unstained smear preparations were examined with a phase contrast microscope. Control and treated roots were photographed under exactly the same conditions.

The rate of penetration of colchicine into the cells is important, as the cells in the outer layers of the root will be exposed to the threshold concentration for spindle inhibition before the cells in the centre. Consequently, variability is encountered between different cells from the same root, and this is especially marked in treatments of one hour or less. The actual concentration of colchicine in any cell at any one time cannot be estimated and, in the absence of data on rates of penetration of colchicine into roots, it is difficult to compare concentration effects with those for single animal cells. Problems of diffusion and penetration are serious difficulties encountered when whole tissues are used for this type of investigation.

Results.

(*a*) *The spindle in normal roots.*

In all preparations of untreated roots, normal dividing cells with clearly visible spindles at metaphase, anaphase and telophase were observed (Plate xi, A, B).

Small groups of spindle fibres were seen in some late prophase cells, but in most cells earlier than metaphase there was no indication of spindle formation. The spindle first became apparent on either side of the nucleus at the ends of the cell about the time the membrane disappeared.

Cell plates were found in various stages of formation. When the chromosomes began to lose their distinctness, cell plates appeared as a row of dark spots in the centre of the cell. These spots joined together to form a plate which extended towards the sides of the cell, frequently pushing the spindle material to the side walls and leaving a clear area in the centre.

At metaphase, the spindle was a typical double cone which increased in length during anaphase and at telophase was a narrow cylindrical structure separating the two new nuclei, which were then at the ends of the cell. The spindle regained its original cone shape as the cell plate formed across the cell (Darlington, 1937).

At telophase the spindle appeared to be quite separate from the reforming daughter nuclei, and there was a clear area without fibres at each end of the spindle next to each telophase nucleus.

(*b*) *Treatment with 0·1% colchicine.*

No abnormalities were observed in the cells of roots treated for 5–10 minutes. After 15 minutes' treatment, most of the cells were unaffected and normal cells with well-developed spindles were numerous. In the affected cells, abnormalities occurred in the usual regular arrangement of the chromosomes at anaphase and telophase, though these cells had visible spindles. In some telophase cells, where no spindle could be observed, the two daughter nuclei which normally move to either end of the cell were closer together towards the centre.

After 30 minutes' treatment there were few anaphase and telophase stages. A few of these had spindles, but in most, the spindles had disappeared from the cells. In some abnormal anaphase stages, no spindle was visible but the chromosomes were separated into two groups which were closer together and lacked the regular V-shape and uniform arrangement of normal anaphase chromosomes. Late telophase stages without spindles or cell plates, binucleate cells and dumbbell-shaped interphase nuclei were observed (Plate xi, E–H). No normal metaphase cells were found after 30 minutes' treatment. Metaphase chromosomes were short and thick and scattered at random in the cytoplasm but no spindles were seen in these cells. Prophase was also affected as prophase chromosomes were shorter and thicker than normal.

Blocked metaphases, and prophases with thick chromosomes, were found in the cells of all roots treated longer than 30 minutes, but no spindles (Plate xi, C, D). Binucleate cells, dumbbell-shaped interphase nuclei, abnormal metaphase, telophase without spindles but with chromosome groups close together in the cell were observed up to 60 minutes (Plate xi, G). Division of the centromeres to give a tetraploid chromosome number

was not found in any cell during the first hour of treatment. However, colchicine treatment for longer than one hour produced the well-known sequence of events where polyploid cells are the result of chromosome division without chromosome separation.

This continued succession of prophase, metaphase and then interphase, without any visible spindles, demonstrates that colchicine prevents spindle formation and the result is blocked metaphase with the complete absence of anaphase and telophase.

These results also indicate that the immediate effect of colchicine on dividing cells is on spindles already formed in metaphase, anaphase and telophase. The "untidiness" noticed in anaphase and metaphase after 15 minutes is probably the first indication of a vanishing spindle. This is supported later by the occurrence of anaphase and telophase without spindles but with chromosome groups close together in the cells.

Dumbbell-shaped interphase nuclei probably result from the destruction of anaphase and very early telophase spindles and binucleate cells result from telophase cells where the nuclear membrane is formed or forming when colchicine enters the cell.

The hyaline globule observed by Gaulden and Carlson in animal cells when the spindle disappeared, was not seen in any cells of treated root tips. The fixative used in these experiments was extremely acid and it is possible that the globule could have been formed and either destroyed, or masked by the granulation of the cytoplasm.

(*c*) *Recovery after treatment for one hour.*

The effects of colchicine persisted for at least 72 hours in cells of roots transferred to water after treatment, but there was a gradual return to the normal cell division process during that time. In the first hour in water, the centromeres of blocked metaphase cells divided forming tetraploid cells with daughter chromosomes lying parallel in the cytoplasm. Some irregular dumbbell-shaped nuclei were seen in early prophase but there were no anaphase or telophase stages and no spindle formation. Ten to fifteen hours later the cells looked very much the same as at one hour, and no cells had passed metaphase which was still "blocked".

After about 19 hours of recovery, however, some cells showed spindles, but most of these were in abnormal cells with multipolar spindles, as described by Levan (1938). These cells would have been unbalanced and would probably have degenerated.

In cells of roots in water for 23 hours, spindles were clearly visible in tetraploid (Pl. xi, M) and diploid cells at metaphase, anaphase and telophase, all of which appeared to be perfectly normal. Cell wall formation was observed in all roots where spindles were formed again. Some prophase cells appeared to be quite normal but prophase with thickened chromosomes persisted to about 19 hours' recovery (Plate xi, J).

These recovery results show that colchicine does no permanent damage to the spindle mechanism which is restored to treated cells when the colchicine is removed, but they do suggest that recovery occurs more slowly than inhibition. The significance of this point will be discussed later.

Discussion.

The results described in this paper confirm the observations of Levan and others that colchicine prevents spindle formation in plant cells. In addition, the results show that colchicine destroys spindles already formed at metaphase, anaphase and telophase. This latter observation has not been reported previously for plant cells, although Gaulden and Carlson (1951) showed a similar result for animal cells.

Cells treated before metaphase, which is the earliest stage obviously organized by the spindle, did not form a normal metaphase plate. The complete absence of a spindle resulted in a blocked metaphase. Centromere division in these cells was followed by the formation of a tetraploid interphase nucleus, as anaphase separation did not occur. These effects indicate the usual sequence of events in colchicine treated material.

When the metaphase spindle was destroyed, the chromosomes clumped together in the cell. These probably form a typical blocked metaphase later, but that would be difficult to ascertain in a multicellular tissue in which blocked metaphase cells produced from suppression of the pre-metaphase spindle are numerous.

The results of spindle suppression in cells at later stages than metaphase are visible abnormalities in the interphase nuclei. When anaphase cells were treated, the two chromosome groups moved towards the centre of the cell and were finally incorporated in one large, irregular, frequently dumbbell-shaped nucleus. Sometimes one or more chromosomes become separated from the chromosome group and form micronuclei. Early telophase stages blocked by colchicine produced the same result as spindle destruction at anaphase. If the membranes of the daughter nuclei were initiated before the spindle disappeared, that is if late telophase was treated, the result was a binucleate cell but the two nuclei usually occupied the centre of the cell.

