutrient-dextrose agar	do	7.4	82
Czapek-Dox agar	do	7.3	322
W-L agar	Difco	5.5	82
W-L differential agar	do	5.5	82

a. Actidione made available by the Upjohn Co., Kalamazoo, Mich.

b. Chlormycetin made available by Parke, Davis & Co., Detroit, Mich.

c. Prepared by heating 70 grams of dehydrated malt rootlets

Table 1. - Continued

in 500 ml. of distilled water, strain through cheesecloth, add 15 grams of agar and 15 grams of dextrose, heat to dissolve agar, bring up volume to 1,000 ml. with distilled water, autoclave and bottle.

Table 2.- Surface-disinfecting treatments employed in the study of the influences exerted by surface-disinfection method on barley microflora.

Type of surface-disinfecting treatment [a]	References
None ("Ulster" method)	280, 281
Sterile water--no agitation	---
A.S.B.C. method	5
Modified method of author [b]	300
NaOCl and sterile water (1:9)	307, 308, 322
70% ethyl alcohol	322
3% hydrogen peroxide	307, 308, 322
3% formalin	322
2% $KMnO_4$	322
1% carbolic acid	322
$HgCl_2$ - water (1:1,000)	322
$HgCl_2$ (1:1,000) - two rinses in sterile water	308, 322

a. All treatments disinfected for two minutes and plated on PDA.

b. Modified by increasing disinfection time to two minutes.

for 4-6 days. After the incubation period the microflora are identified, the number of colonies are counted, and the percentage of seed germination is ascertained. Identification of the fungi is made by direct examination of the colony with the microscope or after re-isolation of the fungus in pure culture. The latter procedure is necessary in the event that the fungus fails to sporulate. With few exceptions identification of fungi is made only to genus; bacteria and yeasts are routinely recorded on the basis of their gross colony color (300).

In the evaluation of the various surface-disinfectants, a standard disinfection period of two minutes was employed with all treatments. In this series of experiments all barley samples were plated on potato-dextrose agar (Difco). The two methods of surface-disinfection employed with the varying disinfection times were: (1) the method of the author described above, and (2) the method recommended by the American Society of Brewing Chemists for the determination of barley microflora (5).

The use of ground barley 'flour' and dilution plates for the quantitative determination of seed microorganisms is a commonly employed method. The accuracy of the method was studied by plating portions of barley kernels decreasing in size from the whole kernel to aggregates held on a 1/64" sieve. Surface-disinfected kernels were aseptically sectioned with a sterile razor blade to provide half- and quarter-kernels. Aggregate sizes of 1/32" and 1/64" were

obtained by grinding surface-disinfected kernels in a LabConCo burr-type mill. Dilution correction-factors were employed in order to accurately compare the population counts obtained with each reduction in aggregate size. The dilution-plate technique employed in this phase of the study was carried out by suspending the 'flour' in sterile water and plating 0.1 ml. aliquots at three dilutions in agar maintained at 40°C. in a water bath. Four separate media were used in conjunction with the dilution-plate procedure: potato-dextrose agar (PDA), PDA plus actidione (100 p.p.m.), PDA plus chloromycetin (50 p.p.m.), and nutrient agar (Difco).

The location of microflora associated with barley kernels was studied. Transverse sections of surface-disinfected kernels were plated on PDA, and the developing colonies were counted separately for three regions of the kernel; embryo end, middle, and awn end. Surface-disinfected barley kernels were also pearled for one minute in a barley pearler which had been previously cleaned by passing a large quantity of disinfected grain through the machine. The pearls were surface sterilized briefly and plated on PDA. A corresponding sample of whole barley kernels of the same seed lot was plated at the same time, and the microfloral contents of pearls and whole kernels were compared. Histological procedures were also employed in the study of the location of microflora in barley kernels. This work is discussed in another section (Chapt. 3).

EXPERIMENTAL RESULTS

The choice of culture media used in the plating of barley kernels is of some importance in influencing the kinds and amounts of microflora isolated from the seeds (Table 3). The mode of surface-disinfection also exerts a selective influence on type and amount of microflora recovered from plated seeds (Tables 4 and 5). For the sake of simplicity the microflora detected in these experiments are categorized into broad groups. The "field" fungi include species of Alternaria, Helminthosporium, Fusarium, Epicoccum, Cladosporium, and several others. "Storage" fungi refer to species of Aspergillus, Penicillium and members of the Mucorales. The bacteria included white, yellow, creamy, pink, and various other morphological types. The yeasts were largely pink in color, although some white yeast colonies were observed. The barley germination figures are for the Hannchen sample only since the feed sample was excluded because of erratic germination. Linear increases in the number of bacteria and fungi per kernel were obtained in the plating of dilutions of barley kernel aggregates of decreasing size (Fig. 1). The various media also showed a selective action on the microflora of the kernel in the grinding-plating technique (Fig. 1).

The percentages of microorganisms detected on plated transverse sections of barley kernels were plotted as

Table 3. - The influence of culture medium on microflora isolated from barley kernels

Type of medium	Percentage of discrete colonies from 100 kernels								Percent barley germination (a)	Remarks
	Field fungi		Storage fungi		Bacteria		Yeasts			
	a	b	a	b	a	b	a	b		
Potato-dextrose agar (PDA)	66	15	1	5	75	85	1	17	88	Profuse bacterial slime production
PDA plus chloromycetin	90	25	2	8	10	14	6	4	83	Bacterial growth decreased, fungal growth increased
PDA plus actidione	14	--	0	--	59	--	0	--	45	Sparse fungal growth, barley germination reduced
PDA acidified	94	--	1	--	2	--	5	--	88	Bacterial growth decreased, fungal growth increased
Water agar	29	5	1	0	2	7	0	1	94	Poor microfloral growth, good barley germination
Barley agar	67	12	0	0	53	74	0	11	87	Similar to PDA counts
Malt-extract agar	72	23	0	7	80	57	1	4	89	Bacterial slime produced, fungal growth stunted
Malt-sprout agar	71	--	1	--	104	--	2	--	91	Bacterial and fungal growth good
Malt-salt agar	30	3	0	22	0	0	0	0	0	Storage fungi grew well, field fungi very stunted
Lima-bean agar	68	15	1	4	97	89	2	9	85	Bacterial growth good, fungal growth fair

Table 3. - continued

Type of medium	Percentage of discrete colonies from 100 kernels								Percent barley germination (a)	Remarks
	Field fungi		Storage fungi		Bacteria		Yeasts			
	a	b	a	b	a	b	a	b		
V-8 agar	58	14	0	4	95	56	1	16	90	Bacterial and yeast growth heavy, fungal growth poor
Prune agar	65	22	0	10	42	32	0	3	36	Bacterial growth reduced
Cornmeal agar	47	--	0	-	42	--	0	--	82	Microfloral growth poor
Czapek-Dox agar	53	20	0	18	90	77	1	1	87	Storage fungi growth good
Nutrient agar	45	22	0	7	112	130	0	4	91	Fungal growth poor
Nutrient-dextrose agar	47	--	0	-	119	--	5	--	89	Fungal growth poor, bacterial slime produced
W-L agar	77	--	0	-	55	--	2	--	79	Gives acid-base reaction
W-L differential agar	50	--	0	-	103	--	2	--	76	Fungal growth very sparse, bacterial growth good

a. 1959 moderately stained Hannchen sample grown at East Lansing

b. 1958 severely stained feed sample grown in Michigan

Table 4. - The effect of various surface-disinfectants on the isolation of microflora and on barley germination

Type of surface disinfectant treatment	Percentage of infection: Field fungi a	Field fungi b	Storage fungi a	Storage fungi b	Bacteria a	Bacteria b	Yeasts a	Yeasts b	Barley germination a	Remarks
None ("Ulster method")	106	60	0	2	60	96	1	0	98	
Sterile water	96	52	3	4	96	95	23	15	89	Bacterial and yeast growth profuse
ASBC method	78	12	1	0	14	20	1	0	94	
Author's method	77	8	0	0	25	51	2	3	93	
NaOCl-sterile water (1:9)	98	46	0	0	60	83	0	3	98	
70% ethyl alcohol	86	38	0	2	82	104	4	8	83	Acrospires stunted
3% H_2O_2	102	40	2	4	72	95	5	3	98	Barley germination stimulated
3% formalin	60	16	2	0	1	0	0	0	72	Barley stunted
2% $KMnO_4$	103	28	2	4	72	94	5	4	96	cf. H_2O_2 treatment
1% carbolic acid	80	61	0	3	87	102	1	2	89	
$HgCl_2$ (1:1,000)	4	10	0	0	0	35	0	1	63	Bacteria, fungi, and barley germination reduced

Table 4. - continued

Type of surface disinfectant treatment	Percentage of infection: Field fungi a	Field fungi b	Storage fungi a	Storage fungi b	Bacteria a	Bacteria b	Yeasts a	Yeasts b	Barley germination a	Remarks
$HgCl_2$ (1:1,000) followed by two rinses in sterile water	77	38	0	0	11	78	0	2	100	High percentage barley germination due to reduced bacteria?

a. Moderately stained Hannchen sample grown at East Lansing, 1959.

b. Severely stained feed sample grown in Michigan, 1958.

Table 5. - The effect of varying disinfection times with two surface-disinfection treatments on the isolation of microflora and barley germination.

Duration of disinfection (in min.)	Type of disinfecting treatment	Percentage of discrete colonies from 100 kernels: Field fungi a	Field fungi b	Storage fungi a	Storage fungi b	Bacteria a	Bacteria b	Yeasts a	Yeasts b	Percentage barley germination a
0		136	12	3	56	98	96	2	0	94
1/2	c	89	4	2	8	73	110	2	4	97
	d	93	--	1	--	12	--	1	-	95
1	c	79	10	1	6	87	20	5	2	93
	d	88	--	0	--	26	--	0	-	97
2	c	78	6	0	2	88	39	4	1	93
	d	85	--	1	--	7	--	2	-	97
5	c	83	8	3	10	71	30	2	0	89
	d	70	--	3	--	4	--	0	-	92
10	c	73	6	0	6	59	21	1	1	87
	d	71	--	1	--	10	--	2	-	89

a. Hannchen sample

b. Feed sample

c. Method of author

d. ASBC method

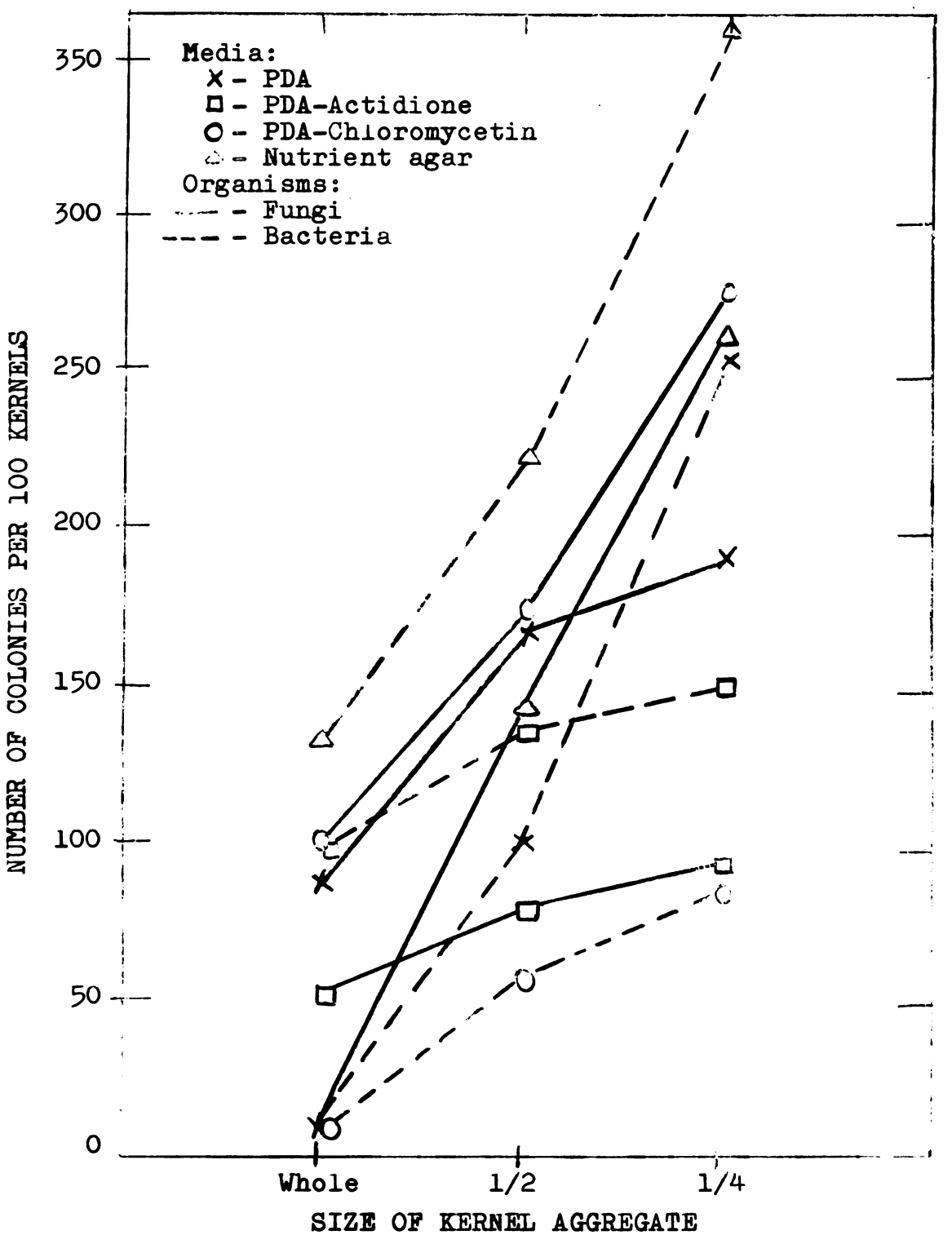


Fig. 1-a. The effect of culture media and size of kernel entity plated on microfloral population.

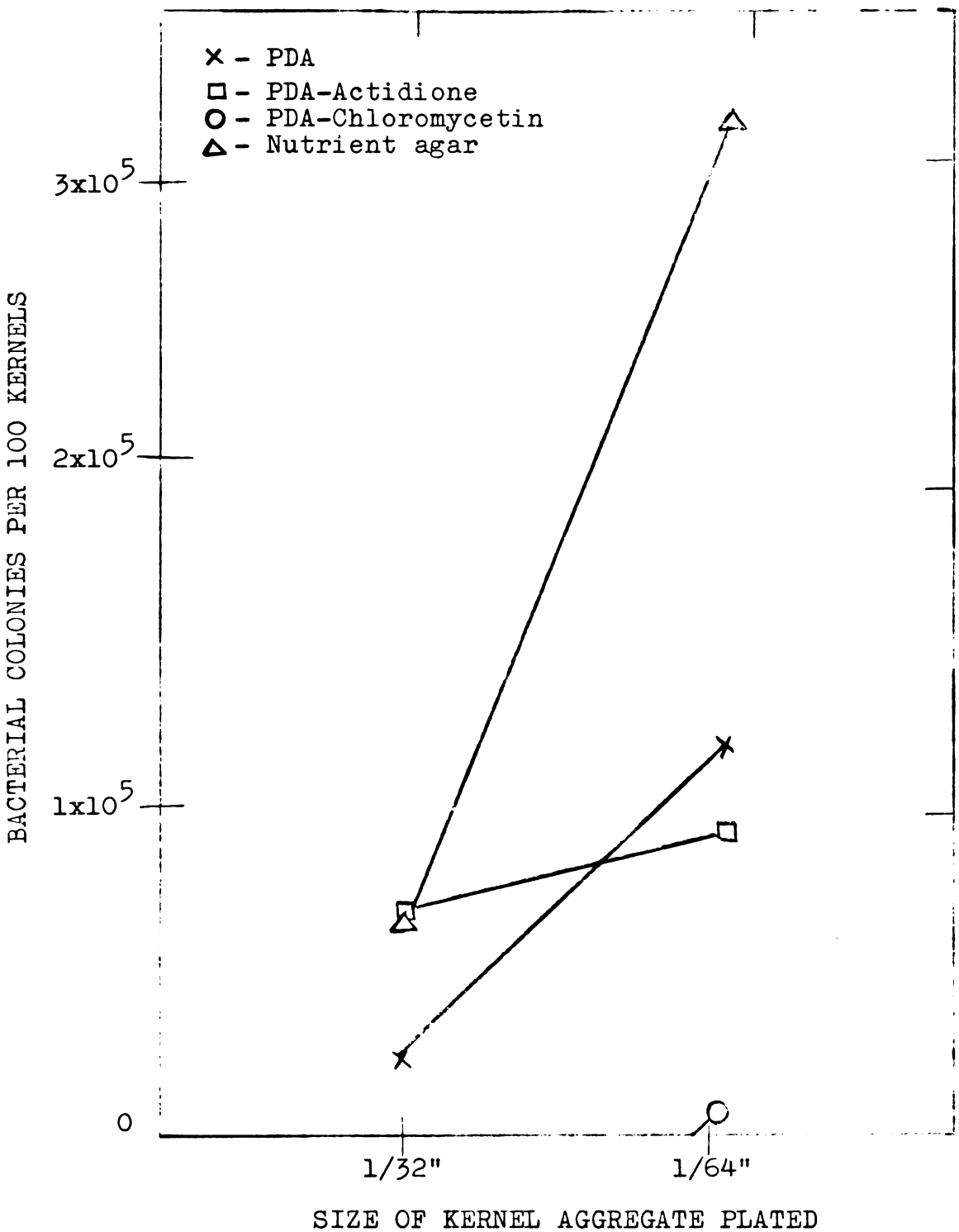


Fig. 1-b. The effect of nutrient media and the size of the kernel entity plated on microfloral population (bacteria only).

functions of the location on the kernel (Fig. 2). The sectioning and plating of the Montcalm sample was performed approximately ten months after harvest, whereas these operations were performed with the Hannchen sample after it had been stored only two and one-half months (Fig. 2). While the fungal determinations in each case are very similar in their patterns, the bacterial counts are considerably disparate. The degree of kernel infection determined by plating disinfected barley pearls differed considerably from that of whole kernel determinations (Table 6). The most radical difference in the two treatments is the large increase in the number of Candida colonies isolated from the plated barley pearls.

DISCUSSION AND CONCLUSIONS

Certain culture media employed in the plating of barley kernels exert a selective effect on the kernel-flora, and the type of media used may also affect the germination of the plated kernel. Most of the media tested in this experimental series are useful for some phase of barley-microfloral work. For the author's purpose a medium (or media) must accurately demonstrate the presence of "field" fungi, bacteria, and yeasts on sound malting-type barley kernels. "Storage" fungi would not ordinarily present a problem in an investigation of this nature since barley carrying any appreciable amount of "storage" mold would not be acceptable to a maltster after even a cursory visual examination. For workers interested in

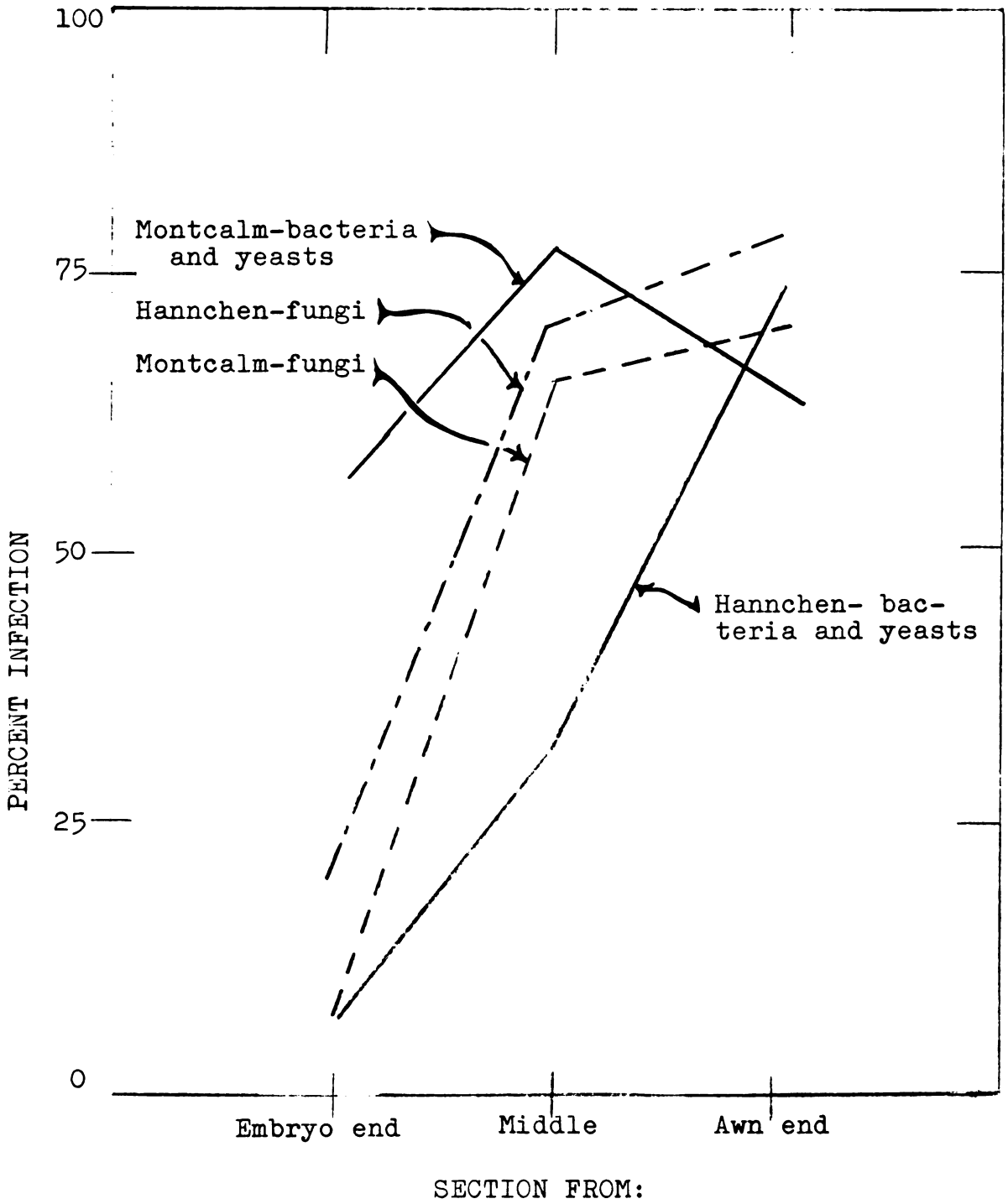


Fig. 2. The location of microflora as determined by plating transverse sections of barley kernels on culture medium.

Table 6. - Percentages of microflora isolated from whole kernels and from 'pearls' of the same barley sample[a].

Type of kernel	Percentage of discrete colonies from 100 kernels						
	Alternaria	Helmintho-sporium	Fusarium	Aspergillus	Bacteria	Candida	Other Yeasts
Whole	78	6	5	0	93	0	0
Pearl	53	4	9	4	100	58	8

[a] A stained Montcalm barley sample was used for both "whole-kernel" and "pearl" platings. The barley pearler was cleaned with brush and air-blast and a sample of sterile barley was run through the machine prior to the experimental pearling.

the storage organisms several media are useful in plating work; one of these, malt-salt agar, was employed in this study and with the feed-barley sample permitted growth of the Aspergillaceae while suppressing other microfloral growth (Table 3). The high salt content (10%) is the active inhibitory principle in this medium and not the reaction of the medium. Prune agar with the same reaction (pH - 3.8) supported other fungal growth and the germination of the kernels was not as drastically affected (Table 3). The "storage" fungi are tolerant of high osmotic pressures and are not able to compete in growth rate successfully with other organisms whereas the converse is true of the "field" organisms. The possibility is also suggested that many of the Aspergillaceae are sensitive to various germination inhibitors released from germinating barley kernels. This hypothesis is lent credence by the observation that "storage" molds only become strongly established on weak and damaged kernels, and the embryo region of the kernel is more resistant to infection after injury than is the endosperm area (188).

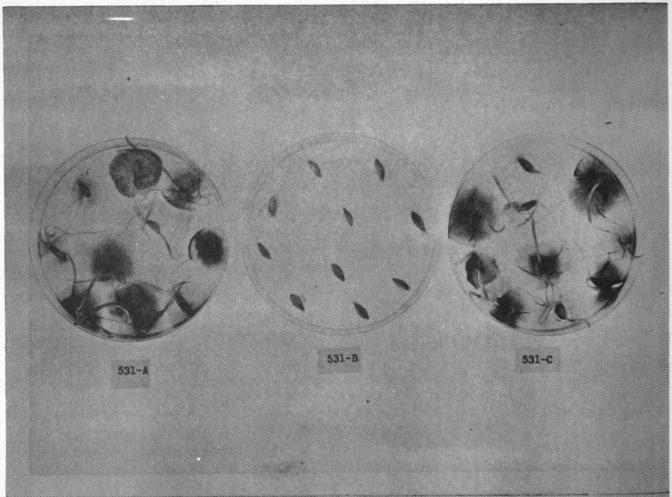
Potato-dextrose agar is a satisfactory medium for the common bacteria and fungi of barley kernels; however, the high sugar concentration (two percent) in this and certain other media, e.g. nutrient-dextrose agar, promoted polysaccharide slime production by several bacteria (Table 3). Barley agar, malt-extract agar, and malt-rootlet agar also prove to be very satisfactory media for the

detection of the "field" organisms. These media have the additional advantage of being cheap and readily available to barley and malt workers. Several media support bacterial growth at the expense of the filamentous fungi. These include nutrient agar, nutrient-dextrose agar, V-8 agar, lima-bean agar, and certain differential media (Table 3). Other media such as water agar, prune agar, and cornmeal agar have limited usefulness and are used for the isolation of certain specialized organisms, for sporulation, kernel germination checks, etc.

While a standard method for the determination of fungi in barley is proposed by the American Society of Brewing Chemists, no accepted method is adopted by the society for the detection of bacteria in barley. The Subcommittee on Methods of Barley Analysis for the society states that "the satisfactory determination of the bacterial count of barley and malt is complicated by the many types of bacteria present as well as the interference of certain very common molds, for example - Alternaria . . . An accurate determination would require the development of specific nutritional conditions which would inhibit the development of molds and permit the growth of bacteria . . ."(5). In lieu of an accepted method the society refers the reader to the method for the total bacterial count in flour used in the milling industry (381). The "specific nutritional conditions" are realized in the use of actidione in culture media.

Consequently a differential series of plating media was developed to accurately assess the relative bacterial, yeast, and fungal populations of plated kernels (Table 3, Plate I). This series consists of a basal medium such as potato-dextrose agar, barley agar, or malt agar; the same medium plus chloromycetin (50 p.p.m.) used to suppress bacterial growth; and the basal medium plus actidione (100 p.p.m.) which selectively suppresses the growth of yeasts and filamentous fungi. By plating a barley sample on this series of media one can estimate the numbers of bacteria, yeasts, and fungi while avoiding the problems of microfloral antagonism and the "masking" of small or slow-growing colonies by more rapid-growing species. These additives are available in powdered form, are easily added to culture media, and are not greatly affected by subsequent heat sterilization (375). The phytotoxicity of actidione inhibits the germination of barley kernels on plates, consequently the germination readings, if required, must be made from either the basal medium or the chloromycetin-medium (Table 3).

The failure of certain surface-disinfectants to eradicate "storage" flora from the surface of the Hannchen sample is an indication that the treatment fails to properly disinfect the surface of the kernel (Table 4). This interpretation is based on the assumption that the "storage" organisms are generally present on the surface of well-conditioned samples only as fortuitous post-harvest contaminants. This assumption is not valid in the


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531-B
531-C

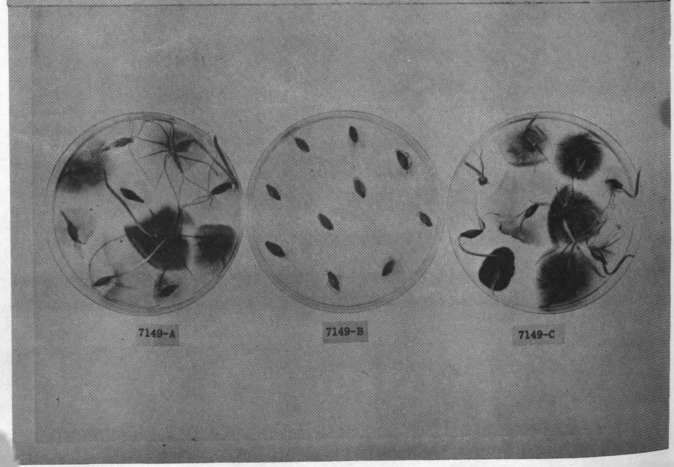

7149-A
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7149-C

PLATE I

The use of differential media in the plating of barley kernels. In both pictures; 'A' represents the basal medium - potato-dextrose agar, 'B' is the basal medium plus Actidione (100 p.p.m.), and 'C' is the basal medium plus Chloromycetin (50 p.p.m.). Plates incubated at room temperature for five days.

Upper - Discolored Hannchen kernels plated on the differential series of media after surface-disinfection.

Lower - Discolored Montcalm kernels plated on the differential series of media after surface-disinfection.

case of poorly-conditioned samples, as exemplified by the feed-sample employed in this study. All of the hypochlorite treatments were satisfactory in removing surface contamination without materially reducing the true microfloral population or injuring barley germination (Table 4). The mercuric chloride treatment used without subsequent rinsing and the formalin method had too severe an effect on both microflora and barley germination. The mercuric chloride disinfectant followed by two rinses in sterile distilled water compared favorably with the hypochlorite treatments (Table 4). Certain compounds had a stimulatory effect on barley germination, notably hydrogen peroxide and potassium permanganate. This effect has been noted by other workers (384) and is probably the result of increased oxygen supplied by the disinfectant.

Evidence for the mild action of the hypochlorite-disinfectant treatments is provided by barley germination figures (Table 5). While a steady decrease in both fungal and bacterial counts occurred as disinfection time increased, barley germination was not severely impaired. The added advantage in the use of chlorine compounds is the ommission of a rinsing step in the plating procedure. Hypochlorite germicides rapidly lose the free chlorine and thus possess little or no residual action (366). Fungal populations did not vary significantly between surface disinfectant treatments, but the A.S.B.C. method significantly reduced the bacterial counts in all cases

(Table 5). Surface-disinfecting solutions containing hypochlorite and ethyl alcohol have been used by several workers (5, 18, 56, 115, 300, 415). The alcohol serves as a wetting agent, dissolves lipoidal material, and is slightly germicidal (especially at a 70 percent concentration). In conjunction, however, with hypochlorite compounds the alcohol (reducing agent) combines with the hypochlorite (oxidizing agent) to yield several reaction products, viz. acetaldehyde and sodium chloride. Thus the efficacy of both compounds as surface-disinfectants is somewhat lessened in combination.

The ground barley flour and dilution plate method for bacterial determinations, unless rigidly standardized, is subject to criticism for several reasons: (1) the results cannot easily be correlated with the whole-kernel plating method used in fungal determinations, (2) the size of the flour-particles will determine the number of colonies that develop on the dilution plates, (3) fungi that produce copious numbers of conidia and spores will yield population figures all out of proportion to their actual presence in terms of comparative area and weight figures. It is reasonable to suppose that as the kernel is sub-divided into smaller and smaller portions the numbers of bacteria and fungi will increase to the point where all viable spores, mycelial fragments, and bacterial cells have been separated into discrete units. Thus a kernel containing the mycelium of Alter-

naria, for example, might yield one fungal colony with the whole-kernel method and 1,000 or more colonies if ground to a fine flour and plated. The data in Figure 1 demonstrates that this is indeed the case. Some preliminary work has also indicated that if the size of the aggregate is further reduced, a point is reached at which the populations rapidly fall off, or are even reduced in number, perhaps indicating that heating and/or mechanical disruption of the cells may take place in the grinding process (Figure 1). It is postulated that this technique, if properly refined, might be used to determine a correlation factor which could be employed with whole kernel plating on the differential media described earlier. This would give a reliable index of the total number of viable cells, spores, and mycelial entities as well as a relative percentage value.

Some measure of the foci of invasion of barley kernels by microorganisms is given by the data in Figure 2 and Table 6. In the Hannchen sample the percentage of infection by bacteria and yeasts was markedly lower than that of fungi at the germ end of the kernel. A steady increase in the number of organisms of all three types was also noted in transverse sections taken successively from the germ end to the awn end of the kernels (Fig. 2). Observations made on numerous platings of barley kernels confirms this thesis. Since bacteria and yeasts are generally less efficient than fungi in the infection and

establishment of tissues in which natural openings and wounds are reduced in number, this is perhaps not surprising. It must be emphasized that we are dealing here primarily with the "weathering" or "field" organisms and not with kernels afflicted with basal glume rot, black point, or severe kernel blight in which case this generalization does not necessarily hold true. The close similarity of the curves representing fungal infection in the Hannchen and Montcalm samples emphasizes not only the location of infection, but the decrease in fungal viability as well.

The data in Table 6 reinforces the observations of many workers, viz. that the major portion of the fungal microflora are located within the pericarp and hull tissues of barley. The increase in bacterial and yeast colonies on the plated pearls, as contrasted with the whole kernel determinations, is perhaps only of apparent significance since the techniques used in the experiment were semi-aseptic at best. Existing evidence also indicates that bacteria, yeasts, and heavily-sporulating fungi may yield disproportionately large population counts when physical disruption of the kernel takes place. Thus the particles of dust from the pearling operation may have adhered to the pearls and brought about the increase. The decrease in the number of competitive fungi might also explain such an increase in these organisms, whose normal presence is in many cases

masked by the more rapidly-growing and/or inhibitory fungi. More experimental evidence will be presented in Chapter III, with respect to the location of microflora on and within barley kernels.

CHAPTER II

THE CHARACTERIZATION OF MICROFLORA

LITERATURE REVIEW

It is a truism that microflora are usually to be found intimately associated with plants and plant parts. Thaysen and Galloway have stated that:

> All green plants possess an epiphytic microflora which normally subsists on the slight traces of carbohydrates, protein and inorganic salts which dissolve in the water extruding from, or condensing on, the epidermis of the host . . . . It is able to subsist under the most severe climatic conditions to which the host normally becomes exposed, and . . . at periods of damp and rain it develops luxuriantly, spreading over the whole of the epidermis, including the flower and the seed (390).

This "epiphytic microflora" may be comprised of numerous species of bacteria, yeasts, actinomycetes, and higher fungi; and represents a symbiotic spectrum, ranging from saprophytism at one end of the scale to obligate parasitism at the other.

In general, the subject of seed-borne microflora in the cereal crops has been approached from two directions: (1) the storage organisms implicated in the deterioration of stored grain and (2) seed-borne pathogens which injure or destroy plants which develop from diseased seed. Several excellent review papers are available which treat of these two categories.

Semeniuk has dealt with the problems of microflora in the deterioration of stored grain (344). An extensive

list of research papers is reviewed in this article, including reports on cereal microflora not implicated in storage problems. Other review papers, primarily concerned with the storage microflora of cereals, are those of Christensen (69, 70) and Blum (45).

The subject of seed-borne pathogens has also been adequately treated in several publications. The lists of Doyer (106), LeClerg (223), and Noble and her co-workers (290) are especially noteworthy. Older compilations of seed-borne diseases have been authored by Chen (64), Gardner (125), and Orton (293). The references by Dickson (100), Fischer (117), and Sprague (368) are also valuable sources of information on the seed-borne diseases of cereals.

On the other hand, the microflora of the small-grains, and expecially of barley, which are neither storage pests nor obvious pathogens, have been generally neglected by authors of review papers. The literature dealing with barley microflora, in the broad sense, is, hence, voluminous and scattered.

As early as 1702, Lisle described the condition in barley kernels that we recognize today as 'blight' and 'scab' (237). It was not until the latter half of the nineteenth century, however, that significant progress was made in the isolation and identification of seed-borne microflora. This as a result of the pure-culture techniques perfected by Pasteur, Koch, and others.

It was commonly believed by several early workers that microorganisms were always present within healthy vegetative tissue (39, 123). This view was challenged by Buchner (57) and Fernbach (116) who demonstrated that the interior of plant tissue was devoid of microflora. More recently Schanderl (338) has adduced evidence which leads him to conclude that the interior of vegetative tissues is not free from microorganisms. He regards cell mitochondria as bacterial symbionts which under certain conditions become free-living forms. Reichard, in a study of barley exposed to excessive dampness, assigned the decreased germinative ability of the grain to an excessive microflora, chiefly the lower-fungi (321). The same conclusion was reached by Becker in his paper dealing with bacterial flora isolated from barley (29). Chrzaszcz considered the microflora of cereal kernels to consist of 'true epiphytic' organisms and 'secondary' flora; that is, an 'internal infection' as contrasted with those organisms which become mixed with the grain in the form of soil particles (79). Zöbl, in 1892, described the isolation of several fungi from discolored ("Braunspitzige") barley kernels (425). He concluded that the kernel discoloration was caused by Cladosporium herbarum (Pers.) Link. The bacterial flora of plant surfaces was studied by Burri (59). He described a bacterium, later named Bacterium herbicola aureum Düggeli as occurring most frequently on normal-appearing plants (108). This view was supported by Düggeli in a later paper (108). The two workers found Bacterium

fluorescens Lehm. and Neum. [Pseudomonas fluorescens Migula] was next in order of occurrence, followed by Bacterium putidum Lehm. and Neum. [Pseudomonas putida (Trev.) Migula], Bacillus megaterium deBary, Bacillus vulgatus (Flügge) Migula [Bacillus subtilis Cohn emend Prazm.?], Bacterium coli Lehm. and Neum. [Escherichia coli (Migula) Castell.], and several others. The identity of Bacterium herbicola aureum Dügg. has been questioned by Thaysen and Galloway (390) and Beijerinck (30), since many bacteria superficially resemble this organism. It is possible that this isolate is identical to Pseudomonas trifolii Huss (53). In another early study, Ravn isolated Helminthosporium teres Sacc. from discolored barley kernels and attributed the staining of the grain to this fungus (320). Much of the early work, dealing with kernel microflora has been reviewed in papers by Chen and Rettger (65), Atanasoff (21), Mead (264, 266), Hyde (190), and Machacek and Greaney (247). More recently, the microflora of barley has been investigated by a number of workers. The Fusaria of cereal grains, including barley, have been studied by Bennett (34, 35, 36, 37, 38) and by Gordon (131, 132, 133, 134, 135). Fusarium, and other seed-borne fungi of barley, were studied by Christensen and Stakman (77). Roth, in a study of fungicidal action on seed-borne organisms, isolated a number of fungal and bacterial species from barley kernels (330). Greaney, Machacek, Wallace and other Canadian workers have contributed greatly to our knowledge of the flora of cereal grains,

including barley (142, 143, 144, 245, 246, 247). The microflora of barley was investigated by Tervet in two reports (385, 387). Whitehead has reported on the microorganisms found associated with barley kernels in his doctoral thesis (415), as has Pepper in a master's thesis (300).

The fermentation industries have, for some time, been interested in the microorganisms found in barley and in other brewing and distilling raw materials (130, 215, 233). Because of the possible effects on barley and its products, malt and beer, interest in barley microflora has recently been stimulated. At least three groups have been identified with barley microflora and its possible effect on brewing materials; these groups include workers in Minnesota, Wisconsin, and Michigan. Several progress reports dealing with this work have been released from Minnesota (8, 9), and Wisconsin (97, 316). Numerous other papers dealing with grain microflora have appeared during the last half-century. A list of microorganisms, reported in the literature as having been isolated from barley kernels, is presented in the Appendix, Table B.

Several workers have reported the quantitative determinations of microflora found on the surface of, and within, barley kernels. Semeniuk has tabulated populations from several papers ranging from 5,000 to 12,000,000 bacterial cells per gram of barley(344). The number of bacterial cells per gram of barley was estimated by Hoffman to be 7,740,000 (181), while Dügelli's investigation

yielded an estimate of 1,600,000 cells per gram (108). In general, such determinations are made either by plating dilutions of kernel-washings (for external flora) or by grinding or macerating the kernel and plating the flour (to measure internal populations). It is clearly an approximation in either case, and the determination will depend upon the methods employed. This has been demonstrated in a paper by Adams and his co-workers in which the bacterial content of distillers' malt was estimated variously at 70,000,000 bacteria per gram or at 90,000,000 cells per gram depending upon the plating method used (10). In the plating of whole kernels of grain it has been observed that individual kernels may be infected by as many as five different species of fungi and/or bacteria (142).

The location of microflora on and within the barley kernel is determined, to a great extent, by the anatomy of the kernel. The barley kernel is, botanically speaking, a caryocist, i.e., a fruit and is enveloped by a persistent palea and lemma. The fruit with its enclosed seed is termed a caryopsis (40, 252). Collins has shown that the barley grain is, with the exception of the micropyle and chalazal tract, completely invested by a strongly cutinized membrance which is impermeable to strong acids and dissolved salts (81). The same membrane is described in a paper by Dickson and Shands (101), by Pugh and others (318), and by Tharp (388). This membrane and the various layers of the testa have been shown to serve as a protec-

tive barrier against the invasion of wheat kernels by Gibberella saubinetii (Mont.) Sacc. (318). Mann and Harlan presented a comprehensive treatment of the morphology of the barley kernel and the process of germination, especially as it pertained to malting (252). The authors concluded that the aleurone layer served as a protective structure against the inroads of bacteria and fungi and that under moist conditions fungi were invariably to be found investing the kernel and feeding upon the hull tissues. While the hyphae of the organisms extended throughout the envelope, they were unable to penetrate the aleurone layer. It was not determined whether the heavily cuticularized walls or the nature of the protoplasm of the aleurone tissue were responsible for the resistance to microfloral penetration.

