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STUDIES ON *OPHIOBOLUS GRAMINIS* AND A ROOT-ATTACKING STRAIN OF *RHIZOCTONIA SOLANI*

by

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STATEMENT

This dissertation has not previously been submitted for a degree at this or any other University and is the original work of the writer, except where due reference is made in the text.

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PART 1

The saprophytic survival of *Ophiobolus graminis* on artificially infected straw was studied using monosporous F_1 progeny of a wheat isolate and field isolates from wheat and oats. Nitrate-enrichment of soil increased survival, the effect of the amendment outweighing any adverse effects of other soil organisms.

Strongly-pathogenic F_1 progeny survived well on wheat straw, but weakly-pathogenic F_1 progeny did not. All F_1 ascospores from perithecia formed on living hosts were strongly-pathogenic, but only half the F_1 ascospores from a perithecium produced in culture were strongly-pathogenic. Perithecia were only formed in culture by strongly-pathogenic isolates.

O. graminis survived better on barley straw than on straws of wheat, *Hordeum leporinum*, *Lolium rigidum*, and *Vulpia myuros*; survival was higher under laboratory rather than summer field conditions. In the field, survival was greater at one than at six inches below the surface.

On naturally infected material, *O. graminis* survived at a high level on *Bromus gussonii*, *Hordeum hystrix*, and *H. leporinum* but not on *Ehrharta calycina*, *Lolium rigidum*, and *Vulpia myuros*.

Western Australian isolates from oats were similar

to var. avenae; survival of this strain on artificially infected straw was increased by nitrate-enrichment of soil. Nuclear distribution in mycelia of both the wheat and oat attacking strains of O. graminis was similar but attempts to induce hyphal anastomosis between the two were unsuccessful.

South Australian isolates from oats were pathogenically similar to the type variety although some were similar to var. avenae in tolerance of oat extract.

A sterile fungus which produced "Ophiobolus-like" runner hyphae on cereal roots differed from O. graminis in pathogenicity and nuclear content of cells and was incompatible with isolates of O. graminis.

PART 2

Saprophytic activity and survival of a root-attacking strain of Rhizoctonia solani was examined, using the two cultural types differentiated by de Beer. These further differed in ability to utilize various forms of carbon and nitrogen, in optimal temperature for growth, and in sensitivity to above-growth-maximum temperatures. Near-maximum temperatures caused irregularity in appearance of colonies and increased frequency of self-anastomosis.

Nitrogenous soil amendments reduced activity, viability and mycelial density of the root strain in unsterilized Moonta soil (pH 8.5) but urea was the only amendment with an inhibitory effect in gamma-irradiated soil.

Urea exercised restrictive effects over test ranges in soil temperature (15°-30°C) and soil moisture (pF 1.70-2.55). Increasing concentrations of urea up to 200 mg. nitrogen/Kg. soil correspondingly reduced activity and viability of the root strain, but the higher urea concentrations caused root injury to wheat seedlings. Soil amendments of fructose reduced the restrictive effect of urea. Growth by the root strain was not inhibited on a defined medium (pH 7.1) containing fructose and urea (1120 mg. nitrogen/litre) as carbon and nitrogen sources respectively; increasing pH to 8.5 restricted growth without apparent morphological abnormalities.

Other soil organisms influenced ability of the root strain to colonize filter papers over test ranges of soil moistures and temperatures. At 30°C the activity, viability and mycelial density of the root strain was reduced considerably by other organisms.

Soil temperature changes over the range 15°-25°C had little effect upon the activity of the root strain. Viability was not significantly different at soil moistures equivalent to pF 1.70 and pF 2.55, although mycelial density was greater at pF 2.55.

In the field, single applications of urea and ammonium sulphate (17 lb. nitrogen/acre) reduced "bare patch" in barley.

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INTRODUCTION

Ophiobolus graminis Sacc. and *Rhizoctonia solani* Kühn are two of the many organisms which attack roots of the wheat plant. McKnight (1960), when reviewing the relative importance of cereal root pathogens in Australia, concluded that the greatest losses to the wheat industry were caused by *O. graminis*, *R. solani*, *Fusarium graminearum* Swabe, *F. culmorum* (W.G.Sm.) Sacc., *Helminthosporium sativum* Pammel, King and Bakke, *Curvularia ramosa* (Bainier) Boedijn and the nematode *Heterodera avenae* Wollenweber.

Of these, *Ophiobolus graminis* is considered to cause a greater overall reduction in wheat yield than any other root pathogen (Butler, 1961). It occurs in all States, although it is of minor importance in Queensland (McKnight, 1960). The natural host range is species of the Gramineae, although Müller-Kögler (1938) has shown that hyphae of the fungus may grow along the tap root of many dicotyledons and even temporarily infect them. Cereals differ in their susceptibility to the fungus, oats being generally regarded as highly resistant (Osborne, 1919; Hynes, 1937; Willetts, 1961). However, an oat-attacking form was recorded in Wales and described as *O. graminis* var. *avenae* (Turner, 1940a). Occasionally the fungus is reported on oats in Australia (Osborne, 1919; Chambers, 1964) but it has not been established that var. *avenae* occurs here (Turner, 1940a; Butler, 1961).

Rhizoctonia solani is also cosmopolitan in its

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distribution and has been differentiated into many strains on the basis of host range and cultural variation (Kernkamp, de Zeeuw, Chen, Ortega, Tsiang and Khan, 1952; Flentje and Saksena, 1957). Several strains attack wheat, but only three of these have been recorded in South Australia (Samuel and Garrett, 1932; Kerr, 1955; de Beer, 1965) where R. solani is regarded as an important cereal pathogen. It sometimes causes appreciable losses in New South Wales (Anon., 1950).

Of the remaining major root-attacking organisms, Fusarium graminearum is the main pathogen in Queensland where it is primarily a cause of crown and foot rot and seedling blight (Anon., 1964). It is also of importance in north-western New South Wales (Magee, 1957; Anon, 1963) and Western Australia (Chambers, 1964). Fusarium culmorum is confined mainly to southern New South Wales, Victoria and South Australia (McKnight, 1960), being especially prevalent in crops grown after leys (Butler, 1959). Helminthosporium sativum occurs in all States, but is declining in importance under clover ley conditions (Butler, 1959; Chambers, 1964). Curvularia ramosa is of concern only in New South Wales (McKnight, 1960). The nematode, Heterodera avenae is important mainly in Victoria and South Australia, but is restricted in distribution by soil type (Meagher, 1961).

The major investigation reported in this thesis concerns the saprophytic survival of Ophiobolus graminis and a root-attacking strain of Rhizoctonia solani, because few similar studies have been made on cereal root pathogens

in Australia. Some attention is also given to variation in the F_1 progeny of an _C. graminis_ isolate and to the occurrence of this fungus on oats in Australia. While the study provides further information on the two fungi, it should be regarded as introductory because it poses many unanswered questions, particularly on the importance of variation within species upon survival.

PART 1

STUDIES ON OPHIOBOLUS GRAMINIS SACC.

REVIEW OF LITERATURE

(1) Occurrence in Australia

The role of Ophiobolus graminis as the causal agent of the take-all disease of wheat in Australia was first demonstrated by McAlpine (1904), although the disease had been known in South Australia as early as 1852 (Anon., 1868). Between 1852 and 1904, many theories were advanced attributing the disease variously to the effects of soil salinity, lowered soil fertility, mineral deficiencies, weeds, insects, nematodes and fungi (Anon., 1868; Muecke, 1870; Pearson, 1888; Tepper, 1892; Cobb, 1892).

However, unknown to the early Australian workers, Prillieux and Delacroix (1890) reported O. graminis as the cause of a similar wheat disease in France. Although the validity of their diagnosis was not questioned, considerable delay occurred before it was generally accepted. This was due to confusion between O. graminis and the morphologically distinct O. herpotrichus (Fr.) Sacc. by some of the early European workers (Drechsler, 1934). McAlpine (1902) also considered O. herpotrichus as the possible cause of take-all before identifying O. graminis in 1904.

Following the identification of O. graminis in South Australia and Victoria by McAlpine (1904), it was accepted as the cause of take-all in New South Wales,

(Musson, 1907). The first published reference to O. graminis in Western Australia was by Sutton (1920). It was not recorded in Tasmania until 1949 (Anon., 1959) nor in Queensland until 1952 (Simmonds, 1953).

(2) Taxonomy and Nomenclature

Short reviews on the taxonomy and nomenclature of Ophiobolus graminis were given by Drechsler (1934), Garrett (1942) and Butler (1961). Originally the fungus was described as Raphidophora graminis by Saccardo in 1875, but transferred to Ophiobolus by Roumeguere and Saccardo in 1881 because the first generic name was homonymous with a plant genus (Garrett, 1942). Several generic and specific changes have been proposed since (Hara, 1916; Fitzpatrick, Thomas and Kirby, 1922; von Arx and Olivier, 1952; Petrak, 1952), but none has gained wide acceptance. Holm (1948) and Chadefaud (1955) considered the species should be placed within or near the Gnomoniaceae.

The following are generally regarded as synonyms:

Ophiobolus cariceti (Berk. and Br.) Sacc.
in Mycol. 14 (1922) : 30-37

Ophiochaeta graminis (Sacc.) K. Hara in Byôchû-gai
Zasshi Tokyo 3 (1916) : 342-345

Linocarpon cariceti (Berk. and Br.) Petr. in Sydowia
6 (1952) : 383-388

Gaeumannomyces graminis (Sacc.) v. Arx and Olivier
in Trans. Brit. Mycol. Soc.,35(1952)
29-33

Lemaire and Ponchet (1963) have also described *Phialophora radicicola* Cain as the conidial form of *Linocarpon cariceti*.

(3) The Fungus in Culture.

The vegetative morphology was described by Kirby (1925) who observed two general patterns of growth determined by the composition of the medium. One pattern occurred only on 0.2 percent dextrose agar and was characterized by the radiate growth of hyphal strands, whereas in the other the hyphal strands intermixed freely. Garrett (1942) noted that the majority of leading hyphae curled back to give the thallus a whorled appearance which served as a useful diagnostic character on isolation plates. The conditions necessary for perithecial formation in culture were defined by Thrower and Weste (1963). Maximum production occurred on a medium containing one percent glucose and 0.2 percent asparagine when incubated at 20°C and exposed to a light intensity of 60-360 foot candles. Micronidia have been observed on many occasions and are formed either on differentiated conidiophores or budded-off ascospores (McAlpine, 1904; Kirby, 1925; Garrett, 1942; Weste and Thrower, 1963). According to Garrett, microconidia were first recorded in 1899 by Mangin, who claimed to have observed their germination. This claim has not been substantiated by any other worker (Garrett, 1942; Weste and Thrower, 1963).

Studies on nutritional requirements have shown that both biotin and thiamine are essential for satisfactory

growth by O.Graminis (White, 1941). Whereas biotin proved indispensible, thiamine was not, but its inclusion in media doubled growth. White also found that when supplied with biotin, O. graminis was non-specific in its nitrogen requirements, growing well on ammonium, nitrate and organic sources, but best on asparagine. Earlier work had suggested that the fungus could not use inorganic forms of nitrogen (Fellows, 1936).

Other cultural studies have indicated that satisfactory growth will occur over a wide range of pH and the temperature for optimum growth is between 20°C and 25°C (Davis, 1925; Webb and Fellows, 1926; Russell, 1934; Ward and Henry, 1961).

(4) Life Cycle of Ophiobolus graminis.

O. graminis was aptly described as a root inhabitant by Garrett (1956) under his schema for soil fungi, because it is almost completely confined to host tissue in both the parasitic and saprophytic phases of its life. The only recorded exceptions are two reports of its free living occurrence (Anon., 1934; Fellows and Ficke, 1934) and the occasional direct isolation of O. graminis from soil (Warcup, 1957).

(a) Parasitic Phase.

There is ample evidence to show that the fungus only grows actively in the soil along the roots of susceptible hosts (Garrett, 1934, 1936, 1950; Padwick, 1935;

Adam and Colquhoun, 1936; Fellows and Ficke, 1939). During this process, the fungus produces two types of hyphae, viz., dark-coloured macrohyphae on the root surface and internal hyaline microhyphae (Fellows, 1928). The macro- and micro-hyphae are also known as runner- and infection-hyphae respectively (Garrett, 1934). The cells are uni-nucleate (Jones, 1926).

Runner hyphae frequently mass to form a black incrustation around the stem base inside the sheaths of the lower-most leaves, thus producing the most characteristic symptom of the disease. Perithecia sometimes form in the lowermost leaf sheaths and Jones (1926) described the cytology of perithecial development, although Turner (1940a) provided evidence that Jones may have examined perithecia of *O. graminis* var. *avenae* and not *O. graminis*. Detailed descriptions of symptoms are given by Kirby (1925), Fellows (1930), Russell (1934) and Garrett (1934, 1937a, 1942). The histology of infection has been studied by several workers (Davis, 1925; Fellows, 1928; Russell, 1934; Garrett, 1934).

The rate of growth by runner hyphae along the roots is influenced by soil conditions, being especially favoured by loose open soils (Garrett, 1936; Winter, 1939). In Australia, take-all is most prevalent in crops on loose, light-textured soils of an alkaline reaction (Gray, 1913; Hartmann, 1913; Griffiths, 1933; Garrett, 1934) but is not restricted to these soils (Richardson, 1910; Samuel, 1923, 1924).

The effect of soil alkalinity on hyphal growth along the roots was demonstrated by Garrett (1936) who varied the pH of "Slough" soil in England and obtained more growth with rising alkalinity. Garrett postulated a linkage between the concentration of carbon dioxide in the root zone and the growth of runner hyphae. He considered that alkaline soils act as chemical acceptors of respiratory carbon dioxide from the host root, hyphae of Ophiobolus graminis and the soil microflora; in acid soils, however, this carbon dioxide tends to accumulate, thereby restricting growth of runner hyphae.

Much of the available field and laboratory evidence is consistent in indicating that soil alkalinity favours O. graminis (Hartman, 1913; Brittlebank, 1919; Rosen and Elliott, 1923; Kirby, 1925; Fish, 1927; Glynne, 1935; Garrett, 1936, 1937, 1937a; Lal, 1939; Gorter, 1945). Nevertheless, the fungus will grow over a wide range of pH in culture (Kirby, 1925; Webb and Fellows, 1926; Ward and Henry, 1961) and severe outbreaks of take-all have been recorded on acid soils (Rosen and Elliott, 1923). As part explanation of such outbreaks, Garrett (1937) demonstrated that runner hyphae will grow satisfactorily in an acid soil provided it is aerated so as to reduce the accumulation of respiratory carbon dioxide. However, the phenomena may be more complex than indicated by Garrett, as Winter (1940) claimed that the growth of runner hyphae was suppressed more by substances from antagonistic soil micro-organisms than by the accumulation of carbon dioxide.

Soil temperature for optimum infection of host plants can also be influenced by microbial antagonism (Henry, 1932; Garrett, 1934). For example, Henry found that the optimum temperature for infection of wheat was only 13°-18°C in unsterilized soil and infection was reduced considerably with higher temperatures. However, in sterilized soil, the optimum temperature for infection approximated to the optimum of 20°-25°C for growth of the fungus on agar. Henry considered that increased microbial activity was responsible for the decrease in infectivity by _O. graminis_ in unsterilized soils at higher temperatures. Although most evidence suggests that take-all is encouraged by high temperatures, there are some contrary field observations, the most notable being from Japan (Suzuki, Kasi, Nakaya, Araki and Takahashi, 1957) and North America (Anon., 1934).

There is much field data to indicate that take-all is favoured by wet seasonal conditions (Sutton, 1920; Waters, 1920; Russell, 1934; Simmonds, 1939) especially during the spring period (Garrett, 1934; Pittman, 1937; Moore, 1948; Glynne, 1950; Blair, 1953; Sims, 1958). However, controlled experiments on soil moisture have given variable results. For example, Russell (1931, 1934) reported that the disease was favoured by a soil moisture of 30 to 40 percent moisture holding capacity, whilst McKinney and Davis (1925, 1925a) obtained optimum infection at 70 percent moisture holding capacity. Garrett (1936) found the growth of runner hyphae

to be more rapid in two soils at 30 percent saturation than at 70 percent saturation. Sanford (1927) and Winter (1939) reported most infection at 40 to 60 percent moisture holding capacity.

Griffin (1963), when reviewing the ecology of soil fungi in relation to soil moisture, noted that most workers described soil moisture regimes solely in terms of moisture holding capacity. However, this criterion provides no information either on the energy required to remove water from the soil or on the distribution of water and air in the soil; thus at present soil moisture regimes for optimum infection and hyphal growth by *O. graminis* cannot be adequately defined from available field and laboratory data. Further work based on the moisture characteristics (Childs, 1940) of various soils will be necessary to elucidate the problem.

Rate of growth of runner hyphae is not only influenced by soil conditions, but also by the reaction of the host to infection. Davis (1925), Robertson (1932) and Broadfoot (1933) showed that susceptibility of the wheat plant to take-all declines with age. Increased resistance of the host is attributed to lignification of the sub-surface parts of the plant (Fellows, 1928; Robertson, 1932). The overall effect of the disease may also be reduced with maturity of the plant because of an increase in production of new crown roots, which replace the older infected ones (Simmonds and Sallans, 1933). Production of new roots may

be further increased by the application of nitrogenous fertilizer to the growing crop (Clark, 1942; Stumbo, Gainey and Clark, 1942; Garrett, 1948) but susceptibility of individual roots to infection is increased at the same time (Garrett, 1948, 1956). Phosphatic fertilizers are also considered to promote vigorous root development, thereby reducing losses from take-all (Garrett, 1937a, 1948).

The natural host range of O. graminis is extensive but so far as is known it is restricted to the Gramineae. Garrett (1942) concluded that wheat was the most susceptible cereal, with barley less so, whilst rye was comparatively resistant and oats almost immune. Recently Butler (1961) suggested oats might be classified more accurately as highly resistant. The relative susceptibility of many grasses was studied by Kirby (1922, 1925), Padwick and Henry (1933), Russell (1934), Winter (1939) and Garrett (1941). While there was general agreement on the susceptibility of some species, there was also divergent opinion concerning others. Garrett (1942) attributed these differences to the different criteria used by the various workers when assessing relative susceptibility and concluded that a satisfactory method has still to be found for studies with grass species.

One of the inherent difficulties of host range studies is variation in pathogenicity of isolates of O. graminis. Pathogenic variability has been reported on many occasions (Davis, 1925; Russell, 1934, 1939;

Padwick, 1936; Hynes, 1937; White and McIntyre, 1943; Henry and Gilpatrick, 1947; Henry and McKenzie, 1959) and isolates maintained in culture for long periods tend to decline in virulence (Russell, 1934, 1939). Even monosporous isolates derived from one ascus may differ in pathogenicity (White, 1942) despite the fact that the fungus is homothallic (Davis, 1925; Padwick, 1939; White, 1939).

Recent demonstration of infection of wheat roots in unsterilized soil by ascospores of Ophiobolus graminis (Brooks, 1964, 1965) indicates that these spores may be important in disseminating the fungus. Brooks has suggested that air dispersal of ascospores may be responsible for the reported outbreaks of take-all on newly-drained polders of the former Zuider Zee in the Netherlands. Previously Samuel and Garrett (1933) had observed the ejection of ascospores from moistened ripe perithecia and had postulated the spread of take-all by wind-dispersal of ascospores during showery weather. However, Garrett (1939a) was unable to secure infection of wheat seedlings in unsterilized soil to substantiate the hypothesis. Gregory and Stedman (1958) have noted that ascospores of O. graminis were part of the air spora of a wheat field after one or more points of rain.

(b) Saprophytic Phase.

Direct microscopical observations by Garrett (1940) indicated that O. graminis does not become inactive with the death of the host but continues to grow slowly within the

tissue. However, evidence provided by Lucas (1955) suggested that the fungus is unlikely to spread saprophytically from infected material to other host residues in soil even when there is contact between the two tissues.

The first critical data on the effect of soil conditions on the saprophytic survival of *O. graminis* were given by Garrett (1938). He found that in artificially infected wheat straw *O. graminis* declined in viability most rapidly under conditions of medium to high temperature, adequate moisture content and good aeration. Fellows (1941) confirmed these results when investigating the disappearance of *O. graminis* from naturally infested soil. Van der Watt (1965) studied the effect of soil moisture on survival of *O. graminis* in South African soils and obtained survival values similar to but higher than those of Garrett. In all of these studies, however, soil moisture regimes have been described solely in terms of the moisture holding capacity of each soil and are therefore unacceptable for exact comparisons. Garrett also found that saprophytic survival was not affected by soil reaction, although Lal (1939) reported that *O. graminis* persisted better in light alkaline soil than in heavy acid soil.