Effects on pre-metaphase stages were more conspicuous in colchicine treated material than those on later stages, because the absence of a spindle does not prevent new divisions. In colchicine cells continue to come into prophase and pass through blocked metaphase to interphase, but all anaphase and telophase stages soon disappear from treated material and are not replaced. The duration of the cell division stages has been worked out for pea roots at 20°C. (Brown, 1950) where it is about 5 and 13 minutes for anaphase and telophase respectively. Barber (1939) measured the rate of division in Tradescantia staminal hairs and found that anaphase took 25 minutes and telophase 4–7 minutes at 25°C. In both plants the total time for anaphase and telophase is half an hour or less. As we have shown, 0·1% colchicine affects the spindles of all dividing cells in a root tip in one hour, so that abnormal cells produced by the destruction of spindles in anaphase and telophase cells would not be found after 1½ hours' treatment. Probably this is why earlier observations on colchicine treated plant cells have not shown that stages later than metaphase could be affected. Abnormal interphase nuclei persist, but these are easily overlooked among the recurring blocked metaphase cells. Levan (1938) examined root tips 7–30 minutes after the beginning of treatment and observed anaphase chromosomes which remained in two groups and were later included in one large nucleus. This can be explained by the removal of an anaphase spindle.

Barber and Callan (1943) have suggested the abnormalities induced in dividing cells of newt can be explained as the inactivation by colchicine of the centromere or the centrosome or both. They postulate inactivation of the centromere only to give "exploded" metaphase, of centrosome only to give "star" metaphase and of both centromere and centrosome to give complete spindle suppression resulting in "prophase" —metaphase and "ball" metaphase. Gaulden and Carlson have shown that "star" metaphase is formed during the destruction of fully formed spindles by high concentrations. Abnormalities grouped as "unorientated" metaphase by Barber and Callan could be the result of lack of spindle formation. This would explain why Barber and Callan found mainly unorientated metaphases in colchicine treated material as "star" metaphases would be produced only as long as anaphase and telophase were being affected and would quickly be replaced by unorientated metaphase as colchicine prevents spindle formation in new cells coming into division. It is possible that centrosomes or centremeres or both are responsible for the organization of the spindle, and it is also possible that colchicine inactivates these cell centres, but it does not seem necessary to postulate degrees of effect on these centres to produce the different abnormalities.

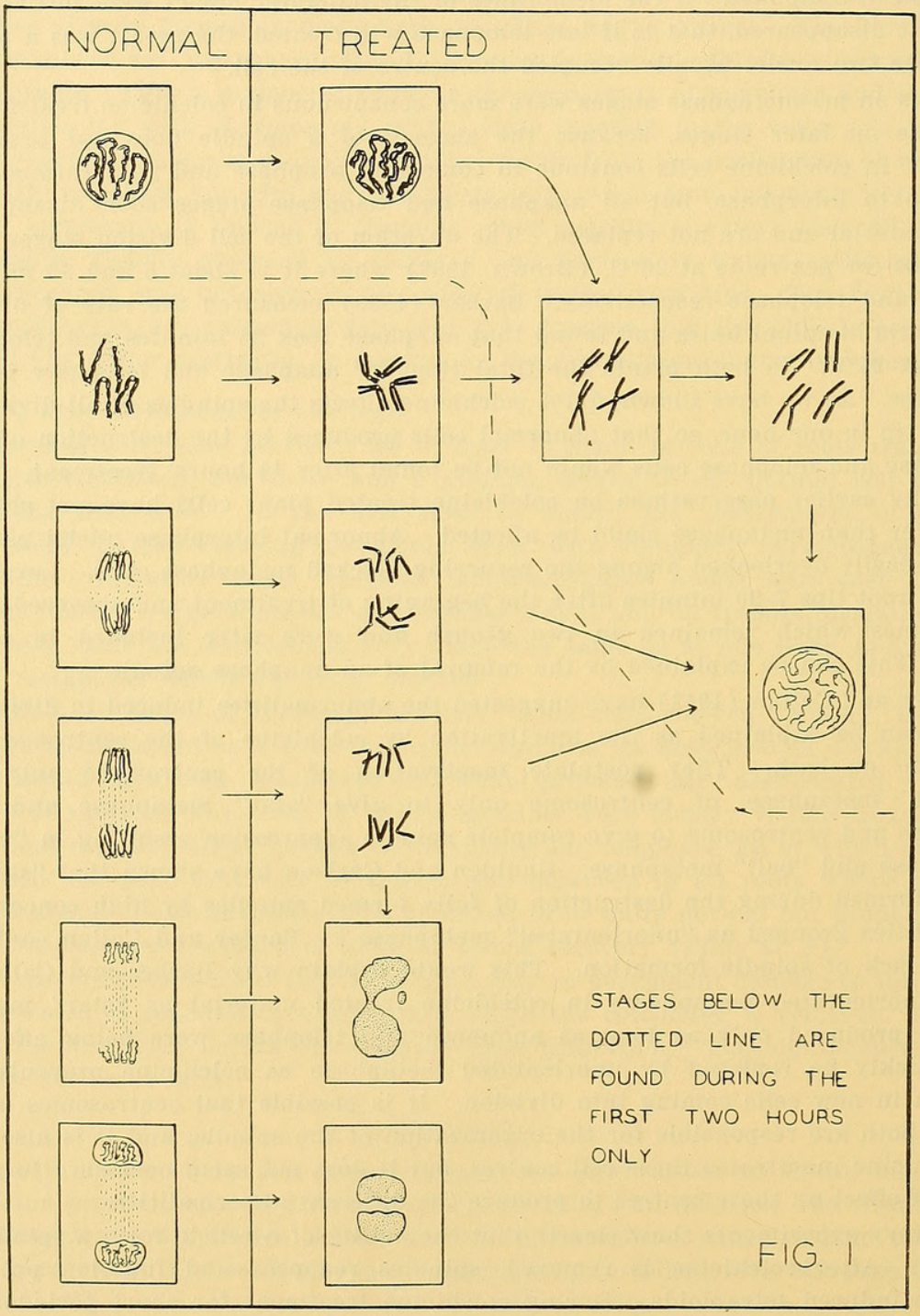
Recovery experiments show clearly that the ability of a cell to form a spindle is not destroyed. After colchicine is removed, spindles reappear and function properly in colchicine induced tetraploids. During colchicine treatment for short periods chromosomes undergo a division cycle without a spindle, so that in recovered cells the spindle lags one division behind the chromosomes.

Spindle suppression is seen in all dividing cells one hour from the beginning of treatment, but spindles are not reformed for about 16 to 20 hours after colchicine is removed. There are a number of possible explanations of this time lag.

(1) Washing in water is not efficient enough and a very low concentration of colchicine might maintain induced abnormalities in the cells.

(2) Colchicine may be adsorbed on to sensitive centres of the cell, perhaps the centromeres, and not readily removed by washing but slowly utilized in the cell.