It has been generally observed that the microflora of cereals are confined to the surface of the kernel or within the tissues exterior to the testa and aleurone layer (42, 47, 48, 68, 88, 104, 126, 190, 191, 230, 253, 258, 264, 280, 295, 301, 346, 390, 397, 398, 413, 415, 416, 423). The endosperm and embryo are also invaded by various cereal fungi (118, 199, 414, 424). The mycelium of Helminthosporium gramineum Rabh. is especially profuse in the region of the germ end of the kernel (423). Helminthosporium sativum Pamm., King, and Bakke was found by Mead to be located mainly at both ends of the barley kernel (264). The same distribution of Fusarium sp. mycelium was described on barley by Dounin (104). Hyde and Galley-

more stated that the mycelium of fungi found on wheat kernels was expecially abundant wherever the epidermis was loose, i.e. at the two ends and in the region of the 'crease' and the 'breast' (191). The authors also reported that the mycelium was less profuse at the germ end of the kernel. The kernels of wheat and barley, infected with basal glumerot caused by Pseudomonas atrofaciens McCull., are stated to be more heavily infected at the germ end of grain (258). The fungal flora of cereals exists mainly in the form of 'dormant' or 'resting' mycelium; only rarely are spores found within the husk of the kernel (191, 264, 280). This 'dormant' mycelium is characteristically irregular in shape, dark, and has thickened walls (264) (Plates II, III).

In general, the microflora of cereal grains are capable of surviving for long periods of time in a dormant state. It was indicated by Burri (59) that the resistance of bacterial cells on and within grain kernels to dessication was due to the production of mucous sheaths by the individual cells. This protective layer also served to fix the organism to the plant tissue. The bacterial blight bacterium, Xanthomonas translucens (J.,J., & R.) Dows., is reported to survive for at least two years in diseased barley seed (206, 207). The mycelium of the barley loose smut fungus, Ustilago nuda (Jens.) Rostr., remained viable in barley seed embryos for eleven years according to Porter (310). Christensen reported no living mycelium in wheat seed infested with storage molds after eight years

of storage (68). Whitehead et al. reported living mycelium of Microascus trigonosporus in barley kernels stored in bottles for 25 years (416). Barley seed stored under proper conditions retains high germinability while the microflora more rapidly lose their viability. Haferkamp obtained high germination percentages with barley stored for periods of 24 to 29 years (155). The kernels, with or without hulls, had little or no mold present after the termination of the storage period [species of fungi were not named]. Machacek and Wallace stated similarly that barley seed retained its germinability and the associated microorganisms lost viability after a ten year storage period (249, 250). By testing at intervals over this period they reported that only Helminthosporium teres Sacc. was still present at the conclusion of the experiment, while Alternaria tenuis sensu Wilts. and Helminthosporium sativum P., K., & B. were no longer present, having died out at a comparatively rapid rate. Russell compared the longevity of several lots of wheat seed with cracked seed-coats over a 17 year period (352). All of the seed lots germinated well until eight years had elapsed, then viability decreased rapidly. Nearly all the seed was dead at the end of the testing period. Alternaria tenuis disappeared after about seven years while Helminthosporium sativum decreased more slowly, with eight percent of the original amount still present after 17 years. Christensen reported a longevity period of approximately seven years for H. sativum in barley kernels

(73). Mead stated that the dormant mycelium of the same fungus remained viable in the barley grain for two to five years (264).

Shands furnishes longevity data for several barley seed-borne organisms. He reported that Gibberella saubinetii (Mont.) Sacc. G. zeae (Schw.) Petch survived for periods ranging from eight to twenty-seven months and lost its viability completely after 30 months (346, 347). Alternaria sp. had completely lost its viability after 63 months and Helminthosporium spp. were apparently non-viable after 51 months (347). Aspergillus sp., Cephalothecium sp., Trichothecium sp., Chaetomium sp., and Helminthosporium gramineum Rabh. all remained viable after periods ranging from 75 to 123 months, Alternaris sp. from 57 to 75 months; and Fusarium culmorum (W.G. Sm.) Sacc. and F. avenacium (Fr.) Sacc. were not found at the end of 28 months (346). The author indicated that longevity of the seed-flora appeared to be affected by storage conditions. The longevity of H. gramineum in barley kernels has been variously reported as being slightly over 17 months (120) or in excess of five years (230). Paxton obtained conidia and conidiophores of the same fungus from 16 year old herbarium specimens of barley placed in a moist chamber (299).

Many investigators have suggested that the qualitative and the quantitative nature of seed microflora may be strongly influenced by interaction-effects between

these various organisms. Hess isolated an antibiotic substance produced by H. sativum in culture and active against Bacillus mesentericus [Bacillus subtilis Cohn emend Prazm?] (176). He also attributed plant injury primarily to this substance. Conversely, several workers have shown that certain bacteria display marked inhibitory effects against H. sativum and other seed-fungi. Christensen and Davies found that isolates of Bacillus mesentericus produced a heat-stable substance which suppressed growth, inhibited or retarded germination, caused abnormal hyphal growth, and induced mutation in certain races of H. sativum (75). Ledingham and others found that treatment of wheat seed with formalin prior to inoculation with H. sativum, increased the amount of lesioning (225). They attributed the cause of increased infection to the removal of the surface bacteria which otherwise interfere with the fungus. The existence of a bacterial surface-flora on cereal seeds antagonistic to H. sativum was also demonstrated by Simmonds (355). Chinn and Russell controlled covered smut of barley [Ustilago hordei (Pers.) Lagerh.] and loose smut of barley [U. nuda] by soaking the infected seed in broth cultures of the bacterium, Pseudomonas viscosa (Fr. & Fr.) Migula, or of an unnamed filamentous yeast (66). Soaking seed, under similar circumstances, in cultures of Bacillus subtilis or in water failed to give satisfactory control. Leben et al. examined the filtrates from cereal seed soaked in

water and found that they all contained several volatile acids (222). They concluded that these organic acids, anaerobiosis, and perhaps antibiotic principles were involved in the control of grain diseases using the 'water-soak' method.

Species of Fusarium, primarily F. poae (Pk.) Wollenw and F. culmorum, are antagonistic to H. sativum, and this antagonism may express itself in lower percentages of kernel-infection by the latter organism (224, 352). The pathogenic activities of H. sativum are strongly inhibited by Phoma humicola Gilm. and Abb., Epicoccum purpurascens Ehr., and Trichoderma viride (60). Alternaria tenuis inhibits H. gramineum, H. sativum, Pullularia pullulans, and Stemphylium botryosum and is inhibited in turn by Chaetomium erectum, Curvularia trifolii, Fusarium miniliforme, F. poae, and Gibberella zeae (52). Christensen and Tuite isolated a slow-growing species of Aspergillus which proved to be strongly antagonistic to other sub-species of the A. glaucus group (72). The "storage" fungi, viz. species of Aspergillus, Penicillium, Mucor, and Rhizopus, have been observed by Wallace to invade cereal seed already infected with "field" fungi and to inhibit their growth (409).

The epiphytic flora associated with the aerial portions of grain plants, and especially the kernels, may be termed the "aerosphere" (182). Certain pronounced similarities exist among these organisms and have been noted by many workers. In general, most investigators are agreed

that the ubiquitous, small, gram-negative rods commonly isolated from grain kernels are not characteristic of the flora associated with fecal material (65, 325). The microflora of the aerosphere has been shown to contain many gram-positive cocci and gram-negative rods (53). Some authors have emphasized the similarity existing between kernel-flora and soil-flora (246). Others have concluded that this flora is distinct and separate from chance soil contamination (355, 410). Wallace and Lochhead found a greater similarity between seed bacteria and those of the "rhizosphere", which appeared to be intermediate in type between seed- and soil-bacteria (410). The similarity between the microflora of barley kernels and that of the air is well-known and will be discussed in a later section. That a more or less constant bacterial and fungal population exists, with respect to species, has been suggested by many workers (222, 246, 355, 410). Some of the commonly-occurring microorganisms are obvious pathogens, others are indicative of suppressed or inhibited parasitism (253). According to the hypothesis formulated by Hollis (182), many of these organisms involved in "nonreactive" (i.e. not producing disease symptoms) relations with higher plants may become "reactive" (i.e. pathogenic) through the agencies of mutation and natural selection. Many of the associated microorganisms are undoubtedly fortuitous saprophytes and intergrading symbiotic relationships of various degrees are probably in evidence. Little has been done in this research area and

the kernel-microorganism relationship awaits further elucidation.

MATERIALS AND METHODS

On the basis of information gained from the evaluation of various plating methods, routine plating procedures were adopted for the detection of barley microflora. In general the following methods were employed:

1. Plating whole kernels, ten kernels per plate, ten plates per sample on:
   a. Potato-dextrose agar
   b. Nutrient agar
   c. Barley agar
   d. Any of the above three plus actidione (100 p.p.m.) for bacterial determinations.
   e. Any of the above three plus chlormycetin (50 p.p.m.) for fungal determinations
2. Plating suspensions of ground kernels with suitable dilutions on any of the above-listed media

All kernels were routinely surface-disinfected with the standard method of the author (300). Plates were incubated for four to six days, at room temperature (ca. 25°C.), and colonies were identified under low and high magnification. As indicated, isolates were transferred to other media and subsequently identified. In addition to the identification and enumeration of microflora other factors were noted. These included: the association of microflora with injured or dead kernels, the location of organisms on the kernel, the interactions between organisms, and the ger-

minability of the plated kernels. Additional germination tests were carried out using the method outlined by the American Society of Brewing Chemists (5).

For routine population determinations, colonies of bacteria and yeasts were listed according to colony color and/or morphology. When these organisms were to be identified, they were isolated in pure-culture using poured-plate, streaking, or single-cell techniques. Bacterial identification requires the use of a number of diagnostic tests. The following tests were routinely employed in the identification of selected bacteria: Gram's staining, cell form, cell size, cell arrangement, motility, spores (location and size), indole production, hydrogen-sulfide production, action on litmus milk, reduction of nitrates, Voges-Proskauer test, methyl-red test, utilization of citrate, catalase activity, hydrolysis of starch, liquefaction of gelatin, and growth on potato slants, agar slants, agar plates, and nutrient broth (82).

Microflora for population counts and for identification were obtained from the following sources: (1) four varieties of malting barley grown at various locations in the United States in 1958, (2) four varieties of malting barley grown at East Lansing in replicated nursery plots in 1959, (3) five varieties of barley grown at two locations in Michigan in rod-row nursery plots in 1959, (4) 84 samples of barley lots being malted and brewed in cooperative tests conducted by the Malting Barley Improvement Associa-

tion and the malting and brewing industry in 1960, (5) rough and smooth awned iso-genic lines of barley grown in 1955 and 1956 at various locations, (6) exposed plates in barley plots at East Lansing in 1959, (7) assorted barley samples collected and received from various locations in Michigan.

EXPERIMENTAL RESULTS

The percentages of microflora obtained by plating barley kernels of several varieties from diverse locations and from different crop years are listed in Table 7. The germination capacities of these barley samples are also included in this table. The population determinations obtained for the Malting Barley Improvement Association samples are tabulated in Appendix C. The great number of bacterial isolates obtained in these plating studies precluded the possibility of making specific identifications except in a few cases. Some of the more significant reactions to standard physiological tests are recorded for bacterial isolates from exposed agar plates, plated kernels, and barley leaves (Table 8). No attempt was made to establish the relative number of bacteria of varying morphological and physiological characters which are present in the air, on foliage, and infect barley kernels. The data in Table 8 merely demonstrates the bio-chemical potential of randomly-selected bacterial isolates. The various cultural tests performed with the bacterial isolates are not tabulated in this section since they are mainly of taxonomic value. Relatively

Table 7. - The isolation of microflora from barley kernels of differing variety and origin. [a]

Identity of barley	Origin of barley	Crop year	Alternaria	Helminthosporium	Fusarium	Cladosporium	Epicoccum	Bacteria	Yeasts	Aspergillus	Phycomycetes	Other	Germination capacity (%) [b]
Kindred	Frankenmuth, Mich.	1958	64	0	1	0	2	27	1	0	0	3	99
Hanna	West Coast	1958	3	0	5	4	0	4	0	0	0	2	98
Montcalm	Saskatchewan	1958	10	0	0	0	0	46	1	1	0	0	98
Kindred	Caro, Mich.	1958	87	0	8	0	6	59	0	0	0	3	99
Hannchen	E. Lansing, Mich.	1959	101	1	18	1	0	64	0	0	1	1	95
Montcalm	do	1959	107	0	22	0	0	56	0	0	1	0	96
Traill	do	1959	98	1	18	0	0	59	4	0	0	0	100
Kindred	do	1959	96	1	18	0	0	58	0	0	0	1	99
Parkland [c]	do	1959	88	1	1	0	2	58	2	0	0	0	97
Traill [c]	do	1959	85	1	4	5	1	22	6	0	0	0	94
Moore [c]	do	1959	89	0	9	1	1	83	2	0	0	0	92

Table 7. - Continued

Kindred[c]	E. Lansing, Mich.	1959	87	2	5	4	2	72	1	0	0	1	90
Montcalm[c]	do	1959	78	0	4	0	2	51	4	0	0	2	95
Parkland[c]	Tuscola Cty, Mich.	1959	68	0	3	0	0	38	4	0	0	3	87
Traill[c]	do	1959	52	2	0	0	2	76	17	0	1	0	87
Moore[c]	do	1959	74	1	4	2	2	52	1	0	1	4	91
Kindred[c]	do	1959	56	0	6	2	1	65	1	0	1	0	89
Montcalm[c]	do	1959	49	1	1	1	0	77	1	0	5	2	95
39-R-11[d]	Madison, Wis.	1955	23	4	0	0	0	69	2	0	0	0	94
39-r-11[d]	do	1955	19	7	0	0	0	73	2	0	0	1	81
41-R-12[d]	do	1955	31	2	2	0	0	4	0	0	0	0	99
41-r-12[d]	do	1955	23	1	0	0	0	8	0	0	0	5	94
42-R-11[d]	do	1955	18	4	0	0	0	35	0	0	0	0	94
42-r-11[d]	do	1955	10	3	2	0	0	24	0	0	0	0	78
42-R-11[d]	do	1956	20	2	1	0	0	47	0	0	0	0	93
42-r-11[d]	do	1956	5	1	1	0	0	60	4	0	0	0	85

[a] Expressed as the number of discrete colonies per 100 plated kernels.

[b] Determined from plated kernels.

Table 7. - Continued

[c] Barley Nursery samples courtesy Dr. J. E. Grafius, Mich. State Univ., from rod-row plots; plated eight months after harvest.

[d] Barley samples courtesy Dr. R. G. Shands, Univ. of Wis., isogenic barley lines - "R" equals rough awn, "r" equals smooth awn.

Table 8. - Morphological and physiological characteristics of bacteria isolated from the air, barley foliage, and barley kernels.

Morphological and physiological reactions	Source and total number of isolates tested					
	Air (57)		Foliage (32)		Kernels (140)	
	(+)	(-)	(+)	(-)	(+)	(-)
Gram's stain[a]	30	23	23	9	37	103
Rod-shaped	45	3	26	2	136	4
Motility	48	3	28	2	101	34
Spores	6	47	15	17	18	105
Indole production	1	13	1	23	4	60
H_2S production	10	6	7	2	49	11
Nitrate reduction	--	--	10	19	40	9
Voges-Proskauer	12	5	23	5	43	19
Methyl red	13	4	6	22	23	39
Citrate utilization	39	9	25	4	69	25
Catalase activity	--	--	28	--	40	3
Starch hydrolysis	--	--	16	12	27	31
Gelatin liquefaction	18	--	--	--	9	--

[a] Read Gram's stain as positive or negative, with all other tests read yes for (+) and no for (-). Blank spaces indicate that an insufficient number of tests were performed.

few bacteria have isolated and identified from barley kernels (Appendix B). The bacteria which were identified to species in this study are listed below (Table 9). Other organisms which have either, not been previously reported as associated with barley kernels, or which have been rarely isolated are listed in Appendix B.

DISCUSSION AND CONCLUSIONS

With the exception of the obligate microorganisms, it would seem that any organism possessing a broad-spectrum enzyme system is capable of infecting barley kernels. Successive qualitative investigations invariably reveal the presence of previously unreported microflora on barley kernels. The mycoflora of barley grains are, by far, better characterized than the bacterial and yeast flora. Certain technical difficulties have prevented an equivalent advance in the knowledge of the bacterial flora of grain kernels. The extremely large populations of these organisms on a single grain, as well as their rapid reproductive and physiological potential, are difficult to accurately assess. This is in part due to a lack of suitable methods for the study of bacterial populations. The identification of only a few bacterial isolates is time-consuming, the task becomes nearly impossible when the flora of a small sample of grain is considered. Various quantitative methods have been devised to circumvent a part of this difficulty (cf. Chapt. 1). By using stand-

Table 9. - Bacterial species isolated and identified from barley kernels and barley leaves.

Source of organism	Name of organism	Frequency of occurrence (a)	Frequency of occurrence (b)
From: Leaves & kernels	*Bacillus cereus Fr. & Fr.	Rare	Rare
do	*B. megaterium D'Bary	Seldom	Seldom
do	*B. pumilus Gotth.	Common	Rare
do	*B. subtilis Cohn emend Prz.	do	Seldom
do	*Pseudomonas spp.	Rare	Common
do	*Xanthomonas translucens (J.,J.,&R.) Dows.	Common	do
Leaves only	*Bacillus coagulans Hamm.	Rare	--
Kernels only	*Achromobacter sp.	--	Rare
do	*Aerobacter aerogenes (Kr.) (Beij.)	--	Seldom
do	*A. cloacae (Jord.) Berg. et al	--	Common
do	*B. cereus var. mycoides (Fl.) Smith et al	--	do
do	B. megatherium-cereus intermediates	--	Seldom
do	*B. polymyxa (Prz.) Migula	--	Common
do	*Brevibacterium linens (Weigm.) Breed	--	Rare
do	*Kurthia zopfii (Kurth) Trev. (?)	--	do
do	*Corynebacterium sp.	--	do
do	Erwinia rhizogenes (Rk. et al) Dows. (?)	--	do

Table 9. - Continued

Source of organism	Name of organism	Frequency of occurrence (a)	Frequency of occurrence (b)
do	*Pseudomonas atrofaciens (McC) Stevens	--	Common

[a] On leaves

[b] On kernels

* Species accepted by Breed et al (54)

ardized procedures an investigator can accurately determine the number of viable single cells occurring in a given sample of grain. These methods, however, do not yield qualitative information, nor do they provide an index to the biochemical potential of a bacterial population.

The bacterial isolates identified in this study do not represent the dominant flora of barley kernels (Table 9). These identifications were dictated by two factors: (1) the common occurence of the organisms, and (2) the inherent morphological and/or physiological properties of the organisms which facilitated their identification. Thus the large number of Bacillus species, commonly found in soil and water, is not a true index to the predominant bacterial flora of grain kernels.

Varietal resistance to kernel-infecting microorganisms is poorly understood. If such resistance exists, it is probably manifested quantitatively rather than qualitatively (cf. Chapt. 3 and Appendices B and C). The origin of the seed is of great importance with respect to both the numbers and kinds of microflora present on and within barley kernels. Barley grown under irrigated conditions and having a bright appearance is relatively free of fungal infection. The one exception to this statement is seen in the case of superficial infection by Cladosporium spp. High counts of this fungus may be made from bright barley kernels, showing no visible evidence of fungal infection (see Chapt. 3 and Appendix C). Generally, bacter-

ial infection is less severe in bright barley kernels than in discolored grains; however, occasional bright samples may show a higher percentage of bacterial infection than a comparable stained sample (see Appendix C). In the case of discolored kernels, a positive correlation exists between the origin of the seed and the number of field fungi present on and in the seed. So much so that the origin of the kernels may be determined from a microfloral analysis of the barley sample (280, 281) (Appendix C).

The field organisms, both fungi and bacteria, are more abundant in the middle and awn regions of the kernel. This has been established by observations made on many plated kernels, as well as by the plating of transverse sections of barley kernels (see Chapt. I) and the use of histological procedures (see Chapt. 3). As indicated earlier, this generalization does not hold for certain bacterial and fungal pathogens, nor is it valid in the case of the storage fungi (Chapt. I).

The barley samples, provided by Dr. Shands, yielded low fungal and bacterial populations, when contrasted with the place of origin and the color of the kernels. It is very likely that this is a reflection of the age of the kernels, viz. four and five years old, since the viability of kernel-infecting fungi decreases rapidly after several years. The bacterial counts made from these samples did not, apparently, decrease in the same order as the fungal counts. No information exists on the longevity of the

bacterial flora of barley kernels. The data indicates that little or no differences exist between the bacterial species found in the air, on barley foliage, and on barley kernels (Table 8). This may be an unwarranted assumption inasmuch as the isolation methods are themselves selective and may, therefore, preferentially screen out the less-specialized bacteria. The ability of many of these isolates to utilize sodium citrate as the sole carbon source, to hydrolyze starch, and to liquefy gelatin indicates that these organisms are able to infect and colonize a wide range of host substrates (Table 8). The more commonly occurring fungi, found on barley kernels, have also been shown to possess broad enzymatic properties which suggest that the fungal flora are similarly capable of attacking a variety of host substrates (300).

CHAPTER III

THE ETIOLOGY OF BARLEY KERNEL INFECTION

LITERATURE REVIEW

Developing barley kernels are subject to infection, invasion, and establishment by microorganisms. These kernel invaders comprise four general classes: the parasitic fungi and bacteria, the "field" fungi, the "storage" fungi, and the non-parasitic bacteria and yeasts. The "field" fungi are those that invade the developing and mature kernel prior to harvest. They include species of Alternaria, Helminthosporium, Fusarium, Epicoccum, Cladosporium, and several others. The "storage" fungi rarely, if ever, are found on well-conditioned kernels prior to harvest. These species of Aspergillus, Penicillium, and certain Phycomycetes invade the kernel after harvest, expecially in damaged grain stored at high moisture levels. The "field" and "storage" fungi have been treated by Christensen (69, 70) and Tuite and Christensen (398). While certain barley pathogens are included in the "field" fungi group, viz. Helminthosporium and Fusarium, other parasitic species are generally considered separately. These include Claviceps purpurea (Fr.) Tul., the bacterial pathogens Xanthomonas translucens (J.,J., & R.) Dows. and Pseudomonas atrofaciens (McC.) Stevens, and the three Ustilago species. General discussions of the life-cycles, symptomology, etiology, and control of these organisms are found in the works of

Dickson (99, 100), Leukel (229), and Fischer (117). The non-parasitic bacteria and yeasts have not been comprehensively dealt with and references to their etiology are widely scattered through the literature.

Previous studies on the etiology of the kernel- and floret-infecting organisms of barley and other cereals have employed several methodological procedures. Whitehead in a study of the seed-borne organisms of barley and other cereals employed spore-traps to determine the numbers and kinds of available air-borne inocula (415). He also used plating- and histological-techniques to determine and/or confirm the mode of infection by these organisms. Similar techniques were employed by Whitehead and his co-workers in a study of cereal and legume seed-infection by Microascus trigonosporus (416). Koehler used a combination of histological and plating techniques to determine the developmental stage at which corn ears were infected by Fusarium moniliforme and other species (216). Natural field inoculum has provided infection in several of these studies (9, 216, 336, 415) while other workers have inoculated developing kernels with suspensions of the organisms under study (11, 12, 174, 175, 192, 264, 334, 341, 396, 404, 416, 423).

The time of infection, location and form of the microflora, as well as the qualitative and quantitative differences in the microorganisms of barley kernels are conditioned by several factors: the anatomy and physiology of the 'host'-kernel or -floret, the availability of inoculum,

the macro- and micro-environment, and the nature of the microorganisms themselves. Thaysen and Galloway stated that grain florets provide particularly good growth-centers in that they furnish exudations for the nourishment of the infecting organisms and the glumes provide protection against the dessication of the microflora (390). A further protective function of the glumes is noted in cereals, such as barley, in which the glumes fuse with the caryopsis during ripening. The cementing of the barley hull effectively shields the enclosed microflora against the external environment. The relation of kernel-anatomy to the microflora of cereal grains is also discussed by Hyde and Galleymore (191). Harlan has discussed the developmental anatomy of barley kernels in some detail (165) and has shown that the moisture content of barley grains decreases from flowering to maturity (167). Similar data are presented in a paper by McLean (261). At the time of flowering the floret has a moisture content of approximately eighty percent. A steady decrease in moisture results in a moisture content of fifteen percent or less at harvest. As the moisture content of the kernel decreases, a sticky substance is secreted by the caryopsis which causes the glumes to adhere thereafter to the developing kernel. As the kernel increases in size the underlying tissues of the pericarp are subjected to severe compressive forces. These tissues become compressed and distorted and are greatly reduced in thickness at maturity (165, 167, 191, 261). All of these fac-

tors influence the infection process by microflora.

The microflora infecting barley kernels are predominantly those species found in the atmosphere as spores, cells, and mycelial fragments. The occurrence of air-borne inoculum in relation to plant disease has been discussed in a review paper by Stakman and Christensen (372). Hirst has presented a general discussion of the liberation and the dispersal of fungal spores (179). The trapping and identification of air-borne organisms generally is carried out by means of mechanical spore-traps, coated-glass slides, or by exposing petri-plates containing culture-media. These methods have been discussed and evaluated by Gregory (148). A large number of papers have been written in which the air-spora of various locations is described. Almost without exception, these determinations have shown that the most commonly-occurring fungal spores in the air are species of Cladosporium (148, 149, 189, 219, 296, 370), Alternaria (219, 296, 370, 371), Sporobolomyces (148, 149, 219), Helminthosporium (370, 371), and Tilletiopsis (219). Other frequently-occurring air-borne spores are those of Erysiphe, Ustilago, Penicillium, Aspergillus, Epicoccum, Fusarium, Pullularia, Stemphylium, Rhizopus, Mucor, and several other yeast and fungal species. Gregory and Hirst (149) reported that fungal spores appeared to be more numerous in the air than bacterial cells. They concluded that soil-microorganisms did not appear to constitute a significant proportion of the air-spora. Fluctuations in the number of spores

present in the atmosphere have been reported for many of the commonly-encountered fungi. Diurnal periodicity has been noted for the spores of Helminthosporium (371), Alternaria (219, 371), Epicoccum, Stemphylium (371), Cladosporium (189, 219), and several other species. Meteorological conditions may also affect the number of spores present in the air; e.g. Sporobolomyces (219) and Fusarium (163) appear to be favored by wet weather, while Cladosporium (189) and Alternaria (219, 371) are more prevalent on dry days. Seasonal variation in spore numbers has been noted for most of the commonly occurring fungi (189, 219, 371). Spurr noted that conidia of H. sativum did not occur on exposed slides until late in the growing season (369). He concluded that air-borne inoculum originating from straw was the principal source of inoculum for barley kernel infection in Michigan. Simmonds and others (358) have reported that H. sativum spores were rarely present on spore-trap slides exposed in western Canada. The authors claimed that development of the fungus in the field was restricted by indigenous bacteria and that the spores were not well adapted for air dissemination. The use of disease-free seed was advocated by Steinkraus and his co-workers in order to produce disease-free plants which would in turn produce larger quantities of disease-free seed (376). In the case of several of the barley kernel-infecting organisms this supposition may not necessarily hold true since air-borne inoculum is capable of traversing large areas while re-

taining its viability (372). Additional inoculum sources exist also in the form of over-wintering organisms. Several of the barley organisms, including H. sativum, and F. culmorum, have been shown to overwinter readily under natural conditions as far north as Edmonton, Alberta (119).

Many workers have noted the correlation between humid weather and the increased incidence of kernel-infection by both fungi and bacteria. Presumably high humidity and the presence of free moisture influence infection by altering the composition of the air-spora and by creating a micro-climate suitable for the invading microorganisms. The subject of micro-climate and infection has been generally discussed by Yarwood (422). Hyde has presented evidence that the degree of wheat kernel infection by "field" fungi is dependent upon the atmospheric humidity during the ripening of the grain (190). Whitehead noted that drier climatic conditions reduced the percentage of barley infection by Alternaria spp. (415). Machacek and his fellow-workers observed that harvested or threshed grain from eastern Canada possessed higher percentages of fungi, both parasitic and saprophytic species, than did western grown seed (246). Greaney and Machacek found that severe infection by Helminthosporium and Fusarium was usually associated with wet years and more particularly with years in which wet weather had prevailed during the later portion of the growing season (144). Gordon has reported that Fusarium infection of the cereals is more prevalent in eastern areas

than in western Canada (133, 134). Hind has stated in his brewing text that bacteria are most abundant in dry weather, the fungi occur in greatest quantity in damp weather, and that yeasts are most prevalent during the time of fruit ripening (178).

The etiology of infection by species of Helminthosporium has been intensively studied. Leukel and his coworkers have discussed the mode of infection of barley kernels by H. gramineum (230). The kernels were shown to be infected by air-borne spores of the fungus. Infection occurred for a considerable period after flowering. According to Yu, the most susceptible period for infection by the fungus was the milk or green mature stage or at any intervening period (423). Andersen has shown that infection of wheat kernels by H. sativum is conditioned by the developmental stage of the host (12). Little infection occurred on inoculated wheat heads prior to flowering. Susceptibility increased from the flowering to the past-flowering stages. Heavy infection during the flowering and after-flowering stages resulted in reduced yield while infection in the later stages of host development affected only quality. Henry found that H. sativum invaded wheat kernels chiefly during the milk stage after inoculating wheat heads with the fungus (175). Scott and Sallans also sprayed spore suspensions of H. sativum on developing wheat heads (341). They concluded that most infection occurred during the blossoming period of the florets but that infection could occur at any later stage in kernel development. Vendrig

reported that many seed of barley and wheat failed to develop following inoculation by H. sativum at the time of flowering and that susceptibility continued,until twenty days after flowering (404). Mead intensively studied the infection of barley kernels by H. sativum (264). He showed that if inoculation of barley occurred at, or soon after flowering, the ovaries were killed. If inoculation occurred from five to ten days later, the glumes, pericarp, and lodicules were invaded and areas of necrotic tissue occurred in these organs. Usually there was some shriveling of the grain at this stage of inoculation. Some infection of fifteen to twenty day kernels was also obtained, but it was confined mainly to the pericarp and glume tissue at both ends of the kernel and no shriveling of the grain occurred at this stage.

Atanasoff concluded that the greatest amount of infection of grain kernels by Fusarium species was obtained after inoculation of wheat heads during the period after flowering (21). In an early paper, Schaffnit showed that infection of grain kernels by F. nivale was caused by airborne spores lodging between the grain and the glumes of the kernel (336). He reported that infection could occur from the late-dough stage until maturity of the kernel. Andersen found that wheat headblight infection, caused by Gibberella zeae, varied with the stage of seed development (11). Little infection was obtained on inoculated heads prior to flowering. Susceptibility increased from

the flowering to past flowering stages with decreasing susceptibility at later developmental stages. Fusarium head-blight infection occurred from pre-flowering until the soft dough stage in small grains inoculated with spore suspensions of the fungus (395, 396). Haskett inoculated spring barley with G. zeae and reported that the kernels were susceptible at any developmental stage with the critical period for infection occurring from flowering to the milk stage (174). Dounin found that many of the scabbed kernels of barley and other cereals were infected with Fusarium in shocks after harvesting (104). The kernel-infecting species of Fusarium have been reported by several authors to be favored by hot, moist weather (11, 21, 201, 336). Crosier and Waters have attributed the high incidence of Fusarium infection of small grains in New York state to cool, humid climatic conditions during the growing season (86). Dickson (99, 100) and Haskett (174) have reported that kernel infection by Fusarium spp. is initiated by entry of the fungus through dehisced anthers. Atanasoff (21) asserts that the fungus invades the kernel at any point at which contact is initially made between the kernel and the pathogen. Tu concluded that the flower or anther was not necessarily the point of initial infection and that the glume seemed to be the more common site of infection by Fusarium (396).

Wheat kernels were inoculated with spore suspensions of Alternaria by Sallans (334). He obtained kernel

discoloration most frequently when the kernels were inoculated during or after the soft dough stage. Whitehead found that approximately one-half of the infection of barley kernels occurred from the period of flowering to the time of the cementing of the glumes to the caryopsis (415). Johnson and Hagborg (203) stated that the main course of infection of wheat kernels by Alternaria tenuis was from spores entering the florets usually at the tip of the lemma. Dead floral parts such as anthers and stigmas favored the establishment of mycelial growth. Fungal mycelium was rarely found in areas beneath the interior of the lemma. Machacek and Greaney reported that Alternaria spp. do not seem to be as actively parasitic as H. sativum in infecting grain kernels since they do not usually attack the glumes until these tissues have withered and died (247). They speculated that Alternaria infection was a direct-contact process with infection occurring either during the blossoming period, when the florets are expanded, or near kernel maturity, when the glumes are separated. These assumptions were based on the unavailability of inoculum during the early stages of development and the correlation between kernel-plumpness and the presence of "kernel-smudge" microflora. Whitehead and his co-workers observed that blossom-infection of barley and other plants occurred with Microascus trigonosporus (416). The fungus apparently persists in the enveloping tissues of dormant seeds and caused no apparent damage to the development of the host (416). They indicated that

this type of infection might be characteristic of the group of fungi commonly isolated from plated seed. Five varieties of barley were tested for microflora in the milk stage, early dough stage, and at maturity (9). Alternaria was shown to appear soon after the appearance of the heads. Fusarium infection ranged from ten to eighteen percent in the milk stage and increased to forty to fifty percent at maturity. The greatest increase in bacteria and yeasts occurred at about maturity and varietal differences in the percentage of infection by several organisms was also noted.

Varietal differences may exist in susceptibility to the various kernel-infecting bacteria, yeasts, and fungi isolated from barley (9). Five barley varieties were compared as to their relative microfloral populations. In all cases Kindred kernels gave the lowest percentage of infection while Moore yielded the highest number of bacteria and yeasts. More Fusarium infection has been noted on the kernels of oats and barley than on wheat (133, 134). This difference was attributed to the presence of hulls on oats and barley. Greaney and Machacek found some varieties of wheat, oats, and barley were more susceptible to internal infection by species of Helminthosporium and Fusarium than were others (144). Immer and Christensen found that susceptibility of barley varieties to kernel-infection by Helminthosporium and Fusarium species was usually correlated with the date of heading (192). In general, they observed that barley varieties that were resistant to

Fusarium blight were also resistant to Helminthosporium blight. Wallace has asserted that Elite, Foundation, and Commercial seed of small grains is equally susceptible to fungus attack and that hull-less seeds are more susceptible to fungal infection than are seeds with hulls (409). A similar observation has been made by Smith (362). Linke has suggested that smooth-awned varieties of barley are more susceptible to infection by the kernel-staining fungi (236). He attributes this difference to the more accessible space between the kernel base and lemma to rain and dew, in turn facilitating infection by the fungi.

Studies on the mode of kernel-infection by bacteria and by yeasts are not as abundant as those dealing with the fungi. Lund isolated several species of both white and pink yeasts from barley grains by the use of special plating techniques (242, 243). He found that while many yeast cells were present on the kernels prior to harvest, only a few cells were isolated after threshing had taken place. One of these yeasts, Sporobolomyces, has also been isolated in quantity from the surface of barley leaves with the aid of special techniques by Last (218). Lund observed that Sporobolomyces and Rhodotorula were apparently favored by very hot and dry climatic conditions (242). Last concluded that the number of colonies of Sporobolomyces was correlated with high humidity (218). Bamberg has presented an intensive review of the literature dealing with the black-chaff organism, Xanthomonas

translucens (24). The bacterium typically enters the host-tissue through wounds or natural openings. Moderate temperatures, high humidity, light, and the age of the host all influence the infectivity of the pathogen. Continued periods of wet weather were reported to favor epidemics of the disease. Boosalis has stated that this bacterial pathogen is also favored by the root-rot and leaf-spotting fungi which apparently pre-dispose the host tissue to attack (49). Thomas and others have noted that the number of coli-aerogenes bacteria associated with developing grain kernels increased during the growing season (392).

Various other factors have been shown to influence the invasion of barley kernels by microorganisms. The increased use of combine-harvesting of barley and other crops has increased the danger of high moisture contents of the harvested grain as well as the incidence of skinned, broken, and otherwise mechanically damaged seed (140). Grain stored at moisture contents above twelve percent are subject to invasion by the storage fungi which subsequently cause deterioration of the seed (45, 69, 70, 226). Hurd has shown that damaged kernels of wheat and barley permit the invasion of Penicillium and Rhizopus species (188). Exposed embryos of wheat are more susceptible to mold invasion by H. sativum and other species according to Mead and his co-workers (268). Weak-strawed varieties of barley are subject to lodging. The heads of lodged grain may come in contact with the soil thus enabling the infection

of kernels by several species of soil-flora to take place. Swathed and windrowed grain may be infected in a like manner, especially when heavily rained upon (226).

MATERIALS AND METHODS

The progress of infection by "field" fungi, bacteria, and other organisms was studied at successive stages of development of the barley kernel. Four varieties of malting barley: Hannchen, Traill, Kindred, and Montcalm were planted in replicated rod-row nursery plots at East Lansing. The varieties were planted at the rate of 40 grams per plot (1.7 bushels per acre). All plots were planted, harvested, and threshed with standard experimental-nursery equipment and methods. Date of planting was April 16, 1959; date of harvest was July 15, 1959 for Kindred and Traill, and July 20, 1959 for Hannchen and Montcalm. Glass slides, coated with glycerol, and petri plates containing either potato-dextrose or nutrient agar were exposed daily at five locations in the field. The slides were taped to the sides of upright 2x4's at the same height as the barley inflorescences. The exposed plates were placed on the top of a small platform affixed to the same 2x4's. The slides were exposed for 24 hour periods, the plates for a period of approximately ten minutes. The numbers and kinds of available air-borne inoculum were later determined from counts obtained from these exposed 'traps'.

A scale was devised denoting progressive stages of

kernel development from pre-flowering to maturity (i.e. harvest), patterned after the developmental stages of barley discussed by Harlan (165). This scale is listed below:

STAGE NO.	DESCRIPTION OF STAGE
1	Inflorescence in sheath, completely enclosed
2	Inflorescence in sheath, flowering
3	Inflorescence in sheath, awns emerging
4	Inflorescence partly emerged from sheath
5	Inflorescence emerged from sheath
6	Early milk stage
7	Early-intermediate milk stage
8	Late-intermediate milk stage
9	Late milk stage
10	Early (soft) dough stage
11	Intermediate dough stage
12	Late dough stage
13	Mature, one week prior to harvest
14	Mature, at harvest

Florets and kernels were harvested from each variety at each stage of development. Each sample was sub-divided; one half was placed in strong FAA (200) to fix and preserve it for subsequent histological work, the other half was transferred to the laboratory for microfloral determinations by means of kernel-plating techniques. Surface-disinfection time was shortened to one-half minute for stages one through four because of the delicate nature of

the tissues. The lemma and glume awns were not removed from the florets in either the plating or the histological procedures.

The fixed florets and kernels were removed from the FAA, infiltrated, and embedded in a 1:1 mixture of embedding paraffin and "tissue-mat" (Fisher) using standard histological procedures (200). The embedded tissues were serially-sectioned with a rotary microtome at from 10 to 14 μ, depending upon the structure of the tissues. A modification of Conant's quadruple-staining procedure was employed to differentiate the kernel tissue and the attendant microorganisms (200). The staining schedule is listed below:

1. Place slides in xylene for 10 minutes (to remove paraffin)
2. Transfer to xylene bath for five minutes
3. Transfer to absolute alcohol for five minutes
4. Transfer to 95 percent alcohol for five minutes
5. Transfer to 70 percent alcohol for five minutes
6. Place slides in safranin (50 percent alcoholic solution) for two hours
7. Rinse in distilled water
8. Place slides in thionin (1% thionin in 4% aqueous phenol) for one minute
9. Pass slides quickly through three rinses of absolute alcohol
10. Place slides in clove oil plus fast green plus gold orange for two minutes
11. Pass slides through four baths of clove oil plus gold orange for two minutes each
12. Pass slides through two rinses of xylene and store in xylene until cover slips are applied

Following this procedure the slides were cleaned and the cover slips were applied. A total of 56 sets of slides, each set containing an average of 15 slides, and each slide consisting of approximately 13 sections, were prepared. This large number of preparations made it necessary that only a representative sample be subjected to microscopic observation. Consequently a slide was selected from each end of the serial series and these two were read together with the middle slide in the series.

The possibility of chemical control of the "field" organisms was also studied. Five chemical compounds and an untreated check were used in conjunction with the four varieties and three replications discussed above. The chemical compounds, manufacturer, and other information is presented below.

Treatment number	Code name/number	Manufacturer	Rate of application (per 4 gal. water)
1	Form 67 (Actidione)	Upjohn	24.0 ml
2	Untreated check	-----	-----
3	CuP (copper omadine)	Olin Matheson	1.6 gm
4	CR-5009 (Ni fungicide)	Rohm & Haas	1.6 ml
5	O-3818 B (do)	Rohm & Haas	1.6 gm
6	Wettable sulfur	-----	75.0 gm.