Garrett (1938) observed that the saprophytic survival of *O. graminis* could be influenced by soil amendments; decline was hastened by nitrogen-deficient amendments, but delayed by nitrogen-rich material. However, gross

decomposition of the infected straw did not proceed parallel to rate of decline in viability, but was most rapid under nitrogen-enriched conditions. Garrett (1940) postulated that an ample nitrogen supply enhanced the survival of *O. graminis* by enabling it to assimilate more of the undecomposed carbohydrates in the straw. In contrast with an earlier hypothesis (Garrett, 1938) he decided that the relationship between *O. graminis* and other soil microflora was merely competitive for available food material. Garrett (1944) considered that the microscopic observations of Baker (1939, 1939a) on enzymic erosion of tissue by fungi and bacteria might also apply to *O. graminis* and postulated that when nitrogen was unavailable, the mycelium died from carbohydrate starvation after exhausting the zones of enzymic erosion around the hyphae.

The hypothesis that ample nitrogen aids survival of *O. graminis* by enabling it to continue saprophytic growth in infected tissue was supported by observations of Butler (1953) and Lucas (1955). Both found that pre-soaking straws with sodium nitrate increased their saprophytic colonization by *O. graminis* when buried in soil heavily inoculated with this fungus. Butler (1953a, 1959) also confirmed the effect of nitrogen in promoting the survival of *O. graminis* in wheat straw using artificially amended soil and soils differing naturally in fertility. Macer (1961) obtained a pronounced increase in survival of the fungus in wheat straws buried in nitrate-enriched soil. However, van der Watt (1965) reported

that nitrogenous soil amendments increased the longevity of O. graminis in wheat straws only when Rhizoctonia solani was present in the soil. Garrett (1944) also noted that O. graminis survived longer in infected straws buried in seed boxes containing fallow soil than in the same soil sown to oats, mustard or trefoil; he concluded that these non-susceptible crops had reduced survival of the fungus by competing with it for soil nitrogen.

Garrett further modified and generalized his hypothesis concerning the effect of nitrogen after studying the cellulose-decomposing ability of several cereal foot and root pathogens (Garrett 1963, 1966). He assigned competitive micro-organisms an even more subsidiary role than previously and maintained that saprophytic survival can be interpreted mainly in terms of the activity of the fungus on its substrate. Should nitrogen be present in excess, it may cause the fungus to consume the substrate at a rate in excess of that required for survival. This would ultimately reduce the longevity of the fungus.

The availability of food material and the physical soil conditions markedly influence the period of survival of O. graminis in wheat straw. Macer (1961) estimated survival in nitrate-enriched and nitrate-impoverished soils after 25 weeks as being 63 percent and four percent respectively. Butler (1959) recorded traces in a nitrogen-rich soil at 52 weeks but none thereafter. Other workers have found

that it may persist for more than two years (Russell, 1934; Fellows, 1941), but under optimal soil conditions for microbiological activity it may disappear from naturally infested soil within three months (Clark, 1942). It is possible that the period of survival may also be influenced by inherent characters of the fungus. However, most workers give little or no information about the isolates of O. graminis used in survival studies and no-one appears to have compared the survival of isolates which differ either morphologically or physiologically.

There are many published accounts on the relative susceptibility of different cereals and grasses to O. graminis but relative susceptibility does not imply any order of importance in perpetuating the fungus. For example, wheat is considered to be more susceptible than barley (Garrett, 1942; Butler, 1961; Chambers, 1962) yet Chambers (1963) recorded more take-all in wheat following barley than in wheat after wheat.

One of the first detailed studies on the relative importance of different grasses in perpetuating O. graminis was made by Padwick (1935) who planted seeds of four grass species and of ball mustard (Neslia paniculata (L.) Desv.) in pots which were artificially inoculated with the fungus; an additional series was left as fallow. After six weeks, the tops of the plants were cut off and the pots were sown to wheat. Appreciable infection of wheat seedlings occurred

after each grass but none after ball mustard or fallow. However, sowing wheat immediately after cutting the tops limits interpretation of the results.

Much of the work assessing the importance of grasses in the survival of O. graminis has been done in England. Garrett (1941) modified Padwick's technique to study the relative importance of sixteen grasses in the survival of O. graminis. He allowed the grasses to grow in boxes for two months before cutting off the tops and inverting the sods to simulate ploughing; at monthly intervals the degree of survival of O. graminis was determined by planting wheat seeds in some of the sods. The fungus survived on all species to some extent, but there were notable differences in the longevity of the fungus under different grasses. Wehrle and Ogilvie (1955) studied the relative importance of four grass species in the survival of O. graminis in the field. They planted strips of each grass across an area where wheat had been severely affected by take-all and re-cropped an increasing proportion of the grass ley with wheat over the following three years. They found no evidence that any grass carried over O. graminis at a higher level. Brooks (1965a) studied the relative importance of fifteen grasses in perpetuating O. graminis in outdoor plots over a thirteen-month period. He concluded that the spread and survival was greatest on Lolium italicum A. Br., L. perenne L., Festuca rubra L. and least on Alopecurus pratensis L. and Arrhenatherum elatius Mert. et Koch.

Relative importance of alternate hosts in perpetuating _O. graminis_ has not been studied in Australia, although casual observations (Sutton, 1911; Samuel, 1923; Fish, 1927; Griffiths, 1933; Anon., 1937; White, 1947; Anon.1961) suggest that the most important are _Hordeum leporinum_ Link, _Vulpia bromoides_ (L.) S. F. Gray, _V. myuros_ (L.) Gmel. and various _Bromus_ species, notably _B. hordeaceus_ L., _B. madritensis_ L., and _B. sterilis_ L. However, detailed field and laboratory experimentation will be required before the role of these hosts can be defined accurately for Australian conditions.

(5) _Varieties of Ophiobolus graminis_.

Although oats are generally resistant to infection by the type variety of O. graminis, an oat-attacking variety, var. _avenae_, was recorded in Wales in 1937 (Turner, 1940a). Subsequently this variety was identified in England (Garrett and Dennis, 1943), Scotland (Dennis and Foister, 1942), United States of America (Gould, Goss and Eglitis, 1961), Norway (Hansen, 1963) and Northern Ireland (Anon., 1964a).

Turner (1940 , 1940a, 1957) differentiated between the two varieties on the basis of pathogenicity, size and septation of ascospores, and growth on media containing cysteine, cystine or oat sap. The varieties differed pathogenically in that only var. _avenae_ attacked secondary oat roots, although runner hyphae of the type variety grew along seminal oat roots (Turner, 1940a). She also reported

that mean ascospore length of var. avenae (101-117 μ) was greater than that of the type variety (79-86 μ). Ascospores of var. avenae also had a greater number of septa. In culture the varieties differed in growth on media containing either cysteine or cystine, the growth of var. avenae being depressed whereas that of the type variety was increased (Turner, 1957); they also differed in their reaction to expressed oat sap in that, whereas growth of the type variety was inhibited, var. avenae was not (Turner, 1940). From subsequent studies on this phenomenon, Turner (1953, 1956, 1960, 1960a, 1961) determined that there was an enzymic basis for the difference in pathogenicity of the varieties on oats.

Garrett and Dennis (1943), Dennis (1944), Davies (1950) and Willetts (1961) also determined mean length of var. avenae ascospores and confirmed Turner 's results, although Davies reported a wider range (92 μ-142 μ). Willetts also reported perithecia of the type variety on oats, giving mean ascospore length as 88 μ. Thus, mean length of ascospores would seem a more variable and less reliable differential criterion than was originally considered by Turner.

Var. avenae has been recorded on many grasses in various countries (Smith, 1952, 1953, 1956, 1958, 1959; Jackson, 1959, 1961; Doling and Hepple, 1959; Gould, Goss and Eglitis, 1961; Anon., 1964a). Turner (1940a) concluded from pathogenicity tests with nineteen English grass species that in every instance infection was more intensive and

more extensive by var. *avenae* than by the type variety. Smith (1956) noted that the type variety seldom caused severe injury to grass hosts under field conditions, whereas var. *avenae* often killed them. Brooks (1965a) examined the spread and survival of var. *avenae* on fifteen grass species in outdoor plots at Cambridge, England, and concluded that *Arrhenatherum elatius* was the most effective carrier together with *Lolium italicum* and species of *Agrostis*.

In Australia, *O. graminis* has occasionally been recorded on oats (Darnell Smith and McKinnon, 1915; Osborne, 1919; Scott, 1948). Hynes (1937) reported susceptibility of oats to two isolates of *O. graminis* in pathogenicity tests in New South Wales; however, Turner (1940a) discounted these results, suggesting that a toxic effect from excessive inoculum may have caused the stunting observed by Hynes. Butler (1961) stated that var. *avenae* had not been recorded in Australia. Chambers (1964) has since reported an oat-attacking strain in Western Australia, but no attempt was made at the time to establish whether or not it was var. *avenae*.

MATERIALS AND METHODS

(1) Isolates of *Ophiobolus graminis*

Details of the isolates used are summarised in Table 1. Ascospores from perithecia of isolate W1 were also cultured singly to give a range of single-spore isolates. These have been designated by a number followed by the letter A, C or R, depending on whether they were obtained from a single ascus (A), a perithecium formed in culture (C), or a perithecium formed on an oat plant (R).

Stock cultures were maintained on a glucose-asparagine medium (Lilly and Barrett, 1951). The composition of this and other media is described in Appendix A.

(2) Methods of Isolation

For isolation of *O. graminis* from infected roots or straw, small fragments of tissue were immersed in one-percent silver nitrate for 30 seconds. After three ten-second rinses in sterile water, the fragments were placed on freshly poured plates (3 fragments/plate) of potato-dextrose-"Marmite"-agar (PMD) containing 33 p.p.m. Aureomycin hydrochloride. After two to four days' incubation at 20°C, the plates were examined for typical whorled hyphae of *O. graminis* and hyphal tips of the fungus were transferred to PMD slopes. A 30-second immersion period in one-percent silver nitrate gave more satisfactory results than the two-minute period originally suggested

TABLE 1

ORIGIN OF ISOLATES OF *OPHIOBOLUS GRAMINIS*

Culture Numbers	ORIGIN				Isolated by
	Fungal Structure	Host	Locality	Date	
W1	Ascospore	Wheat	Alford, So. Aust.	Feb., 1965	S. C. Chambers
W2	Hyphal tip	Wheat	Glen Osmond, S. Aust.	Oct., 1965	S. C. Chambers
W3	Hyphal tip	Wheat	Glen Osmond, S. Aust.	Oct., 1965	S. C. Chambers
W4	Hyphal tip	Wheat	Moora, W. Aust.	Dec., 1959	S. C. Chambers
W5	* -	Wheat	Wagga Wagga, N.S.W.	Sep., 1961	F. C. Butler
01	Ascospore	Oats	Forest Hill, W. Aust.	Dec., 1963	G. Weste
02	Ascospore	Oats	Williams, W. Aust.	Oct., 1965	S. C. Chambers
03	-	Oats	Woogenellup, W. Aust.	Nov., 1962	G. Weste
04	-	Oats	Mt. Gambier, S. Aust.	Oct., 1962	R. L. Dodman
05	-	Oats	Mt. Gambier, S. Aust.	Oct., 1962	R. L. Dodman
06	-	Oats	Mt. Gambier, S. Aust.	Oct., 1962	R. L. Dodman
07	-	Oats	Mt. Gambier, S. Aust.	Oct., 1962	R. L. Dodman
08	-	Oats	Munabella, S. Aust.	Sep., 1961	R. L. Dodman
09	-	Oats	Woodchester, S. Aust.	Sep., 1961	R. L. Dodman
1A-8A	8 Ascospores from Ascus of W1	Oats	-(Pathogenicity Test)	Sep., 1965	S. C. Chambers
1C-100C	100 Ascospores from Perithecium of W1	-	-(Culture)	Oct., 1965	S. C. Chambers
1R-100R	100 Ascospores from Perithecium of W1	Oats	-(Pathogenicity Test)	Oct., 1965	S. C. Chambers

* = Structure not recorded

by Davies (1935).

Monosporous cultures were obtained by placing a mature perithecium on a flamed microscope slide and adding a drop of sterile water to induce ejection of ascospores. The spores were collected in a capillary pipette and after dilution in sterile water were scattered over the surfaces of freshly poured plates of PMD + Aureomycin. After two days' incubation at 20°C, pieces of agar, each containing a germinated ascospore, were cut out and transferred to fresh plates of PMD.

For the isolation of eight ascospores from a single ascus, a mature perithecium was split open on a flamed microscope slide and transferred to a petri dish containing a layer of water-agar covered by cellophane. A needle with a curved tip was used to move the perithecium over the cellophane surface so as to scatter the asci. Under a dissecting microscope, any perithecial debris was cleared from around an isolated ascus. A piece of cellophane ($4mm.^2$) bearing the ascus was cut out and placed on the surface of a freshly poured plate of PMD + Aureomycin. A drop of sterile water was added to the ascus which was then kept under microscopic observation until the ascospores were ejected. More water was added and the ascospores were dispersed by rotary movement of the petri dish. After two days' incubation at 20°C, pieces of agar, each containing one of the eight germinating ascospores, were cut out and transferred to fresh plates of PMD.

(3) Soils.

Two soils were used for laboratory studies; a calcareous sandy soil from a barley field near Moonta, South Australia, and a lateritic podzolic soil from an oat field at Forest Hill, Western Australia. Each soil was passed through a six-mm. sieve in order to remove coarse gravel and any large pieces of plant debris. Field experiments were carried out in a red-brown earth of the Urrbrae series at the Waite Agricultural Research Institute, South Australia. Particle size distribution, nitrogen content, carbon content and pH of each soil is given in Table 2. The "moisture characteristic" of each soil was determined by the method described by de Beer (1965). Portions of the drying boundary curves of the soils are shown in Fig. 1.

(4) Pathogenicity Tests.

Seed was sown in thin plastic pots, each containing 250 grams of Moonta soil which had been "partially sterilized" with aerated steam at 160°F for 30 minutes (Baker, 1962). Each seed was planted between two agar discs cut out with an 8mm. diameter cork borer from a 14 day-old culture growing on PMD. Five seeds were sown in each pot, but the number of plants was reduced to four after emergence. Moisture content of the soil was adjusted to 15 percent (pF1.6) and maintained at that level by watering the pots daily to a constant weight. Pots were placed in a growth cabinet adjusted to a ten hour

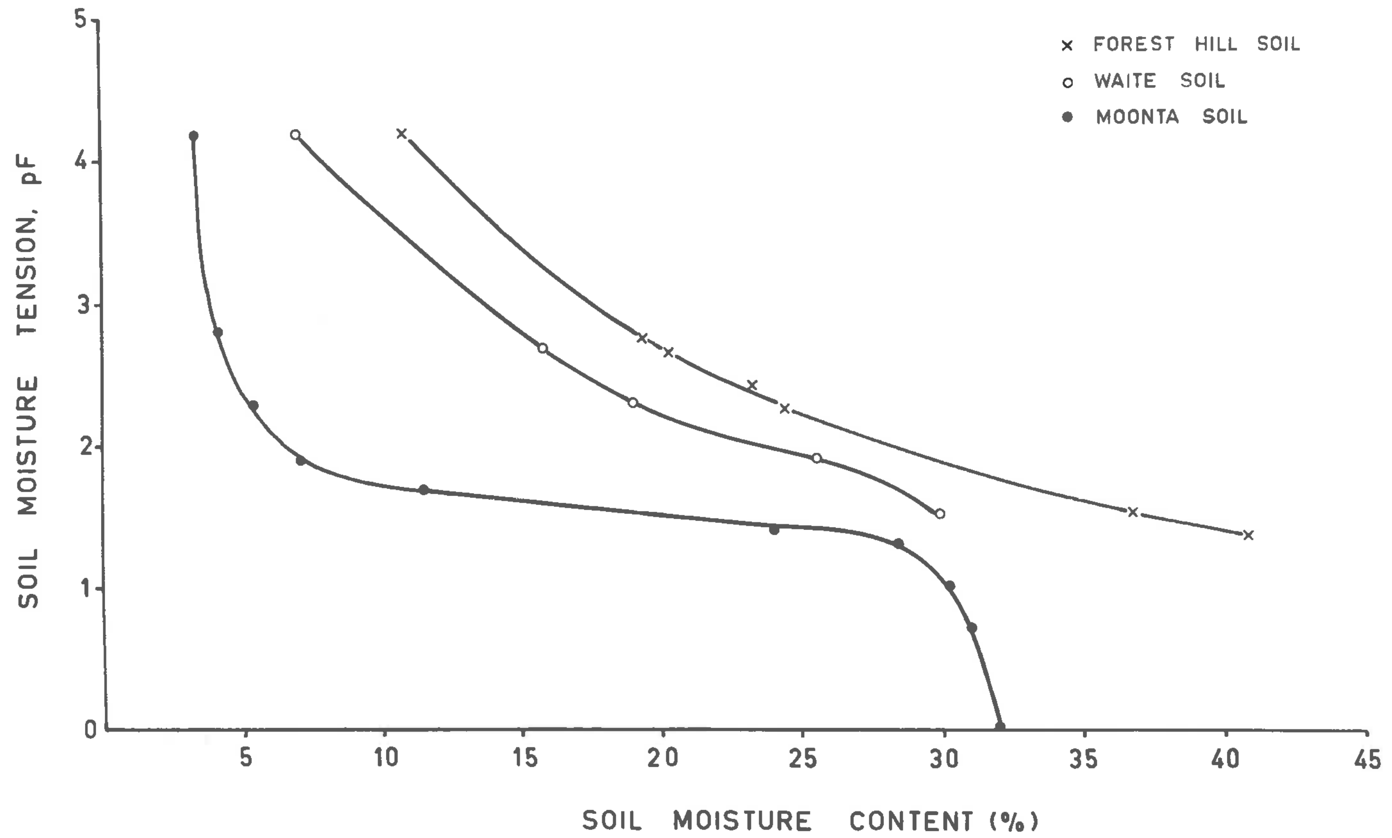
TABLE 2.

ANALYSES OF THE THREE EXPERIMENTAL SOILS

Source of Soil	Particle Size Distribution			Nitrogen			Organic Carbon %	pH
	1-2 µ (Clay) %	2-20 µ (silt) %	Over 20 µ (sand) %	Total % (Kjehdahl method)	Nitrate Nitrogen ppm.	Ammonium Nitrogen ppm.		
Moonta, Sth. Aust.	8	2	90	0.041	7.5	3.9	0.7	8.5
Forest Hill, West Aust.	3	7	90	0.205	76.4	13.2	4.8	7.0
Glen Osmond, Sth. Aust.	20	34	46	0.084	25.9	11.0	1.6	7.6

Fig. 1.

DRYING BOUNDARY CURVES OF THE THREE EXPERIMENTAL SOILS



"day" with a light intensity of 1500 foot candles and day and night temperatures of 20°C and 18°C respectively.

After four weeks, the roots of all hosts other than oats were washed free from soil and the tops were cut off and weighed. The oldest three roots on each plant were examined under a dissecting microscope (magnification x50) and the length of runner hyphae measured (Garrett, 1936). At the same time, measurements were taken of the overall length of the root together with the length discoloured by infection. In the case of oats, the plants were allowed to grow for eight weeks before harvesting as outlined above.

The design for each experiment was a single randomization of treatments within each of either three or four replications.

(5) Production of Perithecia

A method similar to that of Weste and Thrower (1963) was used for producing perithecia in culture. Fertile perithecia were formed by many isolates when incubated at 20°C on the glucose-asparagine medium and exposed to a light intensity of 210-290 foot candles. The cultures were kept on the shelf of a converted shop display cabinet maintained at 20°C under a bracket of four 40-watt fluorescent light tubes.

During pathogenicity tests, perithecia were formed by some isolates on roots in contact with the sides of the translucent pots.

(6) Measurement of Asci and Ascospores.

Measurements for a particular isolate were based on a random selection of asci and ascospores from at least five perithecia obtained from the same source.

(7) Estimation of Saprophytic Survival.

The technique for estimating the survival of *Ophiobolus graminis* in artificially infected straw was similar to that originally described by Garrett (1938).

Fully mature straws were cut into one-inch lengths so as to contain a node at one end and part of an internode covered by the leaf sheath. The segments were sterilized by adding 20 ml. of water to 500 straws in a litre flask and autoclaving at one-and-a-half atmospheres for 90 minutes. After cooling, each flask was inoculated with 150 g. of month-old maize-meal sand culture (Butler, 1953) of the test isolate and incubated at 25°C for fourteen days. The flasks were then shaken vigorously and incubated at the same temperature for a further fourteen days. The straws were removed, washed clean in running water and buried in the test soil.