(3) There is no observable effect on interphase nuclei in colchicine treated material, but neither is there any real evidence about the time of initiation of the spindle, nor about the earliest point at which spindle suppression can occur. Cells treated during a division cycle do not reform a new spindle when colchicine is removed until they have passed through an interphase. Perhaps spindle suppression occurs at late interphase; this could explain why colchicine effects are still visible after 19 hours' recovery time.



Text-fig. 1.—Abnormalities resulting from the destruction of the spindle.

The time of such an action on interphase cells could not be accurately estimated without more information on the duration of stages in colchicine. As some diploid cells result after one hour's treatment the effect must be postulated in the later part of interphase, leaving cells in early interphase unaffected. This possibility would mean that the effect of colchicine is the same on all stages of division rather than one action on pre-metaphase stages and another on anaphase and telophase spindles. If spindle formation begins in interphase then the effect of colchicine is to destroy the spindle from the time of its initiation to telophase.

The nuclear material of cells in which the spindle is destroyed moved towards the centre of the cell. Apparently at anaphase and telophase in normal cells the spindle overcomes the forces which hold the nucleus at the centre of the cell, and keeps daughter nuclei apart until the new cell plate is formed. If the spindle is destroyed the original forces predominate once more and the nuclear material takes up a central position.

A diagrammatic representation of the effect of colchicine on cells in all stages of mitosis is shown in Figure 1.

Cell wall formation is prevented by colchicine, probably as a result of spindle suppression. No cell plates are formed if the spindle disappears at telophase. When the cell recovers, cell plates are formed after the spindle reappears, but not before, and a cell which becomes binucleate during treatment remains in that condition till it divides again during recovery. The observation that numerous cross walls form in recovered colchicine treated cells supports the view that cell plate formation is dependent on the spindle. The division figures of polyploid cells produced by treatment are frequently too wide to fit in the cell in the usual way, so they turn length-wise or from corner to corner in the cell. The cell plate in these cells forms across the spindle wherever it may be, not in the normal position across the cell.

In addition to altering the normal arrangement of chromosomes, colchicine affects their contraction since prophase and metaphase chromosomes are shorter and thicker than in normal cells. Two suggestions have been made to explain this aspect of the colchicine effect. Levan and Ostergren (1943) believe that colchicine, by destroying the spindle, prolongs metaphase and as a result spiralization continues for a longer time. This means thicker chromosomes are secondary effects of spindle destruction.

Ostergren (1944) rejected this explanation because he found thick chromosomes in prophase as well as metaphase, and also that chromosome contraction began at lower concentration than spindle suppression. He observed thickened chromosomes in some cells with normal or nearly normal spindles and concluded from this that colchicine must have a direct effect on the spiralization process. It is difficult to decide between these two views, especially since thickened chromosomes have been observed during this investigation in cells at anaphase after the spindle has been destroyed by colchicine. There seems to be some evidence to indicate a slowing down of some stages of the cell division process by colchicine. Metaphase is longer than normal when colchicine removes the spindle (Levan 1938; D'Amato 1948) and Barber and Callan (1943) found blocked metaphase chromosomes in swollen vacuolated newt cells treated with colchicine. Vacuolation normally occurs at the end of anaphase. Gaulden and Carlson have shown that colchicine not only prolongs metaphase but also prophase; so that extra spiralization at prophase could be due to a prolonged division with chromosome contraction proceeding at the normal rate. However, it is not clear how this could account for thicker chromosomes at anaphase.

All the observed irregularities indicate a disorganized cell division mechanism and can be explained by the absence of the spindle. The spindle appears to be responsible for metaphase plate formation, anaphase separation, holding the chromosomes apart at anaphase and telophase and for cell plate formation. Possibly it controls also the timing of the cell division process. In other words the spindle is responsible for the organization of the cell division and when it is removed the chromosomes continue their part in the division cycle but without the usual organization. It should be stressed that the formation of a tetraploid nucleus during colchicine treatment is not a reversal of any part of the division process, but a forward sequence of events following the normal path as closely as is possible without the spindle.

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EXPLANATION OF PLATE XI.

All photographs × ca. 350.

A, B.—Normal cells showing spindles. C, D.—Abnormal metaphase. Treated for 30 and 45 minutes with colchicine. E, F.—Colchicine treated telophase. E shows a partly developed cell wall but the spindles have been destroyed in both cells. G.—Two nuclei close together in the cell after colchicine treatment for 45 minutes. H.—Abnormal interphase nuclei—a dumb-bell-shaped nucleus and a binucleate cell. Thirty minutes' treatment. J, K.—Prophase in irregular nuclei after one hour in colchicine followed by 19 and 48 hours' recovery respectively in water. L.—Prophase showing early development of the spindle on either side of the nucleus. One hour in colchicine and 48 hours in water. M.—Spindle and cell plate in colchicine induced tetraploid telophase. One hour in colchicine, 48 hours in water.

A STUDY OF THE MICROFLORA OF WHEAT GRAINS IN NEW SOUTH WALES.

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[Read 26th November, 1952.]

Synopsis.

The microflora of surface sterilized wheat grains from 49 samples harvested in 1949 and 1950 was examined by means of plating tests. Germination tests in soil and brief non-quantitative examinations of the surface flora were also carried out.

Bacteria and *Penicillium* spp. constituted a large part of the surface flora, while *Alternaria* spp. were the fungi most commonly isolated from surface sterilized grains, exceeding the total of the other organisms isolated by almost three times. Of the other organisms isolated, only *Septoria nodorum*, *Helminthosporium sativum* and *H. tritici-repentis* are known to be pathogenic to wheat, and of these only *H. sativum*, which was isolated from approximately 0·5% of the grains, has been observed to cause a seed-borne disease. *Fusarium* spp. were isolated only very rarely and these isolates were not pathogenic to wheat under glasshouse conditions.

Studies were made of the distribution of mycelium within the grain, and of the internal floras of various atypical types of grain, viz., pinched, mustard, pink and black-pointed grains. A marked association was observed between a pink discoloration of the grain, and the presence of *H. tritici-repentis* within it. *Alternaria* spp. were isolated from a greater proportion of black-pointed grains than from apparently normal ones, but further work is needed to establish the cause of this condition as it occurs in New South Wales.

The factors affecting the population present and the economic importance of the microflora are discussed.

Introduction.

Numerous organisms have been recorded on and in wheat grains from all parts of the world. The literature relating to this subject is extensive and in this study is reviewed as briefly as possible, an attempt being made to discuss the various aspects in the light of results obtained from investigations on grain samples from New South Wales. The work is concerned mainly with organisms isolated from surface sterilized grains.

The aims of the study were

1. to examine the microfloras of samples from the 1949 and 1950 harvest, noting differences, if any, between samples from different parts of the State;
2. to observe any relationships which might exist between the organisms present, the appearance and germination of the samples, and any diseases which might develop on seedlings grown from these samples; and
3. to determine the position of fungi within the grain.

Organisms Previously Recorded.

One of the earliest observations was made by Bolley (1913) who stated that certain fungi could be obtained from the surface and interior of wheat grains from almost any wheat-growing region of the world.