It was decided that, if chemical control of the staining or weathering organisms was to be economically feasible, only one application of the compound could be made. This

premise is dictated with field crops as a result of the relatively low cash return per acre. Consequently the application was made, using a portable field sprayer, on June 27, 1959. This date coincided approximately with the developmental stage of the four barleys designated number seven, i.e. the mid-point of kernel development.

Analyses of variance were run with all four varieties in order to determine whether significant differences existed between 1,000 kernel weights, bushel weights, yields, treatments, varieties, replications, and dates of harvest.

EXPERIMENTAL RESULTS

The exposed-slide technique employed in this portion of the study was not refined enough to use as a quantitative estimation of the air-borne inoculum present in the air around the experimental plots. Such a method is of great value in determining the diurnal and seasonal variations in air-spora but only when some procedure is used for measuring time intervals on the slides. The following data were obtained from the exposed slides: (1) Alternaria sp. spores were present in moderate amounts throughout the period of kernel development, (2) Helminthosporium spores were not observed on the coated slides until late in the season at a time corresponding to developmental stage eleven (see p. 82), (3) large numbers of unidentifiable hyaline fungal spores (powdery mildew?)

were observed throughout the experimental period (Table 10).

No significant differences were noted between plates exposed at the same time at different locations in the barley plots. The choice of media exerted a selective effect on the type and number of colonies developing on the exposed plates. For the determination of bacteria and yeasts by this method, nutrient agar plates gave the best results (Table 10). Nutrient agar was decidedly inferior to potato-dextrose agar for the trapping of fungal air-inoculum. Exposure periods in excess of ten minutes had two unwanted effects: (1) the culture media dried out rapidly, and (2) the number of developing colonies became too numerous to accurately count. In general all of the species of microflora commonly isolated from barley kernels were present on the exposed plates. Differences in the amount of airspora present in the field and the amount of infection obtained on kernels by certain of these organisms were pronounced in several cases. Cladosporium sp. was generally the most prevalent fungus obtained on exposed plates and slides. However, the amount of infection obtained with this fungus on barley kernels was negligible. Similar differences were obtained with Dematium pullulans, Candida, and other fungi.

In addition to platings of the fourteen developmental stages of kernel maturation, a plating determination was made of samples which had remained standing for several days after harvesting (Fig. 5). All barley varieties

Table 10. - The occurence of microflora in the air about the grain plots at East Lansing as determined by exposed petri plates.

Date of plate exposure	Remarks	Average number of colonies per plate per 10 minute exposure period[a]									
		Alternaria	Cladosporium	Fusarium	Epicoccum	Phycomycetes	Yeasts	White bacteria	Yellow bacteria	Aspergillus Penicillium	Other
6-17-59	---	4.0	18.0	3.5	1.2	0.6	1.8	9.8	0.2	0.0	0.0
6-18-59	---	3.8	30.2	2.8	0.0	1.0	3.6	9.8	0.4	0.0	0.0
6-19-59	___	2.2	12.2	0.0	0.4	0.4	2.2	25.8	0.6	0.6	0.4
6-20-59	Aspergillus niger present	1.0	4.8	0.2	0.2	0.0	1.6	14.4	0.8	4.4	0.6
6-21-59	---	No plates exposed									
6-22-59	---	4.0	59.4	0.8	0.0	0.2	11.4	12.1	0.3	0.0	0.3
6-23-59	---	Plates contaminated									
6-24-59	---	9.6	20.4	0.4	1.4	0.4	6.4	13.0	2.0	0.4	1.0
6-25-59	First appearance of H. sativum	9.8	3.2	0.2	0.4	0.0	1.4	9.4	1.2	0.0	1.0
6-26-59	Phoma sp. present	5.2	5.6	1.4	0.2	0.6	0.6	4.6	1.0	1.0	3.8

Table 10. - Continued

6-27-59	---	No plates exposed									
6-28-59	---	16.4	1.0	1.6	0.0	0.2	1.2	11.8	3.4	0.0	1.4
6-29-59	---	10.2	6.0	2.4	0.2	0.0	1.6	13.4	1.0	0.0	0.6
6-30-59	---	4.8	37.0	0.4	1.2	0.0	3.4	5.6	4.0	4.6	1.6
7-1-59	Heavy rain - preceeding 3 hours	3.4	16.0	0.4	0.6	0.8	1.0	6.2	2.6	1.2	1.8
7-2-59	Periodic rain Phoma present	15.6	44.6	0.6	2.0	0.0	2.2	13.0	4.0	0.0	1.8
7-3-59	Actinomycetes and Phoma present	19.8	189.6	1.2	7.0	0.2	0.2	24.8	8.0	0.0	1.4
7-4-59	Exposed nutrient agar plates	20.8	22.3	0.0	0.0	0.0	12.0	22.3	36.6	1.6	4.0
7-5-59	---	16.6	35.6	2.6	1.0	0.0	1.0	17.4	0.4	0.0	0.4
7-6-59	---	12.8	27.4	0.4	5.0	0.4	1.6	23.4	1.4	0.4	1.2
7-7-59	---	No plates exposed									
7-8-59	---	20.6	19.6	3.8	3.4	0.0	9.0	32.0	3.6	0.0	0.4
7-9-59	Actinomycetes present	5.8	7.4	0.8	0.8	0.6	1.2	10.6	0.4	0.2	1.4

[a] Non-acidified potato-dextrose agar used, unless otherwise noted; number of colonies represents an average of five plates in each case.

performed similarly in their susceptibility to microfloral infection (Figs. 3a, 3b, 3c, 3d). The progress of fungal infection was very similar in all cases. Fungal infection began at a later stage than did bacterial infection. The shape of the curves for bacterial and yeast infection was more similar for the six-rowed varieties than for the two-rowed variety (Fig. 3d). In all cases, bacterial and yeast infection decreased after the milk stage in the six-rowed barleys and after the intermediate-dough stage in Hannchen. The optimum stage for bacterial and yeast infection in the six-rowed varieties was the intermediate- to late-milk stage and for Hannchen the intermediate-dough stage. Fungal infection increased in all varieties up to harvest. Little or no fungal infection was obtained prior to the early milk stage.

The plating data were corroborated by the histological evidence. Slide preparations however, gave little information as to the degree of infection. In almost every case, fungal mycelium was found in abundance only at the awn end of the kernels i.e., at the point of juncture between the palea and lemma (Plate II). Hyphae were also observed in tangential sections of the glumes which indicated that infection and ramification of the mycelium takes place on the sides of the kernel as well as at the ends (Plates III & IV). In several cases mycelial fragments and/or spores were found lodged under the epidermal barbs of the hull in the mid-kernel region (Plate IV).

Figure 3a-Infection by microflora at progressive stages of kernel development (Kindred)

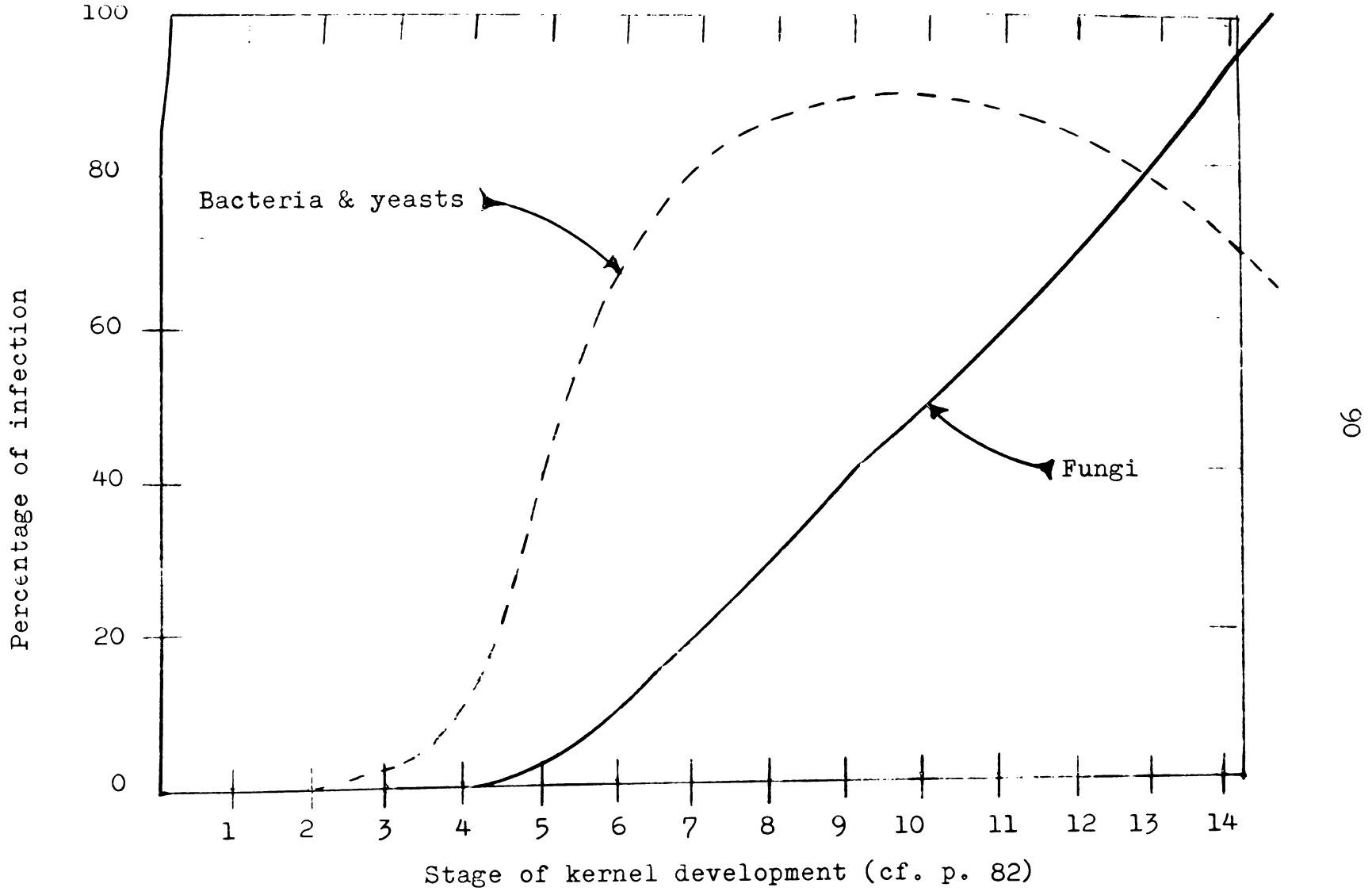


Figure 3b-Infection by microflora at progressive stages of kernel development (Traill)

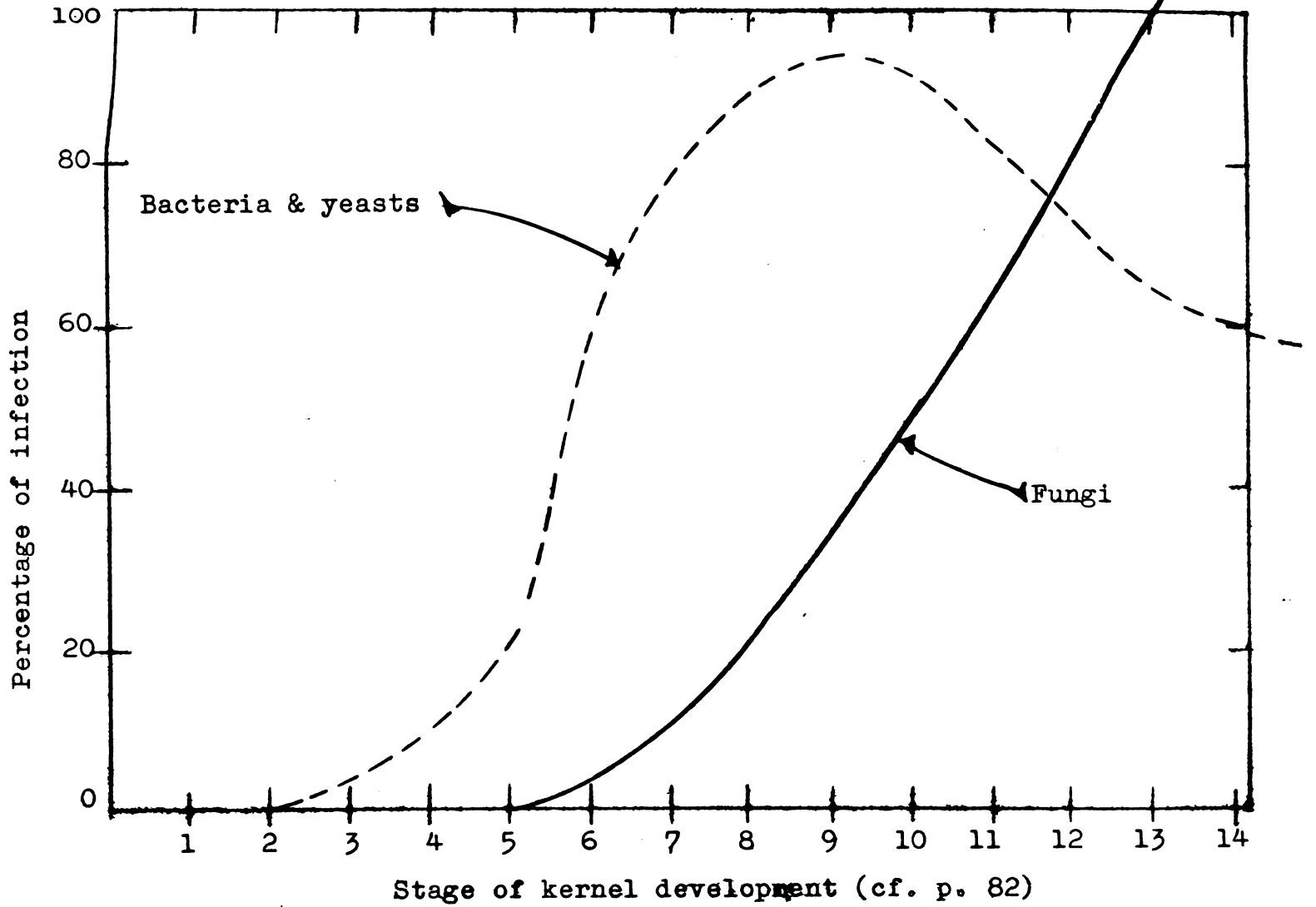


Figure 3c-Infection by microflora at progressive stages of kernel development (Montcalm)

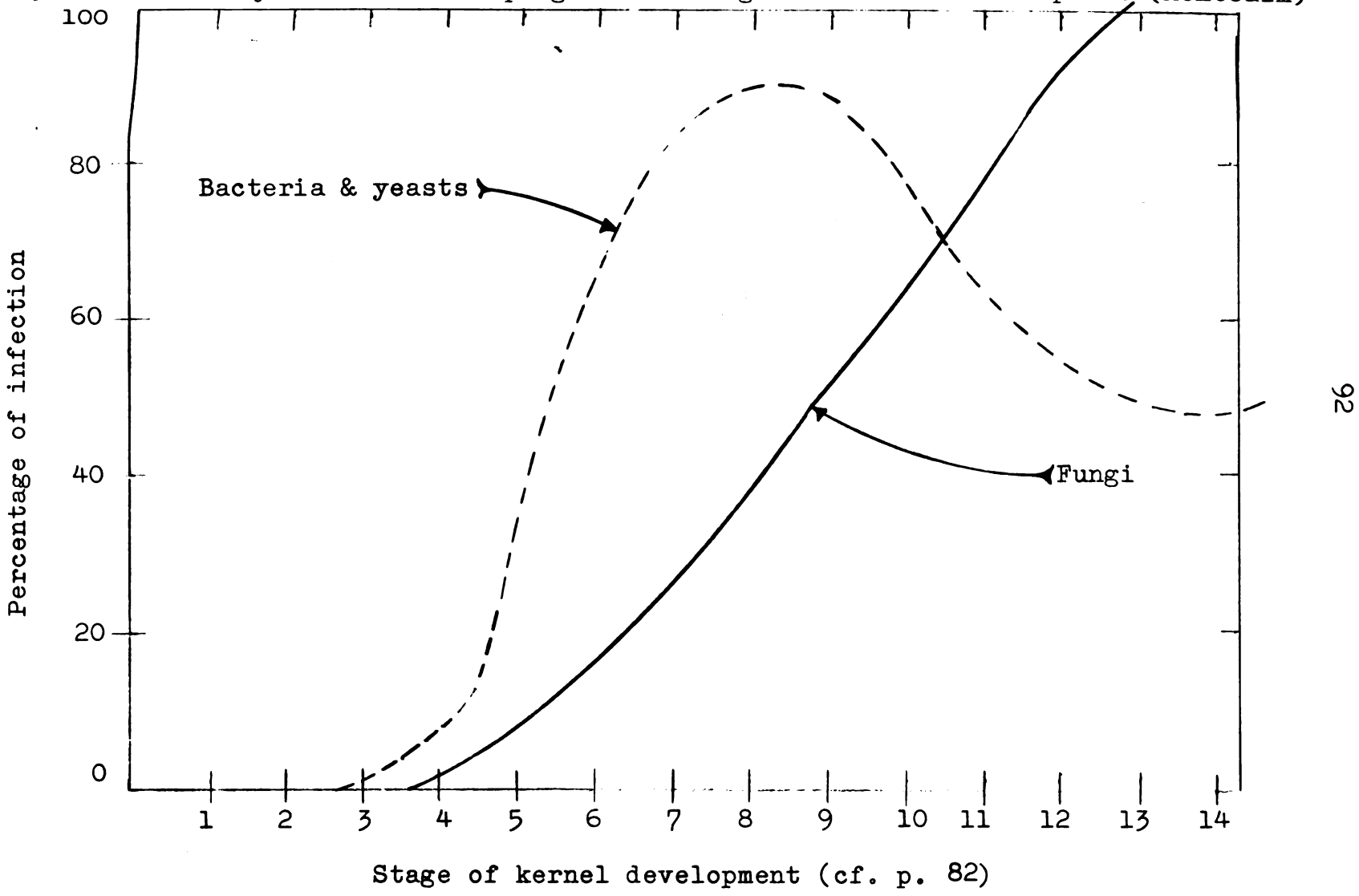


Figure 3d-Infection by microflora at progressive stages of kernel development (Hannchen)

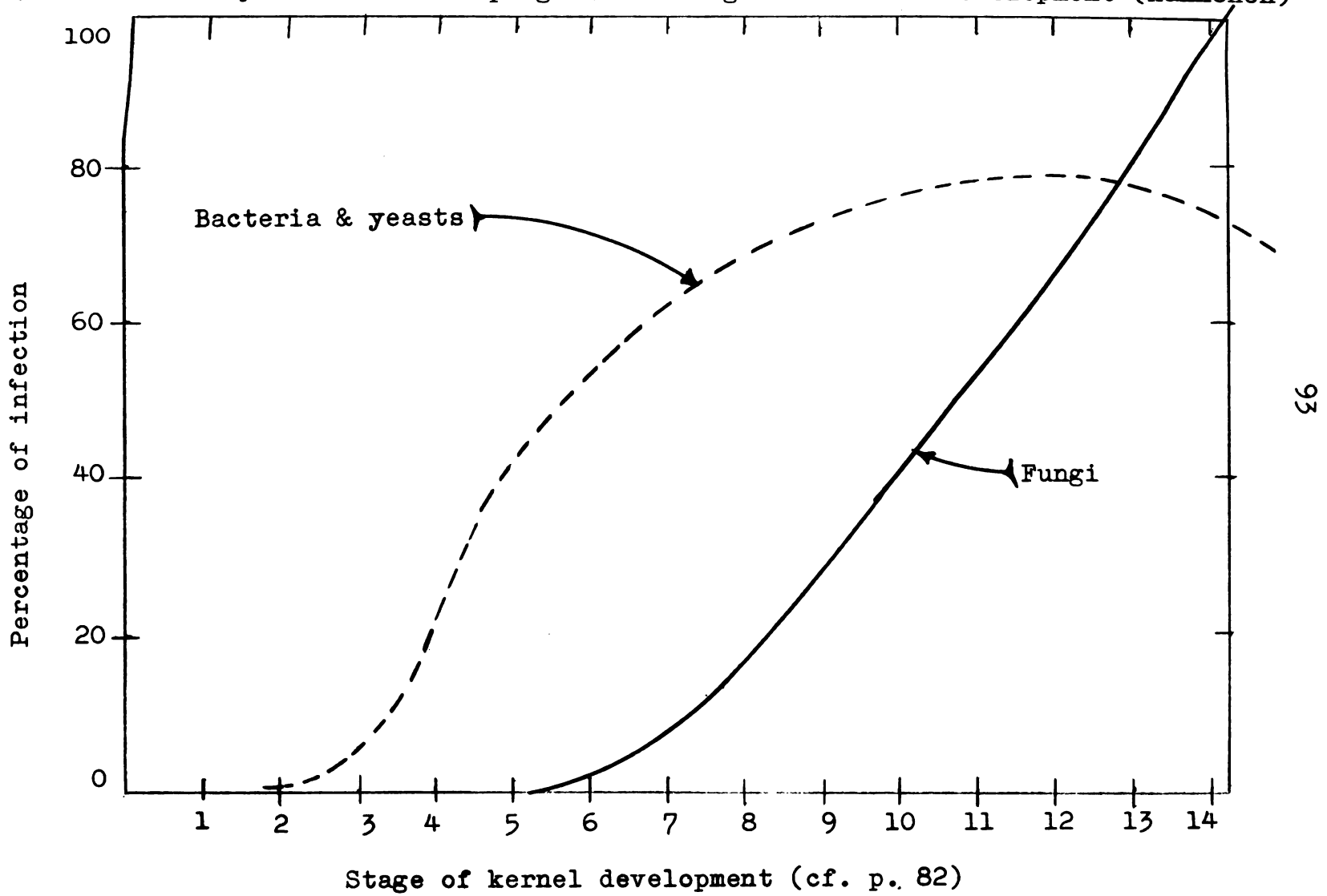






PLATE II

Fungi in the glume tissue of barley kernels.

Upper - Fungal mycelium ramifying through the glume tissue of a longitudinally-sectioned kernel of Kindred barley, developmental stage 13 (magnified ca. 1,000 X).

Lower - Fungal mycelium located in the glume tissue, at the distal juncture of the lemma and palea, of a longitudinally-sectioned kernel of Traill barley, developmental stage 11 (magnified ca. 1,000 X).

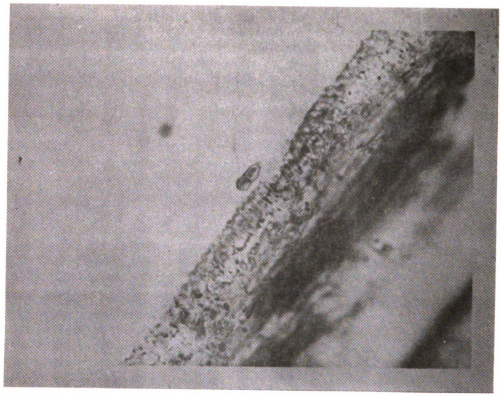


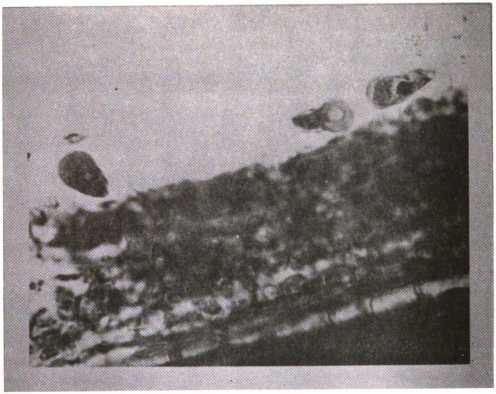


PLATE III

Spores and mycelial fragments on the surface of barley kernels.

Upper - A two-celled spore on the surface of a longitudinally-sectioned kernel of Traill barley, developmental stage 12 (magnified ca. 1,000 X).

Lower - Spores and hyphal fragments on the surface of a longitudinally-sectioned kernel of Traill barley, developmental stage 12 (magnified ca. 1,000 X).



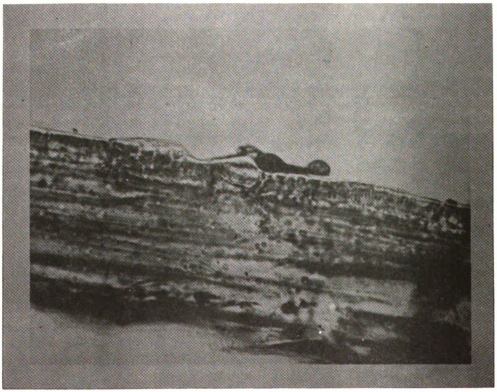


PLATE IV

Spores and mycelial fragments on the surface of barley kernels.

Upper - Fungal hyphae and/or spores on the surface of a longitudinally-sectioned kernel of Traill barley, developmental stage 3 (magnified ca. 1,000 X).

Lower - Germinating (?) fungal spore lodged against a barb on a longitudinally-sectioned kernel of Traill barley, developmental stage 12 (magnified ca. 1,000 X).

Fungal spores were rarely observed within the glumes (Plate V). The spores were identified as Alternaria and Helminthosporium conidia. Mycelium was never observed to penetrate the kernels deeper than the inner pericarp layer (Plate VI). Slides of glume-strips stained with aniline blue in lactic acid (191) also demonstrated that fungal infection by the field organisms was superficial and scattered over the kernel surface (Plates VII and VIII). Stained entities which morphologically resembled bacterial cells in shape and in size were observed to increase in frequency with each progressive increase in kernel development (Plate IX). The accurate assessment of bacterial numbers and location was impossible because of the similar artifacts obtained in the sectioning and staining process.

In the chemical control experiments several properties of the harvested barley were analyzed to determine whether significant differences existed between varieties of barley, replications, chemical treatments, and the time of harvest (Table 11). Varieties differed significantly at the one percent level, with respect to 1,000 kernel weight, bushel weight, and yield. This difference is only an apparent one since one of the varieties, Hannchen, was rated against the six-rowed varieties. The five-day difference in harvest time made an obvious difference in the percentage of kernel infection by fungi, bacteria, and yeasts (Table 12). Varieties differed significantly at the one percent level in their susceptibility to fungal

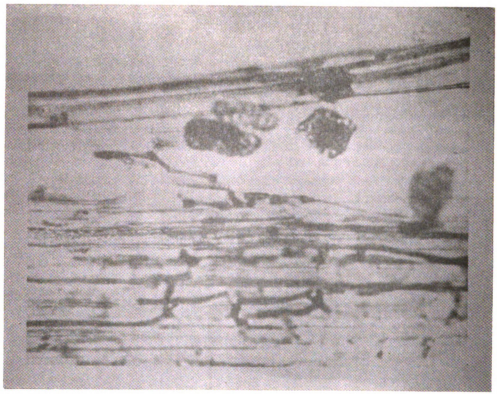




PLATE V

Characteristic "dormant" fungal mycelium in glume and pericarp tissues.

Upper - Fungal hyphae located in pericarp tissues of a longitudinally-sectioned kernel of Traill barley, developmental stage 14 (magnified ca. 1,000 X).

Lower - Segmented spore or hyphal fragment located in pericarp tissue of a longitudinally-sectioned kernel of Traill barley, developmental stage 12 (magnified ca. 1,000 X).





PLATE VI

Characteristic "dormant" fungal mycelium in glume and pericarp tissues. Glume tissue injured at the point of fungal infection.

Upper - Mycelium in glume tissue of a longitudinally-sectioned kernel of Traill barley, developmental stage 13 (magnified ca. 1,000 X).

Lower - As above, different section of same series.

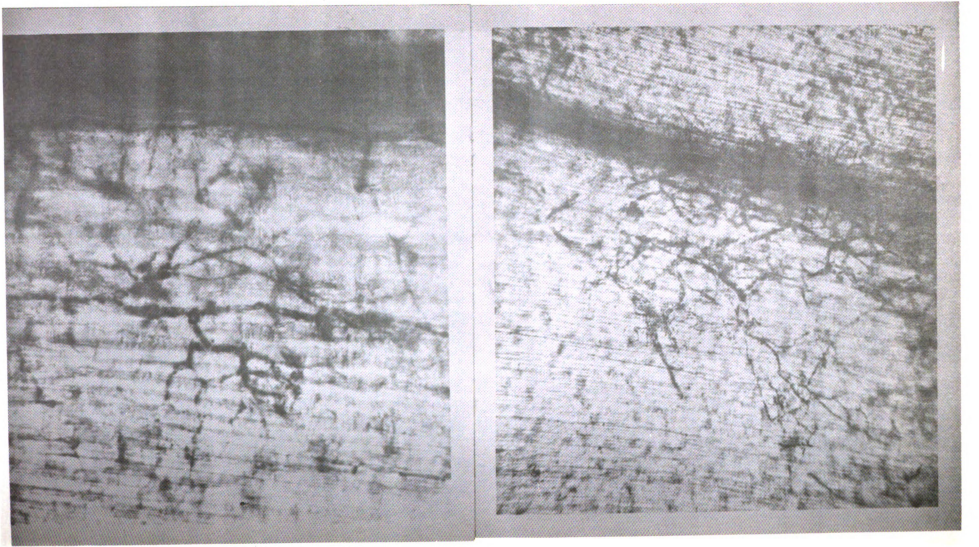


PLATE VII

Fungal mycelium in glume tissues of mature, weathered Hannchen barley. Mycelium stained with aniline-blue and lactic acid method (191).

Upper - Mycelium in glume tissues near middle portion of the kernel (magnified ca. 500 X).

Lower - Detail of above (magnified ca. 1,000 X).

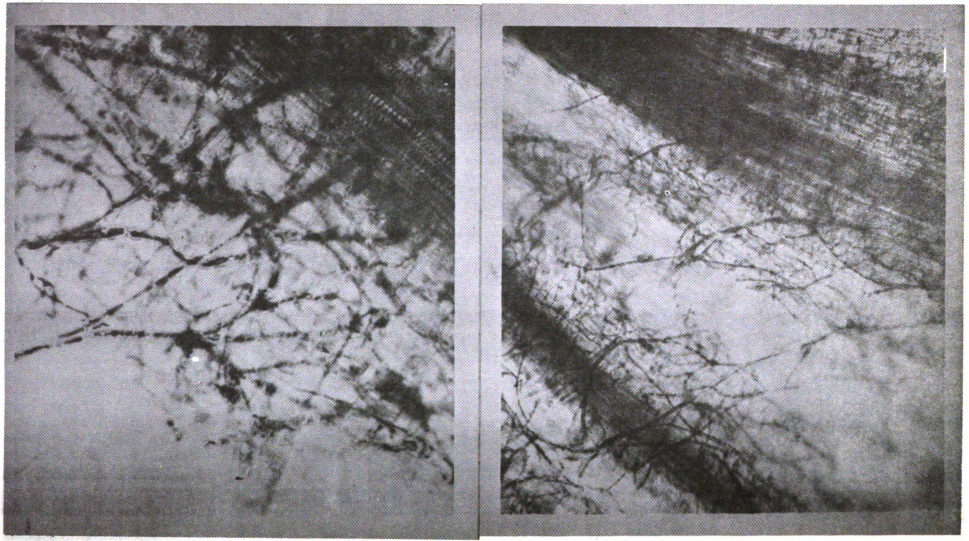


PLATE VIII

Fungal mycelium in glume tissues of mature, weathered Hannchen barley. Mycelium stained with aniline-blue and lactic acid method (191).

Upper - Mycelium in glume tissues near awn end of the kernel (magnified ca. 500 X).

Lower - Detail of above (magnified ca. 1,000 X).



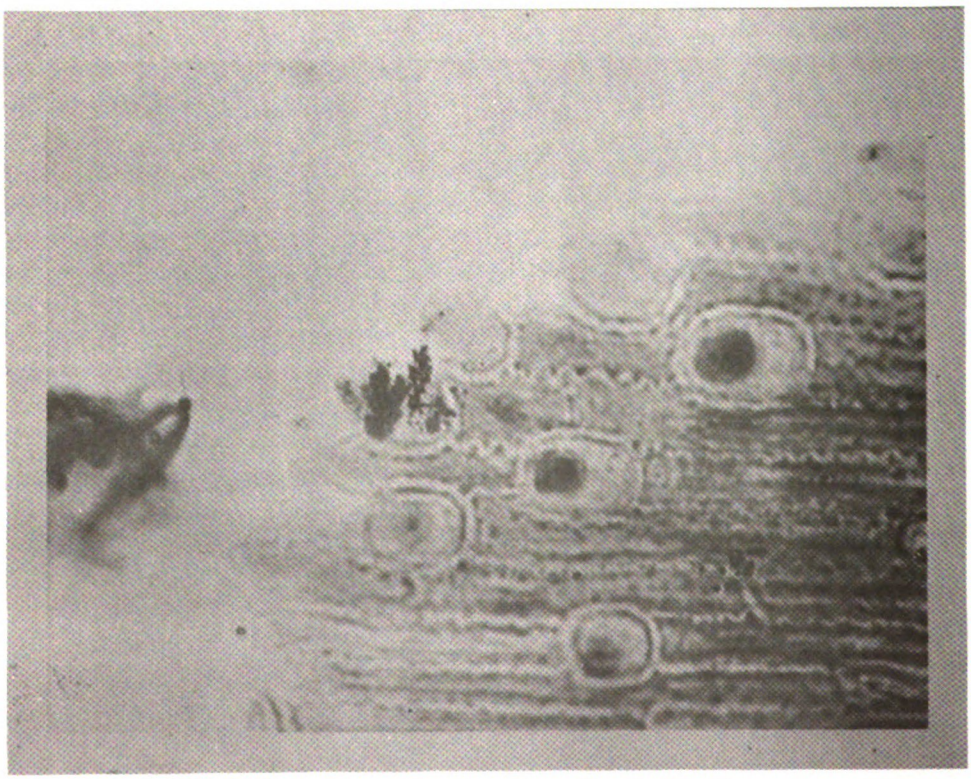


PLATE IX

Bacteria in the glume tissues of barley kernels.

Upper - Bacterial cells located at a natural opening of the hull of a longitudinally-sectioned kernel of Traill barley, developmental stage 10 (magnified ca. 1,000 X).

Lower - Bacterial cells located in the glume tissues, at the base of the lemma-awn, of a longitudinally-sectioned kernel of Traill barley, developmental stage 3 (magnified ca. 1,000 X).

Table 11. - The effect of variety, chemical treatment, and harvest time on several properties of barley. [b]

Property / Variety	Chemical treatment [a] 1	2	3	4	5	6	Variety Total
1,000 kernel wt. (dry basis)	(Expressed in grams)						
Montcalm	32.04	30.89	31.75	30.27	31.05	31.68	187.68
Kindred	30.46	30.87	30.34	31.88	30.59	32.09	186.23
Hannchen	35.75	35.74	37.81	37.24	35.67	37.49	219.70**
Traill	32.37	31.72	30.31	32.25	31.45	31.61	189.71
	130.62	129.22	130.21	131.64	128.76	132.87	
Bushel wt.	(Expressed in pounds)						
Montcalm	44.1	43.9	43.9	44.5	44.5	44.6	265.5
Kindred	46.5	46.3	46.7	46.3	45.7	45.8	277.3
Hannchen	50.5	49.8	51.1	51.4	50.6	50.7	304.1**
Traill	48.4	47.5	48.5	49.3	47.6	47.9	289.2
	189.5	187.5	190.2	191.5	188.4	189.0	
Yield	(Expressed as grams/2 harvested rows)						
Montcalm	509	357	425	491	511	724	3017
Kindred	526	559	617	609	595	583	3489
Hannchen	549	581	681	605	639	612	3667
Traill	805	589	669	667	699	719	4148**
	2389	2086	2392	2372	2444	2638	

[a] See page 84 for explanation of chemical treatments used.

[b] Double asterisks indicate statistically significant differences at the 1 percent level between varieties.

Table 12. - The percentage of microfloral infection of four barley varieties harvested at two different times [a].

Time of harvest / Barley variety	Percentage of infection by:													
	Fungi [c]							Bacteria & yeasts						
	Chemical treatment [b]													
	1	2	3	4	5	6	Total	1	2	3	4	5	6	Total
Early harvest (7-15-59)														
Kindred	70s	74	77	74	60	83	438	23	69	59	73	88	89	401
Traill	88s	95	90	78	85	92	528**	99	100	97	88	87	70	541
	158	169	167	152	145	175		122	169	156	161	175	159	
Late harvest (7-20-59)														
Montcalm	116	110	115	109s	115	112	677**	120	114	110	115	109	105	673*
Hannchen	100s	102s	94	104s	102	105	607	74	86	95	91	99	106	551
	216	212	209	213	217	217		194	200	205	206	208	211	

[a] Microfloral counts from the average of the combined replications.

[b] See page 84 for explanation of chemical treatments used.

[c] 's' indicates the presence of "storage" fungi in the plated kernels.

[d] Single asterisk indicates statistically significant difference at the 5 percent level between varieties. Double asterisks indicate statistically significant differences at the 1 percent level between varieties.

infection in both early and late harvested material (Table 12). The least susceptible varieties were Kindred and Hannchen respectively. No significant differences were noted in bacterial and yeast infection on the early-harvested varieties. Hannchen showed significantly less bacterial and yeast infection at the five percent level in the late-harvested material (Table 12).

DISCUSSION AND CONCLUSIONS

A positive correlation exists between the number and types of kernel-infecting microflora found in the air and isolated from barley grains. This correlation is further strengthened by the simultaneous appearance of certain organisms, e.g. H. sativum, on kernels and exposed-plates at a particular stage in the growing season. The absence of typical soil-inhabiting microorganisms on barley kernels plated from the experimental plots and from other samples which have not suffered "ground-damage" is taken as evidence that the bulk of kernel infection arises from airborne inoculum. In these trials only Montcalm lodged excessively and also supported a much higher microfloral population than did the other three varieties. Large numbers of white bacteria, primarily aerobic, gram-positive, spore-forming, rods (Bacillus spp.) were isolated from this variety in the final stages of kernel maturation. These organisms are typically soil-inhabitants, indicating that bacterial soil-inoculum may be of importance in the infection

of lodged grain. It seems probable that Cladosporium sp. is an ineffectual "parasite" under the humid growing conditions encountered in the Midwest. The low percentages of infection obtained with this organism determined from a large number of plating of stained barley samples, indicates that this effect is primarily due to inhibition and/or suppression by the more efficient inhabitants of grain kernels. This hypothesis is supported by the plating data in Appendix C. In no case was Cladosporium found to be present in excess of four or five percent on samples grown east of the Rocky Mountains, i.e. samples which support moderate to large populations of other fungi. On the other hand, samples grown under irrigated conditions may show percentages of Cladosporium as high as forty-five percent but are relatively free of other fungi.

The data obtained from the plating and sectioning of barley kernels at progressive stages of development support the observations made by previous workers. The ability of the bacteria to infect the maturing florets prior to or during flowering may be explained by the high moisture content of these spikelets (about eighty percent) (167, 261), the opening afforded by the separated glumes, the succulent tissues of the inflorescence, the protruding awns, and the sheltered micro-environment within the enclosing sheath. Any or all of these factors may favor bacterial invasion and establishment of the immature spikelets. The bacteria isolated during the early stages of kernel growth

most frequently developed from the lemma awn or from the awn-end of the kernel. The slower invasion of developing kernels by the field fungi is thought to be primarily due to the preference shown by these organisms for a senescent nutritional substrate as well as for a less-moist environment. The author is in accord with Atanasoff's interpretation of the foci of infection (21), viz, that the kernel-infecting organisms can attack any portion of the kernel, but are generally to be found in the most-freely exposed areas. The points which seem to furnish the most accessible infection loci for fungal spores and mycelial fragments are the separated glumes at the base of the lemma awn, the base of the kernel at the point of attachment to the rachis, the epidermal barbs on the exterior of the hull, and wounded glume tissue. The data have indicated that many of the field organisms preferentially attack and establish themselves in the awn end of the kernel. Infection frequently takes place along the ventral crease as well. With those organisms causing "black-point" and "basal glume-rot", i.e. H. sativum, P. atrofaciens, etc., this observation does not hold and the embryo end of the kernel is usually attacked. Why these organisms behave in this manner remains to be explained although there are some indications that these differences are reflections of varying responses of the pathogens to a higher concentration of diffusible sugars and amino acids in the embryo region of the kernel (300).

The stage of kernel development at which susceptibility to bacterial and yeast infection declines seems to be related to three separate phenomena: (1) the adherence of the glumes to the caryopsis with resultant compression and destruction of the underlying tissues, (2) the decrease of the moisture content of the grain to about sixty percent or less, and (3) the increase of fungi on and within the kernel. That the fungi continue to infect the kernels up to the time of harvest is again a reflection of their preference for less free moisture and for dead and dying tissue.

The microfloral determinations and subsequent statistical analyses have given some indication that varietal differences may exist with respect to kernel infection. This supposition has not been supported by data obtained from field observations and the plating of barley varieties over the last several years. Montcalm was the only smooth-awned variety employed in this experimental series. The microfloral content of Montcalm was also the highest of the three varieties. Shands has reported that smooth-awned varieties are more susceptible to "weathering" and generally show more undesirable hull color than do rough-awned varieties (348). The high correlation between hull-color and microflora seem to indicate that if varietal susceptibility differences do exist, they may be in some way related to the smoothness or roughness of the awns. Rough- and smooth-awned isogenic barley lines were obtained from Dr. Shands, and were plated in an attempt to determine whether

the reported color differences were due to varietal differences in susceptibility to kernel-infecting flora. The results of the plating test have been listed in Table 7, (see Chapter II). The seed furnished by Dr. Shands was four to five years old and consequently the microfloral determinations were only an approximation. In all cases however, the germination capacity of the smooth-awned barleys was significantly lower than that of the rough-awned lines. This, barring a genetic difference in germinability, indicates that the smooth-awned lines may have had a considerably higher microfloral content than the rough-awned barleys at the time that the color differences were recorded.