In laboratory studies, straws were buried fifty at a time in 200 grams of soil in a wide-mouthed glass jar. Straws were first mixed thoroughly with three-quarters of the soil and afterwards the remainder of the soil was added to the surface. Soil and straws were then compressed to a bulk density of 1.3. When only a low moisture content was required, the necessary water was thoroughly mixed with the soil before

adding the straws, but when a high moisture level was used, water was added by pipette after mixing soil and straws. In both instances, the soil surface was covered with a thin polythene film before sealing the jar with a screw cap lid and storing in a growth cabinet. The cabinet was adjusted to a ten-hour "day" with day and night temperatures of 20°C and 18°C respectively. In most experiments, a second series of jars was prepared differing from the above only in that a solution of a nitrogenous compound was used for adjusting the soil moisture level instead of water. Unless otherwise stated, sodium nitrate was used and was applied at the rate of 100 mg. nitrogen per Kg. air dry soil. In field studies, straws were buried at one inch and six inches below the surface in batches of 100 and their position marked.

In all experiments, 100 straws of each treatment were unearthed after four, eight, twelve and twenty-four weeks' burial and a seed was inserted into the lumen of each straw. The seed was placed with its embryo downwards in the lumen to ensure that the roots of the seedling grew through the straw and thus came into contact with any surviving mycelium of Ophiobolus graminis. Wheat seeds were used for experiments with wheat and oat straw, but silver grass (Vulpia myuros (L.) Gmel.) seeds were used for grass straws. Each straw was planted upright in moist coarse sand in a disposable paper cup of 36 c.c. capacity and placed in a glass house with a temperature of 20°C to 30°C for four weeks. The seedlings were then removed from the straws and examined under a dissecting micro-

scope (magnification x12.5 and x50) for runner hyphae of O. graminis. The number of infected seedlings represented the percentage of straws containing viable mycelium of the fungus at the time of testing.

(8) Preparation of an Aqueous Oat-leaf Extract.

For comparative studies with Australian oat and wheat isolates, an oat extract was prepared (Janes, 1947). Freshly harvested leaves from four week-old plants of the variety Ballidu were dried overnight at 80°C and then ground to pass through a one-mm. sieve. An aqueous extract was obtained by adding 5 g. of the ground material to 500 ml. of distilled water and steaming for one hour. Upon cooling, the insoluble portion was filtered off on a Buchner funnel with No. 42 Whatman filter paper to give a clear yellow solution.

(9) Preparation of Mycelium for Nuclear Studies.

For studies on hyphal anastomosis and the distribution of nuclei in hyphae, isolates were grown on cellophane overlying PMD in Petri dishes. The cellophane (British Cellophane Ltd., non-moistureproof film 22 µ thick) was cut into 8 cm. diameter discs, boiled in distilled water for one hour to remove the surface film and autoclaved in distilled water for 20 minutes. It was then placed aseptically over the surface of freshly poured plates of PMD.

For determining nuclear distribution in mycelium,

inoculum was taken from the periphery of an actively growing colony and placed centrally on the cellophane. After 3-5 days' incubation at 20°C, a sector was removed and stained with HCl-Giemsa (Robinow, 1945) adapted from the method described by Hrushovetz (1956).

For studies on hyphal anastomosis, the inocula of two isolates were placed 25 mm. apart and incubated at 20°C until the two colonies came into contact. The common segment of intermingling hyphae was cut out and stained with HCl-Giemsa. Material for staining was fixed in Carnoy's fixative (three parts 95 percent ethyl alcohol to one part glacial acetic acid) for ten minutes. It was then immersed in cold 1N.HCl for five minutes, hydrolysed in 1N.HCl at 60°C for eight minutes and washed in three changes of distilled water for five minutes. The material was then immersed in phosphate buffer for five minutes and placed in Giemsa staining solution for 20 to 40 minutes. After 20 minutes in the staining solution, the material was transferred to phosphate buffer on a slide and examined under a microscope. If the nuclei were insufficiently stained, the material was returned to the Giemsa solution. Temporary mounts were made in phosphate buffer and the cover-slip sealed with a colourless nail polish. Mounts which were properly sealed and stored in a refrigerator at 45°F were useful for up to nine months.

The Giemsa stock solution contained 3.8 g. Giemsa's stain powder in 125 ml. glycerine and 375 ml. methyl alcohol.

The staining solution was made by mixing 20 drops of Giemsa stock solution with ten ml. phosphate buffer. The phosphate buffer contained 0.1 percent KH_2PO_4 and 0.2 percent Na_2HPO_4 and was adjusted to pH 6.8.

E X P E R I M E N T A L

Consideration of the available data on the take-all disease and its causal organism suggests there are several aspects which require further study. For example, while there is much detailed data on the effects of soil conditions on the survival of C. graminis in wheat straw, no-one has studied whether different isolates survive equally well under the same environmental conditions. Thus there is no information on whether any specific morphological and physiological variations of the fungus affect its survival. Furthermore, the observations on the effect of nitrogen enrichment of soil do not clearly differentiate between direct effect of amendments as distinct from indirect effects through the growth of other micro-organisms. In Australia there is also need for further information on the relative importance of some of the more common pasture species in the survival of O. graminis and also on the identity of isolates from oats. It was the purpose of this investigation to provide additional data on these points.

The initial work was designed to study some of the variation within the progeny of a single isolate before comparing the survival of variants.

(1) Studies with F_1 Progeny of an Isolate from Wheat.

(a) Colony Characteristics and Pathogenicity of Monosporous Isolates from a Single Ascus.

Heritable variations in colony colour and pathogenicity of monosporous isolates from a single ascus were noted by White (1942) and it is possible that such isolates differ also in other characters. Eight monosporous isolates were therefore obtained from a single ascus of a perithecium which developed on the roots of a living oat plant inoculated with culture W1. Initially the isolates were similar in cultural appearance, each colony being flat and composed entirely of hyaline hyphae which gave them an off-white colour. However, by three weeks all showed slight differences in strand development and colour.

Perithecial formation in sub-cultures of the original eight monosporous isolates was induced and by 21 days ripe perithecia had developed in all cultures. Measurement of asci and ascospores from these perithecia indicated that their mean lengths were greater than those of the asci and ascospores from perithecia on the field material from which W1 was originally isolated (Table 3).

When tested, the parent culture and its progeny were all found to be strongly pathogenic on wheat seedlings (Fig.2). Furthermore, there was no significant difference in the extent of hyphal growth along roots nor in the mean length of root discoloured by infection by different isolates (Table 4). These results therefore contrast with those of White (1942),

TABLE 3.

ASCUS AND ASCOSPORE MEASUREMENTS OF EIGHT SINGLE SPORE ISOLATES FROM ONE ASCUS OF *O. GRAMINIS*

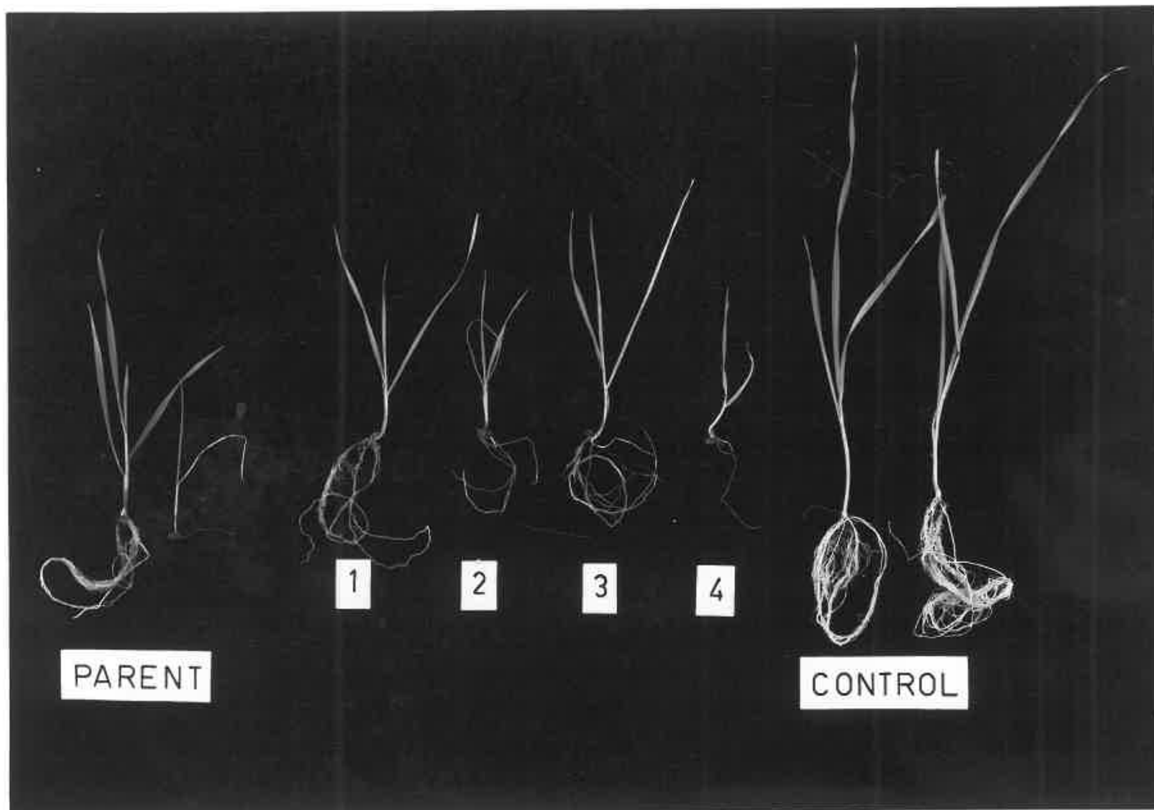
ISOLATE	ASCI		ASCOSPORE MEASUREMENTS				
	SOURCE	MEAN LENGTH (in µ)	LENGTH (in µ)			NUMBER OF SEPTA	
			MEAN	MODAL	RANGE	MEAN	RANGE
1A	Culture	116 ±0.67 *	85 ±0.66 *	84	71-102	7.1	5-11
2A	"	114 ±0.67	90 ±0.66	92	73-107	7.2	5-11
3A	"	117 ±0.81	87 ±0.67	84	73-105	7.2	5-11
4A	"	116 ±0.73	90 ±0.60	92	76-110	7.3	5-11
5A	"	116 ±0.67	90 ±0.57	89	79-105	7.3	5-11
6A	"	118 ±0.80	88 ±0.59	92	73-105	7.2	5-11
7A	"	115 ±0.76	88 ±0.73	84	68-105	7.4	5-11
8A	"	115 ±0.76	87 ±0.63	92	73-107	7.3	5-11
#1 (Parent)	"	115 ±0.75	87 ±0.60	92	73-102	7.2	5-11
#1 (Parent)	Original material	106 ±0.91	83 ±0.58	81	68-94	7.0	5-9

* Standard error.

Fig. 2a

Fig. 2b

Figs. 2a and 2b. General appearance of wheat seedlings, four weeks after inoculation with strongly-pathogenic monosporous isolates (1A-8A) from a single ascus of <u>O. graminis</u>. Seedlings inoculated with parent isolate (left) and control seedlings (right) are included for comparison.


1
2
3
4
PARENT
CONTROL

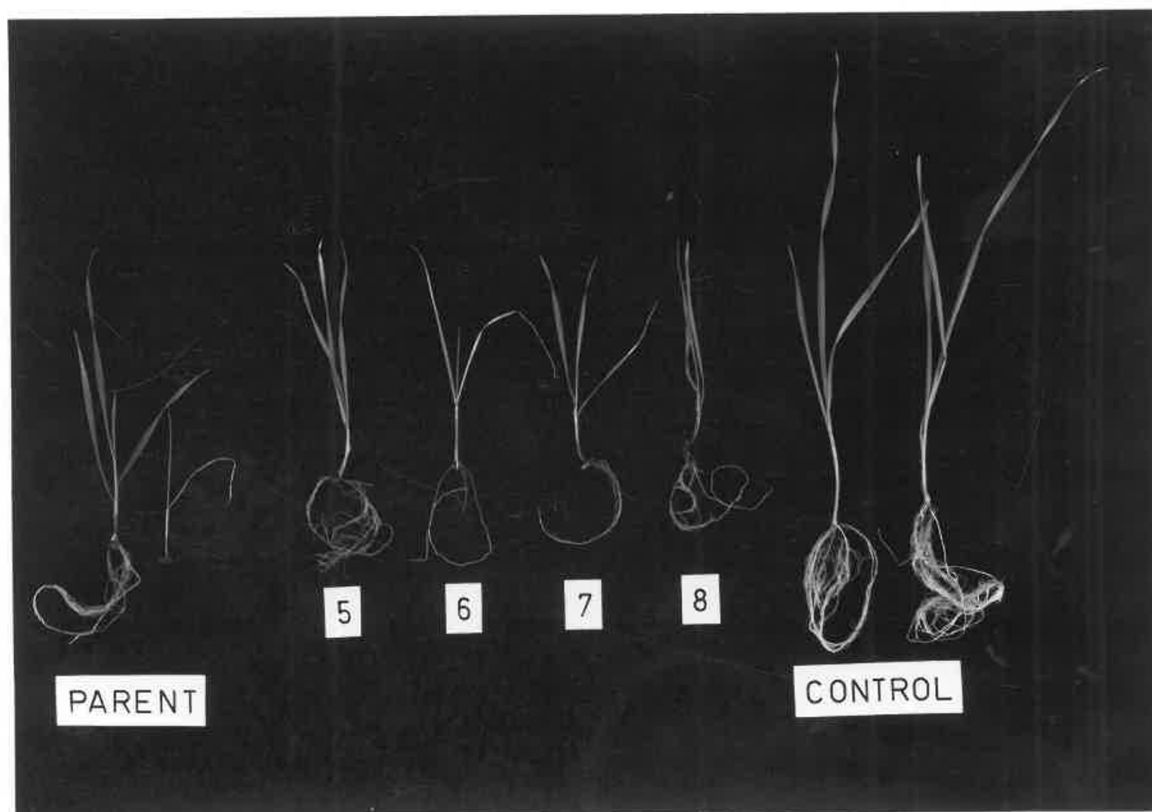

5
6
7
8
PARENT
CONTROL

TABLE 4.

PATHOGENICITY ON WHEAT OF EIGHT SINGLE-SPORE ISOLATES FROM ONE ASCUS OF *OPHIOBOLUS GRAMINIS*

Isolate	Fresh Weight of host (mg)	Length (in mm.) of:			Analysis of Root Measurements Using Transformation $\log_e$		
		Discoloured Root	Runner Hyphae along root	Whole Root	Discoloured Root	Runner Hyphae along root	Whole Root
1A	282	22	40	131	3.1312	3.7171	4.8773
2A	333	21	38	113	3.0890	3.6621	4.7318
3A	257	21	41	117	3.0890	3.7279	4.7644
4A	343	23	43	110	3.1781	3.7730	4.6848
5A	266	25	43	109	3.2445	3.7756	4.6933
6A	308	21	44	122	3.1045	3.8086	4.7868
7A	281	24	40	89	3.1917	3.7134	4.5002
8A	345	21	39	118	3.0910	3.6952	4.7605
W1 - Parent	287	21	41	119	3.0889	3.7221	4.7828
Control	361	0	0	184			5.2184
		Difference for significance P = 0.05			N.S.	N.S.	0.2987

who found that four of the monosporous isolates from an ascus were strongly pathogenic, whilst the remaining four were weakly pathogenic. However, the perithecia were formed under different conditions in these two investigations. In my case, the perithecium was produced on a root of a living plant, whereas those used by White developed on roots of dead plants (White, 1939). This suggests virulence of ascospores may possibly be influenced by nutriment derived from the substrate, but more likely by the relationship (parasitic or saprophytic) of the fungus with the substrate at the time of perithecial formation. An alternative explanation is that some of the variants of the fungus may produce only strongly pathogenic ascospores whilst others form ascospores which differ in virulence.

(b) Colony Characteristics and Pathogenicity of Monosporous Isolates from Perithecia Produced on Different Substrates.

In order to further examine the effect of the substrate, monosporous isolates were obtained from perithecia formed by isolate W1 in culture and also on the roots of a living oat plant. A random selection of 100 monosporous isolates was made from a single perithecium from each source.

Isolates from the perithecium on the oat plant were all similar in cultural appearance. Twelve isolates were selected at random and tested for pathogenicity on wheat seedlings. All proved to be strongly pathogenic and there were no significant differences between the extent of hyphal growth

along roots nor in mean lengths of root discoloured by different isolates (Table 5). Fertile perithecia were formed in culture by the same twelve isolates. Mean lengths of their asci were greater than that for asci on field material from which W1 was originally isolated. Mean lengths of their ascospores were either equal to or greater than that for ascospores on the original plant material (Table 6).

The monosporous isolates from the perithecium formed in culture showed slight variations in cultural appearance (Fig. 3a) The majority (68 percent) were characterized by pronounced white strands and another 25 percent differed only in that the strands were dark brown to black in colour. Of the remainder, thirteen had no pronounced strands, whilst the other four were characterized by slowness of growth. However, after the first sub-culturing, all isolates were similar in cultural appearance and in growth rate and indistinguishable from those obtained from the perithecium on the oat root (Fig. 3b).

The parent W1 and three isolates of each of the four variant groupings were tested for pathogenicity on wheat seedlings. Six isolates were strongly pathogenic but the remainder were only weakly pathogenic (Fig. 4). The strongly pathogenic isolates were equally as virulent as the parent and there was no significant difference in the amount of root discolouration caused, nor in the extent of their runner hyphae along roots (Table 7). However, the six weakly pathogenic

TABLE 5.

PATHOGENICITY ON WHEAT OF TWELVE SINGLE-SPORE ISOLATES FROM A SINGLE PERITHECIUM OF <u>O. GRAMINIS</u> FROM AN OAT ROOT

Isolate	Fresh Weight of Host in mg.	Length (in mm.) of:			Analysis of Root Measurements Using Transformations		
		Discoloured Root	Runner Hyphae along root	Whole Root	$\log_e(X+1)$ Discoloured Root	$\log_e(X+1)$ Runner Hyphae along root	$\log_e X$ Whole Root
2R	78	25	33	65	3.2576	3.5261	4.1472
4R	65	28	35	57	3.3601	3.5666	3.9970
11R	60	28	38	67	3.3525	3.6532	4.1988
25R	121	31	44	81	3.4589	3.7820	4.3730
31R	153	30	41	78	3.4203	3.7223	4.3323
54R	16	24	26	30	3.0951	3.1596	3.2006
62R	71	28	39	69	3.3276	3.6384	4.1612
69R	84	28	41	68	3.3621	3.7321	4.1972
74R	62	26	35	63	3.2819	3.5757	4.1261
92R	67	31	42	60	3.4654	3.7554	4.0834
98R	62	20	26	47	2.9142	3.0822	3.4457
100R	113	30	43	95	3.4223	3.7910	4.5157
Control	384	0	0	170			5.1382
Difference for Significance P = 0.05					N.S.	N.S.	0.8497

TABLE 6.

ASCUS AND ASCOSPORE MEASUREMENTS OF SINGLE SPORE PROGENY AND PARENT ISOLATE OF <u>OPHIOBOLUS GRAMINIS</u>

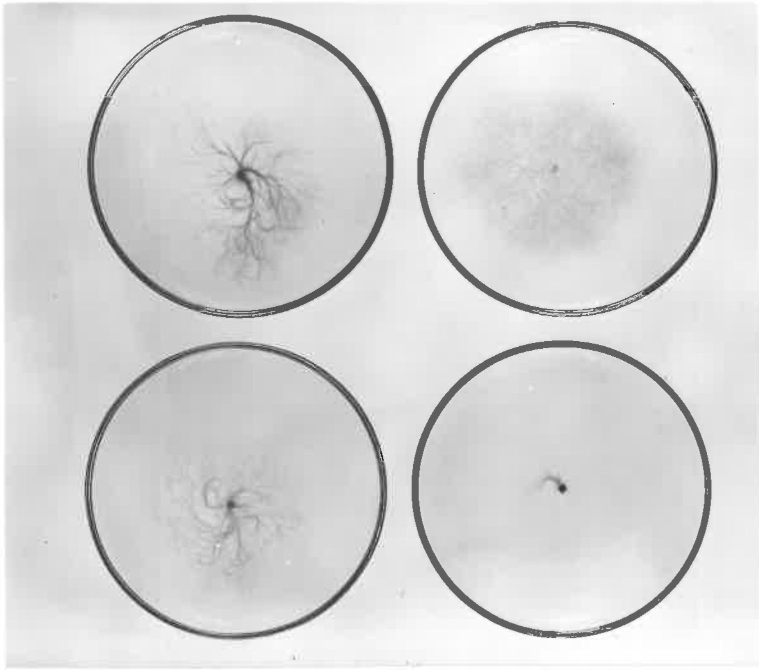
ISOLATE	ASCI		ASCOSPORE MEASUREMENTS					
	SOURCE	MEAN LENGTH (in μ)		LENGTH (in μ)			NUMBER OF SEPTA	
			MEAN		MODAL	RANGE	MEAN	RANGE
2R	Culture	116	±1.18 *	89	±0.87 *	92	68-107	7.2	5-11
4R	"	118	±0.91	88	±0.68	92	73-105	7.1	5-11
11R	"	119	±0.85	85	±0.73	86	68-102	7.1	5-11
25R	"	120	±0.90	88	±0.62	92	71-105	7.2	5-11
31R	"	116	±0.86	88	±0.77	89	68-113	7.0	3- 9
54R	"	117	±0.90	88	±0.56	92	79-105	7.2	5-11
62R	"	120	±0.96	83	±0.56	79	71-100	7.0	5- 9
69R	"	117	±0.84	84	±0.69	79	73-105	7.1	5-11
74R	"	118	±0.88	85	±0.93	86	68-105	7.1	5-11
92R	"	117	±0.83	84	±0.55	84	68-100	7.1	5-11
98R	"	117	±0.88	87	±0.55	92	73-102	7.2	5-11
100R	"	116	±1.11	83	±0.55	79	71-97	7.2	5-11
W1 (Parent)	Original material	106	±0.91	83	±0.58	81	68- 94	7.0	5- 9

*Standard error.