The surface flora has usually been determined by shaking the grain with water and examining the washings under the microscope. Various workers have recorded numerous common moulds, mainly species of *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium* and bacteria, together with pathogens such as *Tilletia caries*, *T. foetida*, *Ustilago tritici*, *Helminthosporium sativum*, *Septoria nodorum*, *S. tritici*, *Fusarium* spp. and rusts (Carter and Young, 1950; Christensen, 1951; Crosier, 1936; Greaney and Machacek, 1942, 1946; James et al., 1946; Pollack, 1945; Rice, 1939; Russell and Ledingham, 1941; Schnellhardt and Heald, 1936). The fungi are usually present as spores and generally are regarded

* Work undertaken while a student in the Faculty of Agriculture, University of Sydney.

merely as contaminants, but James et al. (1946) suggest that since bacteria occur in such large numbers they constitute an epiphytic flora.

The organisms recorded within wheat grains include species of *Alternaria, Aspergillus, Botrytis, Cephalosporium, Chaetomium, Cladosporium, Colletotrichum, Curvularia, Epicoccum, Fusarium, Helminthosporium, Mucor, Nigrospora, Penicillium, Phoma, Podosporiella, Pullularia, Rhizoctonia, Rhizopus, Sclerotium, Septoria, Stemphylium, Torula, Trichoderma, Trichothecium, Verticillium*, together with various other moulds, yeasts, bacteria and actinomycetes (Atanasoff, 1920; Blair, 1937; Bolley, 1924; Brentzel, 1944; Brittlebank and Adam, 1924; Carne, 1927; Christensen, 1951; Curzi, 1926, 1929; Dastur, 1928, 1942; Davidson and Den Shen Tu, 1950; Drechsler, 1923; El-Helaly, 1947; Fomin and Nemlienko, 1940; Galloway, 1936; Greaney and Machacek, 1942, 1946; Hagborg, 1936; Henry, 1923; Laumont and Murat, 1934; Machacek, 1945; Machacek and Greaney, 1938; Machacek et al., 1951; McCulloch, 1920; Milner et al., 1947*a*, 1947*b*; Minz, 1943; O'Gara, 1915; Peyronel, 1926; Rosella, 1930; Russell and Ledingham, 1941; Weniger, 1935; Ziling, 1932).

Most of the studies on the internal flora have been made using discoloured samples, *H. sativum* and *Alternaria* spp. of the *A. tenuis* type being the organisms most commonly present. The general finding with ordinary, sound, market samples has been that *Alternaria* spp. are the most common internal fungi, in some cases occurring in almost every grain (Christensen, 1951; Davidson and Den Shen Tu, 1950; Greaney and Machacek, 1942, 1946; Hyde and Galleymore, 1951; Laumont and Murat, 1934; Machacek et al., 1951; Milner et al., 1947*a*, 1947*b*).

Greaney and Machacek (1946) made a thorough study of a large number of samples from Manitoba over the period 1937–42, and found that 70·2% of the grains were infected with *Alternaria* spp., 3·5% with *H. sativum*, 0·6% with *Fusarium* spp., 3·2% with other fungi, and that 23·9% of the kernels were fungus-free. Bacteria, yeasts and actinomycetes were also found.

Pleosphaeria semeniperda, Penicillium glaucum, Penicillium sp., *Cladosporium* sp., *H. sativum, Alternaria* spp., *Fusarium* spp., *Pseudomonas atrofaciens* and other bacteria have been recorded in Australia, but little detail is given (Adam, 1950; Anon., 1939; Brittlebank and Adam, 1924; Carne, 1927; Noble, 1924, 1933)

Materials and Methods.

Wheat samples were obtained from various parts of New South Wales. Samples 1–20 were from the 1949 harvest and samples 21–49 from the 1950 harvest. Samples 1–17 were from silos and were deliberately selected to contain a high proportion of discoloured grains, whereas Nos. 18, 19 and 20 were random samples. Numbers 21–49 were random samples collected from various sources.

The 1949 samples were subjected to an agar plate test six months after harvest. The 1950 samples were examined one month after harvest and, following Canadian proposals (Greaney and Machacek, 1946; Machacek and Wallace, 1942; Mead et al., 1950; Russell and Ledingham, 1941), were subjected to the following:

(*a*) a macroscopical examination to observe the degree of pinching and the proportion of discoloured, shot, sprung and mechanically injured grains;
(*b*) a qualitative examination of seed washings;
(*c*) an agar plate test;
(*d*) a non-sterile soil test to determine the germination and to observe any seed-borne diseases.

No attempt was made to detect loose smut infection.

The presence of the groove and the brush make the wheat grain admirably suited for carrying a large surface load of organisms, and for this reason a method of thorough surface sterilization is needed if the internal flora is to be examined by means of an agar plate test.

Various techniques have been employed (Machacek and Greaney, 1938; Mead, 1933; Simmonds, 1930*a*; Simmonds and Mead, 1935) and their relative merits are discussed

by Machacek and Greaney (1938) who devised what they describe as a simple, practical and efficient method, which they later (Greaney and Machacek, 1946) modified slightly. Except where otherwise indicated, this modified method, which is described below, has been adopted throughout this study.*

The grains were immersed in a solution of alcohol and mercuric chloride (1 part of 95% ethyl alcohol to 3 parts of 1:1000 mercuric chloride solution) for four minutes, washed in three changes of sterile water, and planted in freshly poured P.D.A. plates, 10 grains per dish. The dishes were held at room temperature for 8–10 days. At the end of this period the organisms growing out from each kernel were either identified at once or isolated for further study and identification.

The germination tests in non-sterile soil were carried out according to the method described by Machacek and Wallace (1942) and Mead et al. (1950). 100 grains from each sample were planted in pots in a glasshouse and left for 10 days, the temperature ranging from 20 to 30° C. during that period. The seedlings were then counted, taken from the soil, washed, and examined for lesions. The diseased tissue, where present, was then surface sterilized and planted in P.D.A. plates, to determine the organisms responsible.

General Results.

Only a brief qualitative examination was made of the surface flora. Microscopical examination of the washings revealed spores of *Alternaria*, *Cladosporium*, *Epicoccum*, *Stemphylium*, numerous unicellular spores, uredospores and hyphal fragments. Occasional spores of *Tilletia* were also found. When the washings were streaked on to P.D.A., the organisms which developed were mainly bacteria and species of *Penicillium*, and it is probable that these constitute a large part of the surface flora.

The results of the plating tests to determine the internal flora of samples 1–17 are shown in Table 1. Species of *Alternaria* were the organisms most commonly isolated, exceeding the total of the other organisms isolated by almost three times. *Helminthosporium tritici-repentis* was isolated from 8·5% of the grains, *Septoria nodorum* from 2% and *H. sativum* from only 0·4%, while *Fusarium* spp. were not isolated at all. There appears to be little relation for these samples between germination in agar, the proportion of grains from which organisms were isolated, and the number and nature of the organisms, except that the two lowest germination figures were obtained with samples 11 and 12, from which were isolated the most colonies of *Aspergillus* spp. This is in keeping with the work of various authors (Laumont and Murat, 1934; Thomas, 1937) who found that *Aspergillus* spp. were able to reduce germination. Samples from which *H. tritici-repentis* was isolated were all from the northern half of the State, and it is interesting to note the high figure for *S. nodorum* obtained with the sample from Ladysmith.