The chemical control trials recorded in this section were of no measurable use in controlling the infection of barley kernels by the field organisms. Other workers have reported some success in the use of chemical applications, but these trials have employed more than one application of the material in the growing season (9). As is the case with most field crops, the cash return per acre will not permit the repeated use of chemical fungicides and/or bactericides. Further, the presence of chemical residues on barley kernels would render the grain unmarketable, if offered for use in the malting and brewing industries. Consequently it would seem that if control of kernel-infection, caused by those organisms which reduce quality rather than yield, is desired it must be

sought, at present, in the direction of varietal resistance. The rapid development of improved pesticides may within the near future provide an effective and economical control method for the field-infecting flora of barley kernels.

CHAPTER IV.

THE EFFECTS OF MICROFLORA ON THE BARLEY KERNEL

LITERATURE REVIEW

Barley microflora may affect the associated kernels in several ways. The nature of these effects is, in most cases, determined by the use to which the kernels are put, i.e., whether the barley is to be malted, planted, or used as livestock feed. In general, these effects are manifested as quality defects, seedling diseases, or as storage deterioration.

The most obvious quality factor affected by grain microflora is the color of the kernel. The color of grain kernels is determined by their genetic composition, the temperature and moisture conditions under which the crop is grown, the way in which the harvested grain is stored, and the number and kinds of microorganisms associated with the kernels. Harlan studied the pigmentation of barley kernels and found that all kernel color was the result of two pigments; anthocyanin and a melanin-like pigment (164). The four basic barley colors; white or yellow, blue, purple, and black, are the result of the absence or combination of these pigments. These heritable colors may be present in the aleurone layer and/or the barley glumes. Wiggans has also discussed the genetic basis of barley coloration (418). The author indicates that coloration may also be influenced by immaturity, weathering, or pre-

mature harvesting of barley. That discoloration of grain kernels may arise as a result of genetic factors controlling the production of melanin pigments is well known in the case of certain wheat varieties, e.g. Hope, H-44, and their derivatives (197, 203, 204). This form of glume discoloration has been termed "pseudoblack chaff" because of the resemblance to the condition caused by Xanthomonas translucens infecting wheat kernels (55, 197). Campbell has noted that wheat kernels of some varieties may take on a translucent pink appearance, without the presence of fungi, if harvested prematurely (61).

The influences of temperature and moisture on the discoloration of grain kernels are difficult to separate from the influences of genetic composition and associated microflora. In both cases, suitable temperatures and sufficient moisture must be present for discoloration to occur, regardless of its orgin. The term "weathering" has been much used and abused with reference to the staining of grain kernels. Authors have usually considered weathering as a process distinct from microfloral infection and discoloration; e.g. Preece has stated that ". . . a darkening or discoloring from the desired golden hue is frequently met with as a result of more or less unfavorable harvest weather with too much rain and too little sun" (313). Lejeune and Parker have indicated that "Severely weathered barley may be infected with molds and other microorganisms . . ." (226). The Official Grain Standards

also distinguish between "weather damaged" and "mold damaged" in the definition of "damaged [barley] kernels" (6). Hind has suggested ". . . that many barley corns described as weathered have been attacked by Helminthosporium teres (177).

Several workers have reported that grain discoloration may be caused by suitable weather conditions, without the intercession of kernel microflora. Thorpe reported that samples of California-grown barley contained kernels with blackened ends and masses of a sugary, glutinous material. These kernels were not infected by the "black-point" fungi (394). The author suggested that the condition was a result of the inability of the kernels to convert the translocated sugar to starch as well as the effect of dew and rain collecting at the angle formed by the kernel and the rachis. Hagborg (156) and Johnson and Hagborg (204) have reported that wheat kernels of some varieties may be discolored after exposure to high temperatures and high humidities under greenhouse conditions. The authors concluded that the staining resulted from a combination of genetic composition and suitable environment and not as a result of pathogenic organisms. They did isolate "non-pathogenic" bacteria, however, but concluded that these organisms were simply secondary invaders favored by the discolored, "degenerate" tissue. Hagborg has also presented a thorough analysis of the various discoloration symptoms of wheat kernels caused by microflora and by

physiologic staining (158). Ledingham and his co-workers have also attempted the artificial weathering of wheat kernels by placing maturing wheat plants, previously grown under dry conditions, in moist chambers (225). The difficulty of growing plants under moist conditions, while avoiding microfloral infection, is emphasized in this paper since later platings of the kernels showed high bacterial concentrations. Wheat kernels may also present a bleached appearance due to severe weathering. According to Cayzer this is the result of the swelling of wet grain with concommitant formation of numerous air spaces between the endosperm cells (62). The author also states that badly-weathered wheat may frequently be dirty and grayish in color as a result of mold development.

Another undesirable type of kernel discoloration is found in heat-damaged barley. The Official Grain Standards define heat damage as those ". . . kernels and pieces of kernels of barley, other grains, and wild oats, which have been materially discolored and damaged by heat." (6). This condition is usually caused by storage organisms which generate heat in barleys stored at high moisture levels (226).

It is a well-known fact that grain discoloration is frequently associated with the presence of microorganisms. Few workers have, however, indicated how closely kernel staining and the microfloral content of the grain are associated. Hanson and Christensen reported that the prevalence of wheat seed discoloration was generally correlated

positively with the prevalence of microorganisms (161). Greaney and Wallace found that in most years wheat samples showed a higher percentage of internal fungal infection than was indicated by the number of discolored kernels (141). In years when leaf rust was not a complicating factor the amount of internal infection was always positively correlated with the incidence of stained kernels. James and his fellow-workers found that when kernel washings of wheat seed were plated, the number of bacteria and fungi increased proportionately with the visible increase in grain damage, from vitreous to weathered to immature to frosted kernels (195).

Workers at the University of Minnesota have reported that fungi were more numerous in several stained samples of barley as compared to bright, western-grown lots although in one bright sample they isolated a much higher percentage of bacteria and yeasts than were present in light and heavy stained barleys (8).

Investigators have employed a number of symptomological terms to describe the staining of grain kernels by specific organisms. "Blighted" barley usually refers to kernel discoloration caused by Helminthosporium spp. (226) although some workers have also used the term for barleys discolored by Alternaria and other fungi (78, 115). Barley kernels infected by Gibberella and Fusarium species are frequently termed "scabbed", but some authors group these infected kernels together with those in the "blight-

ed" category. The term "blackpoint" has been applied to those grains which are discolored at the embryo end. This condition is typical of kernels infected by Helminthosporium sativum and Pseudomonas atrofaciens (47, 88, 110, 112, 158, 247, 258, 333). Zöbl applied the similar term "braunspitzige" to discolored barley kernels (425). He attributed the cause of the discoloration to Cladosporium herbarum, but several other fungi were also isolated from the kernels. Peyronel concluded that C. herbarum was not responsible for the basal blackening of grain kernels, a condition he termed "puntatura" (301). This conclusion was further supported by Bockmann who found that C. herbarum, Alternaria spp., and other so-called "blackening fungi" did not cause the brown discoloration that is typical of black-point (48). He further suggested that these barley organisms are of little economic importance, except insofar as they tend to lower the market value of the grain. The word "moucheture" has been used by Rosella to describe the discoloration of wheat kernels caused by two species of Alternaria (328), and barley staining caused by H. sativum and H. gramineum (329).

Fomin and Nemlienko employed the term "black radicle" in a study of cereal grains showing basal discoloration (118). The authors concluded that the condition was due to a number of factors, the chief causes being H. sativum and an undetermined species of Alternaria. Ziling found that "black germ" of wheat was produced by

H. sativum, Alternaria tenuis, and Fusarium spp., and that the causal agent could be determined by the location and the intensity of the discoloration (424). Drechsler found that while both H. sativum and Alternaria spp. caused cereal seed discoloration, the symptoms induced by these fungi differed slightly (107). Christensen and Stakman inoculated barley plants with 200 isolates of eight genera of fungi and found that many of these organisms caused kernel discoloration (77). They concluded that, while species of Fusarium and Helminthosporium were the most serious blighting fungi, other organisms were also capable of causing kernel defects.

Canadian workers have coined the term "kernel smudge" to describe kernel staining brought about by a number of organisms. Machacek and Greaney have reviewed the literature dealing with the types of cereal seed discoloration (247). They concluded that "black-point", "black-tip", and similar descriptive terms, while suitable for kernels infected by certain bacteria, were not appropriate for the symptoms produced by fungal infection. They suggested that the appellation "kernel smudge" be applied to the disease of cereal grains in which a dark or pale brown discoloration occurs on the kernel, particularly in the embryo region, and which is produced by fungi, expecially those members of the Form-family, Dematiaceae. Mead inoculated developing barley heads with several commonly occurring barley organisms (262). "Smudged" kernels were ob-

tained from the inoculations made with Helminthosporium sativum, 84 percent; Gibberella saubinetii, 45 percent; Fusarium sp., 60 percent; and Alternaria sp., 35 percent. Symptomological differences in staining also were noted among the inoculations used. Russell described two types of smudge on wheat kernels which can be visually distinguished: the "mild" type caused by Alternaria, and the "severe" type caused by H. sativum (331). "Alternaria blotch" has been applied to wheat kernels discolored by Alternaria tenuis, in a paper authored by Johnson and Hagborg (203). The authors found that the fungus incited a grayish-brown discoloration of the lemma.

The microflora of barley and other grain kernels may adversely affect the germinability of the seed. The early work dealing with this effect has been reviewed by Thaysen and Galloway (390). Early workers thought that seed-germination was reduced by the excessive growth of the associated microflora which subsequently clogged the pores of the embryo and thereby prevented respiration. This explanation was questioned by Thaysen and Galloway who postulated a multiple-enzymatic inhibition of germination by the kernel microorganisms. Bishop has discussed the problem of barley germination in a series of three memoranda (40, 41, 42). These memoranda include intensive reviews of the literature dealing with the effects of microflora on germination, as well as the results of the author's original researches. He found that the ear-

lier explanations of Reichard and Becker were at least partially correct, viz. that bacterial slime and fungal growth retarded the entrance of oxygen into the embryo (40). This type of dormancy was overcome by either removing the hull or by using various disinfectant treatments. Thiourea did not affect dormancy, but it did suppress fungal growth on the germinating kernels (41). Attempts were also made to determine the cause of germination inhibition "at a distance" by kernel microflora (42). While the effect resembled that caused by the evolution of certain chemical vapors, no naturally produced lethal vapor was detected. The author also suggested the possibility of enzymatic inhibitors released by the microflora. The presence of a microfloral "gelatinous film" as a germination inhibitor has been further noted in a discussion following a paper presented by Crafa and his co-workers (84). According to this interpretation, the infected barleys may present a sound appearance and yet germinate as low as 60 percent. Leukel has stated that kernel microflora, even those not considered as pathogens, may prevent or inhibit germination by attacking the food material stored in the kernel (228).

Helminthosporium sativum has frequently been implicated as an inhibitor of kernel germination, as have species of Fusarium (74, 306, 404, 424). Rosella reported that barley kernels, discolored by H. sativum, germinated normally, but the author indicated that the lack of inhibition may

have been caused by the drastic surface-disinfection employed (329). Dastur attributed the normal germination of many discolored wheat grains to the freedom of the embryo from infection by H. sativum (88). Alternaria rarely, if ever, caused a reduction in germinability of infected kernels according to Christensen (74), Ziling (424), and Machacek and Greaney (247).

Nilson, in 1905, stated that bacteria prevent normal germination in barley by their biochemical activity (287). Messinger reported that culture filtrates of Achromobacter sp. retarded barley germination, but the inhibitory material soon lost its power in contact with the kernels (273). Barley steep liquor was found to be inhibitory to barley germination by Gilbert and his fellow-workers (127). They concluded that this effect was caused by barley microflora which competed with the barley for dissolved oxygen and by the production of a bacterial exotoxin. This exotoxin was produced from pure cultures of certain of the bacteria, and its properties suggested that it was a salt of an organic acid of high molecular weight. In a later paper, Gilbert and Blum reported that barley, germinated for as little as twelve hours under conditions unfavorable for microfloral growth, was able to complete germination after inoculation with these organisms (128). Minnesota workers have also shown that barley steepwater is inhibitory to barley germination (9). The effect was associated with the presence of large numbers of bacteria and yeasts and the inhibitory

material was at least partially heat-stable. The same workers have reported that certain bacteria isolated from steep-liquor produced a volatile germination-inhibitor which is especially active against pre-steeped barley. Toxic substances were isolated from Cephalothecium sp. and species of Fusarium. These inhibitory materials were not recovered in subsequent experiments, and the authors attributed this loss to either a fungal mutation or a dependence on specific cultural conditions.

Closely related to the topic of germination inhibition and injury, are the categories of seedling and post-emergence diseases. Alternaria species, commonly found on barley kernels, have generally been absolved of guilt in these categories (202, 247, 262). Species of Cladosporium have also been shown to be non-parasitic in the sense that they do not initiate either seedling diseases (202) or head diseases (33). Stakman (373) and Bolley (47) have claimed that certain species of Alternaria isolated from grain kernels may cause seedling defects. Various species of Fusarium and Helminthosporium, on the other hand, are implicated in the lesioning of emerging coleoptiles, the production of seedling-blight, and foot- and root-rots (34, 202, 262, 264, 311). Greaney and Machacek have stated that soil bed tests have shown a high positive correlation between the percentage of cereal seed infected by H. sativum and the incidence of seedling diseases (143). Mead has demonstrated that the greatest amount of seed-

ling blight caused by H. sativum occurs under environmental conditions that are unfavorable to the barley plant, i.e. extremes of temperature and moisture (265). General discussions of the diseases caused by the seed-microflora of barley are also found in the references by Dickson (100), Sprague (368), and Leukel (229).

Several workers have noted that cereal grains, discolored by microflora, are frequently plumper than normally-colored seed (161, 247, 301, 328, 408). Jodidi and Peklo have asserted that certain symbiotic fungi, identified as smuts, produce the aleurone layer in infected cereal grains and penetrate the peripheral cells of the endosperm to produce the gluten (199). This observation is not supported by other reports in the literature.

The subject of grain storage deterioration has been intensively investigated and several comprehensive reviews, dealing with this topic are available (45, 69, 70, 344). Early workers attributed storage losses to heating caused by the biological oxidation of carbohydrates, especially in the embryo of the kernel (22). Gilman and Barron, in 1930, suggested that certain species of Aspergillus were probably responsible for the heating of stored grain (129). Thaysen and Bunker cited the work of Cohn who in 1889 regarded the enzymatic action of Aspergillus fumigatus as responsible for the spontaneous heating of germinating barley (389). These authors stated that Cohn's assumption was not very well substantiated. More recent evidence

indicates that the assumption was not only substantiated but was indeed a very far-sighted observation. Robertson and his co-workers noted that the viability of stored cereal seed decreased in storage with increased humidity (324). Heavy fungal and bacterial growth were observed, but the data did not indicate what influence these organisms had on the stored grain. Leach (221) and Denny (92) have shown that respiration rates of germinating grain kernels may be altered significantly by the attendant microflora. Oxley and Jones arrived at the same conclusion as Leach, viz. that the respiration of stored grain is almost entirely produced by microorganisms growing in the pericarp tissues (295). Roth reported that in barley stored under humid conditions the number of fungi and bacteria initially increase (330). Further increases in humidity caused a decline in the number of bacteria and an increase in the number of certain fungi. The minimum humidity requirements for germination of the conidia of several grain storage fungi have been reported by Armolik and Dickson (17). Hummel and others have shown that respiration is greatly increased by fungi on stored cereals (186). Loss of viability, increases in fat-acidity, and decreases in non-reducing sugar content were also noted by the authors. According to Geddes the increase in fat-acidity caused by fungal lipases is a measure of the soundness of stored grain (126). Armolik and his co-workers found that storage molds reduced the germination of stored barley and

that the variety of barley as well as the geographical location of the crop-producing area greatly influenced the amount of the grain deterioration (18, 19). Tuite and Christensen have stated that no significant amount of invasion of barley kernels by storage fungi takes place prior to harvest (398). Species of Aspergillus and Penicillium increased at moisture contents above 14 percent, and the field fungi disappeared at moisture levels above 15 or 16 percent (397, 398). Bacteria have not been considered of great importance in the deterioration of stored grain (70, 344), although bacterial decomposition of grain products is of some importance (344). Hofer and Hamilton have implied the possibility that bacteria, especially spore-formers, may be of importance in spoilage of food products in which ordinary processing would not destroy the bacteria (180).

The effect of certain seed-borne organisms has been variously noted with reference to the feeding value of infected barley. The most intensively studied example is that of scabbed barley infected by species of Fusarium and Gibberella. When scabbed barley is fed to swine and certain other animals, an emetic principle in the infected grain induces vomiting or death in the animals (99). The principle remains active for at least 26 months after the fungus loses its viability (347) and is not produced in culture media by the fungus (76). If the percentage of Fusarium-infected grain is too high, the animals will fre-

quently refuse to eat the poisonous feed (76, 379). The feeding of scabbed barley has been discussed at length by Dickson (99). Several species of Aspergillus have been reported to cause deterioration of barley and to produce toxic effects in laboratory animals (350). Rhizopus nigricans rendered feed-barley unfit for use by cattle according to Wagn (405). Miessner and Schoop found that while only Gibberella-infected barley was toxic to swine, the animals showed some reluctance in accepting Alternaria-infected barley (274). Jones and his co-workers found that moldy wheat infected by species of Aspergillus, Penicillium, Alternaria, Cladosporium, Actinomyces, Rhizopus, Mesobotrys, and Trichoderma was more digestible by swine than non-moldy wheat (205). The infected grain was not unpalatable to either swine or sheep. German workers have shown that the presence of certain unidentified bacteria in barley produced copious amounts of gas and caused a putrid smell (1). The investigators concluded that these bacteria caused protein decomposition in the stomachs of animals and that the decomposition products were toxic to swine.

MATERIALS AND METHODS

Several approaches were used in the study of kernel discoloration and its relation to the associated microflora. The 84 barley samples furnished by the Malting Barley Improvement Association, mentioned previously (cf. Chapt. II), were visually evaluated for color intensity.

The visual evaluation scale ranged from 1 to 10, with 1 representing extremely bright samples and 10 designating severely discolored samples. Additional notations were made with respect to: the number of "black-pointed" kernels, under-sized kernels, green kernels, and varieties with pigmented aleurones and glumes. Microfloral determinations for these samples are described in the aforementioned section.

Surface-sterilized kernels of bright Montcalm, Hannchen, and Traill barleys were plated on nutrient agar which had been previously seeded with the following bacterial cultures: B-5, B-210, B-226, and B-529 (see Table 18 for identity of bacteria). The surface-disinfection treatment was the standard method of the author (cf. Chapt. I). One hundred kernels of each variety were used with each bacterial treatment. The plates were incubated for eight days at room temperature (ca. 25° C.); and the degree of growth of kernels and the amount of kernel discoloration, were then determined. A check series consisting of the three barley varieties was also plated on nutrient agar without the addition of the bacteria. The degree of staining was rated on an arbitrary scale of one to five, where one denotes no staining and five represents severe staining. Notations as to the location of the discoloration on the kernel were also recorded. The amount of barley growth was measured by removing and weighing the extended acrospires, after drying to a moisture content of approximately

10 percent (by weight). Color differences in the inoculated barleys, both before and after germinating, were also noted. Several other methods were used in the over-all study of barley kernel-staining. These are treated in Chapter VI.

The inhibition of barley germination by associated microflora was investigated by means of three general methods: (1) the recording of germination and/or kernel injury in conjunction with the attendant microorganisms on agar plates, (2) the use of culture filtrates of a number of barley organisms as the steeping medium in the routine A.S.B.C. germination test (5), (3) the A.S.B.C. germination procedure used with the inoculated barleys prepared for pilot-malting. In all cases the barleys were read at the appropriate time for germination energy and germination capacity (5). The first method has been routinely employed by the author with all barley samples plated in the years 1958-1960. The second method, outlined above, consisted of the culturing of fifteen fungal species on a reciprocating shaker for seven days. The fungi were grown in Czapek's sucrose-nitrate solution, 50 ml. of medium per each 250 ml. Erlenmeyer flask. The details of this cultural procedure have been outlined elsewhere (300). At the conclusion of the growth period, the reaction of the media, color of the media, odor of the media, color of the fungi, growth form of the fungi, and the amount of growth were noted. The fungal mycelia and/or spores were removed

by coarse filtration and the liquid portion was passed through a Seitz filter. The filtered liquid was diluted 1:1 with sterile distilled water and divided into two portions. One portion was used without autoclaving for the steeping liquor; the other portion was autoclaved for 30 minutes at 15 pounds pressure before being used as a steep. The same procedure was followed with check tubes of sterile distilled water, Czapek's solution without inoculum (stationary), and Czapek's solution without inoculum (on shaker). All cultures were replicated three times. The barley used in connection with the above culture filtrates was a bright Kindred sample, 1958 crop, held on a 6/64" sieve. The third method used in this section of the study was employed with the inoculated barleys after they had remained in storage for eighteen or more days. The inoculation and storage methods are presented in the section on pilot-malting (Chapt. V). This procedure also provided some information as to the effects of certain barley organisms on storage deterioration of the inoculated kernels.

A sample of feed barley, grown in Michigan in 1958, was submitted by a grower who stated that the grain had been refused by swine. The barley was plated with the routine procedure outlined previously. A sample of the grain was also sent to the Barley and Malt Laboratory for an evaluation of the abundance of Fusarium "emetic principle".

EXPERIMENTAL RESULTS

The color and microfloral properties of the 84 barley samples, received from the Malting Barley Improvement Association, are tabulated in Appendix C. Microfloral determinations have been made on most of the same samples by workers at the University of Minnesota (9). The color of these samples is correlated positively with the number of organisms isolated from the kernels. A positive correlation also exists between the number of microflora in a seed-lot and the climatic conditions which prevail in the location where the seed was grown.

Barley kernels plated on agar, seeded with bacteria, were both discolored and inhibited by the bacteria (Table 13). In all cases the barley kernels were severely discolored by this treatment. The intensity and the location of the discoloration varied slightly between varieties and treatments. In general, the staining was more intense along the ventral crease and at the embryo end of the kernels. The staining was expecially pronounced in the region of the scutellum, forming a dark brown band.

The inhibition of kernel germination by associated microorganisms was noted in several hundred barley lots plated during the past several years. In general, the following fungi have proved to be inhibitory to barley germination: Helminthosporium sativum, H. teres, Fusarium roseum f.sp. cerealis, Aspergillus spp., Penicillium spp., and several members of the Mucorales. Many

Table 13. - Kernel discoloration and germination inhibition of surface-disinfected barley kernels plated on nutrient agar plates inoculated with five bacterial isolates.

Identity of inoculum[a]	Varieties					
	Montcalm		Traill		Hannchen	
	Color[b]	Growth[c]	Color[b]	Growth[c]	Color[b]	Growth[c]
Check[d]	2	2.93	2	4.16	3	2.11
B-5	5	1.64	5	1.37	5	1.34
B-210	5	0.18	5	0.69	5	0.82
B-226	5	4.88	5	6.37	5	2.15
B-522	5	0.79	5	0.83	5	1.24
B-529	5	0.50	5	0.48	5	0.75

[a] See Table 18 for identity of bacterial isolates.

[b] Color rates as (1) equalling very bright, (5) corresponding to severely stained; all varieties equalled (1) before plating.

[c] Growth determined by weighing extended acrospires, calculating mean weight of acrospires in grams, and multiplying by 100.

[d] Check treatment made by plating surface-disinfected kernels on non-inoculated nutrient agar plates.

species of seed-borne bacteria, both white and yellow types have also proved to be virulent germination-inhibitors. Species of Alternaria, Cladosporium, Stemphylium, Epicoccum, Candida, and several less commonly occurring fungi and yeasts have not seriously affected the germination of the associated kernels. Frequently, the germination of the plated grains appears to be adversely affected by the "non-pathogenic" fungi, but closer inspection usually reveals the additional presence of bacterial colonies. The effects of associated microflora on barley germination are tabulated elsewhere (Appendix C. and Table 7). In most cases, a negative correlation exists between bacterial and/or fungal populations and barley kernel germination.

The reaction of the culture medium was significantly altered by Aspergillus niger and a Penicillium isolate (Table 14). The culture filtrates from only three fungal isolates exerted marked deleterious effects on kernel germination under the experimental conditions described above. These species, Aspergillus niger, Fusarium roseum f.sp. cerealis, and an unidentified species of Penicillium; also proved to be inhibitory when the work was repeated. While both the autoclaved and the unheated filtrates from the first two isolates were toxic to the barley grains, only the unheated medium was inhibitory in the case of the Penicillium isolate used in the study. The data indicate that the inhibitory principle produced by Aspergillus niger and Fusarium roseum f.sp. cerealis

Table 14. - The germination of barley kernels steeped in culture filtrates of selected fungal species[a].

Source of filtrate	Reaction of filtrate	Percentage germination			
		Filtered only		Filtered & autoclaved	
		b	c	b	c
Check (distilled water)	7.8	92	96	95	99
Check (Czapek's - stationary)	7.4	100	100	98	100
Check (Czapek's - shaker)	7.3	89	100	99	99
Rhizopus sp.	6.3	97	98	97	98
Gliocladium sp.	7.5	97	98	98	99
Chaetomium sp.	7.4	99	99	98	99
* *Aspergillus niger*	1.9	69	75	59	67
Nigrospora sp.	7.4	99	99	98	99
Cephalosporium sp.	7.5	97	98	99	99
Verticillium sp.	7.3	99	99	98	99
Aspergillus sp.	7.4	97	97	98	99
Helminthosporium sativum	7.6	98	98	98	98
* *Fusarium roseum* f.sp. *cerealis*	7.9	69	84	88	91
Alternaria tenuis	7.5	98	99	98	98
Epicoccum sp.	7.5	97	98	98	99
Gloeosporium sp.	8.0	97	98	97	98
Curvullaria sp.	7.7	97	98	97	98
Cladosporium herbarum	7.0	98	99	98	99

Table 14. - Continued

* *Penicillium* sp.	4.8	77	94	96	98

[a] All figures listed represent an average of three or more replications.

[b] Germination energy - A.S.B.C. method (5).

[c] Germination capacity - A.S.B.C. method (5).

* Visible injury to germinating seedling, viz. stunting and necrosis.

is heat-stable while that of the Penicillium isolate is either heat-labile or inactivated by combining with other substances. The acid reaction of the culture filtrates may also be responsible for germination-inhibition although other data indicate that barley kernels will germinate at low pH levels (Table 3).

Initial moisture contents and germination energies were determined for the inoculated barleys used in the pilot malting studies (see Chapt. 5). These barleys were dried at room temperatures and humidities prior to storage, and the initial moisture contents ranged from approximately 11.8 percent to 13.4 percent (Table 15). Moisture contents of the grain increased during storage to as much as 17.0 percent in the case of certain inoculated barley varieties after thirty days of storage. The pronounced decrease in germinability with several of the treatments is seen in Table 15. This loss of viability was directly reflected in the moisture increases of these treated varieties. A slight increase in moisture content was noted in the stored check samples. No microflora were isolated from either the Traill or the Montcalm check lots after storage, nor was there any marked decrease in the germinability of these samples. Fungi and bacteria were isolated from both steeped and unsteeped Hannchen check samples. The varieties responded differently to the various inoculations; and the two six-rowed varieties were more susceptible to storage deterioration in this experiment

Table 15. - The germination of inoculated barley kernels after storage in sealed bottles at room temperatures[a]

Identity of inoculum[b]	Varieties								
	Montcalm			Traill			Hannchen		
	c	d	e	c	d	e	c	d	e
Check (no treatment)	10.9	93	93	10.9	97	98	11.0	97	96
Check (steeped & stored)	11.9	95	95	11.8	96	92	12.5	92	96
B-5	12.3	91	--	13.1	89	71	12.6	95	--
B-210	13.2	65	--	13.4	79	79	13.9	95	--
B-218[f]	12.4	83	78	12.2	95	94	12.0	95	97
B-226	14.3	85	--	12.5	59	38	12.8	80	--
B-522	13.5	84	42	13.4	15	0	13.8	94	91
B-529	13.7	86	82	13.7	47	2	13.9	78	87
Y-521	12.5	77	79	13.1	91	83	13.8	91	93
F-304	12.2	80	--	12.5	96	--	12.8	93	--
F-310	12.8	78	--	12.7	83	--	12.4	91	--
F-321	12.4	81	--	12.3	94	--	12.5	98	--
F-324	12.2	89	--	12.4	95	--	12.2	97	--

[a] See Chapt. 5 for inoculation procedure. Storage periods of eighteen and thirty days unless otherwise noted.

[b] See Table 18 for identity of species used as inoculum.

[c] Percentage moisture content at time of storage.

[d] Germination energy after eighteen days of storage.

[e] Germination energy after thirty days of storage.

[f] Germination energies determined after 51 and 63 days of storage.

than was the two-rowed variety, Hannchen.

The analysis of the sample of feed barley, conducted by the Barley and Malt Laboratory, showed that the amount of emetic principle present in the sample was not high enough to render it either unpalatable or poisonous to livestock. The barley was however refused by swine. The high bacterial content of this barley lot (Tables 3, 4, and 5) may perhaps explain why the grain was not acceptable to swine when offered as feed.

DISCUSSION AND CONCLUSIONS

Almost without exception, the discoloration of barley kernels signals the presence of fungi, bacteria, and yeasts on and within the kernel. Kernel discoloration is, however, neither a necessary or a sufficient condition for the occurrence of seed-borne microflora. The mechanism of kernel discoloration, either by microflora or by other factors, will be discussed in a later section (Chapt. VI).

The results of numerous studies, especially in the area of storage deterioration, have shown that the loss of germinability of barley and other cereal grains is mainly due to the presence of microflora. The research reported in this section has supported this observation. The inhibitory influence of the seed-borne pathogens of barley has been repeatedly observed on plated kernels. It must be emphasized that germination-determinations made on plated kernels are generally lower than those conducted

on moist blotters; since microflora are favored by the presence of abundant nutrients and moisture. Kernels infected with species of Helminthosporium and Fusarium frequently germinate, but the rootlets rapidly become necrotic and the seedling fails to continue its development. Kernels have repeatedly germinated, in a normal fashion, on agar plates when completely covered with certain fungi, e.g. Alternaria spp. On the other hand, kernels infected, in a similar way, with species of Helminthosporium show marked inhibition. The two genera (Alternaria and Helminthosporium) are phylogenetically related, cause similar staining effects, and infect kernels in somewhat the same manner; the pathological difference seems to indicate the production of a toxin and/or other physiological differences.

Bacterial colonies arising from plated barley kernels are frequently inhibitory to kernel germination. These organisms include white, mucoid, creamy, and yellow types. Not all bacterial isolates are germination inhibitors, a few isolates have demonstrated stimulatory effects (Table 13). Various degrees of kernel-resistance and bacterial-virulence, with respect to the inhibition of barley germination, have also been demonstrated (Tables 13, 15, 34). Most of the commonly isolated bacteria have shown inhibitory effects, especially if the bacterial colony develops at, or near, the embryo end of the kernel. The inhibition effect by these organisms differs from that

of the seed-borne fungi. Kernels infected with bacteria usually show little, or no, evidence of germination. If germination does begin, the acrospire is usually affected less than the developing rootlets. The mechanism of germination-inhibition is poorly understood. The inhibition of kernels plated on agar, seeded with various bacterial species, may take place without visible contact between the kernels and the bacterial cells. Such an occurrence seems to indicate that a volatile and/or diffusible inhibitor may be produced by certain organisms, as has been suggested by other workers (9, 42).

The effects of seed-borne bacteria on barley kernels have not been previously studied in great detail. In addition to their inhibitory properties, as shown by barley plating studies, there is some evidence that bacteria, especially certain Bacillus species, may be of importance in the deterioration of stored grain (Table 15). It has been generally assumed that bacteria are not involved in grain storage injury, since they require free water to grow (70, 344). The data presented above does not entirely support this contention. Traill and Montcalm samples, inoculated with two Bacillus species, were stored at initial moisture contents of approximately 14 percent. The germinability of these samples steadily decreased in storage (Table 15). The moisture content of the samples was calculated, after sixty days of storage, at from 16 to 17 percent. The kernels proved to be non-germinable at this

time. All samples were stored in sealed mason jars. These preliminary observations are subject to several possible explanations: (1) the moisture increase was the result of bacterial respiration and kernel decomposition, (2) the moisture content of the inoculated grain was not homogeneous, (3) the bacterial content of the inoculated grain was not homogeneous, (4) certain bacterial species may be able to multiply and carry out storage deterioration at moisture contents at the "safe" level of 14 percent. The methods employed in moisture and bacterial determinations of these inoculated barleys require further refinement before any general conclusions may be validly drawn.

The refusal of swine to eat barley infected with large numbers of bacteria, and possessing only slight amounts of the Fusarium-emetic principle, indicates that the seed-borne bacteria of barley may exert other important effects on the quality of the grain.

CHAPTER V.

THE EFFECTS OF MICROFLORA ON MALT QUALITY

LITERATURE REVIEW

Barley damaged by microflora has long been implicated in the lowering of malt quality. Edward Lisle, in the early part of the eighteenth century, reported that ". . . Barley, reddish and stained at the germinating or sprouting end . . . will come away untowardly in malting, much of it lying behind on the malting-floor . . ." (237). The same author also indicated that mow-burnt and heat-damaged barley were unsuitable for malting. In a discussion of the malting process Lisle pointed out the necessity of changing the steep water to avoid "souring" and "sliminess" - undoubtedly the result of bacterial action.

Matthews conducted an investigation on *Fusarium*-infected barley and malt (257). He concluded that the fungus reduced malt quality by lowering germination, reducing extract, and by imparting peculiar flavors to the malt. Güssow studied an organism, identified as *Bacterium herbicola rubrum* Dügg., which seriously interfered with the malting process (152). The bacterium was reported by a British brewer to spread from the embryo end of the kernel over the surface of the husk. The husk and rootlets were discolored, the endosperm was converted to a slimy mass, and the rootlets later became necrotic.

The organism was effectively controlled by washing the infected grain with lime water prior to germination.

Lott found that moldy wort gave less extract, less fermentable material (and consequently beer with less alcohol), and was more acid than non-moldy wort (240). He found that, contrary to other reports, fungus-infected malt did not cause a moldy taste or smell in beer and wort. Nilson stated that bacteria lowered the enzymatic power of malt by their biochemical activity (287). Mason found that Fusarium infection of green malt reduced germination capacity and gave uneven growth on the malting floor (255). Hind has indicated in his brewing textbook that, in addition to the loss in germinability, bacteria may cause beer instability by the putrefaction of infected barley kernels (177). Wahl and Henius recommended that an antiseptic material be added to the first steep of barley to destroy fungal growth which might have an effect on beer flavor (406). De Clerck states in his brewing reference that the heavy seed-borne flora will multiply in steep and in germination and bring about the asphyxiation of the barley embryos (90). Germination inhibition by the microflora of severely weathered barleys has also been discussed by Preece (313). According to Leonhardt, weathered barley produces stained malt and off-flavored beer (227). Beaven has also indicated that barley over-exposed to bad weather may be rendered unfit for malting (28).

Fusarium-infected barley was found by Shands to injure the malting quality of the grain by lowering germination, reducing vigor of growth, and altering chemical composition. Fungus growth during malting caused discoloration of the malt (347). Striulcic reported that the starch grains of barley infected by species of Fusarium presented an atypical corroded appearance, as the result of local fermentation and this type of corrosion was distinct from that observed in germinating kernels (379). Hopkins and Cooper have indicated that the beta-amylase content of barley may be contaminated by variable amounts of alpha-amylase secreted by the kernel microflora, eg. Bacillus subtilis, B. mesentericus, and Aspergillus spp. (184). Blackwood found that analytic alpha-amylase, produced by Bacillus subtilis, also contained appreciable amounts of cytolytic enzymes active on barley gum (43). The author tested 114 species of Bacillus for cytase activity; and while all of the isolates produced some cytase, B. subtilis gave the best yields of the enzyme. Meredith investigated the effects of peeling barley on malting quality (270). He found that the peeled kernels were subject to serious mold infection, and the finished malts were of poor quality, probably as a result of infection by Mucor sp. The kernels germinated poorly and abnormally, modification was poor, extract yield and enzymatic activity were reduced and the malts had an unattractive appearance.

A cooperative study of the influence of weather-

ing on malting and brewing quality of barley was carried out by the Malt Research Institute and several malting and brewing companies (3). Pilot scale and commercial maltings were made of paired bright and weathered barley lots from the 1951 and 1953 crops. Pilot scale brewing tests were also conducted with these malts by the participating laboratories. The barleys, malts, and beers differed markedly in their properties, depending on the degree of weathering. The following abnormal properties were, however, almost unanimously agreed upon by the cooperating laboratories for the combined 1951 and 1953 weathered barleys:

1. Weathered barley steeped at a faster rate, had poorer germination energy and uniformity, and usually showed a prolonged post-harvest dormancy.

2. Malts prepared from weathered barley showed an abnormally high protein modification, higher wort color, and generally tended to be lower in extract.

3. Malts prepared from weathered barleys brewed and fermented abnormally only if weathering was severe.

4. Beers prepared from weathered barley malts were abnormally high in nitrogen and were dark in color. Taste and aroma were also less desirable.

5. Certain malt and beer abnormalities had a greater tendency to persist through decreasing degrees of weathering than did others. Abnormally high beer and wort nitrogen and color, while diminishing in intensity with reduced weathering, were apparent with all weathered lots. This was true to a lesser degree of beer aroma

> and taste. Other factors, e.g. gas instability and poor fermenting and brewing response, while obvious with severely weathered barleys, were less apparent as weathering decreased.

The authors of this report acknowledged the association of microorganisms with the complex of weathering, but pointed out that the microflora of the barleys studied in this program were not investigated as a separate factor.

Sheneman and Hollenbeck have investigated the microbial patterns in malt, during the various stages of the malting process (349). The authors state that characteristic groups of microorganisms; including the aerogenes group, psychrophilic gram-negative rods, lactic-acid bacteria, aerobic spore-formers, molds, and yeasts, can be distinguished in these various stages. The increase and decrease of lactic-acid bacterial populations during the malting process are described in detail by the authors.

Smyk has reported on the effects of microflora on the diastatic power of distillery and brewery malts (363). The author stated that brewery malts contained fewer microflora, both qualitatively and quantitatively, than did the distillery malts. Preliminary studies indicated that distillery malts, infected by fungi and other organisms, from 10 to 50 percent, showed a significant reduction in diastatic power. Penicillium expansum, Fusarium herbarum, and Rhizopus nigricans all

reduced barley germination and lowered the enzymatic strength of the grain.

Distillers object to high bacterial counts in grain because the alcoholic yield is lowered and bacterial metabolic products, such as acrolein, may be carried over with the alcohol distillate (50). The effect of the bacterial content of distillers' malt on fermentation efficiency has been discussed by Adams and his co-workers (10). These workers have shown that the pasteurizing effect of conversion temperatures so greatly reduce the bacterial count of barley malt that fermentation efficiency is not lowered.

The reduction of barley germination by steeping has been noted by several workers. It has been indicated by authors that certain chemical compounds, usually polyphenolic in nature, may be removed from the barley kernels in the steeping process, and these compounds exert an inhibitory effect on the germination of the grains (90, 214, 256). Gilbert and his co-workers have attributed the inhibition of barley germination by steep liquor to an exotoxin produced by the bacterial flora of the kernels (46, 127). Piratzky found that the growth of microorganisms was suppressed by the addition of small amounts of formalin to the steep water (303). He also showed that the inhibitory materials present in the husks were not very active in reducing barley germination. Green and Sanger added both hydrogen peroxide and peracetic acid to steep

water to overcome inhibitory effects; they concluded that the action of microflora was of minor importance (145). Jansson and others have reported that "water-sensitivity" of steeping barleys is not due to the action of microflora, as was suggested by Gilbert, Piratzky, and others (196). The authors concluded that the phenomenon of "water-sensitivity" is an intrinsic property of certain barleys, a view shared by Kirsop and Pollock (211). Kudo and Yoshida have taken a stand between these two camps, and assert that "water-sensitivity" is related to both the state of the barley and its microbial flora (217).

Workers at the University of Minnesota have reported that microflora on and in barley kernels may increase very rapidly under temperature and moisture conditions similar to those of commercial steeping and germination (8). These investigators have also analyzed nine samples of barley for malt and beer properties, e.g. gas instability, reduced alpha-amylase, and low wort nitrogen ratio; but the relationship to the microfloral populations of the samples was not completely clear (9).

The malting process is subject to a number of inter-acting variables. Consequently researchers have designed facilities which permit rigid control and evaluation of these variables in order to better understand the process. This has been best achieved by the use of pilot malting equipment, i.e. highly specialized equipment which, step-by-step, duplicates the commercial malt-

ing process. During the past thirty years such equipment has been increasingly employed by industrial, government, and university laboratories. Until recently pilot malting and pilot brewing facilities were most widely used in the quality evaluation of barley hybrids and varieties (96, 97). A more recent use of this equipment has been made in the study of microfloral influence on malting quality (97, 316).