Fig. 3a. Different types of initial growth by single spore isolates from perithecium formed in culture.

Top left: Definite dark strands
Top right: No strands
Bottom left: Definite light strands
Bottom right: Slow rate of growth

Fig. 3b. Appearance of same isolates after the first subculture. There are no definite strands in any and growth of each is now similar.



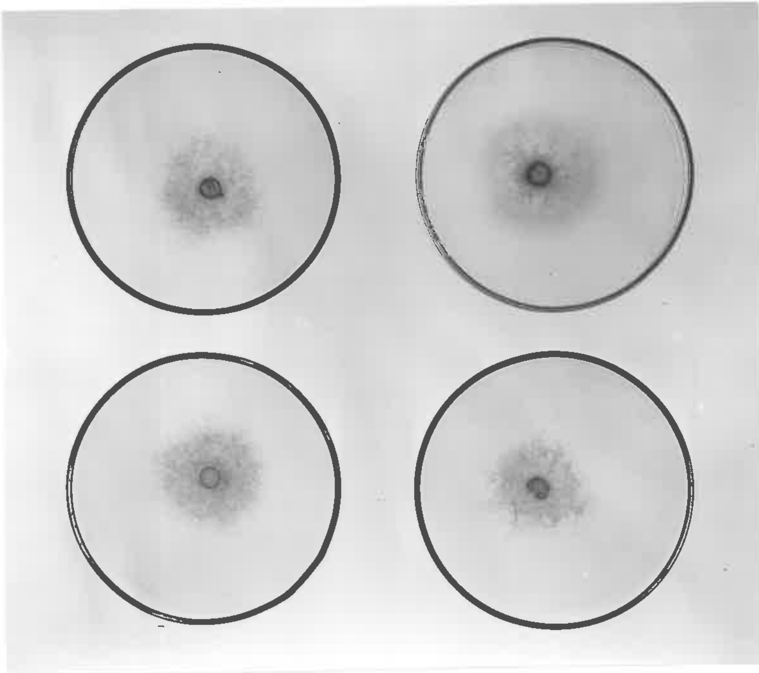


Fig. 4. Wheat seedlings inoculated with strongly pathogenic isolate 2C, weakly pathogenic isolate 74C and parent isolate W1. Note similarity in appearance of cultures 2C, 74C and W1 above seedlings.

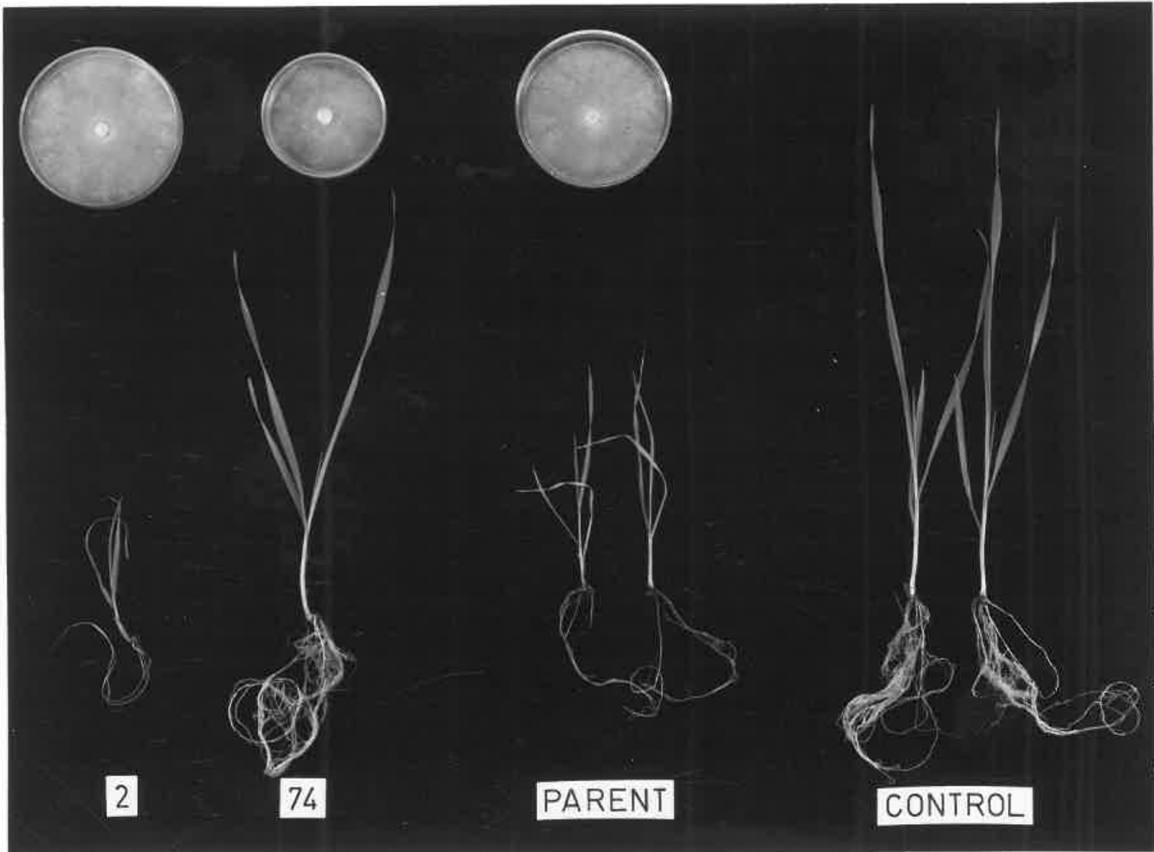

2
74
PARENT
CONTROL

TABLE 7.

PATHOGENICITY ON WHEAT OF TWELVE MONOSPOROUS ISOLATES FROM ONE PERITHECIUM OF *OPHIOBOLUS GRAMINIS*

Isolate	Fresh Weight of Host (mg.)	Length (in mm) of:			Analysis of Root Measurements Using transformation $\log_e X$		
		Discoloured Root	Runner Hyphae along root	Whole Root	Discoloured Root	Runner Hyphae along root	Whole Root
2C	278	29	51	170	3.3958	3.9403	5.1154
4C	249	27	41	132	3.3142	3.7433	4.8774
25C	391	22	40	191	3.1467	3.6924	5.2538
62C	388	26	45	158	3.2657	3.8146	5.0666
92C	333	22	39	179	3.1460	3.6855	5.1867
98C	327	26	47	167	3.3061	3.8767	5.1012
11C	550	3	7	253	1.1945	2.0897	5.5322
31C	458	3	9	252	1.2904	2.2445	5.5330
53C	573	2	6	291	0.8283	1.8661	5.6735
69C	496	2	6	235	0.9986	1.9390	5.4587
74C	490	2	8	264	0.9027	2.1351	5.5782
100C	477	2	10	249	1.0594	2.3404	5.4992
Parent (#1)	316	29	47	139	3.3865	3.8391	4.9298
Control	522	0	0	270			5.5970
		Differences for significance		$P = 0.05$	0.7843	0.4054	0.2747
				$P = 0.01$	1.0678	0.5520	0.3705

pathogenic isolates produced only limited growth along roots and very little discolouration.

Hand sections of roots were examined microscopically after being cleared in lactophenol and stained with cotton blue. In sections of roots infected with strongly pathogenic isolates, infection hyphae frequently branched from the external runner hyphae, entered the cells of the cortex, also penetrated the endodermis and invaded the vascular tissue. With weakly pathogenic isolates, however, penetration by infection hyphae occurred less frequently and was generally confined to the first two or three layers of cortical cells.

In culture mature perithecia were produced by the six strongly pathogenic isolates but not even rudiments of perithecia were formed by the six weakly pathogenic isolates. Mean lengths of asci and ascospores formed in culture were again greater than those of the original material from which the parent W1 was isolated (Table 8). During the pathogenicity tests, the strongly pathogenic isolates, irrespective of origin, formed perithecia on roots in contact with the sides of the translucent pots (Fig. 5); none was produced on roots of plants inoculated with weakly pathogenic isolates.

These results suggest a linkage between virulence and production of perithecia. This was also evident in subsequent studies with isolates from wheat and oats (page 74) when perithecia were only formed by isolates which were strongly pathogenic. It is interesting to note that Davis (1925) only obtained perithecia in cultures of the "New York" strain, which

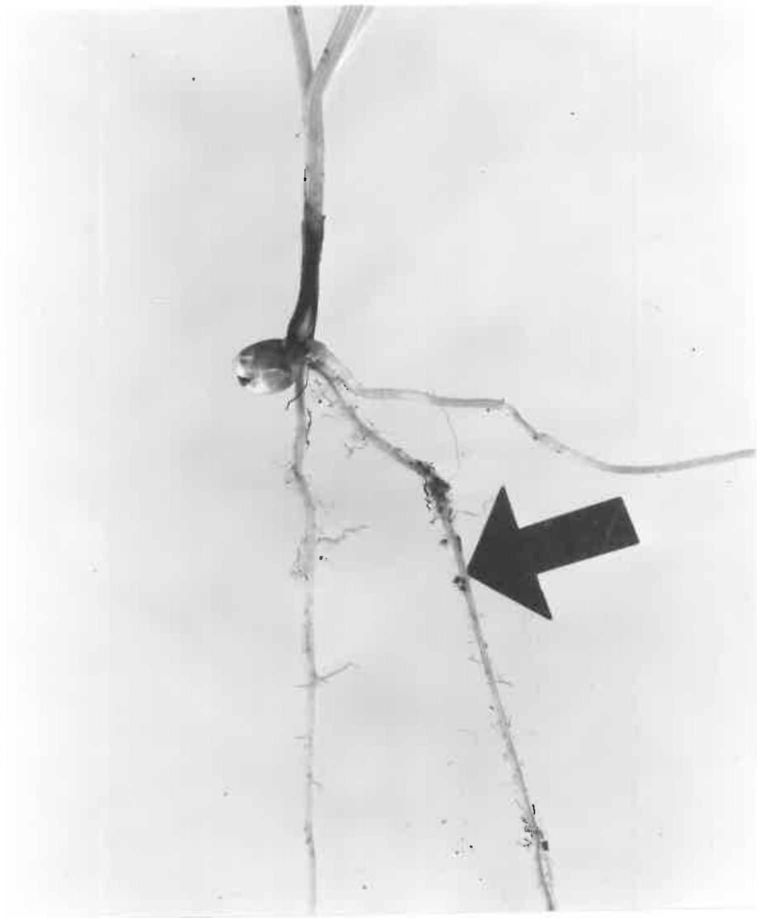
TABLE 8.

ASCUS AND ASCOSPORE MEASUREMENTS OF SINGLE SPORE PROGENY AND PARENT ISOLATE OF OPHIOBOLUS GRAMINIS

ISOLATE	ASCI			ASCOSPORE MEASUREMENTS					
	SOURCE	MEAN LENGTH (in μ)		LENGTH (in μ)				NUMBER OF SEPTA	
				MEAN		MODAL	RANGE	MEAN	RANGE
2C	Culture	117	±0.59*	95	±0.53*	94	84-107	7.7	3-11
4C	"	120	±0.76	95	±0.51	92	81-107	7.6	5-11
25C	"	120	±0.76	94	±0.55	92	81-110	7.6	3-11
62C	"	120	±0.69	96	±0.49	100	81-113	7.9	5-11
92C	"	121	±0.77	96	±0.45	100	84-107	7.8	5-11
98C	"	121	±0.81	94	±0.49	92	81-107	7.4	5-11
W1 (Parent)	"	120	±0.72	95	±0.56	92	81-110	7.7	5-11
W1 (Parent)	Original material (Naturally infected plant)	106	±0.91	83	±0.58	81	68- 94	7.0	5- 9
W1 (Parent)	Pathogenicity test (Artificially infected plant)	106	±0.57	84	±0.51	81	71- 94	7.0	5- 9

* Standard error.

Fig. 5. Perithecia of O. graminis (arrow) formed on the roots of a wheat seedling inoculated with the strongly pathogenic monosporous isolate 31R.



was the most pathogenic of his three test isolates. Willetts (1961), when examining six isolates of O. graminis from wheat and oats, noted that two oat isolates were weakly pathogenic and failed to produce perithecia on either wheat or oat plants. The remaining four isolates formed perithecia on host tissues on which they were actively parasitic. An interesting trend was the increase in size of asci and ascospores formed in culture compared with those on the original field material; this suggests that environmental conditions and substrate may influence their size.

The results also demonstrated that an isolate may produce strongly pathogenic ascospores on one substrate and ascospores differing in virulence on another substrate. However, it is not known whether this was due to a nutritional factor associated with the substrate or to the fungus-substrate relationship (saprophytic or pathogenic) at the time of perithecial formation. It would therefore be interesting to examine the effect of nutrition, especially source and concentration of nitrogen, on the virulence of ascospores of a single isolate in culture. It would also be of value to study perithecial development in the field and determine the comparative numbers of perithecia produced on living and dead plants as well as the virulence of their ascospores.

(c) Pathogenicity Tests with Hosts Other Than Wheat.

In the preceding work, monosporous isolates from a perithecium formed in culture were categorized as weakly or

strongly pathogenic on the basis of their reaction to wheat seedlings. However, these differences may not apply on other hosts.

In order to obtain data on this question, three isolates of each of the two groups, together with the parent #1, were tested for pathogenicity to five different hosts. The hosts were Bromus mollis (L.) (soft brome), Hordeum leporinum (barley grass), H. vulgare L. (barley), Lolium rigidum Gaud. (Wimmera rye grass) and Vulpia myuros (silver grass). The results in Table 9 indicate that isolates which were weakly pathogenic to wheat were also consistently weakly pathogenic on each of the other hosts. Thus the virulence of a specific isolate on wheat indicates its probable virulence on other susceptible hosts (Figs. 4 and 6).

The susceptibility of barley, barley grass and soft brome to the "strongly pathogenic" isolates is evident (Table 9). Silver grass is also considered to be susceptible to O. graminis (Butler, 1961) and it is interesting to note that the hyphal growth along its roots was less extensive than that on the roots of barley, barley grass and soft brome. The reaction of Wimmera rye grass to the "strongly pathogenic" isolates suggests that this grass has some resistance to the fungus. This was also noted by Griffiths (1933).

(d) Survival of Strongly-pathogenic and Weakly-pathogenic Isolates on Wheat Straw in Unsterilized soil.

The occurrence of weakly pathogenic ascospores in

TABLE 9.

PATHOGENICITY ON VARIOUS HOSTS WITH SIX SINGLE-SPORE ISOLATES FROM ONE PERITHECIUM OF <u>OPHIOBOLUS GRAMINIS</u>

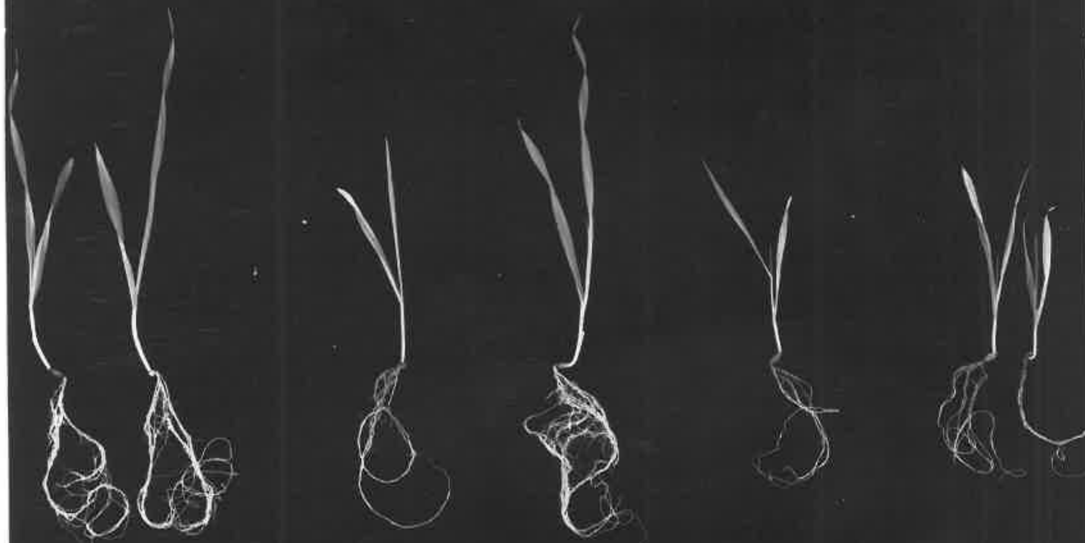
Host	Isolate	Fresh Weight of Host (mg.)	Length (in mm.) of:			Analysis of Root Measurements Using Transformation $Log_e X$		
			Discoloured Root	Runner Hyphae along Root	Whole Root	Discoloured Root	Runner Hyphae along Root	Whole Root
Bromus Mollis	2C	49	13	30	77	2.6374	3.4121	4.3521
	25C	38	15	29	75	2.7362	3.4061	4.3233
	98C	47	14	29	69	2.7268	3.3997	4.2307
	69C	48	0	3	77	0.2310	1.1945	4.3437
	74C	43	0	3	74	0.2310	1.2904	4.3087
	100C	49	0	2	83	0.2310	0.8283	4.4315
	W1-Parent	51	13	26	74	2.6464	3.3026	4.2807
	Control	56			84			4.4372
Hordeum Leporinum	2C	64	22	32	72	3.1120	3.4837	4.2904
	25C	63	22	33	73	3.1349	3.5319	4.2955
	98C	62	23	34	80	3.1093	3.5355	4.3723
	69C	81	1	5	95	0.6932	1.8431	4.5545
	74C	100	2	7	98	1.1945	2.0228	4.5895
	100C	94	2	6	89	0.8283	1.7918	4.5032
	W1-Parent	73	19	29	85	2.9769	3.4078	4.4540
	Control	99			98			4.5772
Hordeum vulgare	2C	334	19	40	165	2.9627	3.6954	5.1113
	25C	337	19	48	165	2.9949	3.8913	5.1093
	98C	313	21	45	153	3.1019	3.8282	5.0338
	69C	383	0	1	196	0.0000	0.8283	5.2836
	74C	378	0	3	234	0.0000	1.3648	5.4567
	100C	384	0	4	212	0.0000	1.6094	5.3610
	W1-Parent	291	21	39	148	3.0718	3.6880	5.0058
	Control	363			236			5.4659

TABLE (9)

Continued

Host	Isolate	Fresh Weight of Host (mg.)	Length (in mm.) of: Discoloured Root	Runner Hyphae along Root	Whole Root	Analysis of Root Measurements Using Transformation $\log_e x$: Discoloured Root	Runner Hyphae along Root	Whole Root
Lolium rigidum	2C	27	3	14	45	1.2904	2.6734	3.8343
	25C	36	0	11	55	0.0000	2.4849	4.0278
	98C	38	2	16	52	1.1552	2.8501	3.9607
	69C	43	0	1	55	0.2310	0.4621	4.0240
	74C	36	0	1	54	0.2310	0.6932	3.9946
	100C	40	0	1	60	0.2310	0.8283	4.0913
	W1-Parent	37	1	12	47	0.6932	2.5383	3.8584
	Control	38			53			3.9903
Vulpia myuros	2C	24	5	15	34	1.8269	2.7875	3.5480
	25C	24	4	14	34	1.5351	2.7125	3.5358
	98C	35	4	18	38	1.6702	2.9331	3.6489
	69C	25	1	1	42	0.4621	0.8283	3.7644
	74C	33	0	1	44	0.2310	0.8283	3.8062
	100C	28	0	1	44	0.0000	0.8283	3.7980
	W1-Parent	23	4	12	36	1.6566	2.5897	3.6061
	Control	32			43			3.7977
Differences for significance $P = 0.05$						0.2001	0.1868	N.S.
0.01						0.2657	0.2480	

Fig. 6. General appearance of barley seedlings inoculated with weakly pathogenic isolate 74C and strongly pathogenic isolates 2C, 98C and parent W1.