The results obtained with random samples from the 1949 harvest are shown in Table 2, and are much the same as those obtained with samples 1–17, except for the high figure for *Penicillium* spp. in sample 18.

In Table 3 are grouped the results obtained with samples from the 1950 harvest. The population of organisms isolated was similar to that isolated from samples of the previous harvest, *Alternaria* spp. again exceeding the total of other organisms isolated by almost three times. *H. tritici-repentis* was prominent as before, being second in frequency to the *Alternaria* spp., this time, however, sharing the position with *Epicoccum* sp. *S. nodorum* was isolated from 2·7% of the grains, *H. sativum* from 0·5%, and a *Fusarium* sp. from only 0·1%.

It is clear, therefore, that the microflora of wheat grains in New South Wales, as revealed in these samples, is, except for the presence of *H. tritici-repentis*, very similar to that found overseas.

Alternaria spp. occurred in every sample examined, the percentage of grains from which they were isolated in any sample ranging from 16 to 95. Nearly all the isolates

* For justification of its use see the appendix.

TABLE 1.

The Organisms Isolated from, and the Germination in Agar of Samples 1–17 from the 1949 Harvest.

Sample.	Source.	Germination in Agar.	Number of Grains Giving—								
			Alternaria spp.	*H. tritici-repentis.*	*Aspergillus* spp.	*Septoria nodorum.*	*Penicillium* spp.	*Rhizopus nigricans.*	*H. sativum.*	Miscellaneous.*	No Organisms.
1	Westdale	38	29	13	—	1	1	—	—	—	6
2	Emerald Hill	46	30	12	2	—	—	1	1	—	5
3	Breeza	37	29	8	3	3	2	—	1	—	5
4	Manilla	41	16	7	—	—	1	—	—	—	27
5	Delungra	42	30	5	—	—	—	1	—	—	15
6	Warialda	42	38	7	—	1	—	—	—	7	1
7	West Tamworth	40	24	14	—	—	—	2	—	1	10
8	Inverell	39	31	—	—	—	—	4	1	7	9
9	Quirindi	34	37	6	—	—	—	4	1	1	2
10	South Harefield	32	33	—	7	—	3	1	—	2	8
11	Brushwood	19	28	—	18	—	—	—	—	1	3
12	Corobimilla	16	20	—	16	—	1	—	—	1	12
13	Ladysmith	31	25	—	14	10	—	—	—	4	2
14	Arajoel	32	37	—	5	—	2	—	—	—	7
15	Illabo	29	48	—	—	—	1	—	—	1	—
16	Old Junee	38	39	—	—	—	—	—	1	2	10
17	Coolamon	32	36	—	—	1	3	1	—	5	10
	Average	34·6	31·2	4·2	3·8	1·0	0·8	0·8	0·2	1·9	7·7
	Average (%)	69·2	62·4	8·5	7·6	2·0	1·6	1·6	0·4	3·8	15·4

50 grains plated from each sample.

* Includes *Epicoccum* sp., *Cladosporium* sp., *Pullularia pullulans*, *Stemphylium* sp., *Botrytis* sp., *Phoma* sp., *Helminthosporium avenae*, together with unidentified fungi, bacteria, and yeasts.

TABLE 2.

The Organisms Isolated from, and the Germination in Agar of Samples 18–20 from the 1949–50 Harvest.

Sample.	Source.	Germination in Agar.	Number of Grains Giving—							
			Alternaria spp.	*Penicillium* spp.	*H. tritici-repentis.*	*Septoria nodorum.*	*Aspergillus* spp.	*H. sativum.*	Miscellaneous.	No Organisms.
18	Castle Hill (Federation)	39	24	18	—	—	1	—	2	5
19	Gunnedah (Gabo)	42	40	—	6	—	—	—	3	3
20	Inverell (Gabo)	37	35	1	4	2	—	1	4	12
	Average	39·3	33·0	6·3	3·3	0·7	0·3	0·3	3·0	7·7
	Average (%)	78·7	66·0	12·7	6·7	1·3	0·7	0·7	6·0	15·4

50 grains plated from each sample.

could be placed in *A. tenuis* auct. as described by Mason (1928), Wiltshire (1933), Groves and Skolko (1944), which is a little more comprehensive than Neergaard's (1945) *A. tenuis* auct. sensu stricto. Some isolates, however, produced only short chains of conidia and there seems to be little provision made in the literature for these forms.

Results of Routine Examination of Samples 21–49.

Sample.	Source.	Macroscopical Characters.						Number of Grains Giving—												Germination Tests.		
		No. Broken Pieces in a Random Sample of 300 Whole Grains.	% Pink Grains.	% Smudged and Black-pointed Grains.	% Shot or Sprung Grain.	% Sound Grain.	Degree of Pinching.**	*Alternaria* spp.	*Epicoccum* sp.	*H. tritici-repentis.*	*Cladosporium herbarum.*	*Septoria nodorum.*	*Penicillium* spp.	*Cephalosporium* sp.	*H. sativum.*	*H. avenae.*	*Aspergillus* spp.	Bacteria.	Miscellaneous.*	No Organisms.	Germination in Agar.	Germination in Soil.	Number of Seedlings with Lesions.***
21	Gunnedah (Gabo) ..	27	9	8	—	83	2	75	4	15	4	1	—	—	—	—	—	1	—	3	44	75	4
22	Edgeroi (Kendee) ..	—	9	4	—	87	5	59	7	14	17	45	2	1	1	1	—	—	1	—	6	93	3
23	Willala (Gabo) ..	3	8	5	—	87	8	70	4	26	8	5	—	—	—	—	—	1	2	—	33	89	1
24	Gunnedah (Cailloux)	6	15	22	—	63	0	76	6	18	6	—	—	1	—	—	—	4	2	—	11	93	—
25	Gunnedah (Kendee)	14	19	9	—	72	1	78	8	9	4	2	—	—	1	6	1	4	1	—	32	78	—
26	Willala	—	10	7	—	83	8	57	11	39	1	1	—	—	—	—	—	—	—	2	73	96	3
27	Maul's Creek ..	—	19	4	—	77	5	51	3	46	1	—	—	—	—	—	—	—	1	2	66	95	1
28	Gunnedah	18	1	3	—	96	1	93	—	3	2	—	2	—	—	—	—	—	1	2	78	83	—
29	Edgeroi (Celebration)	—	—	—	—	100	2	87	4	2	4	—	—	—	—	—	—	—	—	6	37	87	1
30	Gunnedah (Charter)	—	5	4	—	91	1	50	5	19	6	1	—	—	—	1	—	—	—	—	73	91	1
31	Culgoora (Kendee)	2	4	5	—	91	7	80	8	19	5	—	—	—	2	1	—	—	—	—	32	94	—
32	Baan Ba (Charter) ..	7	1	2	—	97	9	71	2	17	1	—	—	—	—	3	—	—	1	8	50	86	2
33	Griffith	14	—	18	—	82	1	79	6	—	6	—	6	1	—	—	—	—	1	4	86	92	—
34	Binnaway	5	8	6	23	63	2	85	6	3	1	3	27	1	—	—	1	1	—	—	40	88	—
35	Baradine	14	3	21	31	46	2	89	12	2	1	—	3	2	1	—	—	2	—	—	69	68	—
36	Pallamallawa ..	2	6	14	27	53	2	71	20	18	—	—	4	—	4	—	2	—	3	—	33	88	4
37	Parkes	21	2	9	21	68	2	87	8	2	1	—	1	2	—	1	—	—	4	1	64	72	2
38	North Star (Charter)	9	2	17	55	26	2	81	24	13	2	2	1	1	3	—	—	1	1	—	43	80	13
39	Hawkesbury College	—	—	4	—	96	4	21	70	—	4	6	1	1	—	—	—	—	4	1	14	77	2
40	Castle Hill	—	2	3	—	95	4	25	51	—	2	2	1	6	—	—	—	—	4	20	5	36	—
41	Narromine	2	1	2	47	50	3	88	6	4	7	—	3	—	2	—	—	2	3	2	66	81	4
42	Gollan (Kendee) ..	—	1	9	57	33	2	95	9	—	—	—	5	4	—	—	—	3	1	—	52	64	5
43	Armatree	8	2	4	57	94	2	72	12	4	—	—	1	—	—	—	—	—	4	8	47	83	1
44	Yeoval (Kendee) ..	117	1	8	17	74	2	84	13	3	—	1	—	2	—	—	6	—	1	1	31	49	—
45	Coonabarabran ..	12	1	17	28	54	2	86	12	2	—	—	2	2	—	1	—	—	5	—	65	71	1
46	Gravesend	8	13	10	14	63	2	56	4	35	1	8	—	—	—	—	—	—	2	2	74	79	6
47	Trangie	9	2	18	16	64	1	82	9	12	5	—	—	—	1	—	1	—	—	—	75	80	4
48	Finley	23	0·3	2·3	—	97·3	1	52	2	2	6	1	—	—	—	—	3	—	1	38	93	90	—
49	Finley (Pinnacle) ..	4	—	1	—	99	0	61	2	—	4	—	—	—	—	—	1	—	5	31	78	94	—
	Average	11·0	4·9	8·1	11·6	75·4	3·0	71·1	11·3	11·3	3·4	2·7	2·0	0·8	0·5	0·5	0·5	0·7	1·7	4·5	50·6	81·7	2·0