In 1937, Anderson described pilot malting equipment in use at the Grain Research Laboratory at Winnipeg (13). The duplicated components of the plant permitted the concurrent processing of eight -350 gram samples. This equipment was modified to allow steeping of 250 gram samples along with several other improvements (14, 15). The germination chamber was changed to accomodate twenty-four -250 gram samples (272) and later a drum-type germinator was introduced into the system to permit greater flexibility (271). The pilot malting equipment of the United States Department of Agriculture Barley and Malt Laboratory permits the processing of 200 to 250 gram barley samples for the evaluation of plant breeders' selections, and ten to fourteen pound samples for pilot brewing studies (95). Davis and Pollock have described an experimental malting apparatus capable of malting several 800 gram barley samples under a wide range of controlled conditions (89). A description of an industrial pilot malting laboratory is given in a paper by Baker and

Burant (23). This facility was an outgrowth from experience gained in the operation of an older pilot plant (154) and was capable of processing barley samples in excess of one-half bushel. Another industrial pilot malting system has been described which differs from conventional experimental equipment in that it provided an approximately adiabatic environment in both the germinator and the kiln (137).

Banasik and his co-workers have devised a micro-malting apparatus and method for the evaluation of barley selections developed by the breeder (25). The apparatus was capable of producing twenty-four micro-malts, each weighing 60 grams. In general, the correlation coefficients for malt properties were high between micro and macro methods. Bendelow (31) and Whitmore and Sparrow (417) have also described micro-malting methods utilizing fifty and thirty grams of barley respectively. Burkhart and his co-workers have compared commercial and laboratory malts prepared from the same barley lots (58). They concluded that laboratory malting was apparently satisfactory for pilot brewing evaluation of barley. The influence of malting environment and the varietal response of barleys to this environment were studied in detail by Shands and others (345). This study emphasized the importance of temperature, moisture, and period of growth on the varieties Oderbrucker, Wisconsin Barbless, Peatland, and Chevron.

Prentice and others (97, 316) have applied ninety-

seven microorganisms, commonly found on barley, to kernels during malting in a pilot plant. Micro-brews were also prepared from these malts. They found that with inoculated malts: (1) wort-nitrogen levels increased, (2) alphaamylase and diastatic power increased, and (3) beer gas-stability decreased. Although several types of organisms were associated with these effects, Fusarium species were most commonly implicated in the production of undesirable characteristics.

Methods of inoculating barley and other cereal grains with microorganisms have been reviewed by Sallans, who found that infectivity of fungal inoculum decreased with the age of the culture (335). A method for inoculating barley with Helminthosporium gramineum Rbh. was devised by Fuchs (120). The author used a needle to insert conidial suspensions of the fungus between the glumes of soaked ripe grains. With large quantities of inoculum, he found that placing the grains in a conidial suspension for twenty minutes, under vacuum, gave the highest percentage of infection. It was recommended that the inoculated grain be stored in an incubator at high humidity (90 to 100%) for two days, followed by drying at room temperature and humidity in order to promote abundant mycelial growth beneath the glume surface. A modification of the 'vacuum method' has been described by Moore for the inoculation of wheat and barley with the loose smut fungus (276).

MATERIALS AND METHODS

To study the influence of certain microorganisms on the quality of malt, a pilot malting unit capable of producing multiple malts under comparable malting conditions was designed and constructed. The unit was constructed so that a maximum of four samples, of approximately one kilogram each, could be run concurrently under conditions as nearly uniform as possible. These samples were placed in the unit in separate compartments to avoid the danger of cross-contamination. A final, but necessary, consideration was the cost of constructing such a unit. Insofar as possible, salvage materials or existing equipment was employed throughout the construction of the pilot plant.

Cleaning and grading

All of the barley samples were cleaned and sized by passing the grain twice over the reciprocating sieves of a bench-type grader. The sized grain was stored in canvas grain sacks until used in the malting runs.

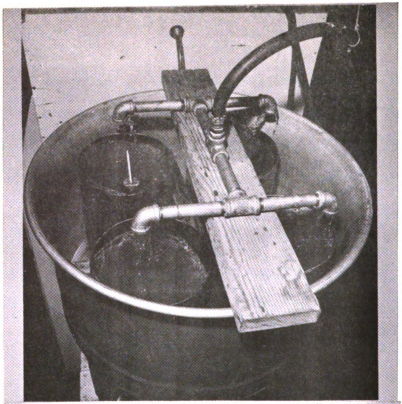
Steeping equipment

A forty gallon aluminum steam cooker was mounted on a wooden platform and adjusted to a height sufficient to ensure water drainage into the adjacent laboratory sink. A plastic tubular hoop covered with a fine-mesh aluminum screening was placed near the bottom of the kettle. This screen served as a trap for spilled kernels, preventing their entrance into the drain outlet.

A wooden framework was constructed to support the individual steeping containers upon the screen. Four - 5 gallon metal fruit containers served as steeping chambers. Cylindrical baskets made of aluminum screening were placed in these containers. These baskets held the steeping grain and facilitated drainage, aeration, and manipulation of the steeped barley. Water was supplied from the university well-water system. Temperatures of the water varied from 54°F., in the winter to 59°F., in the summer. The water was distributed from the sink outlet in a one-half inch rubber hose to a four-way distribution manifold constructed of three-fourths inch galvanized pipe and fittings. Aeration was achieved by the use of a rigid aluminum tube at the end of a rubber hose leading to the laboratory compressed-air outlet. The steeping unit is pictured in Plate X.

Germinating equipment

Paired germinating chambers were constructed from two - 8 cubic feet iceboxes placed back-to-back. The bottom doors were sealed and the upper doors were gasketed to ensure air-tightness. An aluminum shelf was placed in each germinator and openings in the shelves permitted the insertion and removal of the individual germinating pans. The pans were prepared by drilling one-eighth inch holes on one inch centers in the bottom of stainless steel cake pans (2" x 9" x 13"). When the germinating pans were placed in position, all air passing



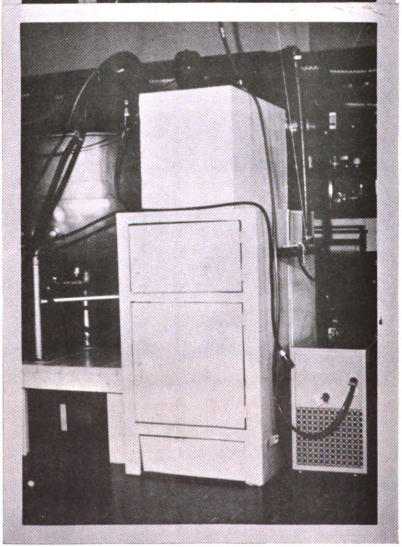


PLATE X

Pilot malting equipment used in the study of the effects of microflora on the quality of malt.

Upper - Aerial view of the steeping equipment; showing water supply, steeping tank, and individual steeping containers.

Lower - Overall view of the germination equipment; showing the spray chamber, paired germinators, and refrigeration unit.

into the upper chamber of necessity, flowed through the pans and was carried out through the exhaust openings in the lower chamber. Two cooling coils were wound from one-quarter inch copper tubing and were placed separately along the rear of each germinator. Aluminum strips were placed under each coil to deflect the condensate away from the germinating pans. A "T"-fitting joined the two coils at each side of the two germinators. Three-eighth inch, flexible, plastic garden hose was connected from each "T"-fitting to a "force-flow liquid coolant circulator" (Daigger model R 5230) which had a cooling capacity, using water initially at 50°F., of 0.90 to 4.5°F. per gallon per minute and a temperature range of from 10° to 90°F. with an accuracy of ± 2°F. The coils, hoses, and refrigerator when connected formed a closed system and stabilized the temperatures within the germinating chambers. The formation of vapor-locks in the system was prevented by introducing a suitable venting line which also permitted the replenishing of lost coolant. Distilled water was employed as the coolant which restricted operation of the unit to temperatures above 32°F. A spray chamber was constructed to attemperate and saturate the air passing into the germination chambers with water vapor. A stainless steel sink (17" wide x 17" deep x 36" long) was fitted with a one-eighth inch thick aluminum cover and served as the spray-chamber. The cover was

gasketed and secured to the sink by means of small machine screws. A Redmond blower of 100 c.f.m. capacity was fitted to one end of the spray chamber top. The air leaving the spray-chamber passed through a system of rigid plastic piping (1-½" diameter) which delivered the attemperated and humidified air to the chambers below. As originally executed, the air was saturated by passing it through a series of glass-wool battens which were in turn impregnated with water by means of a copper-tubing manifold connected to the sink outlet. This system was later discarded because of the heavy bacterial deposition of slime on the battens. A more satisfactory water screen was subsequently employed. This consisted of a brass spray nozzle (Chicago fog jet - Spraying Systems Co., 3/4 7G) which was connected to the water inlet by means of three-quarter inch galvanized piping. The excess spray water was carried by gravity to the laboratory sink below through a sink-drain placed in the bottom of the spray-chamber. A recording thermometer (Weksler with flexible tubing and bulbs) was fitted to the side of the germinating chambers. The two flexible lines from the thermometer were placed, one in each chamber, so as to record the temperatures within the two compartments. Temperatures of the green malt could also be recorded by placing the thermometer bulbs in the germinating pans. Since the temperature and the relative humidity of the germinating chambers were functions of temperatures of both the spray-

water and the coolant systems it was necessary to provide for adjustments of the spray-water temperature. The temperature of the spray-water was varied by introducing a "temperator" (Central Co.), designed for use in reducing the condensate which forms on toilet tanks. Table 16 shows some selected temperatures and relative humidity readings, obtained by varying the temperatures of the two systems. The germination equipment is illustrated in Plate X and XI.

Kilning equipment

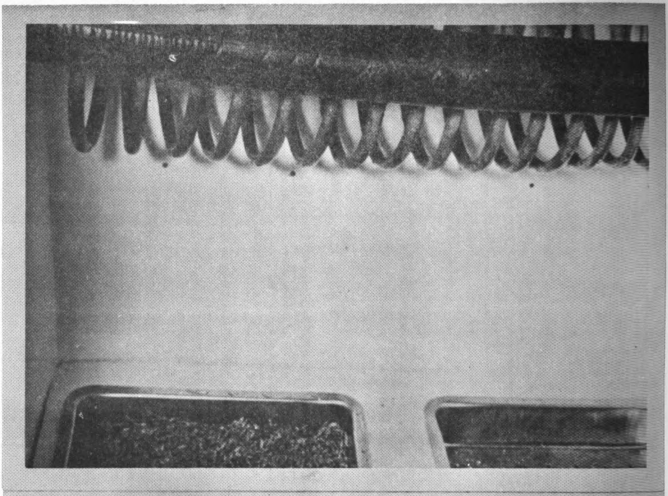
A standard gravity-convection laboratory oven (Modernlab Model 17-12, 800 watts, 180°C. temperature range) with interior dimensions of 20" x 15" x 20" was modified for use as a kiln. A small blower (Redmond - 50 c.f.m.) was installed at the bottom of the oven, and the base of the oven was sealed to prevent air-leakage. An auxilliary blower (Redmond - 180 c.f.m.) was placed at the exhaust opening at the top of the oven. A large container (20" x 15" x 10½") was assembled from one-half inch hardware cloth to hold the individual kilning containers. These containers were fabricated from fine-mesh aluminum screening and measured 7" x 7½" x 8½". The basket assembly prevented physical contact between the various malt samples and at the same time provided for the uniform passage of the heated air through the drying malt. Sulfur-dioxide was applied to the malt by releasing the vapor into the small blower at the bottom

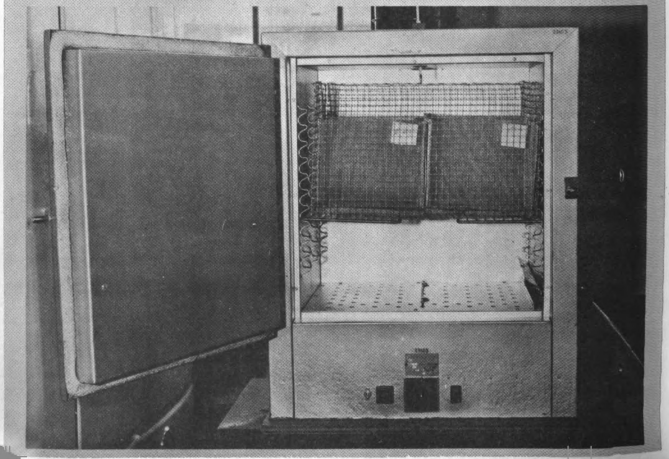
Table 16. - Temperature and humidity readings of germination chamber as a function of spray-water temperature and refrigeration unit temperatures.

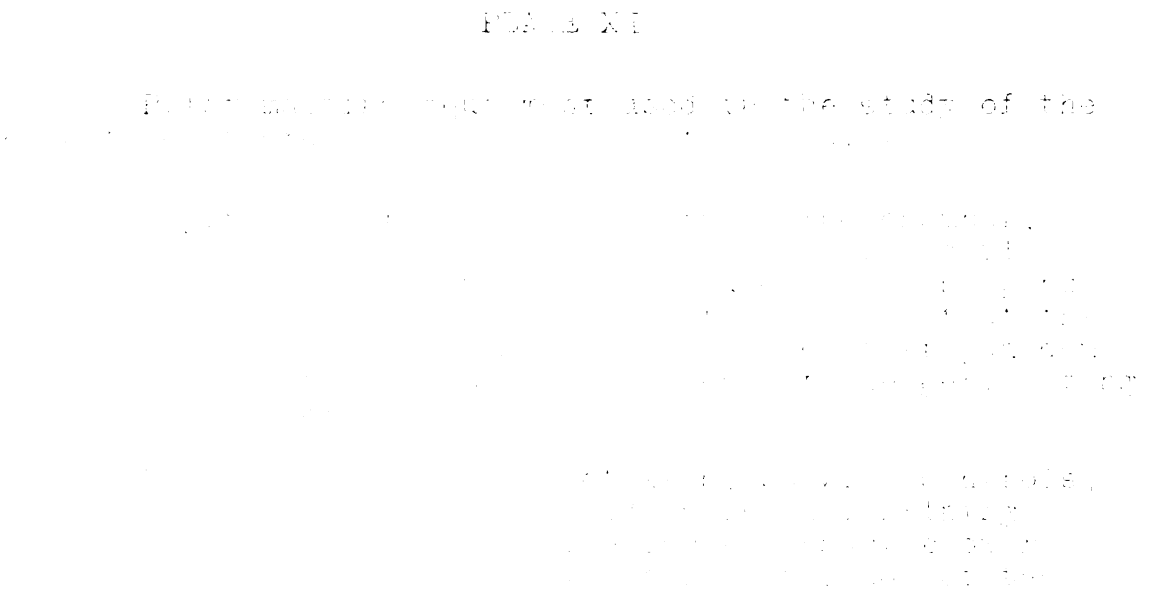
Spray-water temperature	Approximate temperature of refrigeration unit (°F.)											
	40		50		60		70		80		90	
(°F.)	a	b	a	b	a	b	a	b	a	b	a	b
58.0	56	90	60	80	65	74	68	75	72	53	77	46
58.5	54	100	60	81	64	84	68	80	72	54	76	46
59.0	55	100	59	98	63	92	68	69	72	54	77	47
59.5	56	95	61	80	65	87	68	84	72	54	78	44
61.0	57	93	60	87	65	76	69	75	74	64	77	54
62.5	56	100	61	95	66	90	69	95	73	62	78	52
67.0	58	100	62	100	66	100	70	100	75	91	79	68
71.0	58	100	62	100	66	100	71	100	76	100	80	90
74.0	62	100	65	100	68	100	72	100	76	100	80	90
81.5	65	100	68	100	71	100	75	100	79	100	84	100

[a] Temperature of germination chamber in °F.

[b] Relative humidity of germination chamber in percent.







PLEASE NOTE: This is not original copy. Some light and dark type throughout. This page is too light to be readable when filmed.

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of the kiln. The kilning equipment is shown in Plate XI. At the termination of kilning the rootlets were removed by rubbing the kernels in a clean canvas seed sack and the malt was then stored in air-tight metal sample cans.

A number of preliminary malts were run using various steeping, germinating, and kilning procedures. The most satisfactory malting schedule, and the one employed in all of the subsequent runs with inoculated barley, was as follows. Four weighed lots of a single variety of barley were used in each malting. The cleaned and graded barley which passed through the 7/64 inch sieve and was held on the 6/64 inch sieve was used in all runs. The weights of each barley lot ranged from 500 grams to 1,000 grams depending upon the number of analyses desired. The four barley lots were placed in the steeping baskets and the flow of steeping water was adjusted to approximately seven gallons per hour per basket. The steeped barley was inspected periodically and the steep water changed at approximately four, twelve, and twenty-four hours after the beginning of steeping. The water was changed by agitating the steep basket several times to mix the grain and to release adsorbed material, removing and draining the steep baskets, and pouring off the old steep water. As fresh water was being added to the grain the barley was aerated for thirty seconds. When the steeped barley satisfied several empirical tests for the determination of moisture content it was removed from the steep con-

tainers and placed in the germinators. These tests involved: (1) pinching the kernels lengthwise between the thumb and forefinger, (2) pressing the kernel with thumbnail against the flat surface, and (3) sectioning the kernels length-wise and observing water infiltration with the aid of a binocular dissecting microscope (406). A two gram sample of each barley lot was taken as the steeped grain was placed in the germinators and tested for moisture content by the hot air oven drying method (5, 91). Whenever possible the moisture content by weight was brought to 43 percent, as recommended by Shands and his co-workers (345). If the moisture content was below 43 percent the green malt was watered to allow further water imbibition.

The water temperatures of the germination equipment were adjusted to provide a relative humidity of 100 percent and a grain temperature of from 50° to 65° F. These adjustments varied with the temperature and relative humidity of the laboratory. The germinating grain was manually agitated daily to prevent matting of the rootlets, a common occurrence in pneumatic malting (90). The separate samples were watered with a polyethylene spray bottle containing fresh tap water. The grain temperatures, relative humidity of the germinators, temperature and relative humidity of the laboratory, and any manipulations of the equipment and/or grain were noted several times daily. When germination had progressed to the desired stage the

green malt was transferred to the kiln. This stage was reached when the acrospires (plumules) had extended from three-fourths to the entire length of the kernels of the appropriate check lot. The germinated barley was placed in separate wire kilning baskets and dried according to the following schedule: (1) twelve hours at 37° C., (2) twelve hours at 49° C., (3) three and one-half hours at 77° C.[1]. This schedule was approximate; the times and temperatures were adjusted in accordance with the moisture contents of the drying malt. The malt was sulfured during the final drying stage as described above. Immediately after completion of kilning, the malt rootlets were removed and the malt was placed in sample cans and stored.

Three varieties of barley (Hannchen, Montcalm, and Traill), grown in the west in 1959 under irrigation, were used in this study. The three varieties were exceptionally bright and, except for the Hannchen lot, were relatively free of microflora (Table 17). These varieties were variously inoculated with pure cultures of six bacterial species, four fungal species, and one pink yeast (Table 18). A modification of Fuch's method (120) was used for the inoculation of the grain.

The inoculum was prepared by growing three replicates of each organism for one week. The cultures (50 ml of medium in 250 ml Erlenmeyer flasks) were maintained on a reciprocating shaker at 25° C. The bacteria were cultured

[1] R. Bonner - personal communication.

Table 17. - The microfloral contents of the three barley varieties used in the pilot malting studies as determined by whole-kernel platings on agar plates[a]

Barley variety	Number of colonies per 100 kernels						
	Alternaria	Fusarium	Epicoccum	Cladosporium	Bacteria	Yeasts	Other
Montcalm	1	0	0	0	0	0	0
Hannchen	33	3	0	4	2	0	0
Traill	0	0	1	0	0	1	0

[a] Kernels were plated in the manner described in Chapt. I. (cf. ref. 300). The three varieties were all grown in the west under irrigation, and were all extremely bright in appearance. The microfloral counts for untreated check samples (listed above) and for steeped and dried check samples were consistently alike, both qualitatively and quantitatively.
Moisture contents of the untreated grain were between ten and eleven percent (by weight) for all varieties. Germination capacity was 99 - 100 percent for the varieties Traill and Montcalm and 94 - 99 percent for the Hannchen lot.

Table 18. - The source and identity of organisms used to inoculate barley processed in the pilot malting apparatus.

Culture number	Date isolated	Source of isolate [a]	Identity of isolate
B-5	4-59	Kindred; Caro, Michigan	Bacillus polymyxa (Praz.) Mig.
B-210	6-59	Traill; E. Lansing, Mich.	Pseudomonas atrofaciens (McC) St.
B-218	6-59	Hannchen; do	Bacillus megaterium-cereus intermediate
B-226	6-59	Montcalm; do	Flavobacterium sp.
B-522	4-60	do do	Bacillus sp.
B-529	4-60	do do	Bacillus cereus var. mycoides (Fl.) Smith et al.
Y-521	4-60	do do	Rhodotorula sp.
F-304	5-57	OAC-21; Manitoba	Alternaria tenuis Nees.
F-310	4-57	Montcalm; do	Helminthosporium sativum P., K., & B.
F-321	5-57	Parkland; do	Fusarium roseum f.sp. cerealis Snyd. & Hans.
F-324	5-57	Kindred; Fairgrove, Mich.	Cladosporium herbarum Lk.

[a] All isolates obtained from kernels plated in the standard manner (300).

in nutrient broth and the fungi in potato broth (322). The bacterial cells were harvested by combining the contents of the three replications, after establishing their purity, and centrifuging in a laboratory centrifuge for thirty minutes at 3,500 r.p.m. The nutrient broth was poured off and the cells were re-suspended in sterile distilled water. The fungal inoculum was harvested by combing the separate replicates, as above, filtering off the potato broth, and suspending the mycelium and spores in sterile distilled water. This suspension was placed in a Waring blendor and comminuted for one minute. The barley was pre-treated, to remove surface contamination and to increase moisture content, by steeping for one hour in the manner outlined above. The size of the barley samples was, in every case, two and one-half pounds. The steeped barley was placed in a modified pressure cooker after draining off the steep liquor. The suspension of inoculum, either bacteria, fungi, or yeasts, was added to the container and the volume of the liquid increased to one liter (cf. Table 25). With this quantity of liquid the suspension just covered the surface of the grain. The cover was placed on the container and the air evacuated by means of a vacuum line. A manometer, attached to the apparatus, permitted a rapid appraisal of the amount of air removed from the kettle. After the air had been exhausted, a pinchcock on the exhaust line was opened allowing air to flow rapidly back into the container. This procedure was

repeated three times. The inoculation apparatus is pictured in Plate XII. After inoculation the grain was removed from the kettle, drained and spread out in a thin layer on newspaper to dry. The grain was periodically mixed to ensure even drying. A single sheet of paper was placed over the grain to exclude air-borne contamination. The relative humidities encountered in the laboratory at this time ranged from thirty to forty percent. The grain usually reached a moisture content, by weight, of thirteen to fourteen percent after two or three days of drying under these conditions. At the completion of drying the grain was stored in sealed, two-quart mason jars until malted. Check lots of each variety were prepared in the same fashion, except that only sterile distilled water was placed in the vacuum container.

The malting of inoculated barley was carried out using the schedule presented above. In each case only a single variety was malted at one time. A check and three inoculated samples were processed, exercising caution to avoid cross-contamination. The bacterial inoculations were run separately from the fungal treatments. Kernels of the inoculated barleys and of the check lots were plated, both before steeping and after germination, to determine the amount of contamination and the effect of malting on the microfloral populations. Samples of steep liquor were taken periodically and dilution plates run with the steep liquor for the same purpose.

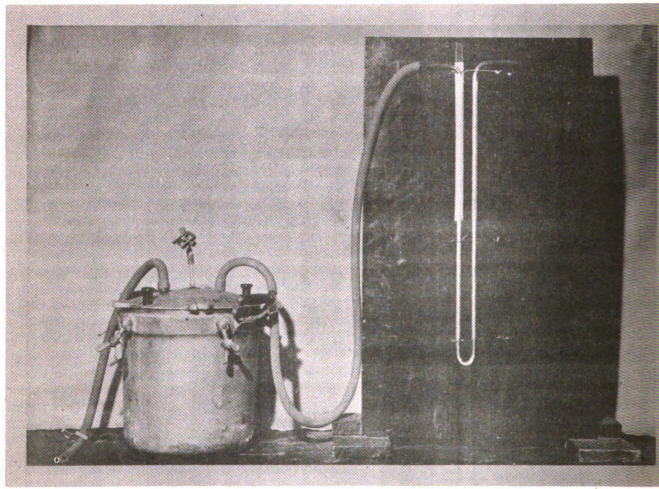


PLATE XII

The apparatus used in the inoculation of bright barleys with bacteria, yeasts, and fungi for use in the pilot malting studies. The modified pressure-cooker, used to hold the steeped grain and the inoculum, is shown on the left; the manometer is supported on the upright board at the right. The tubing on the left is connected to a vacuum line, the center tube is used as an air inlet.

At the time of storage the pilot-malts were given a code number to avoid any subjective bias in their evaluation. These malts were subsequently analyzed, by code number, for physical and chemical properties. The analyses were conducted partly by the author and in part by two commercial malting laboratories. The tests included both standard malt analyses (5) and evaluations dictated by a knowledge of the properties of the inoculated organisms.

Experiments were also conducted to determine differences in the rates of steeping between varieties and samples differing in their microfloral contents. To this end a number of miniature steeping baskets, two inches in diameter and two and one-half inches high, were constructed of fine-mesh aluminum screening. Weighed portions of grain were placed in each basket and the baskets covered with a cheesecloth square. All baskets were submerged in the steep tank and at measured intervals they were withdrawn. The excess water was removed and steeped grain was weighed to determine the amount of water inbibed in this time interval. The moisture content of the grain was computed with the use of De Clerck's formula (90):

$$X = \frac{(H-h \times 100}{100 - H}$$

where X is the necessary increase in weight of 100 grams of barley, H is the desired moisture content to be achieved, and h equals the initial moisture content of the grain.

EXPERIMENTAL RESULTS

Pertinent malt analytic factors of inoculated barleys and the non-inoculated check samples are presented in Tables 19 through 21. Although obvious differences exist between the check samples and the inoculated malts, these differences must be viewed with caution. In many cases these differences are the result of malting conditions rather than a reflection of microfloral effects. Daily records of each malting run were maintained; any malting condition which altered the analytic characters of the finished malt were recorded. This information is included in the tables under "Remarks".

The malting information and the analytic data were examined for differences existing between the inoculated malts and the corresponding check samples (Tables 22, 23, 24). Some of the isolates consistently showed no effects with certain varieties, e.g. Bacillus polymyxa (B-5) on Traill and Montcalm (Tables 22, 24). Alternaria tenuis (F-304) on Montcalm (Table 22), Cladosporium herbarum (F-324) on Montcalm (Table 22), and Helminthosporium sativum (F-310) on Traill (Table 24). Traill and Hannchen malts inoculated with the Flavobacterium isolate (B-226) showed increased 1,000 kernel weight (Tables 23, 24). The kernels of Mantcalm malt, inoculated with the same organism, were reduced in weight (Table 22). Malt prepared from Traill barley, inoculated with Cladosporium herbarum (F-324),

Table 19. - Physical characteristics and chemical analysis of Montcalm pilot malts.

Analytic characters[a]	Treatments[b]								
	Check-1	B-5	B-210	B-226	*Check-2	*F-304	*F-321	Check-3	F-324
1,000 knl. wt.	29.38	29.46	28.60	28.92	28.35	28.51	28.75	--	--
Moisture (%)	4.2	4.6	4.2	4.9	2.5	4.1	3.1	5.8	6.0
Extract (f.g.)	77.3	77.3	76.0	77.1	76.4	78.1	76.2	77.6	77.4
Difference (fg-cg)	1.8	1.6	1.7	1.3	1.8	1.2	2.6	2.2	1.8
Conversion time	<5	<5	5-7	<5	5-7	5-7	7-10	5-7	5-7
Wort color	2.9	2.1	3.2	1.6	14.7	3.0	11.4	1.5	1.3
Wort clarity	cl	cl	sl hz	sl hz	cl	cl	sl hz	cl	cl
Filtration time	nl	nl	sl	nl	nl	nl	sl	nl	nl
Diastatic power	79	80	75	103	42	83	42	125	127
Alpha amylase	35.1	36.0	28.8	37.1	22.4	45.5	23.4	40.8	37.9
Sol./Total protein	43.4	41.0	42.1	42.8	40.4	46.8	34.7	42.3	41.9
Wort acidity	5.59	5.60	5.58	5.61	5.50	5.65	5.55	--	--
Soluble protein	4.68	4.44	4.53	4.66	4.17	4.91	4.21	4.62	4.45

[a] Characters reported on a dry basis. Analytic procedures performed according to A.S.B.C. methods (5).

[b] See Table 18 for identity of isolates used. Abbreviations used are as follows: v - very, sl for slow or slightly, hz - hazy, cl - clear, nl - normal.

* Sample kilned at excessive moisture with subsequent melanoidin formation.

Analyte	Treatments						
	B-210	B-220	Check-2	F-304	*F-310	F 321	

Table 20. - Physical characteristics and chemical analysis of Hannchen pilot malts.

Analytic characters[a]	Treatments[b]							
	Check-1	B-5	B-210	B-226	Check-2	F-304	*F-310	F-321
1,000 knl. wt.	29.42	30.00	29.69	37.96	36.80	37.13	34.33	37.54
Moisture (%)	5.1	6.3	8.4	5.6	3.8	4.8	2.8	3.6
Extract (f.g.)	80.1	80.8	80.2	79.8	80.5	80.3	77.8	80.0
Difference (fg-cg)	3.2	4.3	3.5	4.0	2.8	3.8	2.6	3.8
Conversion time	6-8	6-8	6-8	5-7	< 5	10-15	15-20	10-15
Wort color	1.3	1.0	1.4	1.1	2.6	3.4	24.1	3.1
Wort clarity	cl	cl	hz	sl hz	cl	hz	sl hz	hz
Filtration time	nl	nl	sl	sl	sl	nl	nl	nl
Diastatic power	84	92	91	85	64	45	25	51
Alpha amylase	38.0	38.2	30.1	34.9	35.9	24.6	10.7	31.1
Sol./Total protein	43.6	38.5	42.0	37.6	45.1	41.6	39.3	43.4
Soluble protein	4.10	3.80	4.13	3.75	4.47	4.02	3.64	4.22
Wort acidity	5.72	5.80	5.44	5.78	5.60	5.69	5.30	5.70

[a] Characters reported on a dry basis. Analytic procedures performed according to A.S.B.C. methods (5).

[b] See Table 18 for identity of isolates used. Abbreviations used are as follows: v - very, sl for slow or slightly, hz - hazy, cl - clear, nl - normal.

* Sample kilned at excessive moisture with subsequent melanoidin formation.

Analysis	Treatments/1/							
Characters[a]	*B-5	*B-210	B-226	Check-2	F-304	F-310	F-321

Table 21. - Physical characteristics and chemical analysis of Traill pilot malts.

Analytic characters[a]	Treatments[b]							
	*Check-1	*B-5	*B-210	B-226	Check-2	F-304	F-310	F-321
1,000 kn. wt.	32.52	32.69	32.66	33.06	32.51	31.96	32.06	32.42
Moisture (%)	9.0	5.6	8.5	7.6	7.6	4.9	5.8	3.7
Extract (f.g.)	77.1	76.8	74.5	77.8	78.7	78.5	78.5	77.6
Difference (fg-cg)	3.8	2.8	4.1	3.8	2.3	2.2	1.6	3.1
Conversion time	5-7	<5	5-7	5-7	5-7	<5	<5	<5
Wort color	4.1	3.6	4.7	1.3	0.9	1.8	1.2	4.7
Wort clarity	v hz	hz	v hz	hz	cl	cl	cl	hz
Filtration time	nl	nl	nl	nl	nl	nl	nl	sl
Diastatic power	86	65	61	104	122	79	98	52
Alpha amylase	24.8	25.8	17.3	29.9	40.6	37.1	38.4	30.4
Soluble protein	4.65	4.10	4.16	4.25	4.55	4.86	4.65	4.52
Sol./Total protein	42.9	37.2	36.8	41.1	41.5	44.8	42.8	41.9
Wort acidity	5.45	5.60	5.50	5.65	5.80	5.71	5.76	5.70

Table 21. - Continued

Analytic characters[a]	Treatments[b]			
	*Check-3	F-324	Y-521	B-210
1,000 kn. wt.	--	--	--	--
Moisture (%)	3.2	4.6	5.1	4.9
Extract (f.g.)	77.4	78.3	76.8	77.5
Difference (fg-cg)	2.1	2.0	2.2	2.3
Conversion time	5-7	5-7	10-15	7-10
Wort color	8.9	1.8	1.5	1.4
Wort clarity	sl hz	cl	hz	hz
Filtration time	nl	nl	sl	nl
Diastatic power	44	86	78	94
Alpha amylase	24.8	43.8	19.8	22.8
Soluble protein	4.42	4.68	4.69	4.62
Sol./Total protein	41.6	43.1	42.8	41.1
Wort acidity	--	--	--	--

[a] Characters reported on a dry basis. Analytic procedures performed according to A.S.B.C. methods (5).

[b] See Table 18 for identity of isolates used. Abbreviations used are as follows: v - very, sl for slow or slightly, hz - hazy, cl - clear, nl - normal.

* Sample kilned at excessive moisture with subsequent melanoidin formation.

Table 22. - A summarization of the effects of six inoculated organisms on the quality of pilot malts prepared from Montcalm barley[a].

Inoculated with[b]	Effects
B-5	None
B-210	Reduced 1,000 kernel weight Erratic germination Increased number of glassy kernels Increased wort color Hazy wort Slow filtration Reduced diastatic power Reduced alpha-amylase
B-226	Reduced 1,000 kernel weight Erratic germination Hazy wort
F-304	None
F-321	Slight reduction in extract Slow conversion time Slightly hazy wort Slow filtration
F-324	None

[a] Physical and chemical analytic data are listed in Table 19.

[b] See Table 18 for identity of isolates.

Table 23. - A summarization of the effects of six inoculated organisms on the quality of pilot malts prepared from Hannchen barley. [a]

Inoculated with [b]	Effects [c]
B-5	Reduced 1,000 kernel weight Reduced kernel plumpness Slight increase in wort color
B-210	Reduced 1,000 kernel weight Reduced kernel plumpness Increased wort color Slow filtration Slightly reduced alpha-amylase Lower wort acidity (pH)
B-226	Increased 1,000 kernel weight Slightly reduced kernel plumpness Slight increase in wort color Slow filtration Slightly hazy wort Slightly reduced alpha-amylase
F-304	Increased 1,000 kernel weight Slightly reduced kernel plumpness Slow conversion time Increased wort color Hazy wort Slightly reduced diastatic power Slightly reduced alpha-amylase
F-310	Reduced 1,000 kernel weight Reduced extract Slow conversion time ? Increased wort color ? Hazy wort ? Reduced diastatic power ? Reduced alpha-amylase ? Lower wort acidity (pH)
F-321	Increased 1,000 kernel weight Reduced kernel plumpness Slow conversion time Increased wort color Hazy wort Reduced diastatic power Reduced alpha-amylase

[a] The effects noted here may be due to natural micro-

Table 23. - Continued

floral content of the Hannchen sample (cf. Table 17). Physical and analytic data are listed in Table 20.

b See Table 18 for identity of isolates.

c Question marks denote a questionable effect - high kilning temperatures at unsafe moisture levels may be responsible for the effect.

Table 24. - A summarization of the effects of eight inoculated organisms on the quality of pilot malts prepared from Traill barley[a].

Inoculated with[b]	Effects
B-5	None
B-210	Reduced kernel plumpness Erratic germination Slow conversion time Hazy wort Reduced extract Reduced alph-amylase
B-226	Increased 1,000 kernel weight Hazy wort
F-304	Reduced 1,000 kernel weight Reduced diastatic power
F-310	None (under-modified)
F-321	Slight reduction in extract Increased wort color Hazy wort Slow filtration Reduced diastatic power Reduced alpha-amylase
F-324	Reduced diastatic power Increased alpha-amylase
Y-521	Reduced kernel plumpness Slow conversion time Hazy wort Slow filtration Reduced diastatic power Reduced alpha-amylase

[a] Physical and chemical analytic data are listed in Table 21.

[b] See Table 18 for identity of isolates.

demonstrated an increase in alpha-amylase over the corresponding check sample (Table 24). The most consistent defects in the inoculated malts were: increased wort color, haziness of the wort, slower conversion and filtration times, reduced kernel weight and plumpness, and reduced enzymatic power (Tables 19 through 24). The organisms which were most consistently associated with these effects were: Pseudomonas atrofaciens, (B-210), Helminthosporium sativum (F-310), and Fusarium roseum f.sp. cerealis (F-321) (Tables 22, 23 24).

Surface-disinfected kernels were plated with all varieties and treatments, both from storage containers and from the germinators after two to three days of germination. The microfloral content of the stored samples remained relatively constant, i.e. at or near the original inoculum level. With only one exception, the bacteria increased to nearly 100 percent on all of the plated green malt kernels, including the uninoculated check lots (Table 25). The exception was the first series to be run through the pilot plant after the apparatus had remained idle for several months. The check lot of this Montcalm series remained free of both bacterial and fungal infection, although the inoculated samples all showed an increase of the original bacterial inoculum to 100 percent on the plated kernels (Plate XIII). The foreign bacteria were mainly white or creamy in appearance. A number of randomly-selected cells

Table 25. - The amount of original inoculum, initial infection level, increase of original inoculum, and the increase of alien microflora during the pilot malting of three barley varieties.

Identity of inoculum/ On: [a]	Original inoculum level (per 2-½ lbs. of barley) [b]	Initial infection by inoculated organism [c]	Increase of original inoculum during malting [d]	Increase of alien microflora during malting [e]
B-5	50,000,000 cells			
Montcalm	do	68	100	64 ?
Hannchen	do	52	100	25 ?
Traill	do	78	97	20 ?
B-210	56,000,000 cells			
Montcalm	do	54	99	28 ?
Hannchen	do	42	100	5 ?
Traill	do	50	100	10 ?
B-226	20,000,000 cells			
Montcalm	do	30	100	64 ?
Hannchen	do	20	100	46 ?
Traill	do	16	100	56 ?
B-522	10,000,000 cells			
Montcalm	do	12	--	--
Hannchen	do	10	--	--
Traill	do	24	--	--

Table 25. - Continued

B-529	12,000,000 cells			
Montcalm	do	20	--	--
Hannchen	do	12	--	--
Traill	do	18	--	--
Y-521	11,000,000 cells			
Montcalm	do	17	--	--
Hannchen	do	14	--	--
Traill	do	8	31	66
F-304	30,000,000 cells			
Montcalm	do	20	22	98
Hannchen	do	13	13	90
Traill	do	15	16	100
F-310	300,000 cells			
Montcalm	do	8	57	80
Hannchen	do	10	12	84
Traill	do	8	28	98
F-321	4,000,000 cells			
Montcalm	do	22	24	97
Hannchen	do	21	15	100
Traill	do	18	20	98

Table 23. (Continued)

F-324	1,800,000 cells			
Metal	14	12	122	

Table 25. - Continued

F-324	1,800,000 cells			
Montcalm	do	14	10	100
Hannchen	do	20	8	100
Traill	do	8	4	99

[a] See Table 18 for identity of organisms.

[b] Inoculum level computed with dilution-plate series, i.e. count represents the number of viable cells contained in the inoculum suspension.

[c] Number denotes the number of morphologically similar colonies per 100 kernels.

[d] Figure denotes the number of morphologically similar colonies per 100 kernels.

[e] Figure denotes the number of morphologically dissimilar colonies per 100 kernels. Question mark indicates an approximate figure (identification of dissimilar organisms was difficult because of colony mixtures).

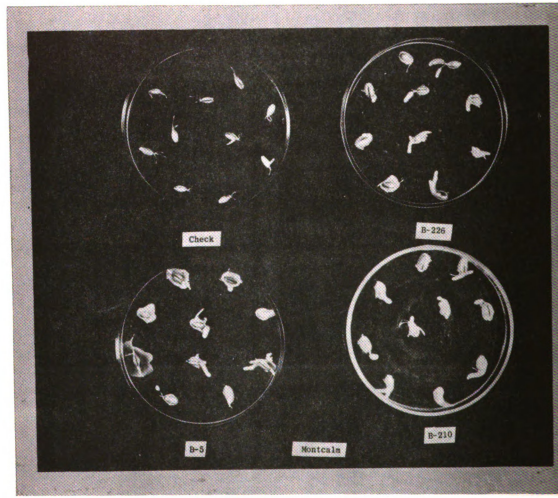

Check
B-226
B-5
Montcalm
B-210

PLATE XIII

Montcalm barley, inoculated with three species of bacteria and an uninoculated check sample, plated after two and one-half days in the pilot malting germinator.

proved to be gram-positive, spore-forming, aerobic bacteria, i.e. species of Bacillus (53, 54). Petri plates containing sterile nutrient media were exposed in the disinfected, empty germinators for ten minute periods. Two to five bacterial colonies appeared on each plate after incubation. The colony color and staining reaction of these bacteria resembled those non-inoculated organisms which appeared on the green malt. A smaller number of bacteria were also isolated from the spray-water source by streaking loopfuls of the water on the surface of agar plates.