HORDEUM VULGARE
CONTROL
2
74
98
PARENT

perithecia formed in saprophytic situations, such as on dead roots (White, 1942) and in agar cultures, raises the possibility that such variants may have a role in the saprophytic phase in nature. An alternative explanation is that weakly pathogenic ascospores are variants well adapted to survival in pure culture but not under natural conditions. The tendency for isolates to lose pathogenicity when maintained in culture for long periods suggests such variants become dominant in this environment. In nature, however, the fungus must infect the living host in order to survive saporphytically on the debris and probably virulent forms become dominant under such conditions. In order to obtain information on this point, the survival of weakly and strongly pathogenic isolates was studied in unsterilized Moonta soil both in presence and absence of nitrate-enrichment of the soil.

Four monosporous isolates of each type, together with the parent W1, were used to artificially infect wheat straws which were buried in unsterilized Moonta soil in two series of jars, one with and the other without added nitrogen. Soil moisture was adjusted to 8.5 percent (pF=1.8) in both series. At intervals straws from each treatment were unearthed and tested for the presence of viable hyphae of O. graminis by the wheat seedling infection test. The percentages of straws containing viable hyphae of the fungus are given in Table 10.

Because of the low figures for survival of weakly pathogenic isolates, these values were checked by attempting to isolate the fungus from test straws which had given negative

TABLE 10.

PERCENTAGE OF WHEAT STRAW IN WHICH VIRULENT AND AVIRULENT ISOLATES OF *OPHIOBOLUS GRAMINIS* SURVIVED WHEN BURIED IN UNSTERILIZED SOIL WITH (+N) OR WITHOUT (-N) ADDED NITROGEN

Survival Period (in weeks)	Strongly Pathogenic Isolates								Weakly Pathogenic Isolates								Parent #1	
	2C		25C		92C		98C		31C		69C		74C		100C			
	+N	-N	+N	-N	+N	-N	+N	-N	+N	-N	+N	-N	+N	-N	+N	-N	+N	-N
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
4	100	97	100	99	98	100	100	99	24	16	45	6	85	44	79	23	100	77
8	99	70	100	59	98	66	99	73	15	1	27	0	21	0	7	3	97	32
12	94	64	95	58	75	41	96	47	0	0	7	0	0	0	1	0	94	22
24	86	22	81	41	95	22	75	30	0	0	0	0	0	0	0	0	88	10

results. The fungus was isolated from only two percent of these straws after four weeks' burial and was not obtained from any after longer periods in soil.

During the experiment, fragments of the straw segments were examined microscopically for mycelium of O. graminis. At burial, mycelial development in the straws was similar for both weakly and strongly pathogenic isolates; the leaf sheath was thoroughly coloured with many dark hyphae, whereas the tissue of the internodal segment contained mainly hyaline hyphae.

After burial, dark hyphae were produced in the tissue of the straw by the strongly pathogenic isolates, development being greater and more sustained in the series buried in nitrate-enriched soil than in the straws in the unamended soil. By twelve weeks, many of the dark hyphae were fragmented and empty in the tissues of straws buried in unamended soil. In straws with weakly pathogenic isolates, no development of dark hyphae occurred in the tissues after burial. Furthermore, many of the dark hyphae in the leaf sheaths were obviously empty and fragmented even after eight weeks' burial. Straws of all treatments still cohered after 24 weeks' burial but those buried in nitrate-enriched soil were darker and softer than those in unamended soil. The leaf sheaths tended to disintegrate in all treatments but were more decayed in straws buried in nitrate-enriched soil.

During the "seedling" test for viable hyphae in straws, the strongly pathogenic isolates produced perithecia in the straw tissue exposed to light above the level of the sand.

In nitrate-enriched soil, perithecia were abundant after four and eight weeks' burial, were present in moderate numbers after twelve weeks' burial, but few were formed after 24 weeks' burial. Perithecia were also produced in straws buried in unamended soil but less abundantly and not after 24 weeks' burial; perithecia were not formed in any of the straws infected with weakly pathogenic isolates. The strongly pathogenic isolates caused severe symptoms of take-all in the test seedlings at the beginning of the experiment, but as viability declined the symptoms on the test seedlings became milder.

The main points arising from this experiment are the rapid decline in viability of weakly pathogenic isolates and increased survival of all isolates in nitrate-enriched soil. The inability of weakly pathogenic monosporous isolates to survive in unsterilized soil suggests they have only a limited role, if any, in nature. However, there is no published information on the occurrence of weakly pathogenic ascospores in the field nor on whether the fungus is a parasite or saprophyte when all or most perithecia are formed naturally. These points would be profitable subjects for further studies. The increased survival of _O. graminis_ in infected straws buried in nitrate-enriched soil is in accordance with previous work (Garrett, 1938, 1940, 1944; Butler, 1953a, 1959; Macer, 1961).

(2) <u>Studies on the Survival of a Wheat Isolate in Soil Previously Sterilized by Gamma Irradiation</u>.

All previous work on the effect of nitrate-enrichment of soil upon the survival of *Ophiobolus graminis* has been with unsterilized soil. While this has approximated field conditions, it has precluded any determination of the direct effect of the amendment as distinct from its effect through the growth of other soil organisms. Use of an initially sterile soil may provide a method of separating these effects. As soil may be completely sterilized by ionizing irradiation with a minimum of side effects (van Groenewoud, 1959; Bowen and Rovira, 1961; Bowen and Cawse, 1962), such a treatment was selected for studying the effect of soil micro-organisms upon the survival of *O. graminis*. The effect was studied both in the presence and absence of nitrate enrichment of the soil.

Sealed polythene plastic bags, each containing 200 g. of air-dry Moonta soil, packed into a metal box, were sterilized by gamma-irradiation at a dosage of five mega-rads at the Australian Atomic Energy Commission, Lucas Heights, New South Wales. After the unopened box had been returned, two bags were checked for micro-organisms by placing some soil on each of ten freshly poured plates of PMD. No growth occurred on any of the plates after ten days' incubation at 20°C.

Wheat straws were sterilized by adding 20 ml. of water to each batch of 50 straws in a 500 ml. Erlenmeyer flask and autoclaving. Each flask was inoculated with a seven mm. agar disc from a 14 day-old PMD culture of isolate W1,

incubated at 25°C for 14 days and then shaken and left at the same temperature for another 21 days.

Two hundred grams of gamma-irradiated soil were then added to each flask by cutting a corner off a polythene bag and allowing the contents to run into the flask. Ten ml. of sterile water was added to half the flasks and an equal volume of a sterile sodium nitrate solution (20 mg. of nitrogen) added to the remainder. This gave the soil an initial moisture content of 8.3 percent (pF 1.8). Half the flasks in each series was then deliberately contaminated by adding 10-14 mg. of air-dry unsterilized Moonta soil to each flask. After covering the cotton wool plugs with polythene to reduce loss of moisture, the flasks were stored in the dark at 20°C. Within four weeks, dense mycelial growth covered the soil surface in flasks which contained only O. graminis. Two of these flasks were exposed to a light intensity of 210-290 foot candles at 20°C for one month and numerous perithecia developed on the sides of the flasks between the glass and the soil (Fig. 7). The mean length of ascospores from these perithecia was similar to that of ascospores on the original field material; the mean length of asci, however, was greater than that of asci on the original material (Table 15).

At intervals, straws from each treatment were tested for viable hyphae of O. graminis by the wheat seedling test. The percentage of straws containing viable hyphae are given in Table 11. Before straws were used for the seedling test, isolations were made from each of 20 straws from the treatments

Fig. 7. Dense mycelial growth of O. graminis on surface of irradiated soil; also numerous perithecia on sides of flask.

TABLE 11.

EFFECT OF SOIL ORGANISMS ON SURVIVAL OF OPHIOBOLUS GRAMINIS
IN STRAW BURIED IN GAMMA-IRRADIATED SOIL
WITH (+N) AND WITHOUT (-N) ADDED NITROGEN

Survival Period (weeks)	Percentage of Straws with Viable Hyphae of O. graminis: in Un-inoculated Soil		in Inoculated Soil	
	+N	-N	+N	-N
0	100	100	100	100
4	100	100	100	96
8	100	99	100	100
12	97	91	96	72
24	100	86	99	67

containing introduced micro-organisms. No attempt was made to surface sterilize the straws but adhering soil was removed with 'Kleenex' tissue. Part of the epidermis was cut away from the node in each straw and three small pieces of underlying tissue were removed and plated on PMD. The plates were incubated for 7-10 days at 20°C and the fungi which were isolated at each sampling are recorded in Table 12. Bacteria were recorded from all straws.

Fragments of the straws were also examined microscopically for mycelium of O. graminis. After four weeks' burial, mycelial development was similar in all treatments, but by twelve weeks dark hyphae were more numerous in straws in nitrate-enriched than in unamended soil. This difference was more obvious after 24 weeks' burial. After the same period, dark hyphae of O. graminis were equally prevalent in nitrate-enriched soil inoculated with other organisms. However, by 24 weeks, in unamended soils dark hyphae were fewer and more fragmented when other soil organisms were present. The straws of all treatments still cohered after 24 weeks, but were darker in colour when organisms other than O. graminis were present.

Several interesting points are evident in these results. Firstly, the decline in viability of O. graminis was very slow in irradiated soil but was increased by the introduction of other organisms. Nitrate-enrichment of the soil, however, outweighed the unfavourable effects from

TABLE 12.

FUNGI ISOLATED FROM WHEAT STRAWS INITIALLY COLONIZED BY *OPHIOBOLUS GRAMINIS* AND BURIED IN NITRATE-ENRICHED OR UN-AMENDED SOIL

Fungi	Frequency of Isolation from Straws buried in:							
	Nitrate-enriched soil for				Unamended soil for			
	4 weeks	8 weeks	12 weeks	24 weeks	4 weeks	8 weeks	12 weeks	24 weeks
Ophiobolus graminis	2	7	14	8	25	51	20	26
Rhizopus nigricans	23	11	39	2	5	1	35	11
Actinomucor repens	20		1	32			2	6
Gliocladium catenulatum	4	13	8	12				
Penicillium spp.		5	1	4	3	2		3
Mortierella sp.				3	5			
Fusarium oxysporum				2			1	
Gliocladium roseum			2				1	
Cladosporium sp.						1		
Fusarium roseum						1		
Unknown (sterile)		4		2	1			6

introducing other organisms so that 99 percent of the straws still contained viable hyphae of <u>O. graminis</u> after 24 weeks; but nitrate-enrichment also increased the frequency of isolation of introduced organisms from straws, thereby making it more difficult to re-isolate <u>O. graminis</u>

This experiment was planned to run for 24 weeks because previous tests in unsterilized soil had given positive results within this period. However, it is obvious that to obtain critical data in irradiated soil, such tests should be continued for longer periods. An unusual feature of the seedling test was the low germination of the wheat seed. Germination ranged between 74 and 81 percent compared with 98 percent for the same seed when used in other tests in unsterilized Moonta soil. This indicates a possible weakness in the technique and should be studied further. Plant growth in irradiated soils has been examined by many workers and has been variously reported as slightly retarded (Bowen and Rovira, 1961), unaffected (McLaren, Luse and Skujins, 1962) and consistently improved (Bowen and Cawse, 1962). Bowen and Rovira also noted in their experiments that irradiated soil became increasingly phytotoxic when stored at room temperature for ten weeks prior to planting. The prolific growth by <u>O. graminis</u> in this experiment, however, indicated that irradiation of the soil had no deleterious effects upon the fungus. This growth is of particular interest in view of the occasional isolation of the fungus directly from soil (Warcup, 1957).

(3) Relative Importance of Some Alternate Hosts in the Saprophytic Survival of *Ophiobolus graminis*.

Little information is available on the role of pasture grasses in the survival of *Ophiobolus graminis*. Laboratory and field experimentation by Garrett (1941), Wehrle and Ogilvie (1955) and Brooks (1965a) has given relevant data on some common pasture species in England. However, there is very little similar information on the common pasture grasses of the cereal areas in Australia. An introductory study was carried out at the Esperance Downs Research Station, Western Australia. The average annual rainfall at the Research Station is 17.20 inches. However, Shier, Dunne and Fitzpatrick (1963) consider the relatively even spread of rain over the May-October period, together with the flat topography and impermeable subsoil, give the area a longer growing season than would be expected from rainfall figures. The soils of the area are mostly grey silaceous sands of variable depth overlying a gravel, gravelly clay or clay subsoil. The soil of the experimental site was of the Caitup series and consisted of 6-15 inches of sand overlying a twelve inch band of gravelly clay with yellow clay beneath.

(a) Incidence of Take-all in Wheat Sown After Pure Stands of Various Grasses.

In 1963, part of a four-year-old pasture was divided into 21 plots, each measuring 60 metres by 2.5 metres. Eighteen of the plots were ploughed, harrowed and sown with

grass seed in June to give pure stands of each of six species in three replications. The grasses were Bromus gussonii (rip-gut brome), Ehrharta calycina Sm. (perennial veldt grass), Hordeum hystrix Roth (barley grass), Hordeum leporinum (barley grass), Lolium rigidum (Wimmera rye grass) and Vulpia myuros (silver grass). The remaining three plots, representing the original pasture, were left undisturbed during 1963. The main grass components of the pasture plots were Avenae fatua (L.) (wild oats), Bromus gussonii, Hordeum leporinum, Lolium rigidum and Vulpia myuros. The remaining dominant species were Trifolium subterraneum L. (subterranean clover var. "Mid-Season") and Cryptostemma calendula (L.) Druce (cape weed). The experimental design was a simple randomization of the seven treatments within each replication. At the end of the season, the plots were left undisturbed.

In 1964, all plots were ploughed, disc harrowed and sown with wheat (variety Gamenya) in June. The seed was sown at the rate of 45 lb. per acre with 112 lb. superphosphate per acre. After 21 weeks, the incidence of take-all was assessed on the number of wheat tillers with obvious symptoms of take-all in 20 random samples from each plot. The individual samples consisted of all the plants in one metre of a row. A central strip was harvested from each plot in January, 1965.

The numbers of diseased and healthy wheat tillers per metre of row, together with the yields, are set out in Table 13. These results clearly demonstrate differences between grasses as perpetuators of O. graminis. Thus, Bromus gussonii and the

TABLE 13.

INCIDENCE OF *OPHIOBOLUS GRAMINIS* IN WHEAT SOWN AFTER VARIOUS GRASSES

Cropping History		Mean Number of Tillers per Metre of Row			Wheat Yield	
1963	1964	Apparently Healthy	With Take-all	Total	Analysis of Actual Yield (lb. per 0.0225 acres) Using Transformation $X^{1/2}$	Estimated (Bushels per acre)
Ehrharta calycina	Wheat	21.7	6.2	27.9	5.03	18.8
Vulpia myuros	Wheat	21.1	8.4	29.5	4.72	16.5
Lolium rigidum	Wheat	17.5	9.5	27.0	4.61	16.0
Pasture	Wheat	8.0	16.6	24.6	3.05	6.9
Bromus gussonii	Wheat	6.7	13.9	20.6	2.88	6.0
Hordeum hystrix	Wheat	4.8	14.2	19.0	2.63	5.2
Hordeum leporinum	Wheat	4.1	17.5	21.6	2.29	4.0
Differences for Significance						
	P=0.05	5.4	5.6	5.2	0.57	-
	P=0.01	7.6	7.8	7.3	0.79	-

two species of _Hordeum_ may be regarded as perpetuators of the fungus, whereas _Ehrharta calycina_, _Lolium rigidum_ and _Vulpia myuros_ appear to be non-perpetuators. It is also evident that pastures will perpetuate the fungus equally well whether all or only some of their main components are perpetuators. Other aspects of the experiment are the significant reductions in both tillering and yield of wheat when the incidence of _O. graminis_ is high.

The ineffectiveness of _Vulpia myuros_ in perpetuating the disease is of particular interest as this grass is generally regarded as being susceptible to _O. graminis_ (Butler, 1961). However, in earlier pathogenicity tests (Table 9) the growth of runner hyphae along the roots of _V. myuros_ was less extensive than on those of _Hordeum leporinum_. Nevertheless, _V. myuros_ can be regarded as being moderately susceptible and therefore survival of _O. graminis_ on a host would appear to depend upon more than susceptibility alone. A factor which may influence the role of grasses in the survival of _O. graminis_ is the rate of decomposition of their straws in the field. _V. myuros_ has a relatively thin straw and it may decompose more rapidly than the coarser straws of other susceptible hosts.

(b) _Survival of O. graminis on the Straw of Various Hosts Buried in Unsterilized Soil in the Laboratory_.

Because of the field results with _V. myuros_ (Table 13), it was of interest to compare the survival of _O. graminis_ on

artificially infected straws of some grasses. Fully mature straws of *Hordeum leporinum*, *Lolium rigidum* and *Vulpia myuros* were used in the first experiment. The straws of barley and wheat were also included because of earlier observations (Chambers, 1963) that more take-all occurred in wheat following barley than in wheat after wheat. Straws were artificially infected with isolate W1 and buried in both nitrate-enriched and unamended Moonta soil. The initial moisture content was adjusted to 9.4 percent (pF 1.75). At intervals, straws were tested for viable hyphae of *O. graminis* by the seedling infection test, using seed of *V. myuros* instead of wheat. The percentage of straws containing viable hyphae is given in Table 14.

During each seedling infection test, perithecia were formed above the sand level in the sheath tissue of all species. As previously, they were produced abundantly after 4, 8 and 12 weeks' burial in both nitrate-enriched and unamended soils, but only a few were formed after 24 weeks' burial. The mean lengths of asci and ascospores are given in Table 15.

Throughout the experiment, fragments of straw were examined for mycelium of *O. graminis*. At burial, the mycelial development was similar in the straws of wheat, *H. leporinum*, *L. rigidum* and *V. myuros*; the leaf sheath tissues contained many dark hyphae but stem tissues had few. After burial, many dark hyphae developed in the stem tissues and were more numerous in straws buried in nitrate-enriched soil than unamended soil; after 24 weeks' burial many of the dark hyphae

TABLE 14.

PERCENTAGE OF STRAWS OF VARIOUS HOSTS IN WHICH OPHIOBOLUS GRAMINIS
SURVIVED WHEN BURIED IN UNSTERILIZED SOIL WITH (+N) AND WITHOUT (-N) ADDED NITROGEN

Survival Period (weeks)	Percentage Survival of *O. graminis* in Straws of:									
	Wheat		Barley		Hordeum Leporinum		Lolium rigidum		Vulpia myuros	
	+N	-N	+N	-N	+N	-N	+N	-N	+N	-N
0	100	100	100	100	100	100	100	100	100	100
4	100	100	100	99	100	100	100	100	100	100
8	99	100	100	100	99	99	100	100	100	100
12	87	85	99	95	87	85	99	96	92	85
24	72	51	96	89	84	49	83	64	70	51

TABLE 15.

EFFECT OF SUBSTRATE ON ASCUS AND ASCOSPORE MEASUREMENTS OF ISOLATE W1 OF <u>O. GRAMINIS</u>

Source of Perithecia	Asci Mean Length (in μ)	Ascospore Measurements: Length (in μ)			Ascospore Measurements: Number of Septa	
		Mean	Modal	Range	Mean	Range
Gamma Irradiated Soil	114 (±0.76)	84 (±0.42)	84	73-92	7.0	5-9
Survival Test						
-Wheat	119 (±0.94)	85 (±0.63)	81	71-118	6.9	5-9
-Barley	117 (±0.79)	84 (±0.52)	86	68- 94	6.9	5-9
-Hordeum Leporinum	122 (±0.96)	90 (±0.63)	92	73-107	7.0	5-9
-Vulpia myuros	123 (±0.94)	88 (±0.59)	86	66-102	7.0	5-11
-Lolium rigidum	124 (±1.03)	91 (±0.47)	92	81-102	6.9	5-9
Original Field Material						
-Wheat	106 (±0.91)	83 (±0.58)	81	68- 94	7.0	5-9

were empty and fragmented in straws in unamended soil. During the experiment, there was no noticeable difference in the colonization of wheat, H. leporinum and L. rigidum straws by O. graminis. After 24 weeks' burial, however, the number of dark hyphae was greater in the straws of V. myuros. At the time of burial, the straws of barley contained fewer dark hyphae than the straws of other species but after 24 weeks' burial barley straws contained the most dark hyphae. While buried, all straws developed a dark discolouration, straws in nitrate-enriched soil being slightly darker than those in unamended soil. Decomposition, as judged by loss of coherence and rigidity, was greatest in straws of H. leporinum, where, after 24 weeks' burial, the tissues became extremely soft and disintegrated readily. Straws of the other species were soft but still cohered, although those of V. myuros were slightly softer than the rest.