100 grains were used in each germination and plating test.

* *Pullularia pullulans*, *Podosporiella verticillata* and species of *Trichoderma*, *Rhizopus*, *Mucor*, *Cunninghamella*, *Botrytis*, *Nigrospora*, *Fusarium*, *Ascochyta*, yeasts and various unidentified fungi.

** The degree of pinching is indicated by allotting each sample a figure from an arbitrary scale of 1–10. The figure for sample 40, which was very badly affected, was fixed at 9.

*** Nearly half these lesions when examined could not be attributed to any parasitic agent. Of the remainder about equal numbers were caused by *H. sativum* and *S. nodorum*.

TABLE 1.

The Organisms Isolated from, and the Germination in Agar of Samples 1–17 from the 1949 Harvest.

Sample.	Source.	Germination in Agar.	Number of Grains Giving—								
			Alternaria spp.	*H. tritici-repentis.*	*Aspergillus* spp.	*Septoria nodorum.*	*Penicillium* spp.	*Rhizopus nigricans.*	*H. sativum.*	Miscellaneous.*	No Organisms.
1	Westdale	38	29	13	—	1	1	—	—	—	6
2	Emerald Hill	46	30	12	2	—	—	1	1	—	5
3	Breeza	37	29	8	3	3	2	—	1	—	5
4	Manilla	41	16	7	—	—	1	—	—	—	27
5	Delungra	42	30	5	—	—	—	1	—	—	15
6	Warialda	42	38	7	—	1	—	—	—	7	1
7	West Tamworth	40	24	14	—	—	—	2	—	1	10
8	Inverell	39	31	—	—	—	—	4	1	7	9
9	Quirindi	34	37	6	—	—	—	4	1	1	2
10	South Harefield	32	33	—	7	—	3	1	—	2	8
11	Brushwood	19	28	—	18	—	—	—	—	1	3
12	Corobimilla	16	20	—	16	—	1	—	—	1	12
13	Ladysmith	31	25	—	14	10	—	—	—	4	2
14	Arajoel	32	37	—	5	—	2	—	—	—	7
15	Illabo	29	48	—	—	—	1	—	—	1	—
16	Old Junee	38	39	—	—	—	—	—	1	2	10
17	Coolamon	32	36	—	—	1	3	1	—	5	10
	Average	34·6	31·2	4·2	3·8	1·0	0·8	0·8	0·2	1·9	7·7
	Average (%)	69·2	62·4	8·5	7·6	2·0	1·6	1·6	0·4	3·8	15·4

50 grains plated from each sample.

* Includes *Epicoccum* sp., *Cladosporium* sp., *Pullularia pullulans*, *Stemphylium* sp., *Botrytis* sp., *Phoma* sp., *Helminthosporium avenae*, together with unidentified fungi, bacteria, and yeasts.

TABLE 2.

The Organisms Isolated from, and the Germination in Agar of Samples 18–20 from the 1949–50 Harvest.

Sample.	Source.	Germination in Agar.	Number of Grains Giving—							
			Alternaria spp.	*Penicillium* spp.	*H. tritici-repentis.*	*Septoria nodorum.*	*Aspergillus* spp.	*H. sativum.*	Miscellaneous.	No Organisms.
18	Castle Hill (Federation)	39	24	18	—	—	1	—	2	5
19	Gunnedah (Gabo)	42	40	—	6	—	—	—	3	3
20	Inverell (Gabo)	37	35	1	4	2	—	1	4	12
	Average	39·3	33·0	6·3	3·3	0·7	0·3	0·3	3·0	7·7
	Average (%)	78·7	66·0	12·7	6·7	1·3	0·7	0·7	6·0	15·4

50 grains plated from each sample.

could be placed in *A. tenuis* auct. as described by Mason (1928), Wiltshire (1933), Groves and Skolko (1944), which is a little more comprehensive than Neergaard's (1945) *A. tenuis* auct. sensu stricto. Some isolates, however, produced only short chains of conidia and there seems to be little provision made in the literature for these forms.

TABLE 3.
Results of Routine Examination of Samples 21–49.