The surface bacteria of steeping barley showed significant quantitative changes, as determined by dilution plating of the steep liquor at successive time intervals (Table 26). These changes were extremely variable, but generally the number of bacteria increased on the inoculated barleys, reaching a peak with the twelve hour sample. A pronounced population decrease took place from the twelve hour period to the final sampling. The steep liquor obtained from the Hannchen check lot yielded the greatest number of surface bacteria in the first sampling; followed by a steady decrease in viable cells in succeeding platings. The pattern of population changes was similar with both Hannchen and Montcalm inoculated barleys.

A few pilot malts, as well as commercial samples, were plated in order to determine the effect of kilning on the kernel microflora. Quantitatively the fungi were affected more severely than the seed-borne bacteria. The

Table 26. - Bacterial colony counts obtained from dilution plates of steep liquor taken at intervals during the steeping of Hannchen and Montcalm barleys[a].

Barley variety	Sample taken after ____ hrs. of steeping	Bacterial colonies per ml. Barley inoculated with: None	B-5	B-210	B-226[b]
Hannchen	4	6,000	300	1,860	800
do	12	570	6,490	500	4,360
do	24	30	6,300	330	100
do	36	90	300	240	60
do	46	30	650	10	100
Montcalm	12	30	10	100	40
do	24	1,080	10	3,600	30
do	36	0	28	20	20

[a] Dilution plate series carried out by mixing 0.1 ml of the steep liquor with liquid nutrient agar (at 40° C.). Steep-baskets were agitated before sample was taken.

[b] See Table 18 for identity of organisms used to inoculate barleys.

bacterial colony counts, obtained with whole-kernel platings, were consistently in the range of 80 to 100 bacterial colonies per 100 plated kernels. These surviving bacteria were generally white or creamy in color, although small numbers of yellow bacteria were also isolated from malt kernels. The fungi isolated from plated malt included: Alternaria tenuis, Helminthosporium sativum, and species of Fusarium, Aspergillus, Mucor, and Penicillium.

Analyses of pilot malts prepared from bright and naturally-infected (severely weathered) Montcalm barleys were made by the Barley and Malt Laboratory (Table 27). The differences in the two malts are characteristic of bright and weathered malts in several ways. The weathered sample, as compared with the bright sample, had a lower 1,000 kernel weight, yielded less extract, produced a darker wort, and showed a higher protein modification.

The pertinent analytic data for the barleys used in the rate of steeping experiments are presented in Table 28. The results from these experiments are plotted in Figure 4. The following observations are drawn from these data: (1) bright Kindred and bright Montcalm samples steeped at a slightly faster rate than the bright Hannchen kernels, (2) the order of steeping rates for the bright barleys was Montcalm: Kindred: Hannchen, while the 1,000 kernel weights taken in the same varietal order were 34.32: 33.74: 35.64, (3) the weathered Montcalm steeped at a faster rate than did the bright Montcalm, (4) the viable

Table 27. - Analyses of two pilot malts prepared from a bright Montcalm and a severely weathered Montcalm barley lot [a].

Analytic data	Bright Montcalm (1957 crop)	Weathered Montcalm (1958 crop)
1,000 kernel wt., d.b. (gm)	30.0	25.5
Moisture (%)	4.4	6.6
Extract, F.G., d.b.	75.5	71.8
Conversion (min.)	less than 5	less than 5
Color of wort (Lov. 52)	1.8	2.3
Clarity of wort	clear	clear
Wort nitrogen (%)	0.654	0.993
Malt nitrogen, total (%)	2.25	2.25
Wort/malt nitrogen (%)	37.1	44.1
Diastatic power (°L.)	200	228
Beta-amylase (Maltose equiv.)	664	773
Alpha-amylase (20°C. dext. units)	40.2	41.1
Ratio, beta/alpha amylase	16.5	18.8

[a] Malt analyses performed by the United States Department of Agriculture Barley and Malt Laboratory, Madison, Wisconsin.

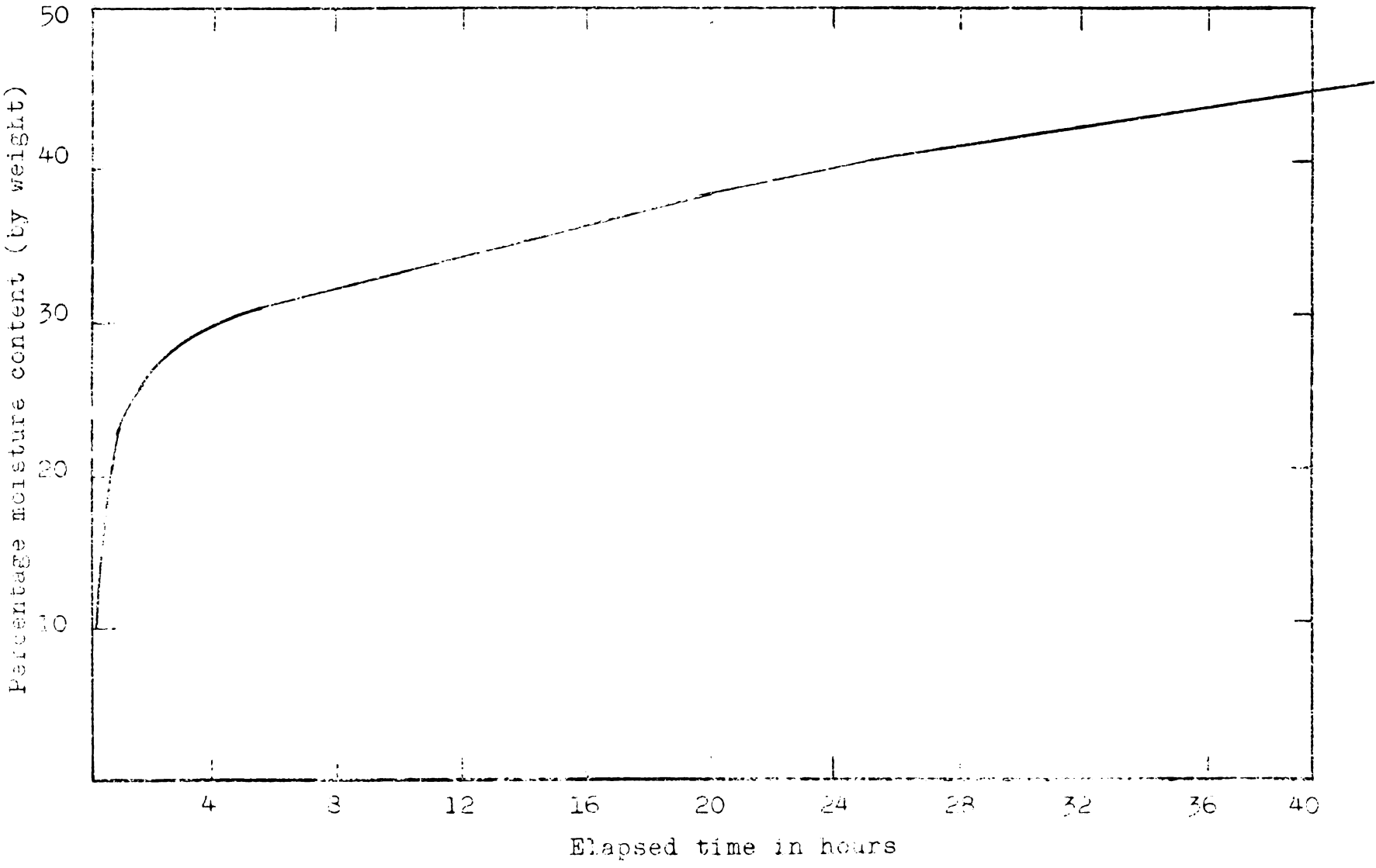
Table 28. - Biological and physical analyses of barleys used in the 'rate of steeping' experiments.[a]

Barley variety	Description of barley	Initial moisture content (%)	1,000 kernel weight (d.b.)	Germination energy (%)	Germination capacity (%)	Elapsed hours to reach 43% moisture content by weight
Kindred	Bright	10.0	33.74	99	99	32¼
Hannchen	Bright	10.0	35.64	92	93	32½
Montcalm	Bright	10.3	34.32	96	97	34½
Montcalm	Dead[b]	14.3	34.62	0	0	39
Montcalm	Weathered	9.8	33.78	84	88	24

[a] Temperature of steep water ranged from 58° to 59° F.; all barleys graded to size - held on a 6/64" sieve.

[b] Barley killed by soaking in a four percent solution of formalin for 20 minutes, draining, and aerating until fumes had been completely dissipated.

Fig. 4a. - Steeping rate of "Bright" Kindred barley.



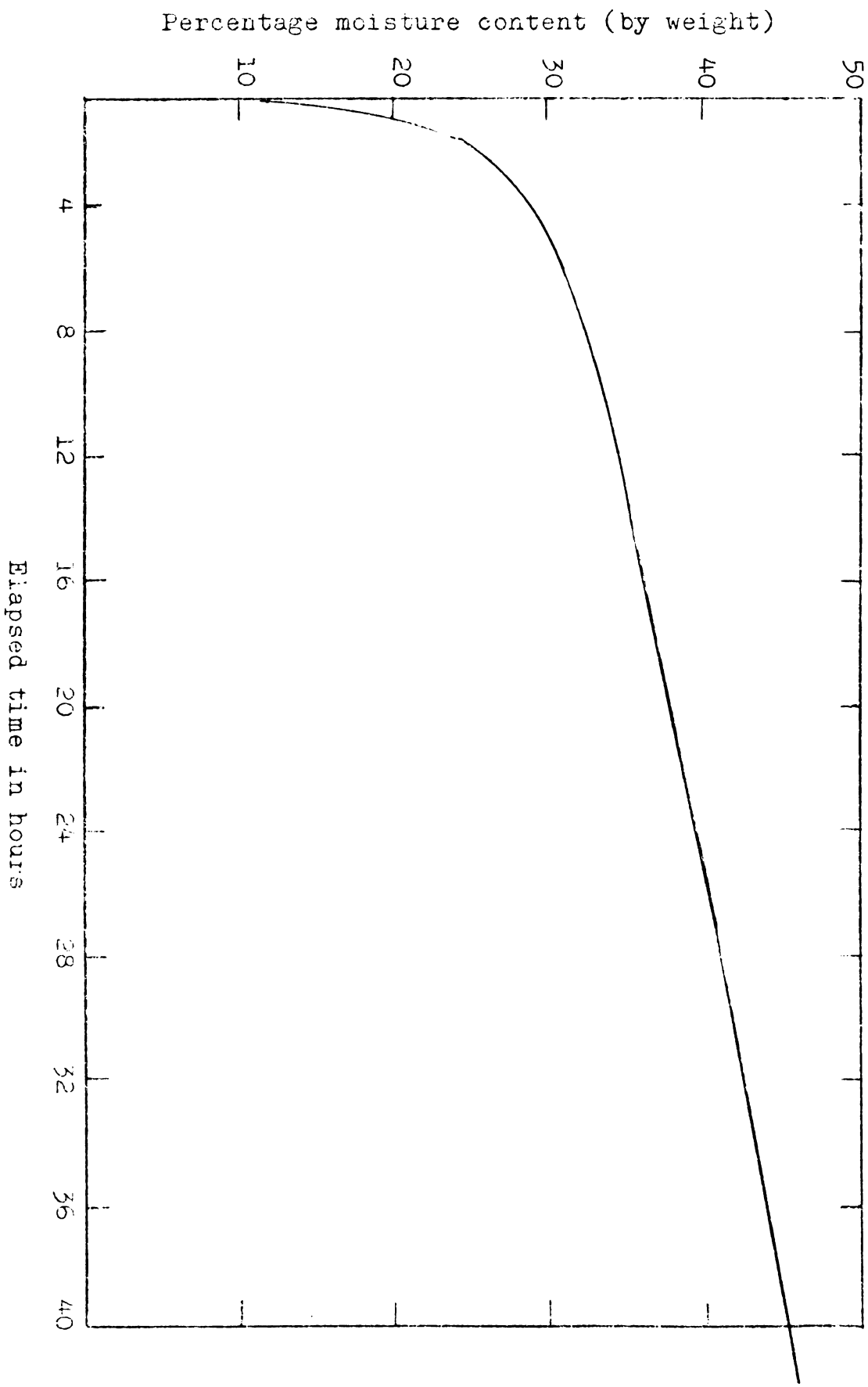


Fig. 4b. - Steeping rate of "Bright" Hannchen barley.

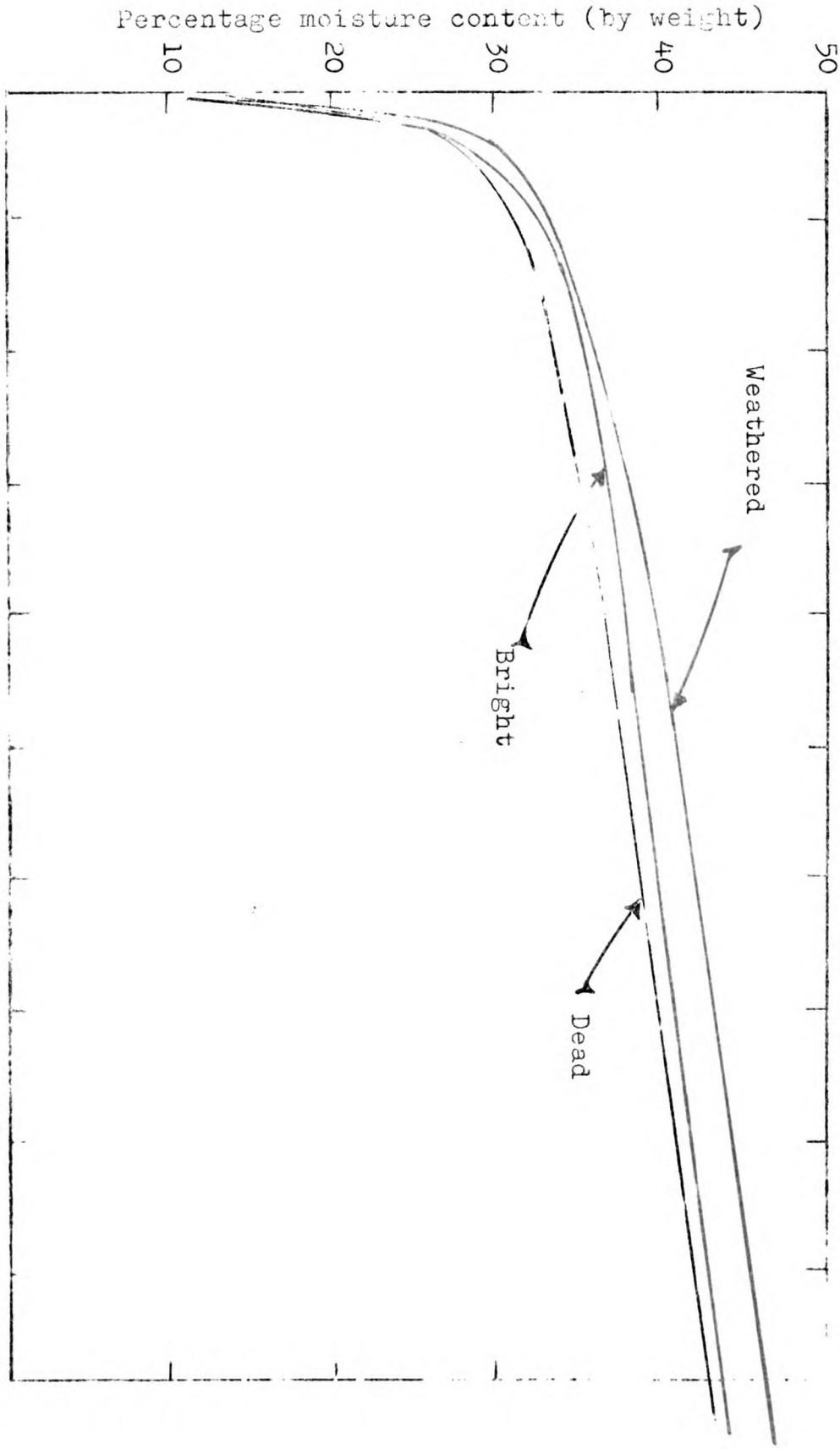


Fig. 4c. - Steeping rate of Montcalm barley.

bright Montcalm steeped at a faster rate than did the dead Montcalm, (5) the overall order of steeping rates, by variety, was: weathered Montcalm: bright Montcalm: dead Montcalm: bright Kindred: bright Hannchen, (6) in the order of increasing 1,000 kernel weights the varieties were: bright Kindred: weathered Montcalm: bright Montcalm: dead Montcalm: bright Hannchen (Figure 4).

DISCUSSION AND CONCLUSIONS

Several problems must be faced in the pilot malting of inoculated barleys. The pilot malting plant must provide an identical malting environment for replicated samples. The apparatus described in this chapter was re-designed, mid-way through the experimental series, to correct such a defect. Condensate from the cooling coils and from the walls of the germinators was unevenly distributed to the germinating pans. This excess moisture increased the moisture content of certain green malts. Consequently these malts were subjected to excessive kilning temperatures, with respect to their moisture contents. This combination of high temperatures and excessive moisture contents was responsible for a decrease in enzymatic power, caramelization, and for the production of melanoidins (351). These compounds, in turn, impart characteristics to the finished malt which may be mistaken for properties engendered by kernel microflora. These include: increased wort color (27, 212,

213, 305), malt flavor (2, 305), and the aroma of the mash (2, 305). There is some preliminary evidence that certain bacteria, e.g. Pseudomonas atrofaciens (B-210), may also increase the moisture content of infected green malt. Elevated grain temperatures were also noted with the germinating barleys infected with this organism. If these effects are not experimental artifacts, it is possible that barley infected with large numbers of certain bacteria may show increased moisture and temperature changes as a result of bacterial respiration. The bacterium cited above is a barley pathogen and is favored by high humidity and by temperatures in the range of 20° to 25° C. (53, 54). Such conditions are provided in germinating drums and compartments. The increases in grain moisture and temperature were observed most frequently in the case of grain infected by P. atrofaciens. These conditions were never found in the case of barleys inoculated with fungi.

Another problem, not completely resolved, is found in the inoculation procedures used in this study. A greater degree of kernel infection may be achieved by incubating the grain at increased temperatures and humidities after inoculation. This solution, however, is not entirely suitable since the moisture content of the grain is dangerously elevated, and germination may be seriously affected. A minimal amount of bacterial inoculum is

required since these organisms rapidly increase during steeping and germination. The fungi do not, however, show this rapid increase (Tables 25, 26).

The infection and subsequent increase of alien bacteria, presumably introduced by means of the humidified air, creates another serious problem. To properly assess the influence of inoculated microorganisms on the quality of malt, these ubiquitous bacteria must be excluded from the germinating compartments. This would necessitate the construction of an apparatus with a filtered-air system. There is evidence to show that the same types of bacteria are commonly introduced into commercial malts in a similar fashion. This evidence is adduced from kernel platings of commercial malts in which the white and creamy types (mainly species of Bacillus and Lacto bacillus) predominate over the bacterial types commonly found on unmalted barley kernels, e.g. white and yellow, gram-negative bacteria. If this is indeed the case, the occurrence of these bacteria in either the malting process or on the finished malt is perhaps not of great importance (from an analytic standpoint) since most, if not all, malts will contain a certain number of these organisms. It is also of interest to note that similar types of bacteria, viz. B-5 and B-226, showed no pronounced quality defects in inoculated malts (Tables 22, 23, 24). Indeed the only inoculated malts demonstrating consistent de-

fects were those samples infected with pathogenic bacteria and fungi, viz. P. atrofaciens, Helminthosporium sativum, and Fusarium roseum f.sp. cerealis. The occurrence of bacteria in malt kernels is of great importance to the maltster, primarily because of the population standards for distillers' malt. Evidence presented in this paper and elsewhere indicates that these bacterial populations may be the result of air- and water-borne bacteria, introduced into the malting process as a function of the malting equipment, rather than as an increase of the seed-borne bacteria of malting barley kernels. These conclusions are based on the morphological and staining characteristics of the malt-bacteria, as well as their resistance to high kilning temperatures.

A few similar quality defects were found in both inoculated malts and naturally-infected malts (Tables 22, 23, 24, 27). These defects included: reduced kernel weight and increased wort color. Additional malt defects such as increased wort acidity, greater protein modification, and reduced extract are frequently found in "weathered" malt, but were not present in the inoculated malts. The similarity of deleterious quality effects in the Hannchen samples (Table 23) and the weathered Montcalm sample (Table 27) suggests that the naturally-occurring microflora of the Hannchen lot (cf. Table 17) exerted a more pronounced effect on quality than the inoculated

organisms.

It seems that the microflora of barley kernels may exert effects on malt and beer quality in several ways: (1) as a direct effect of microorganism on the kernels, e.g. the reduction of germination by toxins or other pathogenic materials (2) as an indirect effect of the organism on the kernel, e.g. reduction of kernel substance and production of degradation products by the attendant microflora, (3) indirectly in the production of host-parasite interaction products, e.g. the pigmented material found in infected barley kernels (see Chapt. VI). To properly evaluate any or all of these possibilities, it would be necessary to infect developing barley kernels with pure cultures of specific organisms, to the exclusion of other microflora, and to rigorously malt these inoculated barleys. Such a procedure would permit the kernel-organism interactions to take place over a period of several weeks, approximating the natural infection process which occurs with weathered or stained barley kernels.

The rate of steeping experiments indicate that weathered barley steeps at a faster rate than does bright and non-viable barley of the same variety. The increased rate of steeping was not a function of kernel weight alone. The order of steeping rates suggests that steeping is not exclusively a physical process and that perhaps enzymatic solubilization of stored kernel materials may also be involved in the process.

CHAPTER VI.

OTHER STUDIES

LITERATURE REVIEW

A number of workers have demonstrated that the seeds of cereals or their attendant microflora may exert an inhibitory effect on certain microorganisms. Smith found that aqueous extracts of wheat chaff were occasionally effective in both the inhibition of seed germination and in reducing the growth of Rhizopus nigricans on shelled seeds (362). Novotel'nov and Ezhov have reported that an antibiotic, isolated from barley grains, was anti-bacterial and inhibitory to Aspergillus niger and Penicillium glaucum (292). Ark and Thompson have shown that wheat and barley seeds contain an antibiotic principle that is active against fungi and gram-positive bacteria when tested in vitro (16). Both seed-extracts and plated whole-kernels were used in this study. Tyner has postulated that quinones, formed in the embryos of wheat or barley during fermentation, may be responsible for the elimination of loose smut mycelium in the water-soak treatment of infected seed (399). Leben and others attributed the control of seed-borne pathogens, by the water-soak method, to the production of organinic acids by the normally-occurring microflora of the seed (222).

The seeds of barley, as well as the seeds of other

cereals, contain substances which, when extracted, may inhibit the germination of these seeds (90). These substances are polyphenolic compounds located, for the most part, in the tissues of the husk, pericarp, and aleurone (256, 304). A number of these inhibitory compounds have been isolated from the husks and rootlets of barley by Massart and his co-workers (256). These phenolic acids and coumarin derivatives were present in a bound state, the nature of which was not entirely understood. Kockova-Kratochvilova and Lukasova-Novotna isolated seven polyphenolic derivatives from barley steep water (214). They found that a chlorogenic acid-like compound and a "tannin" were inhibitory to barley germination.

Other workers have isolated and identified polyphenolic compounds from barley kernels, not necessarily implicated in the inhibition of seed germination. Pollock and Pool found that the barley protein, hordein, was associated with anthocyanogens (leucoanthocyanins) in barley grist but not in whole barley kernels (304). Six anthocyanogens, including cyanidin and delphinidin, were isolated from the crude hordein preparation. Mullick and others have studied the anthocyanin and anthocyanidin content of the aleurone and pericarp tissues of white, blue, purple, and black barley varieties (279). The loosely-defined group of polyphenolic compounds known as "tannins" have been discussed by Preece (314). The author

classifies the "tannins" as: (1) the hydrolyzable galloyl glucoses, (2) the non-hydrolyzable catechins and their derivatives, (3) the related leucoanthocyanins. The occurrence of these compounds in barley and other brewing materials, is dealt with in another paper by Preece (315). Ivanyi (193) and Lüer and Stauber (241) have reported the presence of tannin in the husks of barley grains. "Testinic acid", i.e. compounds of protein and polyphenolic substances, have been shown to occur in the husks of barley (212, 213, 421). Stevens has indicated that these compounds may be artefacts derived from protein and part, or all, of the husk polyphenols (378). The isolation and identification of polyphenolic substances from beer and other brewing materials has been studied by McFarlane and others (259). The authors indicated that many of the tannin-like compounds isolated in studies of this nature may be artifacts because of their susceptibility to oxidative changes. A direct method for the determination of tannins in beer and brewing materials, using ultraviolet spectrophotometry, has been developed by Owades and others (294).

The nature of the discoloration of cereal grains, caused by microfloral infection, is poorly understood. Few workers have attempted to explain this effect, and these with little success. It has been reasonably established, however, that the staining of the kernel is

due to the presence of a brown pigment, or pigments (301). Wiggans (418) and Harlan (164) have both indicated that part of the natural pigmentation of barley kernels is due to a melanin-like pigment. Wood described a brown substance, isolated from wheat kernels, which interfered with chlorophyll determinations (420). While the exact nature of the material was not determined, some of its chemical properties indicated that it was a protein-flavone complex. Lewicki conducted an early study on the pigmentation of wheat kernels (242). He found that the black pigmentation was due to the oxidation of catechol tannins and that the reaction was catalyzed by oxidases. While it was not determined whether the same chemical reaction was involved in both bacterial black chaff and in internodal melanism, the author indicated that in both cases it seemed probable that the black pigment was produced by the oxidation of a chromogen. Hagborg considered that the black pigmentation of bacterial black chaff was a host reaction against the pathogen, and that the same reaction might be brought about, independently of bacterial infection, by purely external causes such as temperature and light (317). The early literature dealing with black chaff and kernel pigmentation has been reviewed by Hagborg (156). The author found that the discoloration associated with bacterial black chaff was apparently not produced directly by the organism, since no black pigment

was produced by the bacterium in culture, nor did the black chaff lesions turn black until several days after the development of maximum areas of infection. While offering no proof, Hagborg suggested that the pigments of bacterial black chaff and internodal melanism were similar in the composition.

Johnson and Hagborg made a detailed study of wheat kernel infection by Bacillus graminis tritici and by Alternaria tenuis (203). Infection of the glume tissue by P. graminis tritici caused a hypersensitive reaction in which the parenchma cells became necrotic and a brown, melanin-like pigment was produced. Lemmas, infected by A. tenuis, revealed the presence of discolored tissue, coinciding with the extension of the mycelial growth. In the discolored tissue, the cells were charged with a yellow or brown pigment, and mycelium was either absent, or rarely present. This observation suggested the action of toxins or enzymes excreted by the hyphae and working at a distance. Since glume discoloration may be caused by parasitic action, by environment and/or genetic factors, the authors suggested that the production of melanistic pigments might be due either to phlobaphene formation by the oxidation of catechol tannins, or to the action of tyrosinase on tyrosine and other amino acids. Attempts to produce melanism by the injection of phenylalanine and tyrosine were negative however. Workers at the University of Minnesota have made water-extractions of brown to black,

viscous substances from barley, stained under laboratory conditions (9). A yellow substance was extracted from a bright barley sample using the same extraction procedure. All of the pigmented materials were inhibitory to barley germination. The degree of inhibition decreasing with the decrease in dark color. Differential adsorption of components of the pigmented substances, varying in their inhibitory properties, was accomplished with activated carbon and ion exchange resins.

The role of plant tannins and other polyphenolic compounds in host resistance to microfloral infection has been discussed by many workers. Cook and Taubenhaus demonstrated that: (1) tannin retarded or inhibited fungal growth, (2) parasitic fungi were more sensitive to the action of tannin than the saprophytic forms, and (3) as the maximum percentage of tannin at which spores will germinate was approached, the mycelium tended to become short, thick, and septate (83). Newton and others have suggested that stem rust resistance in wheat may be due to the liberation of phenolic compounds in the host cells upon entrance of the fungus (285). The authors found that certain phenolic compounds, viz. catechol, vanillin, and salicylic acid, frequently caused a reduction in infection when injected into host plants. The same compounds inhibited growth of Helminthosporium sativum and suppressed rust spore germination. The content of phenolic sub-

stances in wheat varieties was found to bear some relation to rust resistance.

A more certain example of polyphenolic host-resistance is presented by Link and Walker (234). These investigators isolated catechol from the outer scales of pigmented onions and demonstrated its toxicity to the causal organism of onion smudge. The pigmented onions were resistant to the disease, while the susceptible white varieties did not contain the fungi-toxic substance. Stapp has stated, with reference to bacterial plant diseases, that "increased oxidase activity sometimes ensues from disturbance in balance of oxidizing and reducing processes in otherwise healthy cells, manifesting itself in dark discolorations" (374). Japanese workers have shown that the polyphenol content of rice varieties, resistant to "blast", is higher than those susceptible to the disease (407). It was further demonstrated that polyphenol content of the plants was increased by infection with Piricularia oryzae and a species of Helminthosporium. The protective role of the polyphenol-polyphenoloxidase system in plant host-parasite relations has been discussed by Farkas and Kiraly (114). Farkas and Ledingham have demonstrated the presence of polyphenol oxidase and various phenolic substances in the uredospores of Puccinia graminis (113). The oxidation of various substrates and the toxicity of these oxidized materials was investigated. The possible

role of the phenol oxidase-quinone reductase system in host-parasite relations was further amplified by the authors. Kiraly has presented experimental evidence which indicates that the hypersensitive reaction of stem rust resistant wheats is associated with the activity of the polyphenol-phenoloxidase system (210). Cochrane has reviewed the literature dealing with the oxidizing enzymes of various fungi in his recent text (56).

It has been demonstrated by several workers that barley and malt contain tyrosinase (polyphenoloxidase) (160, 305, 411). Tyrosine and phenylalanine which serve as the substrates for the oxidative formation of various polyphenols including melanin also occur in barley kernels (313). The polyphenolic compounds of plant tissues are considered as a part of the respiratory chain, and as a part of the protective mechanism of plants, being powerful protein precipitants and oxidants (309). Hänsel has reported that the presence or absence of tyrosinase in barley kernels is a varietal characteristic, based on the production of kernel discoloration when the grains are steeped in a weak solution of phenol in water (160).

Several quality defects of beer have been shown to originate, at least in part, with the malt and/or the barley used in its manufacture. Two of these defects, "gushing" ("overfoaming", or "wild") beer and unstable or hazy beer, have become increasingly important in recent years.

Beer is said to "gush" when the contents are violently ejected upon opening the bottle (90). The phenomenon has been attributed to several factors, including; poor retention of carbon dioxide, the presence of particles of high surface tension, agitation, and the presence of oxygen, metals, nitrites, and various protein degradation products (90, 138). Nakamura analyzed wild beers and found higher values for gas pressure, for nitrogen-containing substances, and for acidity than for normal beers (282). The author found that some causes of gushing were related to the quality of the malt employed. Malt prepared from weathered barley has been implicated in the production of beer with gushing tendencies (3, 139). Gray and Stone reported that the widespread incidence of gushing which occurred in 1948 and 1949 presumably ceased when new malt became available (139). Thorne and Helm have stated that the main cause of overfoaming is, in all probability, due to the vibration in transport acting together with certain abnormal constituents of the beer (393). These abnormal constituents are presumably derived from the barley; and when the beer is agitated, cause the formation of innumerable minute nuclei which in turn bring about the violent evolution of carbon dioxide immediately after the bottle is opened. The authors stated that overfoaming tended to occur in epidemic outbreaks restricted in both space and time; and they con-

cluded that these epidemics were probably due to some defects in the available barley. An example is furnished by the authors to support this conclusion. A severe outbreak of gushing occurred in Europe in 1955 and was undoubtedly due to the very poor quality of the 1954 crop of barley in the affected area. The poor quality of the barley was attributed to the unfavorable weather conditions which existed at harvest. That other factors in addition to barley quality were operative was evidenced by the quantity of normal beer brewed from the 1954 barley crop. The authors arrived at the conclusion that the primary cause of overfoaming must be sought in the barley used in brewing (393).

Beer instability has been generally categorized as haze of either biological or non-biological orgin (90). Hind has sub-divided non-biological haze in the following manner: (1) protein haze, (2) pasteurization haze, (3) chill haze, (4) metal haze, (5) oxidation haze, (6) starch or dextrin turbidity, (7) oxalate haze (178). According to this worker, chill haze and oxidation haze are normally-occurring turbidities, but hazes due to metals, starch and dextrins, and oxalates constitute beer defects. Beer instability has been dealt with in a number of review papers, including those authored by; Barton-Wright (26), Napier (283), Scriban (342), Schultze-Berndt (340), and Hopkins (183). The enzymatic treatment of beers, i.e. "chill-

proofing", has been treated in a review paper by Wallerstein (412). Napier has discussed the anti-oxidant properties of ascorbic acid and its use in reducing beer instability in his review article (283).

Chill- and oxidation-hazes arise from the aggregation of colloidal particles in the beer, and these particles represent complexes of polyphenolic compounds and fragmented proteins (183). According to Scriban the bulk of the polypeptides responsible for chill-haze formation arise from the malt and barley used in brewing although other sources are known (342). The polyphenolic fraction of chill-haze derives mainly from the malt according to Schultze-Berndt (340), and Hopkins (183). Hopkins stated that the chill-haze of beer shows a tendency to darken on exposure to the air and speculated that the darkening was assoicated with the oxidation of tannin (183). Hartong has attributed the chemical variability of the components of chill-haze to the variation occurring in barley and other brewing materials (173). Preece has indicated that a proportion of the tannins is essential in beer if certain desirable characteristics e.g. flavor are to be obtained (315). While certain polyphenolic materials e.g. the antocyanogens, are more responsible for haze formation, other less harmful tannins may be converted to the harmful anthocyanogens by oxidation processes (315). Kockova-Kratochvilova and Lukasova-Novotna

have also indicated that tannins, being strong reducing agents, are easily oxidized to form dark-colored phlobaphenes which in turn form haze complexes in beer (214).

A comprehensive series of studies have been sponsored by the Brewing Industry Research Foundation on the non-biological hazes of beer (32, 159, 168, 169, 170, 171, 378). The chemical composition of chill- and oxidation-hazes of beer was determined by Bengough and Harris (32). The material was composed mainly of protein and condensed tannins which were derived from both malt and hops. Harris has discussed the separation and identification of malt and hop tannins by means of paper chromatography (168). Harris and Ricketts isolated polyphenolic compounds from malt husks and suggested that the group of compounds known as anthocyanogens, isolated from malt, make the major contribution to the formation of beer hazes (169). Stevens showed that the properties of testinic acid extracted from barley and malt, depended to some extent on the physical state of the sample (378). The author also found that testinic acid recovered from whole barley resembled typical beer hazes in composition. Hall and his co-workers confirmed an earlier suggestion that both malt and hop tannins participate in the formation of beer hazes (159). The authors also bore out earlier indications that the anthocyanogens, effective in promoting haze-formation, were possibly present in beer in bound form. Harris and

Ricketts found at least three separate groups of polyphenols including anthocyanogens, methoxylated lignin-like compounds, and esters containing simple phenolic acids in representative chill-hazes (170). The same authors reported that insoluble polyamide resins served as selective adsorbents for the anthocyanogens in beer (171). Removal of the anthocyanogens, by means of these resins, enhanced the shelf-life of the beer. The problem of beer haze, and its treatment with powdered polyamide resins, has been reviewed in a recent paper by Harris and Ricketts (172).

MATERIALS AND METHODS

Observations made on a number of barley kernels, plated on contaminated culture medium, suggested that the kernels of barley might contain a material, or materials, which exert an inhibitory effect on certain common laboratory contaminants. Consequently, the contaminant was isolated and identified as a variant of Bacillus cereus, a common soil-bacterium /[1]. Potato-dextrose agar was seeded with three concentrations of the bacterium by adding bacterial suspensions to media cooled to ca. 40°C. in a water-bath. Plates were poured with the treated medium, approximately 15 ml of medium per plate, and allowed to solidify. Bright barley kernels of four varieties; Hannchen, Traill, Kindred, and Montcalm, were surface-disinfected in the usual manner and were plated on the

[1] Identified by Miss L. Neu, Department of Microbiology & Public Health, Michigan State University.

seeded agar. A check series of uninoculated media was also plated in the same manner. Small discs, approximately 3/8 of an inch in diameter, were cut from paper toweling. These discs were saturated with the surface-disinfectant and were placed on the surface of the untreated and treated media to serve as additional checks. The plates were incubated for five days at room temperature and were examined, at the conclusion of the incubation period, for both inhibition of barley germination and for suppression of bacterial growth.

A series of experiments were conducted in an attempt to elucidate the nature of barley discoloration, especially staining caused by kernel microflora. Extractions of the pigmented material were made using distilled water and 50 percent ethyl alcohol. The material for extraction was prepared by pearling paired samples of bright and moderately-weathered barley for 30 seconds. The pearled husk material was then sieved in a nest of A.S.B.C. analytic sieves and subsequently divided into three portions: coarse, medium, and fine. Extracts of the coarse husk material were evaporated to dryness, re-suspended in 50 percent alcohol, and chromatographed uni-dimensionally on paper. Other extracts were also chromatographed in a like manner with the omission of the evaporation and concentration steps. The paper (Whatman #1) was cut into six inch strips and the husk extracts were streaked in 0.1 ml. amounts across the top of the paper strips. A descending

system was used and n-butanol, glacial acetic acid, and water (5:4:1 by volume) was employed as the solvent (44). The chromatograms were removed from the jars at appropriate times, dried at room temperatures, cut into one-half inch strips, and sprayed with a number of developing sulutions after visual and ultra-violet inspection. The developing materials used were: $FeCl_3$, ninhydrin, phthalic acid and aniline, Benedict's reagent, Na_2CO_3, basic lead acetate, lead acetate, and alcoholic $AlCl_3$. Color changes and values were variously noted with each paper chromatogram.

Evidence, obtained from several sources, indicated that the discoloration of barley kernels by microflora was an enzymatic process. A series of experiments was consequently devised to test this hypothesis. L-tyrosine was added to distilled water at the rate of 100 mgm of the amino acid to 1,000 ml of the solvent. The solution was dispensed in small test tubes, 10 ml per tube, and autoclaved for 15 minutes at 15 pounds pressure. Ten surface-disinfected kernels of barley were then transferred aseptically to each tube and the tubes incubated at room temperature for periods of from two days to one month. Check tubes were also prepared, either with or without the addition of kernels or microflora. Discoloration of the tyrosine solution and/or the steeping kernels was noted at appropriate intervals thereafter. The

solutions were read for qualitative and quantitative color changes, either visually or with a Coleman spectrophotometer (model 14) at 660 mμ. This wave-length was chosen for maximum absorption after preliminary tests were run over the entire absorption spectrum. Turbidity and precipitation was also noted in each case. This procedure was used with the 84 barley samples, furnished by the Malting Barley Improvement Association, in an effort to relate the degree of enzymatic discoloration to the variables of barley variety and microfloral populations.

Another experimental series was conducted to assess the mechanism of staining produced in the preceding experiments. Two steeping solutions were prepared; Czapek's sucrose-nitrate solution and the same medium with L-tyrosine substituted for the nitrate salt as a nitrogen source. To these solutions, surface-disinfected kernels of bright Traill barley, either with or without the presence of inoculated organisms, and the organism alone, were added in all possible combinations. Cultures of the bacterium and solutions in which non-inoculated barley kernels had been steeped were filtered through sintered glass filters. The test bacterium was added to the filtered barley solution, and surface-disinfected barley kernels were added to the filtered bacterial culture medium. Inoculated and non-inoculated kernels were ground in a laboratory burr-mill, and the grist was placed in the two test solutions. All

of the test combinations were replicated three times. At the termination of a three week incubation period, the tubes were examined for color changes, turbidity, and other effects, as noted above.

In addition to the tyrosine solution, other nitrogen compounds were tested in a similar fashion to determine whether the discoloring was exclusively a "melaninogenic" type of reaction. Using the same manipulative procedures stated above a number of organic and inorganic nitrogen compounds were tested for the production of pigmented material (Table 32). These compounds were dissolved in distilled water, in varying proportions (Table 32). Check of distilled water and L-tyrosine were also run in the series. The quality and intensity of the color of the solutions, and the solutions plus the inoculated and non-inoculated kernels were measured and recorded as previously described.

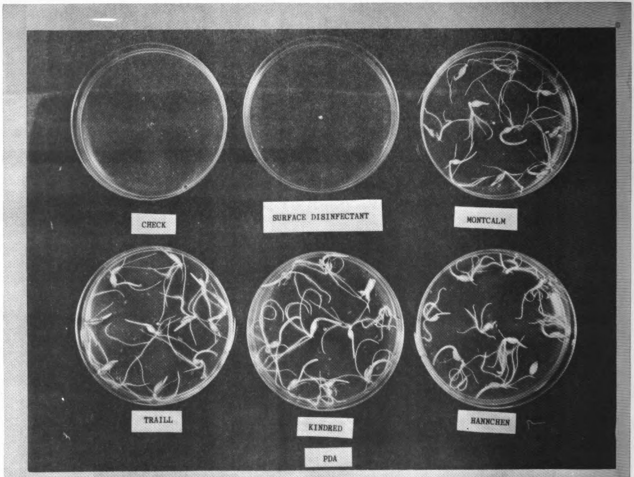
The organisms used in the above experiments as inoculum were also grown in shake-cultures on Czapek's sucrose-nitrate solution and on Czapek's sucrose-tyrosine solution. The cultures were grown in 250 ml Erlenmeyer flasks, containing 50 ml of medium per flask, on a reciprocating shaker at 25°C. for 19 days. The mycelium, spores, and cells were removed by filtration and centrifugation, and the filtrates assessed for color changes and for reaction of the media. The intensity of color was measured as the percent transmission at 660 mμ as described above.