The results in Table 14 provide further evidence that nitrate-enrichment of soil promotes the development and saprophytic survival of O. graminis in infected straw. There is also some evidence that the fungus survives better in barley straw than in wheat straw. This offers a possible explanation for the higher incidence of take-all in wheat following barley than in wheat after wheat (Chambers, 1963). However, some of the data in Table 14 seem contradictory to that obtained from the field experiment (Table 13) and to field observations by other Australian workers. For example, in the field experiment, O. graminis survived at a higher level on

H. leporinum but not on L. rigidum, and Australian workers have described these grasses as being susceptible and field resistant respectively to the fungus (Butler, 1961). Yet in the laboratory, the fungus survived equally well on artificially infected straw of both species and also survived at a high level. One possible explanation is that sterilization of L. rigidum straw inactivated substances which prevent O. graminis from surviving on this species in the field. In any case, it is probable that the importance of various grasses as perpetuators of O. graminis is determined by a number of inherent characters, for example, the physical structure of their straws; the type and availability of chemical constituents which may be used as metabolites by the fungus, etc. The importance of a species in a particular season would be further influenced by its population density and the prevailing environmental conditions.

(c) Survival of O. graminis on the Straw of Various Hosts in the Field.

Straws of wheat, barley, Hordeum leporinum and Lolium rigidum were artificially infected with O. graminis and buried in a bare fallow field at the Waite Agricultural Research Institute. Vulpia myuros had to be omitted from the experiment because there were insufficient straws.

The straws were infected with isolate W2 and buried on February 5, 1966, in two series; one was placed at one inch below the soil surface and the other at six inches. Isolate W2 was chosen in preference to W1 of the previous

experiment because W2 was one of several similar isolates obtained from the experimental area just before commencing the test. Thus W2 probably represented the local "wild type" and would therefore be well adapted to local environmental conditions; also isolate W2 was strongly pathogenic (Table 20). Throughout the experiment, daily minimum and maximum soil temperatures were recorded at the two depths. Rainfall was recorded and the soil moisture content was measured once every seven days. This data has been condensed and presented graphically in Fig. 8. In order to link field and laboratory studies on survival, an additional series of artificially infected wheat straws were buried in jars containing soil from the experimental site. The initial moisture content was 4.4 percent.

At intervals straws were dug up and tested for viable hyphae of O. graminis. The percentage of straws containing viable hyphae is given in Table 16. In contrast with the previous experiment, relatively few perithecia were formed in the tissue of the straws from the field during the seedling test. Some were produced after four and eight weeks' burial but none thereafter.

Examination of straw fragments at the time of burial indicated that mycelial development by isolate W2 was similar to that described for W1 in the laboratory experiment (page 54). However, little mycelial growth occurred in the straws after burial in the field; by twelve weeks many of the dark hyphae in the straws of wheat, H. leporinum and L. rigidum were

Fig. 8.

SOIL TEMPERATURE, SOIL MOISTURE AND RAINFALL DATA

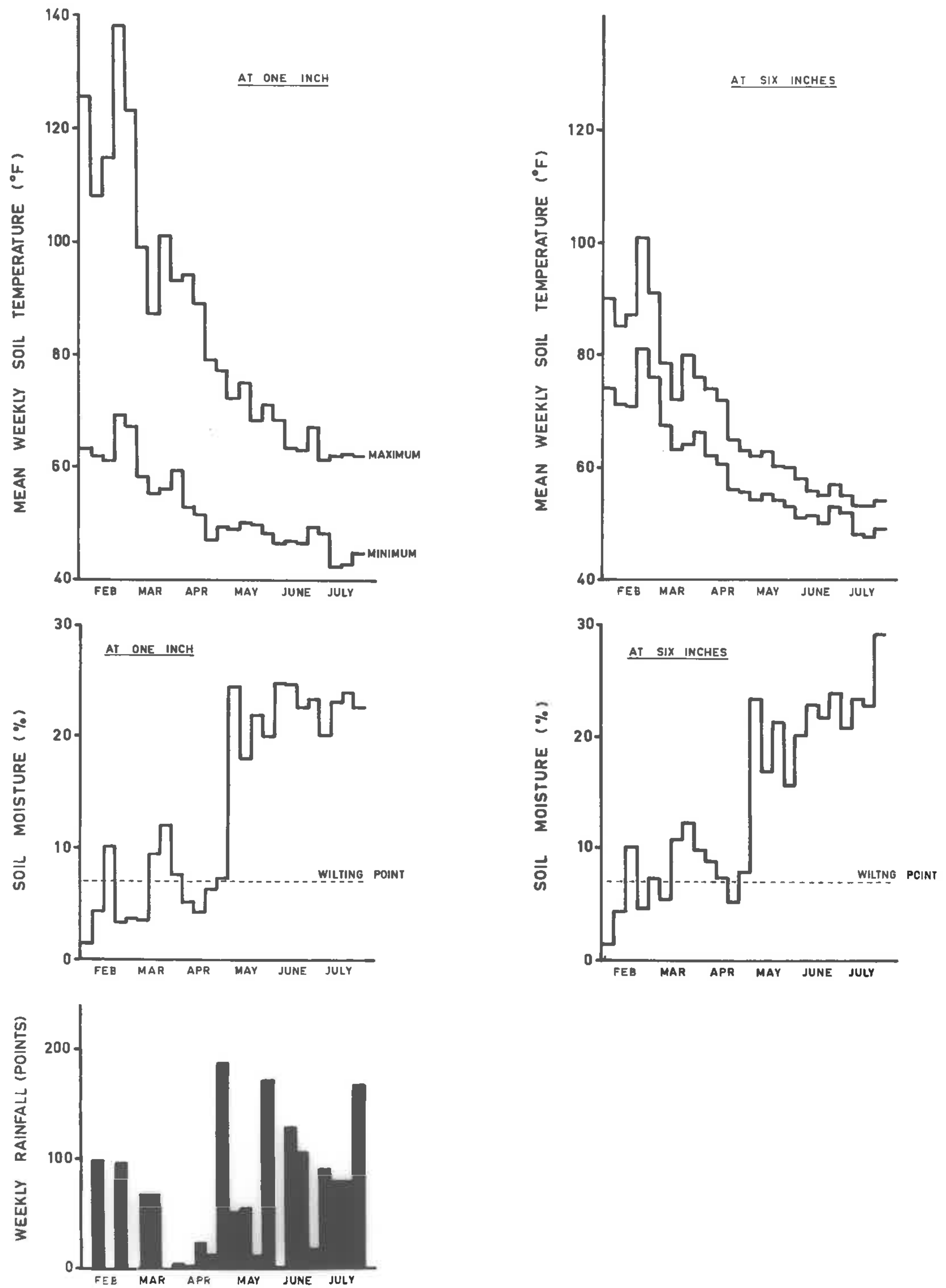


TABLE 16.

PERCENTAGE OF STRAWS OF VARIOUS HOSTS IN WHICH <u>OPHIOBOLUS GRAMINIS</u> SURVIVED WHEN BURIED AT DIFFERENT DEPTHS IN A FIELD AT GLEN OSMOND, SOUTH AUSTRALIA .

Survival Period (weeks)	Percentage Survival of *O. graminis* in Straws of:								
	Wheat		Barley		Hordeum Leporinum		Lolium rigidum		Wheat in Laboratory
	at 1"	at 6"	at 1"	at 6"	at 1"	at 6"	at 1"	at 6"	
0	100	100	100	100	100	100	100	100	100
4	67	34	80	94	85	88	58	47	100
8	24	2	70	18	61	7	67	1	99
12	20	2	74	18	10	7	34	6	94
24	4	14	15	12	1	0	7	2	64

fragmented and empty. At burial, straws of barley contained fewer dark hyphae than those of other species but after twelve weeks' burial, straws of barley had the most dark hyphae.

The test material of L. rigidum consisted of a mixture of narrow and coarse straws which had been obtained from small and large plants respectively. After twelve weeks' burial, it was noted that only the narrow straws were giving positive results in the seedling test. On checking for mycelial development, some dark hyphae were observed in the narrow straws but only a few empty fragments were present in the coarse straws.

Decomposition appeared greatest in straws of H. leporinum and after 24 weeks' burial they were soft and disintegrated. Straws of the other three hosts were soft but coherent, barley straw showing least decomposition. Straws buried at six inches were generally darker in colour and slightly more decomposed than those at one inch. Many straws showed evidence of having been chewed by insects. Ants and wood slaters (Oniscidae) were found among the straws of wheat and H. leporinum both at one inch and six inches after four and eight weeks' burial. An inherent weakness of the experimental method is that the soil must be disturbed in order to bury the straws; the disturbance may change the environment considerably. In this instance, the undisturbed soil was initially compact and dry, whereas the soil covering the straws was relatively loose-textured; it is possible that the open texture encouraged activity of insects, especially wood slaters among the straws. However, this soil was compacted by rain

six weeks after commencement and thereafter was indistinguishable from the surrounding undisturbed soil.

The results in Table 16 indicate a more rapid decline in viability by O. graminis in the field, especially at six inches, than in the laboratory. It would be impossible to define accurately the reasons for these differences as several factors would be varying simultaneously in the three environments. Nevertheless, both soil moisture and soil temperature could be assumed to be relatively important. Garrett (1938) has demonstrated that warm moist conditions similar to those in the field will cause a more rapid decline in viability than cool dry conditions as in the laboratory.

Another important difference between the laboratory and field conditions is the relatively low level of insect activity in the laboratory. A study of the role of insects in the decomposition of straw and their effect upon the survival of O. graminis could be a profitable field of investigation. It would be particularly interesting to know whether insects have any preference for straw infected with O. graminis or for the straw of any particular species.

Although the viability of O. graminis fell rapidly in the field, the fungus appeared to survive better at one inch than at six inches during the first eight weeks. This may have been due to higher soil temperatures and slightly drier conditions restricting microbiological activity more at one inch than six inches. In South Australia, Bainbridge (1966) recorded a decrease in Pythium ultimum propagules at

a depth of 9-12 cm. but not at 0-3 cm. in a fallow pea field over the summer period. He considered the lower soil moisture content at 0-3 cm. was a factor in its greater survival at that depth during high temperature conditions. Garrett (1956) considers O. graminis will survive better in the summer drought conditions of South Australia than when soil temperatures and moisture contents are more favourable for microbiological activity. In any case, it would be necessary to carry out further field experiments to determine whether depth of burial has a consistent effect upon survival of the fungus.

The more rapid disappearance of O. graminis from the coarser straws of L. rigidum was particularly interesting and may be worth further investigation. Such study may provide an explanation for the conflicting results with L. rigidum in the earlier field and laboratory experiments. Again there was evidence that O. graminis survives better in barley straw than in wheat straw, thus supporting the observations made in the laboratory (Table 14).

(4) A Comparison of Isolates of *O. graminis* from Wheat and Oats in Australia.

Although *O. graminis* var. *avenae* has not been identified in Australia, several reports of take-all in oats in Western Australia were investigated by the writer during 1959-1964. In each instance the affected crop contained small patches of stunted plants which had typical black, plate-like mycelium of *O. graminis* at their bases (Fig. 9). Occasionally perithecia of the fungus were found in the lowermost leaf sheaths of affected plants. The disease was restricted in distribution to a narrow zone extending from Williams to Mount Barker along the western fringe of the main southern wheat area (Fig. 10). All the affected oat crops were of the variety Avon. *O. graminis* has been occasionally isolated from oat plants in South Australia (Dodman, pers. comm.) but no attempt was made to identify the variety of the fungus. Australian isolates of *O. graminis* from both wheat and oats were therefore examined by the criteria used by Turner (1940, 1940a, 1957) to differentiate the type and the variety *avenae*.

(a) Pathogenicity of Isolates on Wheat and Oats.

Pathogenicity of isolates O1, W1 and W5 was tested on wheat and oats in unsterilized, "partially sterilized" and irradiated soil. For partial sterilization, Moonta soil was treated with aerated steam at 160°F for 30 minutes and for irradiation a dosage of five megarads of gamma irradiation

Fig. 9. Symptoms of take-all on naturally infected oat plant (variety Avon) collected at Williams, Western Australia in 1963. Note the severe blackening of the lower part of the stem.

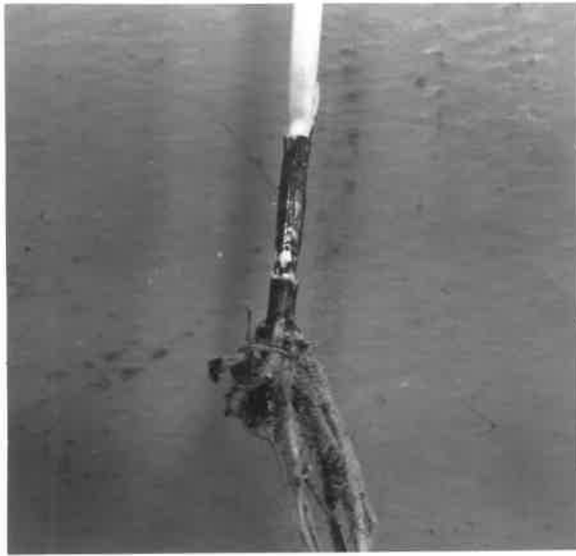
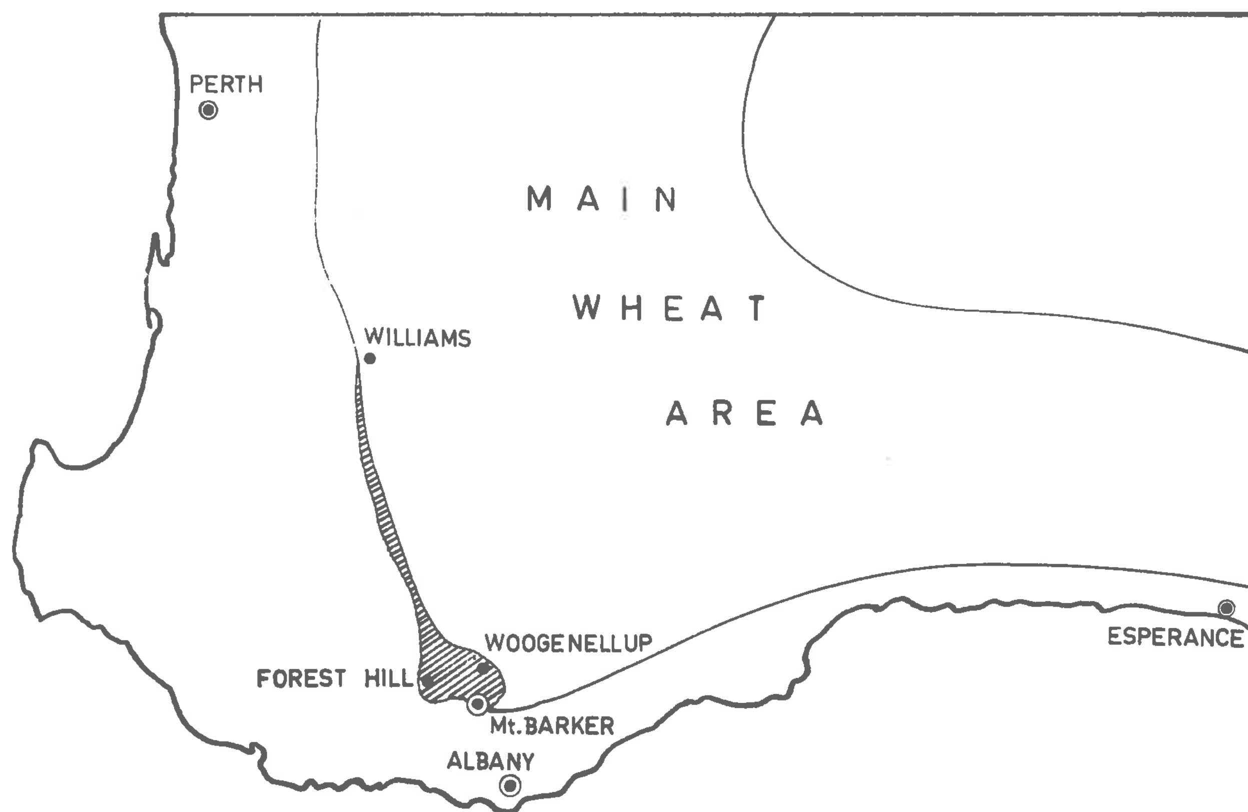


Fig. 10

DISTRIBUTION OF AN OAT-ATTACKING STRAIN OF <u>O. GRAMINIS</u>
IN WESTERN AUSTRALIA



was used. This test differed from all others in that the wheat plants were allowed to grow for eight weeks before being harvested. The results are given in Tables 17 and 18.

All isolates reduced the fresh weight of wheat plants but only isolate 01 reduced the fresh weight of oats; isolate 01 also caused considerable root discolouration of secondary oat roots but the two wheat isolates did not. Sections of secondary roots were stained with lactophenol cotton blue and examined for hyphae. In wheat, hyphae of all isolates were observed in the vascular tissue and inner layers of the cortex, although there were fewer in roots infected with isolate W5. In oats, hyphae of all isolates were found in root tissue, but only those of isolate 01 penetrated to the vascular tissue. Infection of oat roots by isolates W1 and W5 were few and restricted to one or two layers of cortical cells.

These results indicate that isolate 01 is similar in pathogenicity to the var. avenae isolates used by Turner (1940a). Differences in virulence were exhibited by W1 and W5 on wheat but these isolates reacted similarly to the type variety of O. graminis towards oats (Table 17).

Hyphal growth along roots was significantly less in unsterilized soil than in partially-sterilized and irradiated soils, but soil treatments had no significant effect upon the amount of root discolouration caused by O. graminis (Table 18). The roots of plants in unsterilized soil were attacked by cereal eelworm (Heterodera avenae), thereby complicating the interpretation of results. Plants in unsterilized soil were

TABLE 17.

PATHOGENICITY OF ISOLATES OF O1, W1 AND W5 OF O. GRAMINIS ON WHEAT AND OATS

Host	Isolate	Fresh Weight of Host (mg.)	Length of: Discoloured Root (mm.)	Length of: Hyphae Along Root (mm.)	Analysis of Root Measurements Using Transformation $Log_e X$: Discoloured Root	Analysis of Root Measurements Using Transformation $Log_e X$: Hyphae Along Root
Wheat	O1	212	23	32	3.1340	3.4900
	W1	167	23	38	3.1480	3.6353
	W5	290	5	16	1.5567	2.7023
	Control	362				
Oats	O1	275	24	35	3.2033	3.5347
	W1	451	1	7	0.3804	1.9533
	W5	431	3	20	1.1147	2.7350
	Control	457				
			Differences for Significance	$P = 0.05$	0.2678	0.3174
				$P = 0.01$	0.3554	0.4212

TABLE 18.

EFFECT OF SOIL TREATMENT ON RESULTS OF PATHOGENICITY TEST ON WHEAT AND OATS

SOIL TREATMENT	Results of Pathogenicity Test			
	Mean Length of:		Analysis of Root Measurements Using Transformation $Log_e X$	
	Discoloured Root (mm.)	Hyphae along Root (mm.)	Discoloured Root	Hyphae along Root
Untreated	14	21	2.3045	3.9377
Aerated Steam	13	28	2.2085	3.3006
Irradiation	12	24	2.1566	3.1586
Difference for Significance P = 0.05			N.S.	0.1944

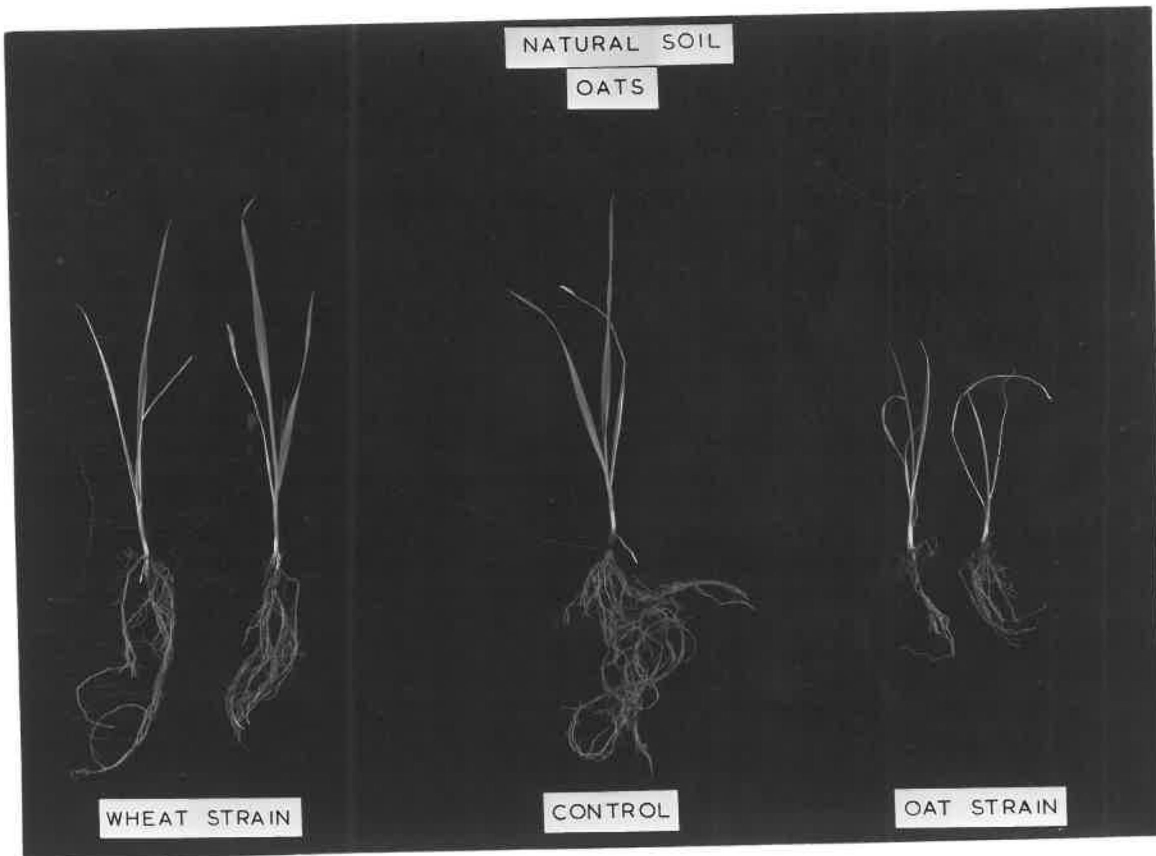
stunted (Fig. 11) compared with those in partially sterilized soil (Fig. 12); irradiation of soil had no apparent phytotoxic effect (Fig. 13). Nevertheless, soil partially sterilized by aerated steam was used for all further tests because it can be prepared easily and is more readily available than irradiated soil.