Sample.	Source.	Macroscopical Characters.						Number of Grains Giving—												Germination Tests.		
		No. Broken Pieces in a Random Sample of 300 Whole Grains.	% Pink Grains.	% Smudged and Black-pointed Grains.	% Shot or Sprung Grain.	% Sound Grain.	Degree of Pinching.**	*Alternaria* spp.	*Epicoccum* sp.	*H. tritici-repentis.*	*Cladosporium herbarum.*	*Septoria nodorum.*	*Penicillium* spp.	*Cephalosporium* sp.	*H. sativum.*	*H. avenae.*	*Aspergillus* spp.	Bacteria.	Miscellaneous.*	No Organisms.	Germination in Agar.	Germination in Soil.	Number of Seedlings with Lesions.***
21	Gunnedah (Gabo) ..	27	9	8	—	83	2	75	4	15	4	1	—	—	—	—	—	1	—	3	44	75	4
22	Edgeroi (Kendee) ..	—	9	4	—	87	5	59	7	14	17	45	2	1	1	1	—	—	1	—	6	93	3
23	Willala (Gabo) ..	3	8	5	—	87	8	70	4	26	8	5	—	—	—	—	—	1	2	—	33	89	1
24	Gunnedah (Cailloux)	6	15	22	—	63	0	76	6	18	6	—	—	1	—	—	—	4	2	—	11	93	—
25	Gunnedah (Kendee)	14	19	9	—	72	1	78	8	9	4	2	—	—	1	6	1	4	1	—	32	78	—
26	Willala	—	10	7	—	83	8	57	11	39	1	1	—	—	—	—	—	—	—	2	73	96	3
27	Maul's Creek ..	—	19	4	—	77	5	51	3	46	1	—	—	—	—	—	—	—	1	2	66	95	1
28	Gunnedah	18	1	3	—	96	1	93	—	3	2	—	2	—	—	—	—	—	1	2	78	83	—
29	Edgeroi (Celebration)	—	—	—	—	100	2	87	4	2	4	—	—	—	—	—	—	—	—	6	37	87	1
30	Gunnedah (Charter)	—	5	4	—	91	1	50	5	19	6	1	—	—	—	1	—	—	—	—	73	91	1
31	Culgoora (Kendee)	2	4	5	—	91	7	80	8	19	5	—	—	—	2	1	—	—	—	—	32	94	—
32	Baan Ba (Charter) ..	7	1	2	—	97	9	71	2	17	1	—	—	—	—	3	—	—	1	8	50	86	2
33	Griffith	14	—	18	—	82	1	79	6	—	6	—	6	1	—	—	—	—	1	4	86	92	—
34	Binnaway	5	8	6	23	63	2	85	6	3	1	3	27	1	—	—	1	1	—	—	40	88	—
35	Baradine	14	3	21	31	46	2	89	12	2	1	—	3	2	1	—	—	2	—	—	69	68	—
36	Pallamallawa ..	2	6	14	27	53	2	71	20	18	—	—	4	—	4	—	2	—	3	—	33	88	4
37	Parkes	21	2	9	21	68	2	87	8	2	1	—	1	2	—	1	—	—	4	1	64	72	2
38	North Star (Charter)	9	2	17	55	26	2	81	24	13	2	2	1	1	3	—	—	1	1	—	43	80	13
39	Hawkesbury College	—	—	4	—	96	4	21	70	—	4	6	1	1	—	—	—	—	4	1	14	77	2
40	Castle Hill	—	2	3	—	95	4	25	51	—	2	2	1	6	—	—	—	—	4	20	5	36	—
41	Narromine	2	1	2	47	50	3	88	6	4	7	—	3	—	2	—	—	2	3	2	66	81	4
42	Gollan (Kendee) ..	—	1	9	57	33	2	95	9	—	—	—	5	4	—	—	—	3	1	—	52	64	5
43	Armatree	8	2	4	57	94	2	72	12	4	—	—	1	—	—	—	—	—	4	8	47	83	1
44	Yeoval (Kendee) ..	117	1	8	17	74	2	84	13	3	—	1	—	2	—	—	6	—	1	1	31	49	—
45	Coonabarabran ..	12	1	17	28	54	2	86	12	2	—	—	2	2	—	1	—	—	5	—	65	71	1
46	Gravesend	8	13	10	14	63	2	56	4	35	1	8	—	—	—	—	—	—	2	2	74	79	6
47	Trangie	9	2	18	16	64	1	82	9	12	5	—	—	—	1	—	1	—	—	—	75	80	4
48	Finley	23	0·3	2·3	—	97·3	1	52	2	2	6	1	—	—	—	—	3	—	1	38	93	90	—
49	Finley (Pinnacle) ..	4	—	1	—	99	0	61	2	—	4	—	—	—	—	—	1	—	5	31	78	94	—
	Average	11·0	4·9	8·1	11·6	75·4	3·0	71·1	11·3	11·3	3·4	2·7	2·0	0·8	0·5	0·5	0·5	0·7	1·7	4·5	50·6	81·7	2·0

100 grains were used in each germination and plating test.

* *Pullularia pullulans*, *Podosporiella verticillata* and species of *Trichoderma*, *Rhizopus*, *Mucor*, *Cunninghamella*, *Botrytis*, *Nigrospora*, *Fusarium*, *Ascochyta*, yeasts and various unidentified fungi.

** The degree of pinching is indicated by allotting each sample a figure from an arbitrary scale of 1–10. The figure for sample 40, which was very badly affected, was fixed at 9.

*** Nearly half these lesions when examined could not be attributed to any parasitic agent. Of the remainder about equal numbers were caused by *H. sativum* and *S. nodorum*.

They may fit *A. peglioni* as described by Curzi (1926) but, except for their shorter chains of conidia, they are similar to *A. tenuis* and could be regarded as extreme types of this very heterogeneous form-species. Simmons (1952), however, warns that the increasingly common practice of treating morphologically similar populations of the Fungi Imperfecti as genetically similar or identical species is based on false premises, in that such similar populations need not necessarily have any great degree of genetic similarity. This is illustrated by his work on three different isolates coming within the modern descriptive limits of *A. tenuis*, each having been derived from an ascospore of a different perfect stage.

Helminthosporium tritici-repentis was found in 34 of the 49 samples, being most abundant in those from the north-west. The percentage of grains from which it was isolated, for any sample in which it occurred, ranged from 2 to 46. This fungus has been recorded in wheat grains only once previously (Galloway, 1936).

A species of *Epicoccum* producing an orange pigment in culture and jet black sporodochia, was isolated from most samples, being obtained from up to 70% of the grains. The highest figures were obtained from samples 39 and 40 from the central coast, the only ones from which *Alternaria* spp. were not the fungi most frequently isolated.

Cladosporium herbarum was isolated from most samples. Frequently it grew out from the brush end of the grain, which suggests that it may not always have been inside the grain but was present deep in the brush and able to withstand the surface sterilization.

Septoria nodorum was found in 22 of the 49 samples, the percentage of grains from which it was isolated in any of these ranging from 1 to 45. It was more abundant in samples from the northern half of the State than the southern, and it is interesting to note that the highest figure was obtained with grain from a crop showing exceptionally bad glume blotch.

Helminthosporium sativum was found in 14 of the samples, highest percentage of grains found to be infected in any sample being 4. The occurrence of *H. avenae*, which was found as frequently as *H. sativum*, is interesting. This is perhaps analogous to the occurrence of *H. avenae* and *H. teres* in wheat grain in Canada (Machacek and Greaney, 1938, Machacek et al., 1951). In inoculation tests the *H. avenae* isolates infected only oats.

Of the fungi isolated, only *H. sativum*, *H. tritici-repentis* and *Septoria nodorum* are known to be actively parasitic on the growing wheat plant. The rest are rarely of any importance though it appears that some may discolour the grain and others, under special circumstances, reduce the viability. *Fusarium* spp. were isolated only very rarely and the isolates were not pathogenic to wheat under glasshouse conditions.