The reaction of the media was determined with a Beckman laboratory pH meter.

EXPERIMENTAL RESULTS

The inhibitory effects of plated barley kernels on the Bacillus cereus variant are depicted in Plates XIV and XV. In two experimental trials, each with ten replications of the four varieties, the order of bacterial inhibition was Hannchen> Kindred> Traill> Montcalm (Table 29). In all cases, the suppression of barley germination was negatively correlated with the ability of the barley variety to inhibit bacterial growth. The surface-disinfectant markedly inhibited the growth of bacteria in every case. The antibacterial effect of Montcalm barley kernels was negligible, or non-existant in all experimental trials.

A brown, pigmented material was extracted from bright and stained husk preparations of Traill, Hannchen, and Montcalm barley with both water and 50 percent ethyl alcohol. The material obtained from the stained barley preparations was a darker brown color and was recovered in greater quantity than from the bright husk material. The alcohol extracts gave greater yields of the pigmented substance; however, the gummy nature of the extracts suggests that other materials, soluble in alcohol and insoluble in water, may have been removed by this procedure. A visible band of yellow to brown pigmented material, with an Rf value of 0.95 to 0.99, was observed on paper chromatograms of bright


CHECK
SURFACE DISINFECTANT
MONTCALM
TRAILL
KINDRED
HANNCHEN
PDA

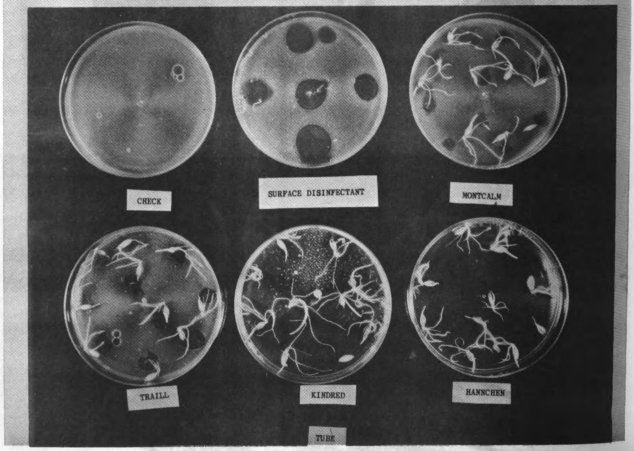
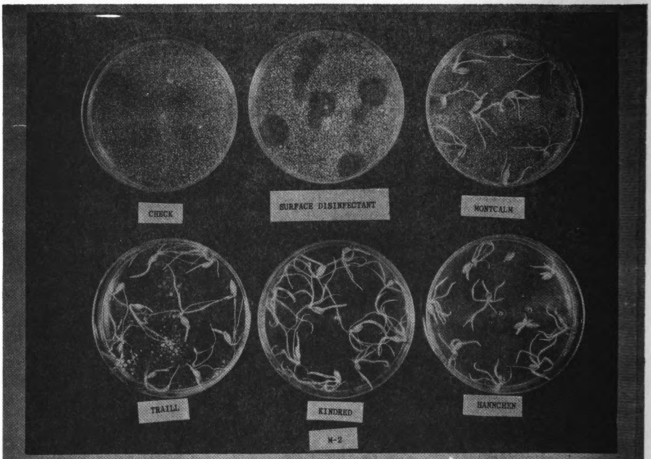

CHECK
SURFACE DISINFECTANT
MONTCALM
TRAILL
KINDRED
HANNCHEN
TUBE

PLATE XIV

Four varieties of bright western barley, surface-disinfected and plated on potato-dextrose agar; and on potato-dextrose agar seeded with different concentrations of a Bacillus cereus variant. The plates demonstrate both the inhibition of bacteria by the barley kernels and the inhibition of barley germination by the bacteria.

Upper - The four varieties plated on potato-dextrose agar check plates (no bacterial inoculum).

Lower - The four varieties plated on inoculated agar at the highest inoculum level.


CHECK
SURFACE DISINFECTANT
MONTCALM
TRAILL
KINDRED
HARNCHEN
M-2

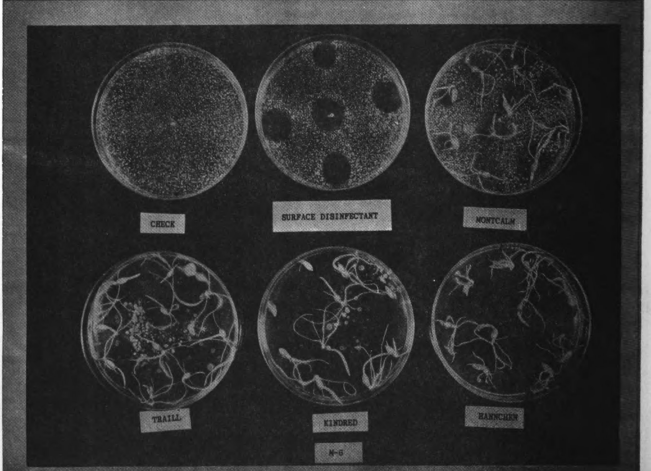

CHECK
SURFACE DISINFECTANT
MONTCALM
TRAILL
KINDRED
HARNCHEN
M-3

PLATE XV

Four varieties of bright western barley, surface-disinfected and plated on potato-dextrose agar; and on potato-dextrose agar seeded with different concentrations of a Bacillus cereus variant. The plates demonstrate both the inhibition of bacteria by the barley kernels and the inhibition of barley germination by the bacteria.

Upper - The four varieties plated on inoculated agar at the intermediate inoculum level.

Lower - The four varieties plated on inoculated agar at the lowest inoculum level.

Table 29. - The inhibition of a Bacillus cereus variant by surface-disinfected barley kernels plated on seeded agar, and the suppression of barley germination by these bacteria.[a]

	Treatment[b]	Bacterial growth[c]	Bacterial inhibition[d]	Barley inhibition[e]	Barley germination[f]
Hannchen	PDA (check)	0	0	0	80
	Tube (high)	1	4	1	80
	M-2 (med.)	1	4	1	80
	M-6 (low)	1	4	0	80
Traill	PDA (check)	0	0	0	100
	Tube (high)	3	2	2	90
	M-2 (med.)	3	2	1	100
	M-6 (low)	2	2	1	90
Kindred	PDA (check)	0	0	0	100
	Tube (high)	2	3	2	90
	M-2 (med.)	1	4	1	100
	M-6 (low)	1	4	2	70
Montcalm	PDA (check)	0	0	0	100
	Tube (high)	4	1	3	90
	M-2 (med.)	4	0	3	80
	M-6 (low)	4	0	3	80

[a] Bacteria isolated from contaminated potato-dextrose agar.

[b] See Plates XIV and XV for concentration of bacterial inoculum.

[c] Rated on a scale of 0 equals no growth, 4 equals profuse growth.

[d] Rated on a scale of 0 equals no inhibition, 4 equals complete inhibition.

[e] Rated on a scale of 0 equals no visible damage, 4 equals necrosis and stunting of shoots and radicle.

[f] As percent germinated on agar plates.

and stained husk extracts, inoculated kernels in an aqueous solution of tyrosine, and of the tyrosine solution itself. The pigmented band fluoresced under ultra-violet illumination in each case. The intensity of both visible color and fluorescence varied with the various extracts. Stained husk extracts showed greater intensity of brownness than did bright husk extracts. An unidentified material, with an Rf value of 0.25 to 0.30, was present in bright husk extracts, but absent in stained husk extracts; this material was initially colorless but gradually turned light-brown in color on exposure to air for several days. Lysine was found, in relatively large amounts, in all husk extracts. A band of material, fluorescing with a blue color under U-V illumination and with Rf values of from 0.81 to 0.94 occurred in all husk extracts and tyrosine-steep liquor but not in the tyrosine-solution chromatograms. The brown-pigmented band, in each case, was apparently phenolic in nature, but occurred in a bound form (44). The blue-fluorescing band also appeared to be a phenolic compound (s), but was not amenable to identification under these experimental conditions.

The production of a light to dark-brown discoloration of aqueous solutions of tyrosine by barley kernels, either with or without attendant microflora, was observed in a number of trials. The intensity of the brown color appears to be a function of both variety and microfloral

population. Attempts were made to correlate color production and the degree of microfloral contamination. Repeated trials with the same barley lot have indicated that a larger sample of a population of kernels is needed to ensure greater accuracy in the determination of microfloral content with this method. The use of a check variety, free of microflora, is also requisite in order to separate the relative influences of barley variety and microflora. That the procedure holds some promise is apparent from the determinations made on the 84 barley samples furnished by the Malting Barley Improvement Association (Table 30). A comparison of these values with the microfloral determinations obtained by plating these samples (cf. Appendix C) shows, in many cases, a definite correlation between the two sets of data.

Whenever bacterial turbidity occurred in the tyrosine solution, the degree of pigment formation was minimal, and conversely, whenever the intensity of the pigmentation was pronounced, no bacterial turbidity was noted. Generally barley samples showed uniform staining of the kernels in the tyrosine solution, with more intense staining of the ventral crease. In some samples, however, the ends of the kernels appeared darker than the rest of the grain. The period of incubation in the tyrosine test is also of importance since increases or decreases in color intensity may occur after some time has elapsed. The most

Table 30. - Association of the production of melanin pigments[a] by surface-disinfected barley kernels steeped in a solution of L-tyrosine for six days and the microfloral populations of these kernels as determined by standard kernel-plating techniques.

M.B.I.A. sample number[b]	Steep		Plated[e]		
	Bacterial turbidity[c]	Color intensity[d]	Number of colonies/100 kernels		
			Fungi	Bacteria	Microflora
59-4	0	91.8	44	90	136
59-7	0	86.9	66	84	151
59-9	3	90.3	74	83	159
59-14	0	93.5	80	22	102
59-16	0	92.3	59	96	164
59-22	0	88.6	55	96	152
59-24	0	88.3	103	83	188
59-25	1	90.7	101	97	199
59-26	0	91.0	108	89	197
59-27	0	88.0	112	96	211
59-28	1	91.0	97	79	176
59-29	0	89.0	88	80	168
59-30	2	89.9	95	82	178
59-31	0	88.9	107	19	126
59-32	2	91.0	105	3	108
59-34	2	87.1	113	27	140
59-35	0	88.8	7	0	7
59-37	0	89.1	7	0	7
59-38	0	91.4	5	1	6
59-39	0	89.0	13	4	17
59-40	0	89.1	6	0	6

Table 30. - Continued

59-41	0	92.5	7	0	7
59-42	0	83.0	5	21	26
59-56	0	89.0	132	26	158
59-57	0	76.1	65	26	92
59-58	0	70.0	104	37	142
59-59	0	87.5	83	43	127
59-60	0	81.0	87	26	93
59-61	0	76.6	78	17	95
59-62	0	83.1	69	9	78
59-63	0	88.9	73	83	157
59-64	0	91.6	83	86	169
59-65	0	89.2	43	89	132
59-68	2	90.2	135	33	168
59-69	1	89.8	103	73	177
59-70	2	89.9	116	76	192
59-71	0	88.3	116	94	210
59-72	1	87.9	106	24	130
59-73	1	88.0	100	68	168
59-74	2	88.2	102	86	188
59-75	0	88.1	55	23	78
59-76	1	87.9	86	21	107
59-77	0	84.8	89	3	92
59-78	0	80.6	93	17	110
59-79	0	86.9	1	0	1
59-80	0	87.6	0	0	0

Table 30. - Continued

59-81	0	85.8	0	0	0
59-82	0	84.1	4	0	4
59-83	0	84.4	0	0	0
59-84	0	70.0	0	0	0
59-85	0	71.9	2	5	7
59-86	0	80.9	7	2	9
59-87	0	86.2	4	0	4
59-88	0	76.9	7	0	7
59-89	2	88.9	24	5	29
59-91	0	73.0	116	40	156
59-92	2	88.8	111	97	208
59-93	3	89.2	107	97	206
59-94	2	89.0	104	37	141
59-95	2	89.4	111	41	153
59-96	0	78.0	127	22	151
59-97	0	91.0	0	0	0
59-98	0	91.0	2	1	3
59-99	0	90.6	0	1	1
59-100	0	91.0	1	55	56
59-101	0	90.0	0	0	0
59-102	0	89.1	4	0	4
59-221	0	85.0	2	0	2
59-223	0	89.6	4	2	6
59-224	0	78.0	5	5	10
59-225	0	89.0	6	5	11

Table 30. - Continued

59-226	0	89.9	5	3	8
59-227	1	89.5	12	0	12
59-228	0	83.9	99	57	156
59-229	0	85.4	75	11	88
59-231	0	69.5	18	1	19
59-232	0	82.9	7	9	16
59-233	0	88.2	3	1	4
59-234	0	84.8	6	0	6
59-235	0	89.9	0	0	0
59-236	1	89.7	6	1	7
59-237	0	89.0	7	0	7
59-238	0	74.1	56	19	75
59-240	0	82.1	8	1	9
L-tyrosine solution	-	99.0	-	-	-

[a] See Appendix C for description of samples, germination, and microfloral determinations.

[b] See Appendix C for identity and orgin of samples.

[c] Rated as 0 equals no turbidity, 1 equals slight turbidity, 2 equals moderate turbidity, and 3 equals heavy turbidity.

[d] Color intensity expressed as percent transmission at 660 mμ on a Coleman spectrophotometer (Model 14).

[e] See Appendix C for microfloral data.

suitable incubation period appears to be six or seven days as determined from tests which ranged in duration from two to thirty days.

Several variables were evaluated in order to determine the mechanism of pigment production in the tyrosine test (Table 31). Some discoloration was obtained without the use of tyrosine in the steeping solution. The degree of discoloration did not, however, differ significantly from the value obtained elsewhere for kernels steeped in water (Table 32). Since the staining principle of barley kernels is somewhat water-soluble, it is not surprising that a certain amount of discoloration occurs in any aqueous solution. In the tyrosine-supplemented solution, significant staining was obtained only when kernels, either with or without inoculated microflora, were present in the medium (Table 31). The addition of the organism to filtered steep liquor, or of kernels to the bacterial culture filtrate, did not bring about a significant increase in pigment production, over the color produced by the leaching out of water-soluble pigments.

The data obtained from the testing of various nitrogen compounds for their pigment-inducing properties are presented in Table 32. None of the inorganic nitrogen compounds demonstrated an ability to promote pigment formation. The decrease of light transmission in the ferric nitrate series is explained by the formation of

Table 31. - The effects of barley kernels, barley grist, culture filtrates,[a] and inoculated barley on the production of "melanin" pigments in culture media, with and without the addition of L-tyrosine.

Culture medium	Treatment[b]	Solution		Kernel	
		Color	Intensity[c]	Color	Intensity[c]
Czapek's (no tyrosine)	Check	Sl. cloudy	0	--	--
do	Non-inoculated kernels	Tannish	2	Sl. stain	2
do	Inoculated kernels	do	3	Mod. stain	3
do	Organism only	White floccu-lent masses	0	--	--
do	Grist from non-inoculated kernels	Cloudy	0	--	--
do	Grist from inoculated kernels	Yellowish	0	--	--
Czapek's (plus tyrosine)	Check	Sl. cloudy	0	--	--
do	Non-inoculated kernels	Mod. tannish	4	Mod. stain	4
do	Inoculated kernels	Mod. brown	5	Dark stain	5

Table 31. - Continued

Czapek's (plus tyrosine)	Organism only	Pinkish-tan	1	--	--
do	Grist from non-inoculated kernels	Cloudy	0	--	--
do	Grist from inoculated kernels	Cloudy	0	--	--
Barley steep liquor	Organism only	Sl. tannish	2	--	--
Bacterial culture filtrate	Non-inoculated kernels	Sl. tannish	3	Sl. stain	1

[a] Incubated in test tubes at room temperatures for three weeks.

[b] The organism used alone and as kernel inoculum was Bacillus cereus var. mycoides (B-529) (cf. Table 18).

[c] Based on a scale of 0 to 5, where 0 equals no discoloration and 5 equals dark brown pigmentation.

Table 32. - The effect of various nitrogen compounds on the formation of "melanin" pigments in solution after the addition of surface-disinfected bright Traill kernels, with and without the presence of inoculated cells of Bacillus cereus a/ var. mycoides

Identity of compound	Concentration (gm/100ml)	Color Quality c	Color Quality d	Color Quality e	Color Intensity b/ c	Color Intensity b/ d	Color Intensity b/ e	
$Fe(NO_3)_3$	26.64	orange	brown	brown	70.7	37.1	36.0	
NH_4NO_3	7.91	clear	tan	tan	100.0	95.5	95.0	
$Ca(NO_3)_2$	23.35	do	do	do	100.0	95.0	95.2	
KNO_3	20.00	do	do	do	99.0	95.8	96.0	
$NaNO_3$	16.81	do	do	do	100.0	93.0	94.3	
$Mg(NO_3)_2$	25.37	do	do	do	100.0	96.9	95.0	
Proline	0.10	do	do	do	99.5	93.2	94.0	
Hydroxy-proline	0.10	do	do	cloudy	99.7	79.5	70.7	*
Phenyl-alanine	0.10	do	do	tan	99.8	92.0	91.0	
Tryptophane	0.10	do	do	do	100.0	85.0	88.2	
"Dopa"	0.10	brown	black	brown	2.0	0.2	13.4	*
Histidine	0.10	clear	tan	tan	100.0	91.0	90.3	
Tyrosine	0.10	do	brown	brown	99.4	72.3	66.0	
Water	--	do	tan	tan	100.0	96.3	95.8	

a Stock number B-529, see Table 18.

b As percent transmission at 660 mu on Coleman spectrophotometer.

c Of solution alone.

d Of solution plus disinfected, non-inoculated kernels.

e Of solution plus disinfected, inoculated kernels. Asterisk indicates cloudiness or precipitate present.

dispersed flocculent material in those solutions containing kernels. It is suggested that the flocculation may have been brought about by iron-complexes formed with the dissolved substances leaching out from the kernels. Several amino acids demonstrated the ability to promote pigment formation; viz. hydroxyproline, tryptophane, and 2,3-dihydroxyphenylalanine (dopa). The results obtained with histidine and phenylalanine are inconclusive. None of the compounds, with the exception of dopa, performed as well as tyrosine. The spectrophotometric readings of the dopa tubes were made after three weeks of incubation. Initially this solution was clear, but the compound was oxidized, non-enzymatically, thus giving the extermely low readings. The color intensity increased initially, and as flocculent masses of a melanin-like material formed and settled to the bottom of the tubes, the solution became lighter. The clearing of this solution, containing the inoculated kernels, was determined to be the result of a limited pigment-substrate.

Microflora used in the several phases of this study were cultured in nutrient solutions with and without the addition of L-tyrosine. Since reports of melanin formation in liquid culture are scanty, the organisms were cultured for extended periods of time. The results of these cultural studies are tabulated in Table 33. The color intensities and medium reaction obtained with the standard

Table 33. - The effects of selected bacteria, fungi, and yeasts on the color and reaction of Czapek's solution, [a] with and without the addition of L-tyrosine.

Identity of organism [b]	Czapek's sucrose-nitrate solution			Czapek's plus tyrosine		
	Color	Intensity [c]	pH [d]	Color	Intensity [c]	pH [d]
Check	Clear	98.0	7.4	Clear	98.0	7.3
B-5	Yellow	89.7	7.8	do	95.4	6.9
B-210	do	73.9	8.7	Cloudy	92.9	4.3
B-218	Clear	98.8	7.3	Pink	93.0	7.0
B-226	Tannish	79.4	8.6	Yellow	87.0	4.7
B-522	Clear	92.0	4.3	Tannish	87.2	4.1
B-529	do	98.0	7.3	Pink	88.0	6.8
Y-521	Yellow	89.4	4.8	Yellow	80.9	4.4
F-304	Pink	83.0	7.4	Tannish	90.3	6.3
F-310	Clear	96.3	7.8	Tan	88.7	6.6
F-321	Yellow	94.0	5.8	Red-brown	75.0	4.9
F-324	Tannish	88.0	5.8	Pink	92.4	6.0

[a] Organisms grown in flasks on a reciprocating shaker for 19 days.

[b] See Table 18 for identity of organisms.

[c] As percent transmission at 660 mμ on Coleman spectrophotometer.

[d] Determined with Beckman laboratory pH meter.

Czapek's sucrose-nitrate medium serve as a standard by which the effect of tyrosine in the other medium may be assessed. A tan to brown color and a markedly reduced reaction was taken as evidence for the possible production of melanin-like pigments in the tyrosine medium. According to this standard, the Alternaria (F-304), Helminthosporium (F-310), and Fusarium (F-321) isolates gave some evidence of possible "melanin" formation (Table 33). The bacteria, yeast, and the Cladosporium isolate did not satisfy the above criterion.

DISCUSSION AND CONCLUSIONS

The cause of the suppression of the gram-positive, spore-forming bacteria, described in this study and elsewhere (16), is poorly understood. Evidence seems to indicate that such suppression may be the result of antibiotic production by the barley kernel. The less-inhibitory nature of the variety Montcalm is of some interest, since this variety also demonstrated a lesser degree of resistance to kernel microflora in the studies described earlier (cf. Chapt. III). It is possible that some resistance to kernel infection may be conferred by kernel antibiotics, and that the production of these antibiotics may be linked with other heritable characters, e.g. the smoothness of the lemma awn.

Chromatographic studies and the use of the "tyrosine test" have indicated that the brown discoloration

of barley kernels, usually associated with microfloral infection and/or weather-damage, is caused by brownish pigments. It further seems that these pigments are members of the so-called class of polymerized, polyphenolic compounds known as "melanins". The "tyrosine test", described in this chapter, has demonstrated that polyphenoloxidase (tyrosinase) is present in both barley kernels and in many of the commonly-occurring barley organisms. While some non-enzymatic oxidation of amino acids and various intermediates has been shown to occur, the conversion of these compounds to the "melanin" precursors is predominately an enzymatic process. When kernels, rendered free of microorganisms by surface-disinfection, are placed in aqueous solutions of tyrosine and certain other amino acids a limited amount of the pigmented material is produced. It appears that the discoloration results from both tyrosinase activity from the embryo and the leaching out of previously formed water-soluble brown pigments. The production of brown pigments, i.e. melanin-type compounds, by several of the common microflora of barley kernels, is negligible or lacking when these organisms are grown on chemically-defined media. This may be due to the lack of certain nutritional factors, precursors, and other materials in culture media. The data indicate that the production of the brown discoloration of the tyrosine solution is mainly an additive process, i.e.

that increased color-production is proportional to the quantity of enzyme or substrate present in the kernel as well as the amount of microfloral enzyme present. While phenylalanine is known to serve as a substrate for melanin production by certain microorganisms, by enzymatic interconversion, the present data suggest that other amino acids, viz. hydroxyproline, tryptophane, and perhaps histidine as well, may also be oxidized and polymerized to form melanin pigments.

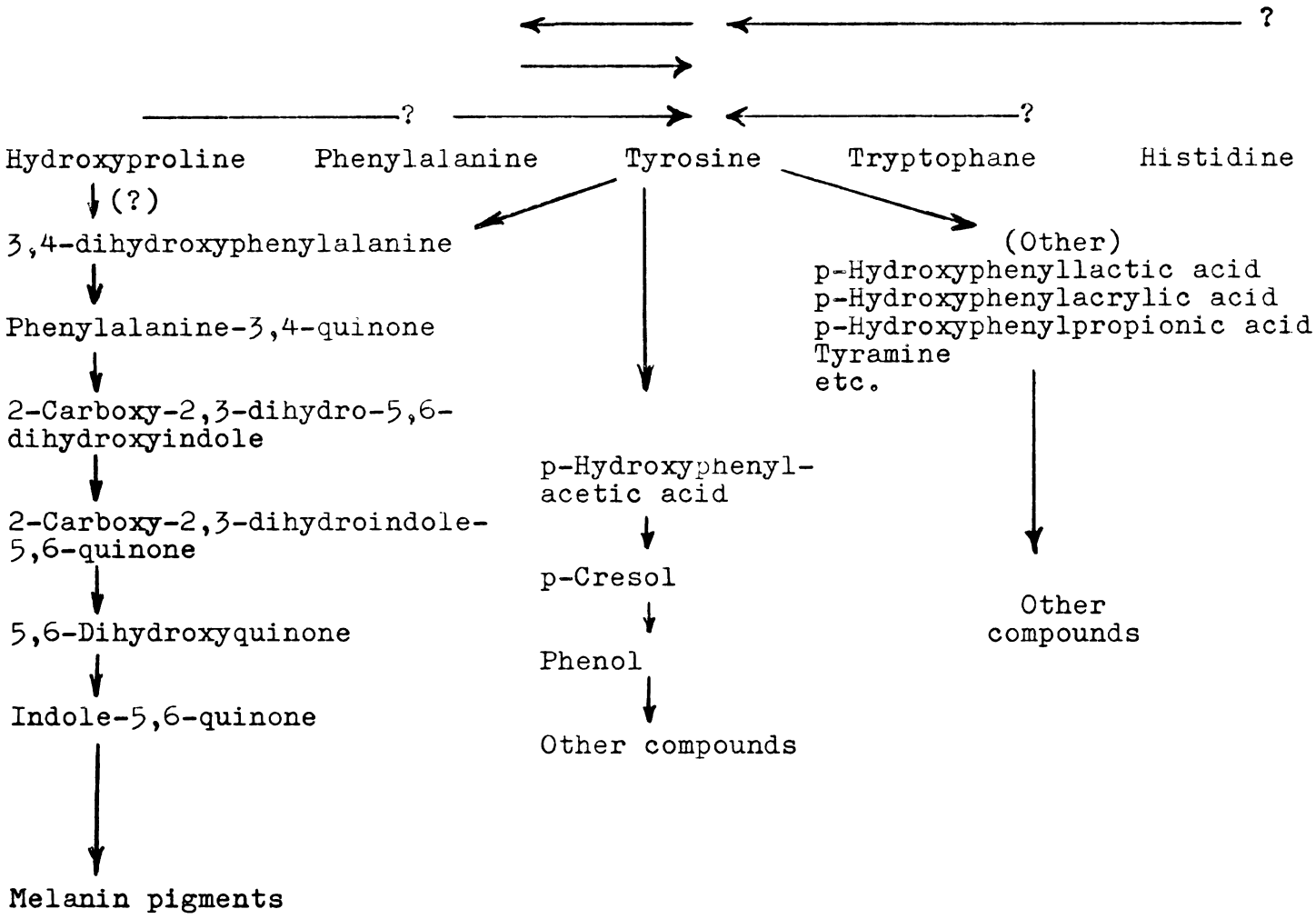
The chromatographic data, with respect to the brown pigments of the various extracts, show a pronounced similarity to the evidence obtained from the tyrosine "model". The presence of an oxidizable material in bright husks, that is absent in the stained husk-extracts, indicates that a precursor (or precursors) exist in bright barley husks; and that this material may be oxidized and polymerized, either environmentally or by microflora, to yield the staining principle found in discolored kernels. The relatively small amount of tyrosine, present in mature kernel husks need not nullify the above conclusions. Other amino acids and oxidized intermediates, as well as a variety of phenolic compounds, each capable of further chemical conversions, are present in sufficient quantity in the mature husk. Further, since staining naturally occurs some time prior to harvest, i.e. when the kernel is at a much higher moisture level and a larger quantity of free amino acids

are present in the still-living husk tissues, the problem of these materials being a limiting factor in mature kernels is obviated.

The pathway of melanin formation, drawn from these investigations and from the existing literature is presented in Figure 5. The innumerable polyphenolic compounds engendered by the enzymatic and non-enzymatic conversion of tyrosine and other ring-compounds, as well as their labile nature, creates many methodological difficulties in the identification and isolation of these materials. Increased interest in the polyphenolic constituents of barley kernels, and expecially barley husks, will undoubtedly shed further light on these problems.

It has been suggested by many investigators that the tannins, melanins, and other phenolic and polyphenolic compounds represent important defense mechanisms in plant tissues. With a few notable exceptions, this contention has received very little experimental support. In the work outlined in this section, some support has been obtained for the "polyphenolic theory of plant disease resistance". It was observed that the presence of bacteria and the degree of pigment formation in aqueous solutions of tyrosine were negatively correlated. While a few bacteria could be recovered from the sharply-discolored steeping liquor, a much higher bacterial population was always demonstrated in equal quantities of a paler solution. Bac-

Figure 5. - A proposed mechanism for melanin formation by microflora [a] on barley kernels.



[a] Taken in part from "General Biochemistry" by J. S. Fruton and S. Simmonds, 1958, Znd. Ed. John Wiley & Sons, New York, 1077 pp., illus.

terial turbidity was never observed in very-dark tyrosine solutions, but cloudiness due to the presence of bacteria was frequent in the solutions showing less discoloration. That this effect was brought about by the inhibitory influence of the "melanin" material is shown in those samples which possessed comparable microfloral populations (cf. Table 30), but differed in their degree of discoloration and in the presence or absence of bacterial turbidity. When strongly-discolored solutions of the tyrosine steep-liquor were decanted into sterile tubes, the "resistant" bacteria frequently continued to multiply at the expense of the pigmented material (and presumably the leached-out kernel nutrients). This was demonstrated by the increase in bacterial turbidity and the accompanying fading of the brown color in the solution. The rapid multiplication rate of bacteria would ensure solution turbidities in all samples in a short time were it not for the presence of some inhibitory material. Varietal differences in the ability to form the "melanin" pigments may be important in this context.

It is postulated that the staining of barley, and other cereal, kernels is brought about by an inefficient resistance mechanism. The developing grain kernel produces a limited amount of melanin pigments, by means of its inherent polyphenoloxidase and substrate content. This enzymatic process is favored by increased levels of

moisture and elevated temperatures. The same environmental conditions favor the infection process by a great number of kernel-infecting microflora; and given a suitable number of air-borne organisms, infection ensues. The interactions of favorable macro- and micro-environments, host nutrients and oxidizing enzymes, and the multiple enzyme systems of the microorganisms all favor an increased production of melanin, with concommitant staining of the husk. The greater prevalence of staining in the immediate area of the infection loci and the ramifying fungal mycelium supports this hypothesis.

The inhibitory nature of the polyphenolic pigments, which seems to be non-specific, as well as certain other physical and chemical changes in the husk of the developing kernel serve to suppress and destroy a certain portion of the invading microfloral population. The effect is, however, only partially effective since resistant strains of the microorganisms apparently exist, and the physiological condition of the host tissues, e.g. moisture content and diffusibility of both nutrients and inhibitors, is also of importance. The reported blighting and death of inoculated florets and young kernels with the attendant production of extremely dark discoloration serves to illustrate the factor of physiological-age of the host tissue. Inoculation with a relatively innocuous organism, such as Alternaria, produces an entirely different

host-reaction on young kernels, as compared to more mature grains. To expand the hypothesis it would seem that this mechanism may have other "side-effects", viz. the inhibition of host-kernel germination. Many of the polyphenolic compounds isolated from barley kernels and husks have been shown to be inhibitory to seed-germination and many of these compounds are soluble in water. The decrease in germination energy and capacity of plump, discolored kernels is commonly observed, even though the seed-borne organisms are not in close association with the embryo. While the inhibitory "action at a distance" has been variously explained as the result of toxin-formation and the production of volatile inhibitory emanations from the kernel organisms, it is suggested that the pigmented materials and their precursors produced by the kernel, may also exert such an effect. In vitro studies have confirmed the inhibitory effects of germination by extracts of the pigmented material (9, 44, 156, 205, 232, 301).

The production of "gushing" or "wild" beer has been circumstantially linked with "weathered" barley used in its manufacture. Weathered barley invariably has a high microfloral content as well as an increase in discoloration. Several features of microfloral action on the quality of malt prepared from weathered barley and inoculated barley indicate that wildness in beer may be due primarily to this microbial action. Both increased proteolysis and

wort-acidity, as well as nitrate-reduction (to yield nitrites) have been demonstrated experimentally with both malts prepared from inoculated barleys and/or physiological studies of the kernel microflora (cf. Chapters II and V). The same properties have been shown to be typical of malts engendering the wildness in beer. Whether the polyphenolic, pigmented materials of weathered barley play a role in beer wildness is questionable. If anything, they may bring about a reduction in gushing by precipitating out the proteinaceous "nuclei" and by reducing the amount of oxygen in the beer. That the latter is a possibility is demonstrated by the strong reducing nature of these compounds.

Another beer defect, related to the microflora of barley, is suggested by the problem of beer instability. It has been amply demonstrated that the conditions known as "chill-haze" and "oxidation-haze" are brought about by the presence of certain polyphenolic compounds and protein degradation products. These materials, if present in sufficient quantity in the beer, form aggregated complexes which in turn produce the aforementioned hazes. Several workers have pointed out that barley and barley malt are, to a large extent, implicated in the production of these hazes. Few, if any, of these investigators have discussed the possible role of barley microflora in this regard. While much experimental evidence, expecially

pilot-brewing studies, is lacking, some of the data reported in this thesis, as well as in other papers, indicate that beer instability may be indirectly influenced by the microflora of barley. It is postulated that many of the polyphenolic compounds, including the many intermediates in melanin formation, produced by the inter-action of kernel and infecting organism, may be carried over in the malt and subsequently into the beer. These compounds, by increasing the normal polyphenolic content of the beer, thus bring about an increase in haze formation. The increase in wort-nitrogen, brought about by weathered and inoculated barleys in the production of malt, also enhance this effect by providing more polypeptides, amino acids, and other proteolytic products with which the polyphenolic materials might combine.

CHAPTER VII.

GENERAL DISCUSSION AND SUMMARY

A number of commonly-used methods for the isolation of the microflora of barley kernels have been described (Appendix A). Several of these methods have been evaluated and new techniques have been devised (Chapt. 1). The purpose for which isolations of microorganisms are made will determine the procedure to be used by the investigator. Qualitative assessments are most rapidly made by means of whole-kernel platings using a differential series of culture media. Existing quantitative methods are not completely satisfactory. The use of suspensions of ground or macerated kernels and dilution-platings will only provide an accurate population figure if the method is rigidly standardized, by combining these techniques with an accurate qualitative method. Proponents of this method assert that it provides an accurate estimation of the total number of organisms present on and within the kernel. This is undoubtedly true, with reference to the uni-cellular bacteria and yeasts, if one considers each cell, endospore, or sporangium as a separate individual. This is not necessarily the case, however, with the fungi located within and beneath the kernel glumes. The dormant fungal mycelium occurs as a network of knotted hyphae with few, if any, spores present. The question arises - is this dormant

mycelium one individual or a collection of many individuals? Since grinding the kernel into smaller aggregates will concommitantly separate the fungal mycelium into correspondingly smaller units, each fragment is capable, in principle, of initiating a new colony on the dilution plates. There is a need for a better quantitative measure of kernel microflora. Such a method should provide an estimate of potential kernel-organism biochemical activity. This might be achieved through the use of wet or dry weight organism/kernel ratios, or perhaps rapid biochemical tests might be devised. The tyrosine test, described in Chapter VI, shows some promise in this regard.

A great number of microorganisms have been isolated and identified from barley kernels (see Appendix B). These include: 68 genera and 148 species of fungi, nine genera and six species of yeasts, 16 genera and 30 species of bacteria, and three strains of a barley virus. The fungal flora of barley kernels are better characterized than the bacteria. The bacterial flora have not been considered of great importance until recently. The difficulty of dealing with very large populations of these organisms and the arduous task of bacterial identification have impeded our understanding of the kernel bacteria and yeasts. Even a small bacterial population, e.g. 10,000 cells per gram of barley, would require some 200,000 or more separate taxonomic procedures to establish specific identifi-

cations. Thus, it is obvious then that new isolation and identification techniques will be required to increase our knowledge of these organisms.

The location of the kernel flora is related to the infection process. The greater number of organisms isolated from the middle and the awn-end of the kernel, as well as their position in the tissues of the husk and pericarp, suggests that infection and establishment of the kernel tissues is largely a fortuitous contact process. That is to say that cells, spores, and mycelial fragments will infect the developing kernel at the point that chance contact is made with physiologically-receptive kernel tissue by the air-spora. Three factors are of importance in this infection process: (1) the presence of potentially-infective cells in the air, (2) floret or kernel tissues in a suitable physiological state, i.e. possessing optimal moisture and nutritive materials, (3) a favorable macro-environment, viz. temperature and humidity (cf. Chapter III).

The microflora of barley kernels are capable of surviving prolonged periods of storage at reduced moisture levels. The fungal and bacterial populations undergo a steady reduction after the first few months of storage. The ability of non-spore-forming bacteria to withstand long periods of storage is surprising, and is perhaps related to the presence of mucilaginous capsular material as well as the presence of entrapped moisture in the

micro-environment of the glume tissue.

The infection process by bacteria and fungi was found to be similar with all varieties tested under field conditions. The time of infection, however, differed with the bacteria and fungi. Generally, the bacteria infected the developing kernels somewhat earlier than did the fungi. The increase of bacterial infection was initially rapid; with a reduction in bacterial populations occurring as the number of fungi increased above the fifty percent level (calculated as the number of fungal colonies per 100 kernels of barley). It is suggested that this relationship is related to both the physiological state of the kernel (i.e. less moisture and reduced nutritive material) and to the inhibitory effects of the attendant mycoflora. The most susceptible stage of barley kernels to infection by both bacteria and fungi has been shown to be in the late milk and early dough stages. There are indications that varietal differences to kernel infection exist in the barleys tested in these experiments. The smooth-awned variety, Montcalm, was more susceptible to kernel infection than were the other rough-awned varieties. Of greater importance is the length of time that the barley remains in the field before being harvested. Prolonging the time of harvest by even a few days may permit a significant increase in the number of infecting organisms, especially if rain falls during this period.

The most striking effect that microflora exert on infected kernels is the discoloration of the pericarp and glume tissues. Both fungi and bacteria are capable of inducing kernel discoloration; although the field fungi are more frequently associated with stained kernels than are the kernel-bacteria. Western grown, bright barley may harbor moderately large populations of bacteria and Cladosporium without being significantly discolored. These organisms may be present in the air; and when brief periods of suitable micro-environmental temperature and moisture occur, they are able to infect the developing kernels. It is further suggested that, since these suitable infection periods are short, because of infrequent rain or varying irrigation applications, the infecting organisms are not permitted to ramify through the host tissue but remain at or near the point of infection in a dormant state. The location of these organisms near the base of the lemma awn on western barleys, and the absence of kernel discoloration, support this hypothesis. The greater prevalence of Cladosporium on western bright barleys may also be due to the absence of the more aggressive fungal species, e.g. Fusarium and Helminthosporium, which may suppress the growth of the less-virulent Cladosporium in barleys grown in the more humid mid-west. (cf. Appendix C). In the case of stained barleys a high positive correlation exists between the degree of microfloral infection and the sev-

erity of the kernel discoloration (cf. Chapter IV and Appendix C).

Six-rowed, Manchurian type barleys are grown in the North Central United States. These barleys are extensively used by American maltsters for their desirable malting and brewing properties. Discoloration of these barley varieties increases concommitantly with an increase in climatic moisture, i.e. as barley cultivation moves eastward from the Dakotas (Appendix C). The correlation that exists between microfloral content of barley and its place of orgin is striking, so much so that the orgin of a barley sample can usually be determined by the organisms isolated from plated kernels of the sample. The determining factors in this close relationship are the climatic conditions, i.e. temperature and rainfall, prevalent in the growing area. An unusual situation occurs in Michigan, whereby barleys grown in the "Saginaw Valley" and "Thumb" areas of the state are typically bright, plump, and free of microflora. Malting barley produced elsewhere in the state invariably shows a high percentage of bacterial and fungal infection. That this isolated area in the state is ideally suited for the production of barley and is at the same time not favorable for kernel infection is explained by the lower day and night temperatures and lower annual precipitation prevailing there and not in other agricultural regions of Michigan[1].

[1] Personal Communication, Dr. J. E. Grafius, Michigan State University.

The prevalence of free moisture and high humidity, just prior to harvest, promotes the so-called "weathering" discoloration of barley and has been directly responsible for the westward shift in barley production. Maltsters and barley buyers have refused to buy stained barley offered for use in malting. While it has been shown that kernel staining may result as an effect of genetic - environmental interaction alone, the most influential cause of kernel discoloration is the presence of associated microflora. This conclusion is inescapable for several reasons: (1) barley grown in humid areas invariably yields large numbers of bacteria, yeasts, and fungi if plated within a year after harvest, (2) large numbers of spores, cells, and mycelial fragments of the same type of microflora encountered on cereal grains are always present in the air during the heading period, (3) the production of "germ-free" weathering has rarely, if ever, been accomplished with barley plants grown from seed to maturity, (4) the climatic conditions which favor "weathering" are also favorable to the infection and establishment of plant tissues by microflora, (5) barley grown in arid regions, under irrigation, typically are "bright" in appearance and possess little, or no, microflora.

If the term "weathering" is used to describe kernel discoloration, without implicitly or explicitly acknowledging the presence of microflora, it is a misnomer.