(b) Differences in Pathogenicity on Other Hosts.

Turner (1940a) reported that infection of nineteen alternate hosts was "more intensive and more extensive" by var. *avenae* than by the type variety. The pathogenicity of isolates O1 and W1 was therefore compared on five alternate hosts: *Bromus mollis*, *Hordeum leporinum*, *H. vulgare*, *Lolium rigidum* and *Vulpia myuros*. The results are given in Table 19 and show that the fresh weights of *B. mollis* and *H. leporinum* were reduced considerably more by isolate W1 than by isolate O1; the two isolates caused similar reductions to the fresh weight of *H. vulgare*, but they had little, if any, effect upon the fresh weights of *L. rigidum* and *V. myuros*. Isolate O1 caused more root injury than isolate W1 on *V. myuros*, but isolate W1 caused more root injury than isolate O1 on *B. mollis* and *H. leporinum*; there was no significant difference in the virulence of the two isolates on *H. vulgare* and *L. rigidum*. The seminal roots of *L. rigidum* were susceptible to both isolates but only an occasional localized lesion was formed on the secondary roots (Fig. 14). Thus the results

Fig. 11. Appearance of oat plants, inoculated with isolates W1 (left) and O1 (right) after 8 weeks' growth in unsterilized Moonta soil. All plants have been attacked by cereal eelworm (Heterodera avenae) and control plants are considerably smaller than those in partially sterilized soil (Fig. 12)

Fig. 12. Appearance of oat plants inoculated with isolates W1 (left) and O1 (right) after 8 weeks' growth in soil partially sterilized by treating with aerated steam at 160°F for 30 minutes. O1 has stunted plants and reduced root development, whereas W1 has had little effect upon the plants.


NATURAL SOIL
OATS
WHEAT STRAIN
CONTROL
OAT STRAIN

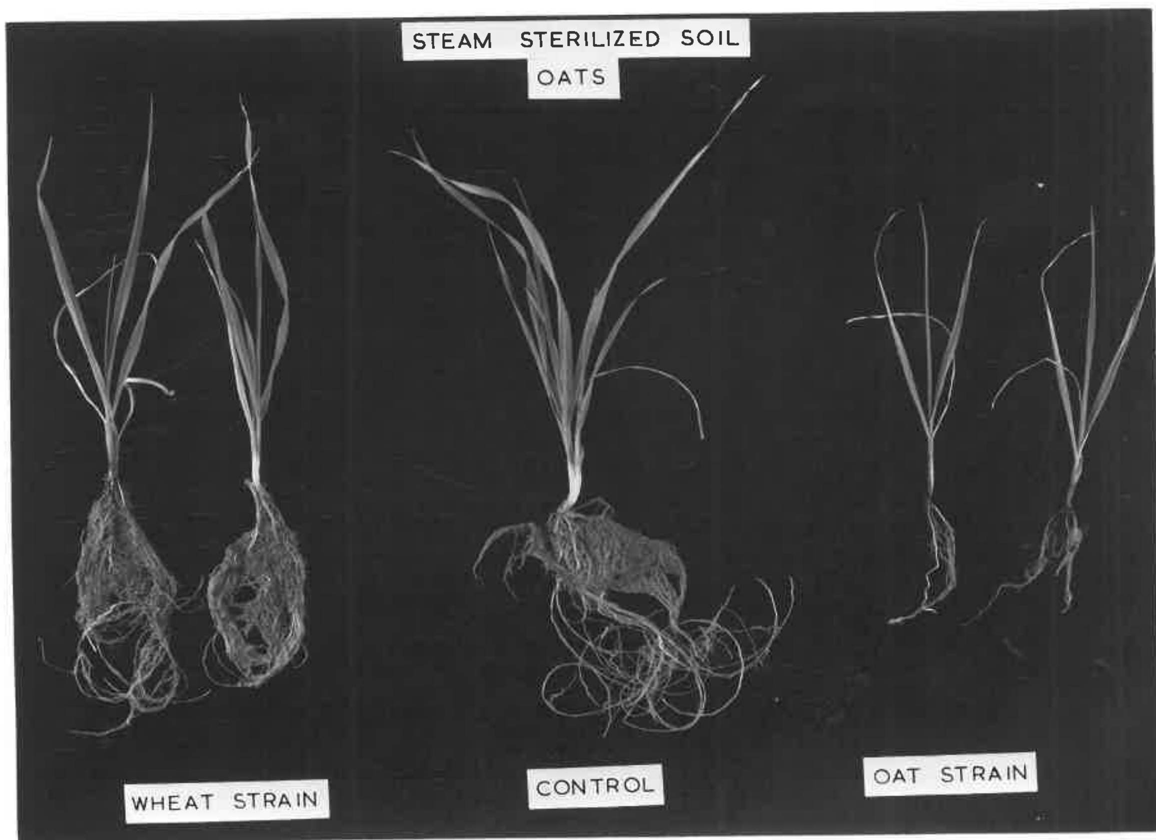
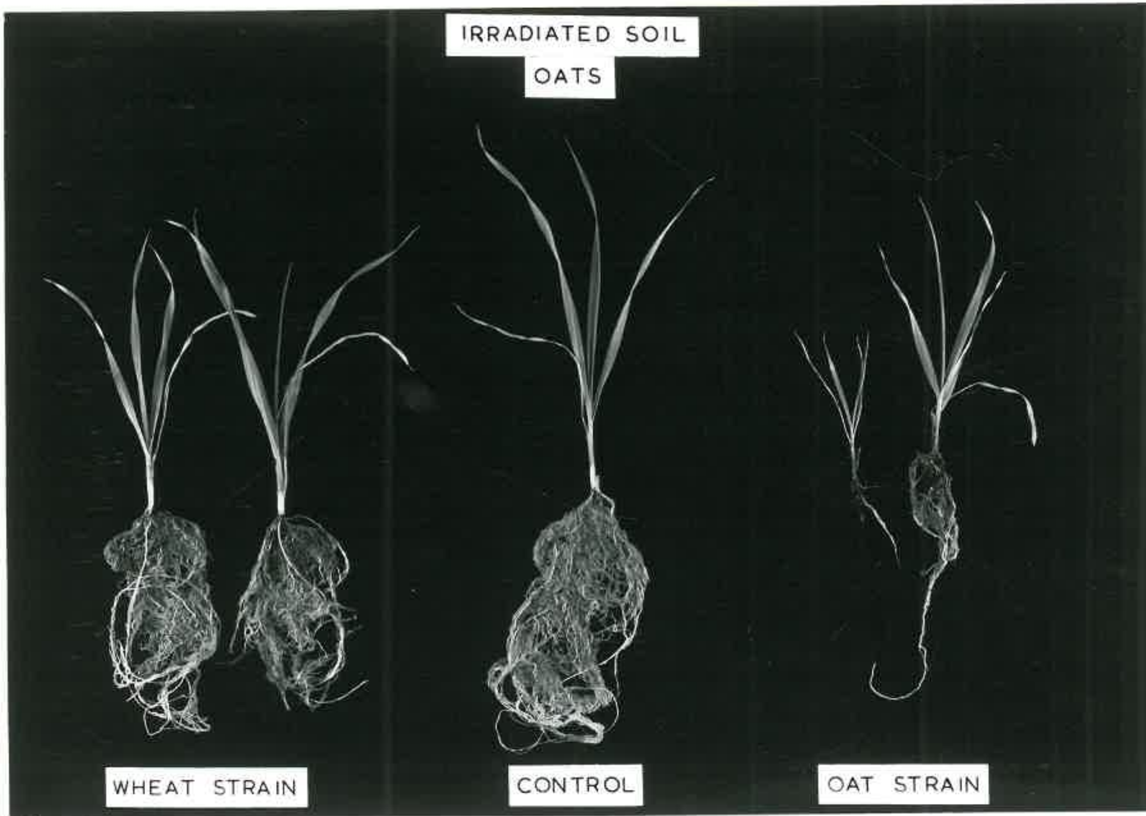

STEAM STERILIZED SOIL
OATS
WHEAT STRAIN
CONTROL
OAT STRAIN

Fig. 13. Appearance of oat plants inoculated with isolate W1 (left) and O1 (right) after 8 weeks' growth in soil sterilized by gamma irradiation. Again O1 has stunted plants and restricted root development, whereas W1 has had little effect upon the plants. There was no evidence of phytotoxicity from gamma irradiation of the soil.

Fig. 14. Appearance of root system of _Lolium rigidum_ seedling, four weeks after inoculation with isolate O1. Although the seminal root and sub-coronal internode have been generally discoloured, only an occasional localized lesion (arrow) is evident on the secondary roots.


IRRADIATED SOIL
OATS
WHEAT STRAIN
CONTROL
OAT STRAIN

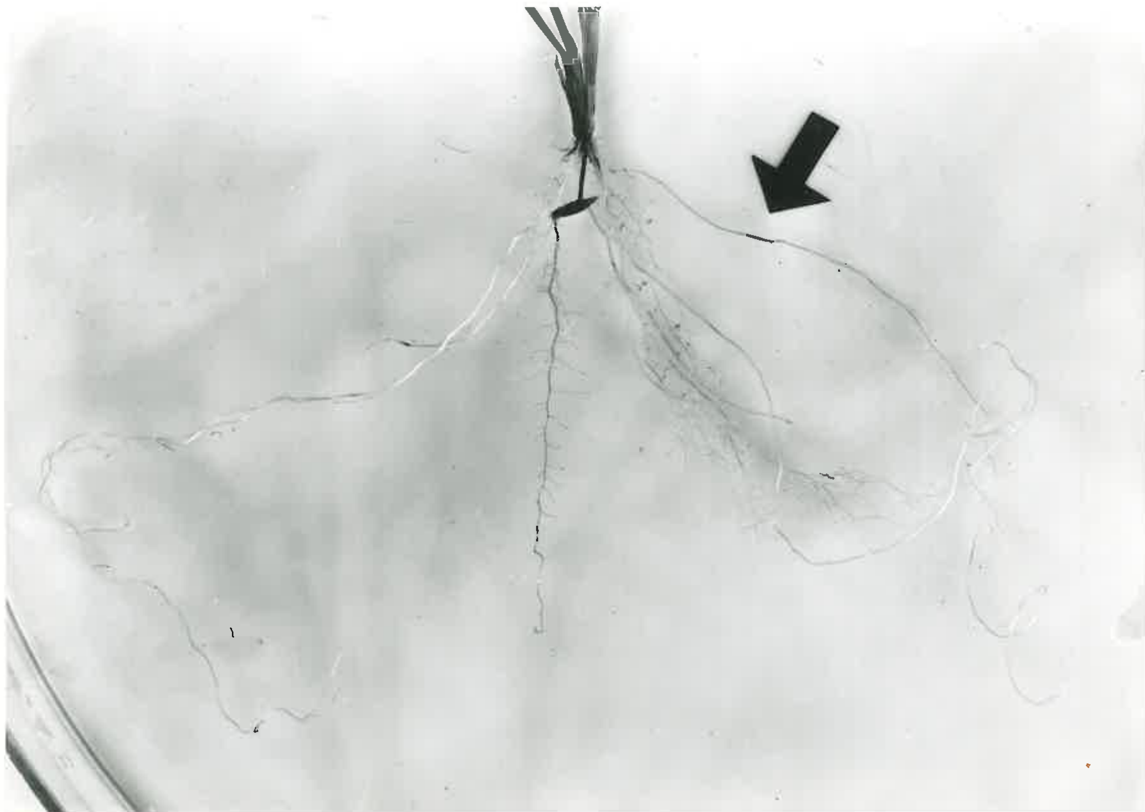


TABLE 19.

PATHOGENICITY OF OAT ISOLATE O1 AND WHEAT ISOLATE W1 OF OPHIOBOLUS GRAMINIS ON VARIOUS HOSTS.

Host	Isolate	Fresh Weight of Host (mg.)	Length (in mm.) of:			Analysis of Root Measurements Using Transformation $Log_e X$		
			Discoloured Root	Runner Hyphae along root	Whole Root	Discoloured Root	Runner Hyphae along Root	Whole Root
Bromus mollis	O1	68	0	9	69	0.0000	2.2236	4.2339
	W1	44	11	25	60	2.4470	3.2642	4.1455
	Control	73			78			4.3690
Hordeum leporinum	O1	198	13	36	136	2.5462	3.5810	4.8930
	W1	65	39	48	88	3.6821	3.8857	4.4714
	Control	258			193			5.2624
Hordeum vulgare	O1	355	28	44	141	3.3311	3.7984	4.9468
	W1	344	23	43	131	3.1768	3.7668	4.8635
	Control	467			188			5.2421
Lolium rigidum	O1	90	9	16	72	2.2232	2.7555	4.2805
	W1	86	5	15	64	1.7006	2.7340	4.1593
	Control	91			71			4.2631
Vulpia myuros	O1	37	9	18	34	2.2443	2.9110	3.5372
	W1	38	6	16	30	1.9702	2.8113	3.4049
	Control	40			40			3.7003
Differences for significance $P = 0.05$						0.3250	0.3186	0.2376
$P = 0.01$						0.4377	0.4290	N.S.

contrast with those of Turner in that the oat-attacking isolate O1 was not more virulent than the wheat-attacking isolate W1 on all hosts. It is interesting to note that perithecia were formed by W1 on inoculated plants but none was produced by O1. This may indicate that isolate O1, which had been in culture for two years, was beginning to decline in virulence.

(c) Pathogenicity of Isolates from Oats from Different Australian States.

The pathogenicity of three Western Australian and five South Australian isolates from oats was tested on wheat and oats. Three South Australian isolates from wheat were also included in the test.

The results (Tables 20,21) indicate marked differences in virulence of isolates; some were avirulent; others weakly pathogenic on both wheat and oats; the remainder were strongly pathogenic on one or both hosts (Figs. 15, 16, 17, 18). Disregarding the avirulent and weakly pathogenic isolates, it is evident that the remaining South Australian isolates were pathogenically similar to the type variety of Turner (1940a). Thus the secondary roots of oat plants inoculated with these isolates were almost entirely free from infection (Fig. 19). However, the two virulent Western Australian isolates (O1, O2) were similar to the isolates of var. *avenae* of Turner and discoloured secondary oat roots (Fig. 20). Isolates O1 and O2 also significantly reduced top growth of oats, but the virulent South Australian isolates did not; all virulent

TABLE 20.

PATHOGENICITY OF ELEVEN WHEAT AND OAT ISOLATES OF *OPHIOBOLUS GRAMINIS* ON WHEAT.

Isolate	Fresh Weight of Host (mg.)	Length (in mm.) of: Discoloured Root	Runner Hyphae along root	Whole Root	Analysis of Data - Using Transformations: $Log_e X$ Fresh Weight of Host	$Log_e(X+1)$ Length of Discoloured Root	$Log_e(X+1)$ Length of Runner Hyphae Along Root	$Log_e X$ Length of Whole Root
01	252	14	20	127	5.5262	2.6870	3.0186	4.8339
02	91	29	38	94	4.3906	3.4066	3.6463	4.5421
03	477	2	4	194	6.1559	1.3648	1.4999	5.2572
04	156	31	41	115	5.0393	3.4460	3.7254	4.7271
05	367	5	9	142	5.9022	1.6566	2.2515	4.9503
06	168	30	46	95	5.1210	3.4167	3.8350	4.5456
07	265	19	26	127	5.5666	2.9679	3.2900	4.8422
08	543	0	0	199	6.2964	0.0000	0.0000	5.2897
W1	188	23	33	99	5.2269	3.1633	3.5141	4.5932
W2	200	22	28	119	5.2430	3.1337	3.3520	4.7796
W3	178	26	37	108	5.1596	3.2865	3.6330	4.6607
Control	484			186	6.1731			5.2169
Differences for significance $P = 0.05$					0.4300	0.3606	0.3355	0.2436
$P = 0.01$					0.5812	0.4887	0.4546	0.3293

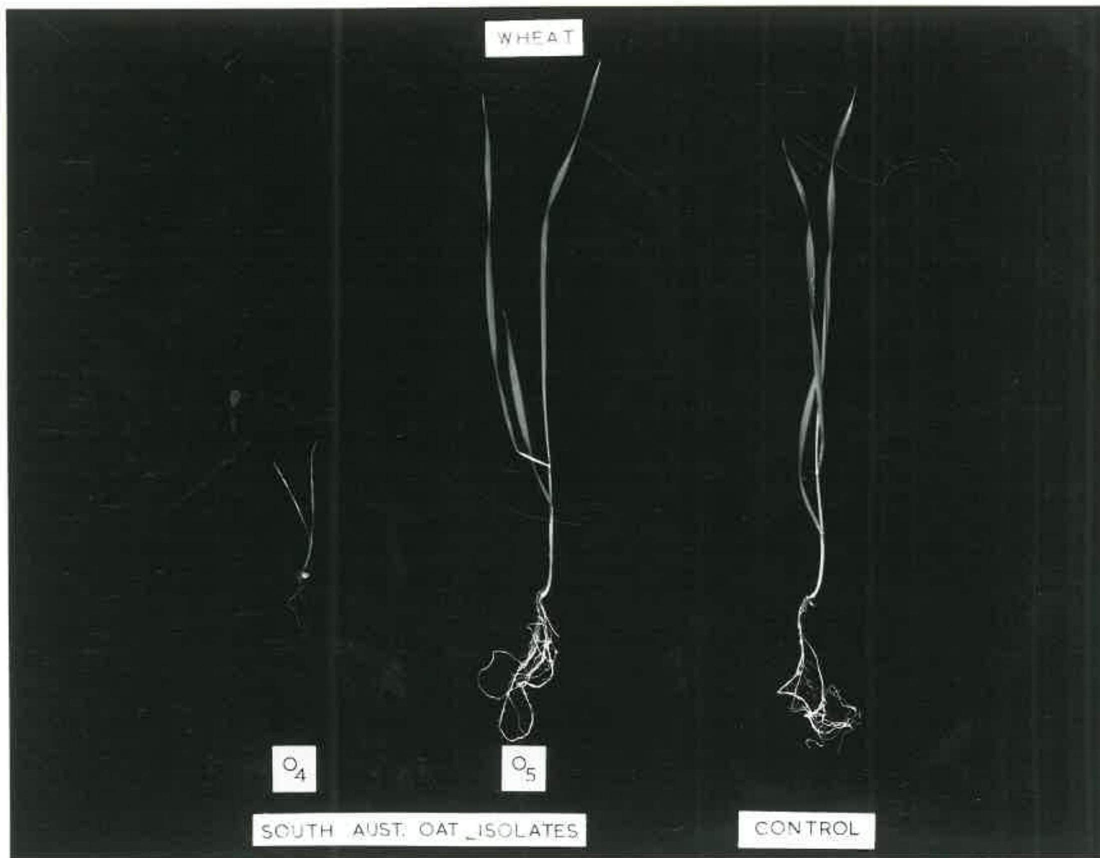
TABLE 21.