From Table 3 no obvious relation can be seen between the number and nature of the organisms isolated from any sample and the degree of pinching, percentage of smudged grains, percentage of pink grains, or number of grain fragments encountered during the macroscopic examination of 300 whole grains.

When the samples showing no shot or sprung grain and those containing such grain are compared, as in Table 4, no very obvious differences can be observed when the variability within each group is considered. However, it does appear that samples with no shot or sprung grains germinated better and contained more grains from which no organisms were isolated and fewer grains from which *Aspergillus* and *Penicillium* spp. were isolated than those samples with shot or sprung grain. This is to be expected when it is considered that the latter samples must have been subjected to moister conditions than the former. Various authors (Christensen and Gordon, 1948; Laumont and Murat, 1934; Milner et al., 1947*a*, 1947*b*; Thomas, 1937) report observations which indicate that invasion of the grain by such fungi as *Aspergillus* and *Penicillium* spp. is associated with high moisture content and cracking of the epidermis.

From Table 3 it can be seen that, except for the difference pointed out above, germination in agar or in soil bore very little relation to the appearance of the grain or its content of organisms. Germination in agar varied enormously from sample to

sample, often differing considerably from the corresponding germination in soil, usually being considerably smaller. This finding is similar to that of Rosella (1930), who found that the germination of mouchété (smudged) grains, with which *Alternaria* spp. were associated, was lower in agar than in sand.

The reason for the enormous variation between the germinations of different samples in agar, particularly between those whose germinations in soil were much the same, is obscure. It may perhaps be related to the depth of the agar, the attitudes in which the grains were placed therein, the overgrowing of the grain by fungal colonies, or the effect of the surface sterilizing agent.

The number and nature of the lesions developing on the seedlings seemed to bear little relation to the presence, prevalence, or absence in the grain samples of the fungi concerned. If, however, the number of grains infected with these organisms had been higher, no doubt a more clear-cut result would have been obtained. Mead *et al.* (1950) point out that production of lesions was somewhat erratic in their experiments owing to differences in temperature, moisture, and other conditions. Hence, until the optimum conditions for development are known for each organism, it is not clear how far the

TABLE 4.

Derived from Table 3 to Show the Average Populations and Germinations of Samples Containing no Shot or Sprung Grains and Samples Containing 14% or More of such Grains.

Samples.	Germination (%).		Percentage of Grains Giving—							
	In Agar.	In Soil.	*Alternaria* spp.	*Epicoccum* sp.	*H. triticirepentis.*	*S. nodorum.*	*Penicillium* spp.	*Aspergillus* sp.	Miscellaneous.	No Organisms.
Containing no shot or sprung grain	47·7	85·1	74·4	11·4	14·6	3·6	0·7	—	8·3	7·0
Containing shot or sprung grain	55·6	74·5	82·2	11·2	8·5	1·3	4·1	3·6	7·0	0·5

number and nature of the lesions developing in the glasshouse can be relied upon as a guide to the amount of seed-borne disease. The lesions developed mainly on the coleoptiles, and, as all the samples were sown and examined at the same time and under the same conditions, the results are comparative. The only pathogens isolated from these lesions were *Helminthosporium sativum* and *Septoria nodorum.*

Little is known of the importance of *S. nodorum* in grain. Machacek (1945) found lesions on the coleoptiles and first leaves of seedlings developing from infected grains, but it has not been shown that the disease can progress beyond this stage. In this study lesions have been observed on the coleoptiles only, and of all the organisms isolated from surface sterilized grain, *H. sativum* is the only one known to cause a seed-borne disease.

It appears from the literature that although fungi can remain viable within the grain for long periods, many species lose their viability relatively quickly (Machacek and Greaney, 1938; Machacek and Wallace, 1952). As can be seen from Table 5, there was a considerable reduction in the viability of the internal flora in sample 20 over a period of seven months. However, the main species were still viable in many of the grains 13 months after harvest.

As samples 1–20 were examined six months after harvest it seems probable that the internal flora had already undergone a decrease in viability. This may explain why these samples contained a higher percentage of grains from which no organisms were isolated than those of the 1950 harvest.

Distribution of Mycelium within the Grain.

Most work on this subject has been carried out with discoloured grains in which the mycelium has been observed to be most abundant in the pericarp (Bolley, 1910; Curzi, 1926; Fomin and Nemlienko, 1940; Laumont and Murat, 1934; Machacek and Greaney, 1938; Minz, 1943; Rosella, 1930: Weniger, 1935). Where *Alternaria* spp. are the main fungi present the mycelium has usually been found to be restricted to the pericarp, but *H. sativum* has been observed penetrating the embryo and endosperm (Fomin and Nemlienko, 1940; Weniger, 1935).

Dastur (1935), when studying black-pointed grains, with which several organisms were associated, found hyphae in great abundance in the funicle and in the pericarp in the central region of the grain furrow, and observed that they creep between the pericarp and seed coat, where they form a kind of stroma, but were not found in the embryo or endosperm.

Table 5.

A Comparison of the Viability of Organisms within Grain Before and After a Storage Period of Seven Months at Room Temperature.

Time of Plating.	Number of Grains Giving—					
	Alternaria sp.	*H. tritici-repentis.*	*H. sativum.*	Bacteria.	Miscel-laneous.	No Organisms.
June, 1950	52	15	—	4	5	23
January, 1951	16	4	1	2	1	75

Sample 20 used, harvested December, 1949. 100 grains were plated on each occasion.

Bockmann (1933) inoculated wheat and barley seeds with *Cladosporium herbarum*, *Alternaria tenuis* and *A. peglioni* and found that they grew in the pericarp but did not penetrate the testa. He observed hyphae of *C. herbarum* to pass from cell to cell through the wall pits. Rosella (1930) observed hyphae of an *Alternaria* sp. to progress in a similar way.

Curzi (1926) found that the mycelium was not restricted to the discoloured area and that it occurred in clean grains as well, while Laumont and Murat (1934) found hyphae in the pericarp of both clean and discoloured grains belonging to a number of wheat varieties of different origins.

Oxley and Jones are reported by Hyde (1950) to have found mycelium in normal wheat grains in the space between the epidermis and the cross layer, and Hyde found subepidermal fungi in a similar position in normal wheat grains from almost all the wheat growing areas of the world, there being, however, a wide variation in the density of the mycelium and the area of the pericarp invaded.

Hyde quotes Marcus as finding mycelium between the pericarp and testa, growing in particular abundance in the large cavities at the side of the crease, which suggests a similar position, and it may be that Marcus and Dastur (1935) mistook the position of the mycelium.

Christensen (1951) found mycelium between the cell layers of the pericarp, on the inner side of the pericarp, and occasionally within the cells of the pericarp. However, as he peeled strips of "pericarp" from the grain, it is possible that the bulk of the mycelium he observed was actually in the space between the epidermis* and the cross layer, as it is usually the epidermis which is detached where grains are peeled.

* The epidermis according to J. M. Hector, Introduction to the Botany of Field Crops, Vol. I, Johannesburg, 1936, fig. 42, includes all layers outside the cross layer.