An alternative term, e.g. "kernel-smudge" or "microfloral stain", should be used with barleys discolored by the field fungi or by bacteria. The Official Grain Standards define damaged kernels as "... kernels and pieces of kernels of barley, other grains, and wild oats which are damaged or materially discolored (by heat or mold) by blight and/or mold, or which are heat damaged, sprouted, malted, frosted, badly ground-damaged, badly weather damaged, or otherwise materially damaged" (4). Since most, if not all, naturally-occurring kernel discoloration is caused by "mold", what constitutes "materially discolored" kernels is problematic. Only by plating a sample of the barley, can one accurately determine the genera and species of microorganisms present on, and within, the barley kernels.

The difficulties involved in the assessment of barley quality on a visual color basis are further multiplied by other observations. Kernels not obviously "black-pointed" or shriveled may support large populations of Helminthosporium and Fusarium species and "black-pointed" and/or shriveled kernels may not contain either "blight" or "scab" fungi. "Ground damaged" grain, characterized by both staining and ground odor, has been generally regarded as another form of environmental injury per se. That this condition is caused primarily by soil microflora is easily demonstrated by the isolation of these organisms from kernels which have contacted the

soil or soil-water and -particles. The "ground odor" of such grain is characteristic of the Actinomycetes which are rarely isolated from kernels not contacting the soil. The microflora of "heat-damaged" kernels frequently is made up of bacteria and members of the Mucorales and Aspergillales. Again, a reliable evaluation method for this type of damage is the kernel-plating technique used in conjunction with the pearling procedure.

Barley infected by storage fungi rarely presents a problem to malting barley buyers since the storage organisms are scarcely ever present on newly harvested and properly stored barley. While the color of barley grains gives some indication of the microfloral population present in the sample, it is less reliable as a qualitative measure. Bright barley, while possessing fewer numbers of the filamentous fungi, may contain populations of bacteria and Cladosporium equal to, or greater than, the number of these organisms in a severely stained sample. Because of the arbitrary use, and the ambiguity, of many of the terms used with reference to barley discoloration, the following nomenclature is proposed.

I. Bright
   A. Western-grown, under irrigated conditions
      1. Free of microflora
      2. Microflora present, mainly bacteria and Cladosporium

   B. Grown under enclosures, e.g. greenhouse, tents, etc.

II. Diseases
  A. Non-microbial
    1. Germ-free, controlled moisture and temperature
    2. Genetic discoloration

  B. Microbial
    1. Bacteria only, e.g. basal glume rot
    2. Fungi only, e.g. smuts, ergot, etc.
    3. Fungi and bacteria
      a. Field organisms
        (1) Blight
          (a) bacterial, viz. Xanthomonas spp.
          (b) fungal, viz. Helminthosporium spp.
        (2) Scab, viz. Gibberella and Fusarium spp.
        (3) Saprophytic or weakly-parasitic, e.g. Alternaria, Cladosporium, Epicoccum, Aerobacter, etc.
      b. Storage organisms e.g. Aspergillus, Penicillium, and certain Phycomycetes
      c. Ground damage, e.g. Actinomycetes, Fusaria spp., Bacillus spp., etc.
      d. Bear damage, e.g. Mucor, Rhizopus, and several bacterial species.

Several species of bacteria and fungi inhibit the germination of barley [illegible] (Chapter IV). This effect may also be of importance in the malting process (Chapter V). The results of [illegible] inconclusive inasmuch as [illegible] in this experimental series were [illegible] from the malting of [illegible] which did consistently show [illegible] were prepared from barleys inoculated with the barley pathogens: Helminthosporium sativum, [illegible] and Pseudomonas atrofaciens. Various [illegible] suggest that both pilot and commercial malts are subject to infection by common air-borne bacteria, especially during germina-

tion. The native barley bacteria, as well as these alien organisms rapidly increase in numbers during steeping and germination. The bacteria which infect the green malt are better able to survive the high temperatures in the kilning process. The microfloral analyses performed on the 84 samples, furnished by the Malting Barley Improvement Association (Appendix C) may be of interest when the results from the cooperative malting and brewing trials of these barleys are published.

Preliminary studies on the nature of barley discoloration by microflora have been carried out. Evidence obtained from paper chromatograms prepared from aqueous and alcoholic extracts of the husks of bright and weathered barleys have established the polyphenolic nature of the brown staining pigments. The steeping of disinfected barley kernels in an aqueous solution of L-tyrosine has shown that barley kernels as well as most of the common kernel-infecting fungi and bacteria possess tyrosinase activity. It is hypothesized that a limited amount of melanin-like pigments are produced by certain barley varieties. Infection by microflora enhances this pigment production, apparently in an additive fashion. The brown pigment possesses a limited anti-microfloral activity, i.e. it seems to represent a defective resistance-mechanism. It is further speculated that the polyphenolic nature of the staining pigment may be of importance in

the production of chill-hazes in beer. Another hypothesis is presented to account for the production of gas-instability in beer by malts prepared from naturally-infected barleys. This explanation is based on the evidence that the commonly-occurring fungi and bacteria on barley kernels are capable of pronounced physiological activity, such as the reduction of nitrates to nitrites and the degradation of barley proteins.

The relationship of barley microflora to the quality of malt and malt products is as yet incompletely understood. The mere presence of bacteria, yeasts, and fungi on barley does not satisfactorily explain many of the quality defects occurring in malt and beer. That certain organisms constitute a menace to the maltster is a truism. Most of these barley pathogens and/or storage organisms are not present, however, on bright or slightly stained barley. From these preliminary studies it would seem that future research in this general area might center on the following aspects of the problem: (1) the effects of the melanin-like pigments in infected barleys on the quality of beer, (2) the effects of the foreign microflora, introduced during malting, on the quality of the malt and malt products, (3) the development of rapid, simple, and accurate tests for the quantitative determination of kernel microflora, especially those organisms which are of commercial importance, (4) control methods for the pre-

vention or eradication of dangerous barley flora, both in the field and in the malting process.

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Appendix A. - Some methods commonly used in the determination of barley microflora.

Method used by	Description of method	References
Brown-Escombe	Disinfected kernels with 1% $CuSO_4$ - one to two hours	419
Schulow	Disinfected kernels with bromine water - 20 minutes	419
Staklasa	Disinfected kernels with 0.1% $HgCl_2$	419
Stoward	Disinfected kernels with $CuSO_4$, $HgCl_2$, CH_2O, chloroform, toluene	419
de Zeeuw	Disinfected kernels with H_2O_2, $HgCl_2$, cleaning fluid (H_2SO_4 and potassium dichromate), potassium dichromate, ammonium persulfate, bromine water - at varying concentrations and lengths of time	94, 419
Wilson	Disinfected kernels with calcium hypochlorite (ca. 2% Cl) - for periods of 7 - 1/2 to 22 hours	419
Porter	Disinfected kernels with one part 30% H_2O_2 to nine parts distilled water - five or more minutes and place on agar plates or blotters	307, 308
Porter	Disinfected kernels with Chlorox (5.25% NaOCl), dilute and use as above	307, 308
Porter	Disinfected kernels with $HgCl_2$ (1:1,000) - 5 to 15 minutes, rinse three times in sterile water, and place on agar plates or blotters	308

Appendix A. - Continued

Porter	Disinfected kernels with dilute 37% formalin (one part formalin to 240 parts water) - 5 to 20 minutes, and place on agar plates or blotters	308
Tu	Disinfected kernels by soaking in 70% ethyl alcohol - 2 to 3 minutes, burn over flame until slightly charred, and plate on agar plates	395, 396
Chen	Disinfected kernels by soaking in water - 10 to 12 hours, shake in an alcoholic solution of $HgCl_2$ (1:500), wash three times in 93% alcohol and three times in sterile water, plate on agar plates or in tubes	64, 291
Selby-Manns	Disinfected kernels by soaking in water - 8 to 10 hours, immerse in an alcoholic solution of $HgCl_2$ (1:500), rinse twice in 93% alcohol, plate on agar plates	343
Tervet	Disinfected kernels by placing in solution of 0.1% $HgCl_2$ and 0.3% Vatsol O.T.C. - 2 to 3 minutes, wash in running water - one minute, soak in solution of 0.35% NaOCl and 0.3% Vatsol O.T.C. - 3 to 5 minutes, plate on acidified potato-dextrose agar plates	385, 386, 387
Ottawa workers	Disinfected kernels by placing in three parts $HgCl_2$ solution (1:1,000) to one part 95% ethyl alcohol - 5 minutes, wash three times in sterile water, plate on soft acidified potato-dextrose agar plates	132, 144 246
Christensen-Stakman	Disinfected kernels by dipping in 70% ethyl alcohol followed by soaking in $HgCl_2$ - 2 to 3 minutes, washed in a 25-50% solution of NaOCl, plate on acidified potato-dextrose agar plates.	78

Appendix A. - Continued

Lund	Wash kernels by shaking them in sterile water, plate shakings on hopped wort agar plates with 0.25% sodium propionate added	243
Minnesota workers	Disinfected kernels by shaking in 1% NaOCl - one minute, rinse in sterile water, plate on acidified potato-dextrose agar plates	8
Shands	Disinfected kernels with a 10% solution of 3.5% NaOCl - approximately 20 minutes, plate on acidified potato-dextrose agar plates.	346
Bruehl	Disinfected kernels by immersing in a mixture of one part 95% ethyl alcohol to one part Chlorox (5.25% Cl) - one minute, plate on cornmeal agar plates	56
Whitehead	Disinfected kernels by immersing in a mixture of one part 70% ethyl to two parts Chlorox (5.25% NaOCl) - one minute, plate on potato-cerulose agar plates	415
Pepper	As above, plate on acidified potato-dextrose agar, barley agar, nutrient agar, and differential agar plates	300
Armolik-Dick-son-Dickson	Disinfected kernels in a mixture of 40$ ethyl alcohol and 2.5% sodium hypochlorite, rinse in sterile water, plate on malt-salt agar or potato-dextrose agar plates	18
Felton	Disinfected kernels by washing in 95% ethyl alcohol - one minute, transfer to a 1% NaOCl solution - 15 minutes, plate on acidified potato-dextrose agar plates	115
A.S.B.C.	Disinfected kernels by immersing them in a mixture of one part 95% ethyl alcohol to nine parts dilute commercial hypochlorite (to give 2% available chlorine) - 2 minutes, plate on malt agar or potato-dextrose agar plates	5

Appendix A. - Continued

Ottawa workers	Plant disinfected (with New Improved Ceresan) and non-disinfected seed in non-sterile soil	144, 246
Ottawa workers	Added aqueous solution of a wetting agent (ester of sulphonated bicarboxylic acid) to seed, shake, decant, centrifuge, and examine sediment under a microscope	144, 246

Appendix B. - A list of microorganisms reported as occurring on and within barley kernels.

Name of organism/[a]	Nature of organism/[b]	Occurrence/[c]	Reported by/[d]
FUNGI:			
Absidia glauca Hagem	non-parasitic	rare	(246)
A. orchidis (Vuill.) Hagem	do	do	(246)
Acremoniella atra (Cda.) Sacc.	do	do	(246)
Acremonium sp. (?)	do	do	(246)
Acremonium hordei Unamuno	do	do	(402)
Alternaria spp.	do ? implicated in staining of kernels	frequent	(9,19,20,74,77,78, 98,115,118,124, 130,142,174,262, 274,290,300,346, 352,373,385,387, 397,398)
Alternaria peglioni Curzi	do	rare	(48, 247, 328)
A. tenuis sensu Wilts.	do	frequent	(48,151,246,247, 253,300,328, 353,415,424)
Aspergillus spp.	storage deterioration	common on "sick" grain	(8,215,300,385, 387, P)
* Aspergillus amstelodami (Mang.) T. & C.	do	do	(17, 18, 330, 398)

Appendix B. - Continued

* A. candidus Lk.	do	do	(330, 398)
* A. flavipes (Bain. & Sart.) T.&C.	do	less common	(246)
* A. flavus Lk.	do	common	(17, 18, 246)
* A. fumigatus Fres.	do	less common	(208, 215, 350)
A. glaucus Lk.	do	common	(130,208,215, 246,385,397, 398,406)
* A. niger van Tiegh.	do	do	(17, 18, 130, 246, 385, P)
* Aspergillus repens (Cda.)DeBary	storage deterioration	common	(17, 330, 398)
* A. restrictus G. Sm.	do	less common	(72, 398)
* A. ruber (Brem.) T. & R.	do	do	(17, 246, 398)
* A. versicolor (Vuill.) Tirab.	do	do	(397)
Basiosporium sp.	non-parasitic	rare	(346)
Botrytis spp.	do	do	(300, 373, P)
Botrytis cenerea Pers.	do	seldom	(106, 246, 330)
Brachysporium spp.	kernel-blighting ?	rare	(74, 385)
Calcarisporium spp.	non-parasitic	do	(P)

Appendix B. - Continued.

Calonectria nivalis Schaf. (C. graminicola (Berk.&Br.) Wr.) (Fusarium nivale (Fr.) Ces. - conidial stage)	snow mold, brown foot rot, head blight	common in Scotland, not seed-borne in U.S.A.	(21, 37 389, 390)
Camarosporium umbonatum Brenck.	non-parasitic	rare	(246)
Cephalosporium sp.	non-parasitic	seldom	(P)
Cephalosporium curtipes Sacc.	do	rare	(246)
C. gramineum	parasitic on wheat	seldom	(56)
Cephalothecium spp.	weakly parasitic ?	do	(8, 9, 142, 300, 387)
C. roseum Cda. (Trichothecium roseum Lk.)	do	do	(130, 246, 330)
Chaetomium spp.	non-parasitic	rare	(246, 300, P)
Chaetomium flexuosum Pall.	do	seldom	(246)
C. funicola Cooke	do	do	(246)
Circinella aspera Lendn.	do	rare	(246)
Cladosporium spp.	implicated in staining of kernels	common	(9,142,300,352, 387,397,398)
Cladosporium herbarum (Pers.) Lk. (Hormodendron cladosporioides Sacc.)	implicated in staining of kernels	common	(33,48,79,106, 124,130,177, 215,246,274, 293,330,425)

Appendix B. - Continued

Claviceps purpurea (Fr.) Tul.	ergot	do	(100,106,111,229, 290,293,368)
Colletotrichum spp.	non-parasitic ?	rare	(246)
C. graminicolum (Ces.) Wils.	anthracnose	do	(228,293,343)
Coniothyrium spp.	non-parasitic ?	seldom	(8, P)
Constantinella sp. ?	non-parasitic	rare	(246)
Curvullaria spp.	implicated in staining of kernels	seldom	(142, 293, P)
C. geniculata (Tr.&E.) Boedj.	weakly parasitic	common	(246, 269)
C. spicifera (Bain.) Boedj. (Helminthosporium tetramera McK.)	do	do	(246,269,309)
Diplodia natelensis Pole-Evans	cane disease	rare	(275)
Epicoccum spp.	implicated in kernel staining	common	(86,142,300)
E. neglectum Desm.	do	do	(124)
E. nigrum Lk.	do	do	(330)
E. purpurascens Ehr.	do	do	(246, 352)
Erysiphe graminis DC. ?	powdery mildew	rare	(177)
Fusarium spp.	seedling blight, root rot, scab, head blight	common	(8,9,20,38,77,78, 86,98,106,115,132, 142,201,229,246, 262,300,346,352, 373,385,387)

Appendix B. - Continued

F. acuminatum Ell. & Ev.	do	do	(134, 135)
F. arthrosporiodes Sherb. ?	do	rare	(21, 134, 194)
Fusarium avenaceum (Fr.) Sacc.	seedling blight, root rot, scab, head blight	common	(21,34,132,133, 134,135,194, 201,255,330)
F. bulbigenum Cke. & Mass.	seedling blight, root rot, scab, head blight	rare	(194)
F. concolor Reink.	do	seldom	(132, 309)
F. culmorum (W.G.Sm.) Sacc.	do	common	(21,34,99,131, 132,133,134, 135,201,255)
F. culmorum var. leteius Sherb.	do	rare	(21, 327)
F. equiseti (Cda.) Sacc.	do	common	(131,132,133, 134,135,246 290,330)
F. equiseti var. bullatum (Sherb.) Wr.	do	rare	(133)
F. graminearum Schw. (conidial stage of Gibberella zeae (Schw.) Petch.)	do	common	(78,86,132, 133,134,135, 228,253,257)
F. herbarum (Cda.) Fries	do	do	(21, 255)
F. hordei	reduces malt quality	seldom	(177, 406)

Appendix B. - Continued.

F. lateritium Nees	seedling blight, root rot, scab, head blight	rare	(134)
F. moniliforme Sheld.	do	seldom	(17,18,19,132)
F. moschatum Kitasoto ?	do	rare	(130)
F. oxysporum Schl.	do	common	(132,133,134, 135,330)
F. oxysporum var. redolens (F. redolens Wr.)	do	rare	(21, 134, 135 255)
F. poae (Pk.) Wr.	do	common	(131,133,134,135 246,290,352,353)
F. roseum Lk. emend Snyd. & Hans. (includes many Fusarium spp. listed in this table)	seedling blight, root rot, scab, head blight	common	(132, 290, 300)
F. sambucinum Fckl.	do	rare	(132, 133, 134, 135)
F. sambucinum form 1 Wr.	do	do	(133)
F. sambucinum var. coeruleum (F. coeruleum (Lib.) Sacc.)	do	do	(134, 135)
F. scirpi Lamb & Fautr.	do	common	(21, 36, 132, 133, 255)
F. scirpi var. acuminatum (Ell. & Ev.) Wr. (F. acuminatum Ell. & Ev.)	do	do	(131,132,133, 194,246,290)

Appendix B. - Continued

F. semitectum Berk. & Rav.	do	seldom	(134)
F. semitectum var. majus Wr.	do	common	(132, 133)
F. solani (Mart.) App. & Wr.	do	rare	(133, 134, 135, 194)
F. sporotrichioides Sherb.	do	seldom	(132, 133, 134, 135)
Gelasinospora tetrasperma Dowd.	?non-parasitic	rare	(246)
Geotrichum spp.	do	do	(397)
Gibberella zeae (Schw.) Petch (B. saubinetii (Mont.) Sacc.) (Fusarium graminearum Schw. - conidial stage)	seedling blight, root rot, scab, head blight	common	(21,35,98,99, 106,115,174,177, 194,201,223,262, 274,290,293,308, 311,330,343,346, 385,387)
Gliocladium spp.	root necrosis (?)	rare	(P)
Gloeosporium spp.	non-parasitic	do	(246)
Gonatobotrys sp.	non-parasitic	rare	(300)
Helicostylum sp.	do	do	(300)
Helminthosporium spp.	seedling blight, root rot, leaf spot, head blight	common	(8,9,20,74,77, 78,246,297,300, 346,385,387)

Appendix B. - Continued

Helminthosporium avenae Eidam (conidial stage of Pyrenophora avenae I to & Kuribay)	leaf blotch on oats	seldom	(246)
Helminthosporium californicum Mackie & Paxton	rusty blotch	seldom	(251, 293)
H. gramineum Rabh. (conidial stage of (Pleospora graminea Died.) or (Pleospora trichostoma Noack)	stripe disease	common	(105,106,111, 153,223,228, 229,246,290, 308,323,329)
H. sativum Pamm., King, & Bakke (H. sorokinianum Sacc.) (conidial stage of Cochliobolus sativus (Ito & Kurib.) Drechsl.)	seedling blight, root rot, spot blotch, head blight	do	(73,74,78,98, 105,106,115, 118,142,143, 153,174,223,228, 229,246,262,268, 269,293,298,300, 308,311,329,352, 353,385,424)
Helminthosporium teres Sacc. (conidial stage of Pyrenophora teres (Died.) Drechsl.)	net blotch	do	(105,106,142,143, 174,228,229,246, 293,300,320,352, 353,385)
H. tetramera McK. (Curvularia spicifera (Bain.) Boedj.)	crown rot on wheat ?	rare	(246)
Heterosporium spp.	parasitic on some grasses	rare, from washing seed	(262)
Macrophomina phaseoli (Maubl.) Ash.	non-parasitic	rare	(246)

Appendix B. - Continued

Macrosporium spp.	do	do	(142)
Mesobotrys spp. ?	do	do	(246)
Microascus trigonosporus	do	seldom	(415, 416)
Monilia brunnea Gilm & Abbott	do	rare	(300, P)
Monilia sitophila (Mont.) Sacc.	do	do	(246)
Mucor spp.	storage deterioration ?	rare on quality seed	(270, 300, 385, 387)
M. christianiensis (?) Hagem	do	do	(246)
M. jansseni (?) Lendn.	do	do	(246)
M. ramanianus A. Moell.	do	do	(246)
M. sphaerosporus Hagem	do	do	(246)
M. spinescens Lendn.	do	do	(246)
M. sylvaticus Hagem	do	do	(246)
M. varians Pispek	do	do	(330)
Nigrospora spp.	do	seldom on quality seed	(9, 142, 300, 387, P)
Nigrospora sphaerica (Sacc.) Mason	Cob and stalk rot of corn	seldom on quality seed	(246, 385)
Paecilomyces varioti Bain.	Non-parasitic	rare	(246)

Appendix B. - Continued

Papularia sphaerosperma (Pers.) v. Höhn.	do	do	(246)
Papulaspora sp. ?	do	do	(246)
Penicillium spp.	Storage deterioration	Common on "sick" grain	(19,79,142,215, 300,330,346, 385,387,397, 398, P)
* P. albidum Sopp	do	rare	(246)
* P. canescens Sopp	do	do	(246)
* P. chrysogenum Thom	do	seldom	(17, 18, 246)
P. citreo-nigrum Dierckx.	do	rare	(246)
* P. citrinum Thom	do	do	(246)
P. columnare Thom	do	do	(246)
* P. cyaneo-fulvum Biourge	do	do	(246)
* P. cyclopium Westl.	do	do	(17, 18)
* P. expansum Lk. emend Thom	do	do	(246)
P. flavi-dorsum Biourge	do	do	(246)
* P. frequentans Westl.	Storage deterioration	rare	(246)
P. glauco-roseum Demelius	do	do	(246)
P. glaucum Lk.	do	do	(130,208,215,406)
* P. granulatum Bain.	do	do	(246)

Appendix B. - Continued

P. hirsutum B. & S.	do	do	(246)
* P. janthinellum Biourge	do	do	(246)
* P. jenseni Zaleski	do	do	(246)
* P. kapuscinskii Zaleski	do	do	(246)
P. matris-meae Zaleski	do	do	(246)
* P. notatum Westl.	do	do	(246)
P. obscurum Biourge	do	do	(246)
* P. purpurogenum Stoll	do	do	(246)
* P. rugulosum Thom	do	do	(246)
P. sanguineum Sopp	do	do	(246)
* P. steckii Zaleski	do	do	(246)
* P. tardum (Bain.) Thom	do	do	(246)
* P. thomii Maire	do	do	(246)
* P. urticae Bain.	do	do	(246)
Phoma spp.	Non-parasitic	seldom	(246,300,387,P)
P. glomerata Wr. & Hoch.	do	do	(246)
Phyllosticta spp.	do	rare	(330)
Puccinia anomala Rostr. (P. hordei Otth.)	Leaf rust	Spores in seed washings	(262)

Appendix B. - Continued

P. glumarum (Schm) Eriks.&Henn.	Stripe rust	rare	(64)
P. graminis Pers.	Stem rust	Spores in seed washings	(262)
Pullularia sp.	Non-parasitic	rare	(P)
Pullularia pullulans (D.&L.) Berk. (Dematium pullulans D'Bary & Löw)	do	seldom	(79, 130, 178, 246)
Pyrenophora teres (Died) Drechsl. (Helminthosporium teres Sacc. - conidial stage)	Net blotch	rare	(223, 290)
Pythium spp.	Seed and root rot	do	(246)
Ramularia sp. ?	Non-parasitic	do	(246)
Rhizoctonia solani Kühn	General parasite	do	(246)
Rhizopus spp.	Storage deterioration	Rare on quality seed	(300, 387)
R. arrhizus Fisch.	do	do	(246)
R. elegans Eid.	do	do	(246)
R. nigricans Ehr. (R. stolonifer)	do	seldom	(130, 215, 246, 362, 405)
Rhizopus tritici Saito	Storage deterioration	rare	(246)
Rhopalomyces sp. ?	Non-parasitic	do	(246)
Rhynchosporium secalis (Oud)Dav.	Barley scald	seldom	(290, 293, 360)

Appendix B. - Continued

Sclerotinia sclerotiorum (Lib.) Mass.	General parasite	rare	(246)
Sclerotium delphinii Welch	Non-parasitic	do	(246)
Septoria sp.	Leaf spots	seldom	(142)
S. avenae Fr. f.sp. triticea Johnson (conidial stage of Leptosphaeria avenae Web.)	Oat leaf blotch	do	(246)
S. culmifida Lind. (Selenophoma donacis var. stomaticola (Bau.)Spr.& Johns.)	Cereal eye spot	rare	(209)
S. nodorum (Berk.) berk. (conidial stage of Lepto-sphaeria nodorum Muller)	Leaf and glume blotch of wheat	seldom	(246)
Septoria oxyspora Penz. & Sacc. (Selenophoma donacis (Pass.) Sprague)	Cereal eye spot	rare	(290)
S. passerinii Sacc.	Leaf blotch	do	(290)
Sordaria fimicola (Rob.) C.& d'N.?	Non-parasitic	do	(246)
Sphaerulina tulasnei	Non-parasitic	rare	(79)
Sporomia sp.	do	do	(328)
Stemphylium spp.	Kernel staining	do	(300, P)
S. botryosum Wallr.	do	do	(246)
S. consortiale (Th.) Gr.&Sk.	do	do	(330)

Appendix B. - Continued

Syncephalastrum racemosum (Chn.) Schr.	Non-parasitic	do	(330)
Tilachlidium sp.	do	do	(373)
Tilletia caries (D.C.) Tul.	Wheat bunt	Spores in seed washings	(262)
T. foetens (B.&C.) Trel. (T. foetida (Wallr.) Liro)	do	do	(262)
T. hordei	Bunt	rare	(286)
T. pancicii Bub. & Ran. (Ustilago pancicii)	do	Rare, not in U. S.	(102,106,238, 286,290,293, 383)
T. texana Long	do	On Hordeum spp., not H. vulgare	(117, 290)
Trichoderma sp.	Non-parasitic	rare	(P)
T. glaucum Abbott	do	do	(246)
T. koningi Oud.	do	do	(246)
Trichoderma viride Pers. ex Fr.	Non-parasitic	rare	(246)
Ustilago spp.	Cereal smuts	common	(268, 352)
U. bullata Berk.	Head smut	On Hordeum spp., not H. vulgare	(117, 290)
U. hordei (Pers.) Lagerh.	Covered smut	common	(63,106,111, 143,223,228, 229,246,277, 290,293,382)

Appendix B. - Continued

U. nigra Tapke (U. avenae (Pers.) Rostr.) (U. medians Bied.)	Black (semi-loose) smut	do	(223,228,229, 246,277,290, 382)
U. nuda (Jens.) Rostr.	Loose smut	do	(63,64,106,143, 223,228,229, 277,290,293, 308)
Verticillum spp.	Non-parasitic	rare	(130,300,P)
V. cinnabarinum (Cda.) Reink.& Bth.	do	do	(330)
Volutella sp. ?	do	do	(246)
YEASTS:			
Unidentified yeasts	Non-parasitic	common	(9,79,300, 397, P)
Pink yeasts	do	do	(246,300,P)
White yeasts	do	do	(246,300,P)
* Candida spp.	Non-parasitic	common	(8,9,242,243, 397,398,P)
C. variabilis (Lind.) Berk. (Monilia variabilis Lind.)	do	do	(130, 246)
* Hansenula spp.	do	seldom	(242, 243)
Mycoderma sp.	do	rare	(233)

Appendix B. - Continued

Oidium sp.	do	do	(233)
* Rhodotorula spp.	do	common	(8,242,243, 300)
* Saccharomyces sp.	do	rare	(8)
S. anomalus Hans.	do	do	(233)
S. apiculatus Reess	do	do	(233)
S. ellipsoideus Hans.	do	do	(233)
* S. pastorianus Hans.	do	do	(233)
* Sporobolomyces spp.	do	common	(93, 242, 243, 300)
* Torula sp.	do	rare	(79)
* T. antennata Pers.	do	do	(246)
* Torulopsis spp.	do	do	(242, 243)
BACTERIA:			
Unidentified bacteria	Variable	common	(9,20,77,118, 142,222,246, 262,385,387)
White bacteria	do	do	(8,300,415)
Yellow bacteria	do	do	(8,300,415)

Appendix B. - Continued

	Unidentified Actinomycetes	Ground damage	Rare on quality grain	(300)
	Streptomyces sp.	do	do	(P)
*	*Achromobacter* sp.	Non-parasitic	rare	(P)
*	*Aerobacter aerogenes* (Kr.) Beij.	do	common	(392, P)
*	*A. cloacae* (Jord.) Berg. *et al.*	do	do	(392, P)
	Aplanobacter hordei Goto & Nak.	Ear burn	rare	(136)
*	*Bacillus cereus* Fr. & Fr.	Non-parasitic	common	(P)
*	*B. cereus* var. *mycoides* (Fl.) Smith *et al.*	do	do	(330, P)
*	*B. megaterium* D'Bary	do	do	(108, P)
	B. megaterium - *cereus* intermediates	do	seldom	(P)
	B. mesentericus (Fl.) Lehm. & Neum.	do	common	(108,184)
*	*Bacillus polymyxa* (Prazm.) Mig.	do	do	(P)
*	*B. pumilus* Gotth.	do	rare	(P)
*	*B. subtilis* Cohn emend Prazm.	do	common	(108,130, 184, P)
	Bacterium cerealinum (Gent.) Ell.	Bacteriosis	do	(106, 293)

Appendix B. - Continued

B. herbicola aurea Dügg.	Non-parasitic	common	(152)
* (Pseudomonas trifolii Huss. ?)			(300)
B. herbicola rubrum Dügg.	Adversely affects malting	do	(53,108,152)
B. herbicola B rubrum Lehm & Neum.	Non-parasitic	seldom	(53)
* Brevibacterium linens (Weigm.) Breed	do	rare	(P)
* Corynebacterium sp.	do	do	(P)
Erwinia rhizogenes (Rik. et al) Dows ?	do	do	(P)
* Escherichia coli Lehm. & Neum.	do	seldom	(392)
* Kurthia zopfii (Kurth) Trev. ?	do	rare	(P)
* Lactobacillus sp.	Contaminant in fermentation industry	common	(187)
* L. delbrueckii (Leich.) Beij.	do	seldom	(130,349)
* Lactobacillus leichmannii Berg. et al.	Contaminant in fermentation industry	seldom	(349)
Micrococcus tritici Prill.	Bacteriosis of wheat	rare	(111)
Pediococcus acetilacti	Beer spoilage organism	do	(349)
* (P. acidilactici Lindn. ?)			
* Pseudomonas spp.	Varies with organism	common	(330, P)

Appendix B. - Continued

* *P. atrofaciens* (McC.) Stev.	Basal glume rot	common	(110,122,258, 290, P)
* *P. fluorescens* Migula	Non-parasitic	rare	(152)
* *Xanthomonas* sp.	Varies with organism	common	(P)
* *X. translucens* (J.,J.,&R.) Dows.	Black chaff of cereals	do	(49,106,206, 207,223,228, 229,290,293, P)
* *X. translucens* f.sp. *cerealis* Hagb.	do	seldom	(413)
* *X. translucens* f.sp. *hordei* Hagb.	do	common	(157)
* *X. translucens* f.sp. *hordei-avenae* Hagb.	do	do	(157)
* *X. translucens* f.sp. *undulosa* Hagb.	do	do	(24, 361)
VIRUSES:			
Stripe mosaic virus (False stripe virus)	Barley false stripe	common	(223,290)
Two related strains of the false stripe virus	Barley false stripe	seldom	(290)

[a] Names preceeded by an asterisk are accepted as valid by the following authorities: *Aspergillus* - Thom and Raper (391), *Penicillium* - Raper and Thom (319), yeasts- Lodder and Kreger Van Rij (239), bacteria - Breed *et al.*(54). Names followed by a question mark are considered as tentative identifications.

[b] The nature of the organism is determined from the reports of the authorities cited in the last column; if no report lists the pathogenic properties of the organism, it is considered 'non-parasitic'. A question mark indicates that the nature of the organism is disputed by several authorities.

[c] The occurrence of the organism on barley kernels is determined from the reports of the authorities cited in the last column.

[d] The authorities, reporting the organisms listed in this table, are cited by number. See "Literature Cited" section for listing of these references. The citation (P) indicates that the organism has been isolated in this study.

Appendix C. - The orgin, variety, color, germination, and microflora of barley samples furnished by the Malting Barley Improvement Association and representing barley lots being malted and brewed cooperatively by industry.

M.B.I.A. Sample number	Identity of sample	Orgin of sample	Visual color score[a]	Germination[b]	Microfloral infection[c]								
					Alternaria	Helmintosporium	Fusarium	Epicoccum	Cladosporium	Bacteria	Yeasts	Storage fungi	Other
59-4	Dayton	Ohio	5	85	40	0	3	0	0	90	2	1	0
59-7	Hudson	Illinois	4 bp	95	49	0	16	1	1	84	1	0	0
59-9	Kenbar	Ohio	9 bp gr	94	61	0	10	2	1	83	2	0	0
59-14	Hudson	New York	7	93	64	1	14	0	1	22	0	0	0
59-16	Hudson	New York	6	92	42	4	9	0	4	96	9	0	0
59-22	Hudson	Indiana	4	88	36	3	4	0	0	96	1	1	11
59-24	B-114	Fargo,N.D.	8 bp	55	90	6	7	0	0	83	2	0	0
59-25	B-115	do	6 gr	83	82	8	6	0	5	97	1	0	0
59-26	B-116	do	4	74	76	13	17	0	0	89	0	1	1
59-27	B-117	do	5	96	76	14	20	0	2	96	3	0	0
59-28	B-118	do	5	90	36	55	2	0	2	79	0	2	0
59-29	B-119	do	6 bp	74	72	14	2	0	0	80	0	0	0

Appendix C. - Continued

59-30	B-120	Fargo, N.D.	7 bp	81	56	33	4	0	1	82	1	1	0
59-31	Traill	do	6	74	83	15	9	0	0	19	0	0	0
59-32	Kindred	do	4 bp gr	82	77	12	9	1	0	3	0	1	5
59-34	York	do	5	95	90	16	2	3	1	27	0	0	1
59-35	Traill	Pullman, Wash.	2	99	3	0	0	0	3	0	0	0	1
59-37	Parkland	do	3 ba	100	1	0	0	0	4	0	0	0	2
59-38	Firlbeck's III	do	3	100	4	0	0	0	1	1	0	0	0
59-39	Heines Hanna	do	3	95	8	0	1	0	4	4	0	0	0
59-40	Haisa II	do	2	100	5	0	0	0	0	0	0	0	1
59-41	Piroline	do	3	100	1	0	0	0	4	0	0	0	2
59-42	Breun's Wisa	do	3	99	4	0	0	0	0	21	0	0	1
59-56	Traill	Crookston, Minn.	10 bp	93	90	40	1	0	0	26	0	1	0
59-57	Traill	Langdon, N.D.	4	98	57	3	2	0	2	26	1	1	0
59-58	B-114	do	7 bp	93	80	3	4	1	1	37	1	0	15
59-59	B-115	do	4	93	56	8	16	1	1	43	1	1	0
59-60	B-116	do	4 bp	97	65	2	15	0	4	26	0	0	1
59-61	B-117	do	4	96	72	2	1	1	0	17	0	0	2
59-62	B-118	do	3	99	35	4	1	0	5	9	0	0	24

Appendix C. - Continued

59-63	B-119	Langdon, N.D.	3		81	60	8	4	0	1	83	1	0	0
59-64	York	do	6	bp	89	72	5	6	0	0	86	0	0	0
59-65	Tschermak	Pullman, Wash.	4		64	15	0	12	0	13	89	0	1	2
59-68	II-51-43	Crookston, Minn.	10	bp	79	103	24	6	0	0	33	0	1	1
59-69	II-51-26	do	9	bp	84	100	0	1	1	0	73	1	0	1
59-70	York	do	10	bp	83	84	20	11	0	1	76	0	0	0
59-71	Traill	St. Paul, Minn.	6		91	84	7	25	0	0	94	0	0	0
59-72	II-51-26	do	5	bp gr	86	99	1	5	1	0	24	0	0	0
59-73	II-51-43	do	5		86	94	1	5	0	0	68	0	0	0
59-74	York	do	7	gr hd?	82	99	0	1	1	1	86	0	0	0
59-75	Traill	Morris, Minn.	3		94	44	0	5	1	3	23	0	2	0
59-76	York	do	3		94	73	2	5	1	3	21	0	2	0
59-77	Traill	do	4		92	85	1	1	0	0	3	0	0	2
59-78	II-51-43	do	5		93	90	0	1	0	0	17	0	1	1
59-79	Piroline	Nezperce, Idaho	1		99	0	0	0	0	1	0	0	0	0
*59-80	Firlbeck's III	do	3		97	0	0	0	0	0	0	0	0	0

Appendix C. - Continued

59-81	Breun's Wisa	Nezperce, Idaho	3		99	0	0	0	0	0	0	0	0	0
59-82	Piroline	Grangeville, Idaho	2		99	2	0	1	0	1	0	0	0	0
59-83	Firlbeck's III	do	3		100	0	0	0	0	0	0	0	0	0
59-84	Breun's Wisa	do	3		100	0	0	0	0	0	0	0	0	0
59-85	Hannchen	Davis, Cal.	2		97	0	0	0	0	1	5	0	1	0
59-86	Cal. 1032	do	1		100	3	0	0	0	4	2	0	0	0
59-87	Cal. 6010	do	2	uk	96	1	0	1	0	2	0	0	0	0
59-88	Cal. Mariout	do	4	ba	97	1	0	2	0	3	0	0	0	1
59-89	Cal. 1096	do	3		98	16	0	1	0	7	5	0	0	0
59-91	Traill	Madison, Wis.	10	bp	76	89	11	14	1	1	40	0	0	0
59-92	Ped. 48	do	7		72	94	1	14	0	1	97	0	0	1
59-93	Ped. 62	do	8	bp	66	88	6	7	0	3	97	2	3	0
59-94	X 573-5	do	9	bp	71	90	9	5	0	0	37	0	0	0
59-95	X 691-1	do	8	bp	87	101	5	3	2	0	41	1	0	0
59-96	York	do	9	bp	69	101	14	6	4	0	22	2	0	2
59-97	A_1 Normal Lemma	Davis, Cal.	2		96	0	0	0	0	0	0	0	0	0

Appendix C. - Continued

59-98	A_1 Orange Lemma	Davis, Cal.	2	94	1	0	0	0	1	1	0	0	0
59-99	A_2 Normal Lemma	do	1	97	0	0	0	0	0	1	0	0	0
59-100	A_2 Orange Lemma	do	2	97	1	0	0	0	0	55	0	0	0
59-101	C_1 Normal Lemma	do	1	98	0	0	0	0	0	0	0	0	0
59-102	C_2 Orange Lemma	do	2	100	2	0	0	0	0	0	0	0	2
59-221	Traill	Dayton, Wash.	4	99	1	0	0	0	0	0	0	1	0
59-223	Firlbeck's III	Bozeman, Mont.	2	99	2	1	0	0	0	2	0	1	0
59-224	Breun's Wisa	do	2	99	3	0	0	0	0	5	0	0	2
59-225	Piroline	do	1	96	5	0	0	0	0	5	0	1	0
59-226	Haisa II	do	2	98	4	0	0	0	0	3	0	1	0
59-227	Svalof 50-109	do	2	99	4	0	0	0	2	0	0	3	3
59-228	X 691-1	Crookston, Minn.	9	97	90	4	3	0	0	57	0	0	2
59-229	Kindred-Traill	N. Dak. Comm. Samp.	5	94	57	11	0	0	1	11	2	0	6
59-231	Kindred	California	3	97	8	0	1	0	8	1	0	0	1
59-232	Betzes	Bozeman, Mont.	1	97	1	2	0	0	1	9	0	1	2
59-233	Alpine	Burley, Idaho	1	99	1	0	0	0	0	1	0	0	2
59-234	Piroline	Tulelake, Cal.	1	98	0	0	0	0	3	0	0	2	1

Appendix C. - Continued

59-235	Firlbeck's III	Tulelake, Cal.	2	99	0	0	0	0	0	0	0	0	0
59-236	Breun's Wisa	do	1	100	0	0	0	0	5	1	0	1	0
59-237	Betzes	do	1	100	1	0	0	0	5	0	0	1	0
59-238	Heines Hanna	do	1	97	5	0	0	0	45	19	0	3	3
59-240	Traill	do	3	96	4	0	0	0	2	1	0	1	1

[a] Color of kernels rated visually on a scale of 1 to 10, where 1 equals very bright and 10 equals severely discolored. Additional observations were made and are abbreviated as follows: bp - black point, gr - immature or green kernels, ba - blue aleurone, hd - heat damage, uk - undersize kernels.

[b] Germination of kernels determined on agar plates. The percentage of germinated kernels is somewhat lower than a blotter determination because of microfloral inhibition and damage.

[c] Number of microfloral colonies per 100 barley kernels, surface-disinfected and plated on non-acidified potato-dextrose agar.

ROOM USE ONLY

JUL 10 1962