PATHOGENICITY OF ELEVEN WHEAT AND OAT ISOLATES OF O. GRAMINIS ON OATS

Isolate	Fresh Weight of Host (mg.)	Length (in mm.) of:			Analysis of Data Using Transformations			
		Discoloured Root	Hyphae along Root	Whole Root	$Log_e X$ Fresh Weight of Host	$Log_e (X+1)$ Length of Discoloured Root	$Log_e (X+1)$ Length of Hyphae along Root	$Log_e X$ Length of whole Root
01	485	19	33	153	6.1822	2.9924	3.4950	5.0302
02	451	32	48	127	6.1075	3.4944	3.8844	4.8412
03	656	0	1	128	6.4826	0.0000	0.4621	4.8478
04	691	0	3	157	6.5375	0.0000	1.2296	5.0561
05	752	0	0	155	6.6218	0.0000	0.2311	5.0389
06	776	1	2	130	6.6501	0.6931	1.1552	4.8696
07	702	0	3	157	6.5528	0.0000	1.2689	5.0461
08	743	0	0	162	6.6048	0.0000	0.2311	5.0630
W1	847	0	2	146	6.7405	0.0000	1.1945	4.9762
W2	847	0	5	151	6.7394	0.0000	1.6702	5.0137
W3	791	0	9	156	6.6720	0.0000	2.2959	5.0353
Control	798			158	6.6693			5.0633
Differences for Significance $P = 0.05$					0.1842	0.3075	0.6906	N.S.
$P = 0.01$					0.2435	0.4659	0.9358	

Fig. 15. Reaction of wheat seedlings to inoculation with South Australian oat isolates O4 and O5.

Fig. 16. Reaction of oat seedlings to inoculation with South Australian oat isolates O4 and O5. Note that O4 is strongly pathogenic to wheat (Fig. 15) but not to oats, whereas O5 is avirulent on both hosts.


WHEAT
O_4
O_5
SOUTH AUST. OAT ISOLATES
CONTROL

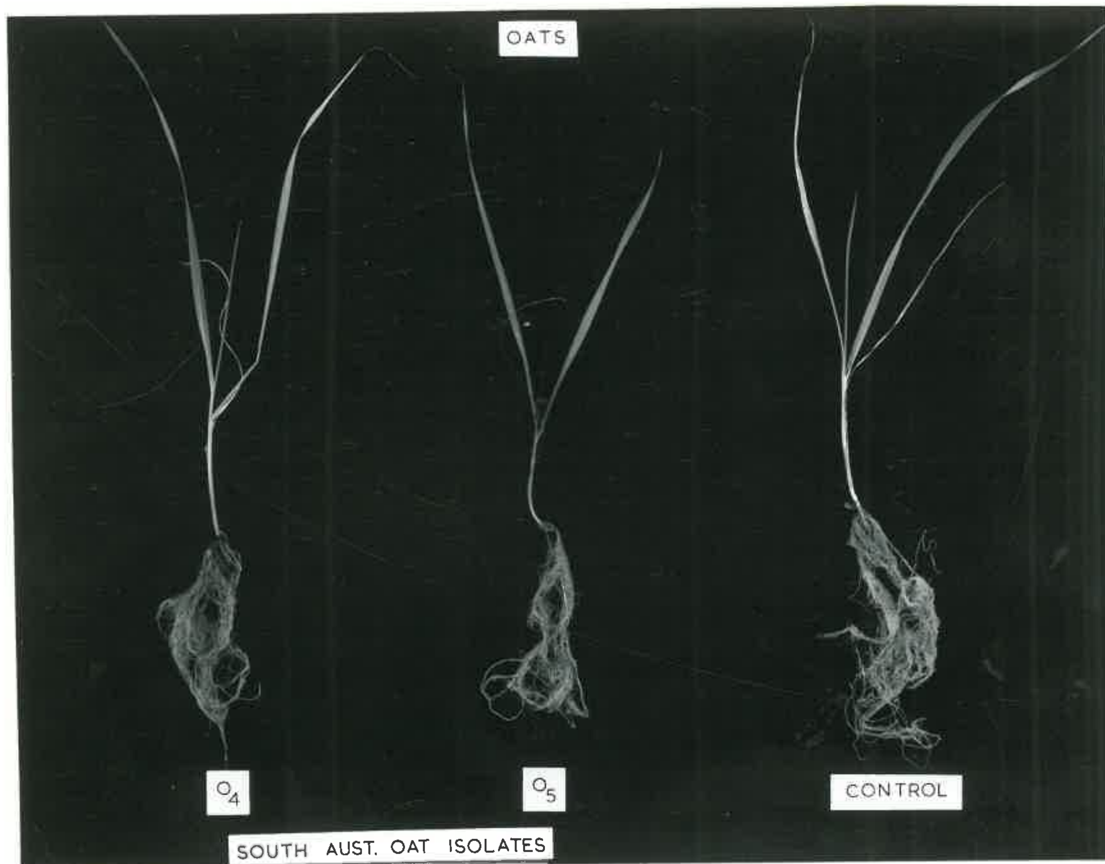
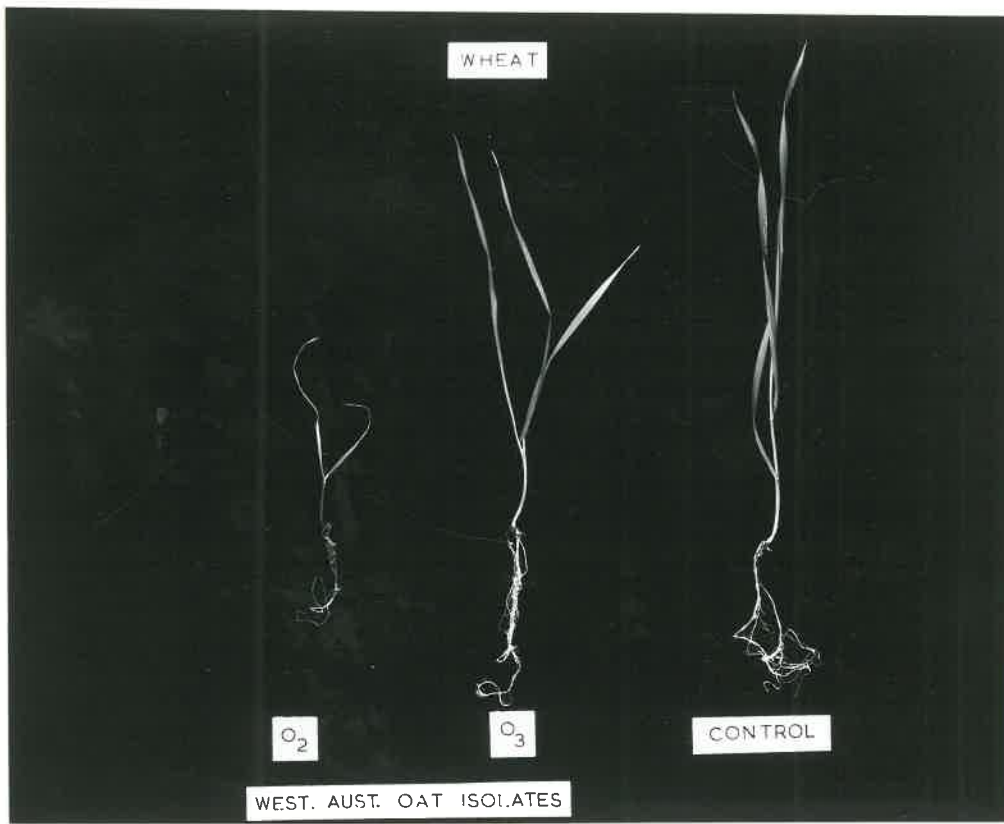

OATS
O_4
O_5
CONTROL
SOUTH AUST. OAT ISOLATES

Fig. 17. Reaction of Wheat seedlings to inoculation with Western Australian oat isolates O2 and O3.

Fig. 18. Reaction of oat seedlings to inoculation with Western Australian oat isolates O2 and O3. Note that O2 is strongly pathogenic to both wheat (Fig. 17) and oats, whereas O3 is avirulent on both hosts.


WHEAT
O_2
O_3
CONTROL
WEST. AUST. OAT ISOLATES

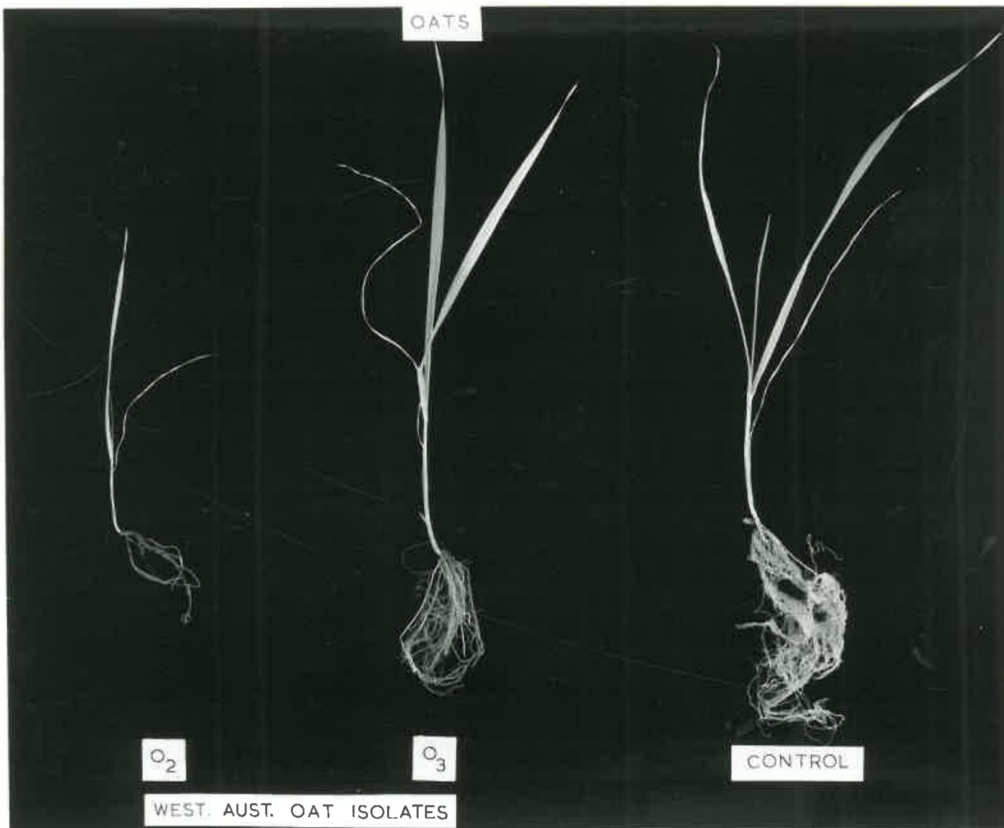
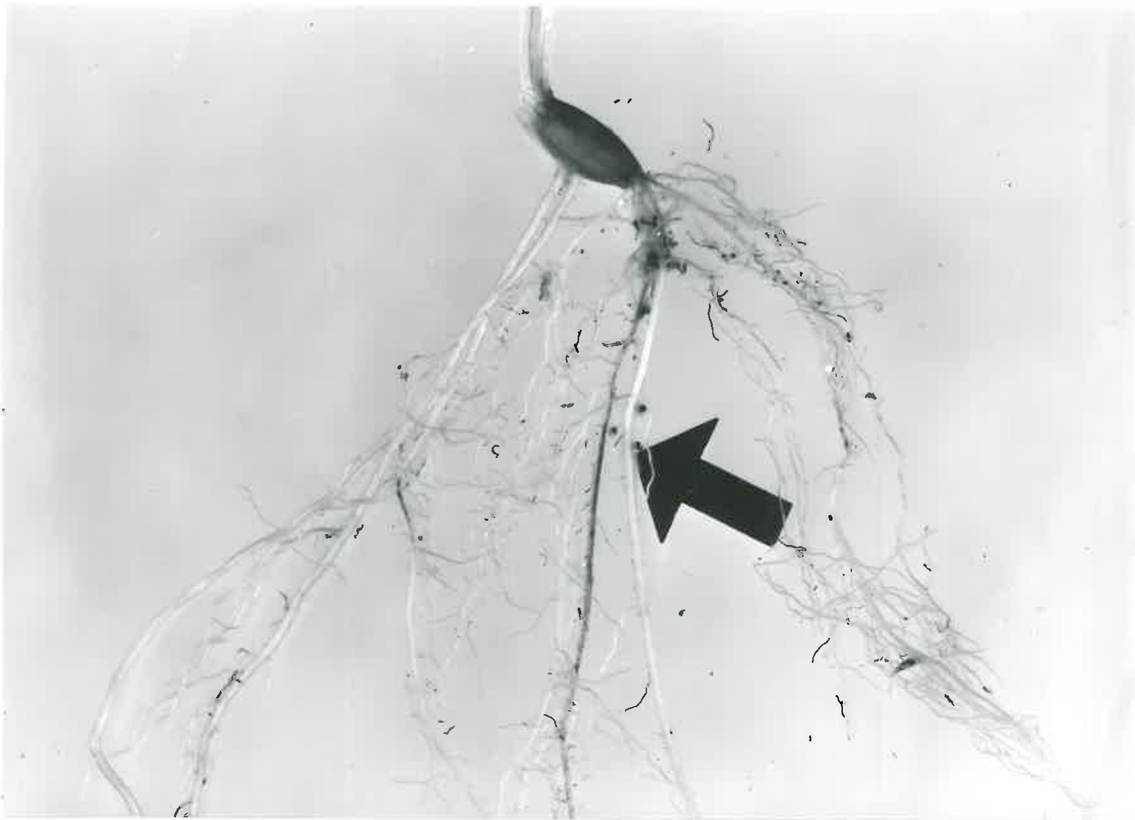
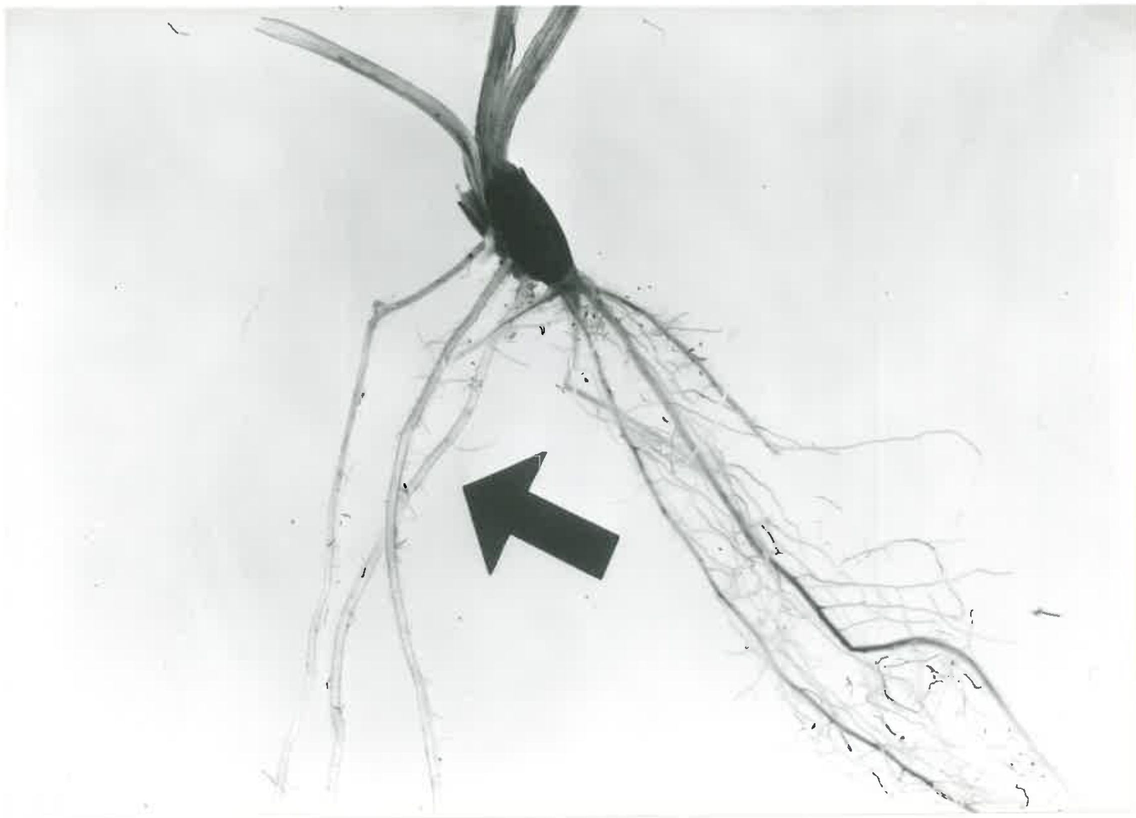

OATS
O_2
O_3
CONTROL
WEST. AUST. OAT ISOLATES

Fig. 19. Root system of an oat plant inoculated with South Australian oat isolate O4. Note the white colour of the secondary root (arrow), which is free from infection. This root contrasts with the adjacent seminal root (left) which is discoloured from infection by O. graminis. Two perithecia can be seen on either side of the secondary root immediately above the arrow.

Fig. 20. Root system of an oat plant inoculated with Western Australian oat isolate O2. The secondary roots (arrow) are infected with O. graminis and are darker in colour than the root illustrated in Fig. 19. The seminal roots (to the right of the arrow) and the leaf sheaths are also discoloured from infection.





isolates significantly reduced the top growth of wheat.

The pathogenicity of O1 and O2 to oats was confirmed by examination of discoloured secondary roots stained with lactophenol cotton blue. Hyphae were seen in the vascular and inner cortical tissues of these roots, being more numerous in roots infected with O2. Infections of secondary roots by South Australian isolates were fewer and hyphae were restricted to the outer cortical cells.

There was also further evidence of a link between virulence of isolates on wheat and the production of perithecia. The strongly pathogenic isolates O2, O4, O6, W1, W2 and W3 formed mature perithecia; the moderately pathogenic isolates O1 and O7 formed none and immature perithecia-like bodies respectively; and the avirulent isolates O3, O5 and O8 formed none.

(d) Ascus and Ascospore Measurements of Isolates from Oats and Wheat.

Turner (1940a) reported that asci and ascospores of the var. avenae are larger than those of the type variety when obtained from host plants. Therefore an attempt was made to obtain perithecial material, preferably from the original field sample, of all the isolates used in the above pathogenicity test.

The writer is indebted to the Western Australian Department of Agriculture for supplying part of the oat

samples from which Mrs. G. Weste had isolated O1 and O3. These samples had been collected by the writer and deposited in the Department of Agriculture herbarium. Unfortunately, none of the original South Australian oat material used for isolations by Dr. R. Dodman was available. Asci and ascospores of isolates O4 and O6 were therefore obtained from perithecia formed on oat plants during pathogenicity tests. However, none could be obtained from this source for the weakly pathogenic O5, the avirulent O8 nor for O7 which formed only empty perithecia-like bodies. Perithecia were obtained from the original field plants for the remaining isolates O2, W1, W2 and W3.

As measurements taken previously of asci and ascospores formed by the F_1 progeny of W1 indicated that those formed in culture were generally larger than those on host tissue, attempts were made to produce mature perithecia of isolates O1-O8 and W1-W3 in culture.

The six isolates O2, O4, O6, W1, W2 and W3, which were strongly pathogenic on wheat, formed many mature perithecia in culture. The moderately pathogenic O1 formed only one mature perithecium and several empty perithecia-like bodies. The moderately pathogenic isolate O7 produced only empty perithecia-like bodies in culture. None was formed by the weakly-pathogenic isolates O3 and O5 nor by the avirulent O8.

Measurements were made of the mean lengths of asci and ascospores from five perithecia from each source. These

are set out in Table 22, together with estimates of the numbers of septa in the ascospores. As only one mature perithecium was formed in culture by O1, no measurements are given for this source.

Turner (1940a) recorded the ranges in mean ascospore length as 101-117 μ for the var. avenae and 79-86 μ for the type variety. However, the measurements of ascospores on host material given in Table 22 do not present a similar clear differentiation. Although the ascospore measurements for O2 and W1 fall within Turner's ranges for var. avenae and the type variety respectively, the remainder are intermediate between the two. Similarly, the mean lengths of asci on host material given in Table 22 are between the two ranges recorded by Turner. Davis (1950) reported an even wider range of ascospore lengths for var. avenae, viz., 92-142 μ. Thus it would be difficult or impossible to classify the majority of isolates used in the present study on the basis of ascus and ascospore measurements alone.

The results in Table 22 also provide additional evidence that asci and ascospores formed in culture are generally larger than those on host tissue. This was further substantiated by measurements of the asci and ascospores of three other wheat isolates (Table 23). It is interesting to note that the mean ascospore length (86 μ) of one isolate was within the type variety range of Turner when formed on host tissue. However, when formed in culture, the mean ascospore length (110 μ) was within the var. avenae range.

TABLE 22.

ASCUS AND ASCOSPORE MEASUREMENTS OF ISOLATES OF *O. GRAMINIS* FROM WHEAT AND OATS.

Isolate	Asci		Ascospore Measurements				
	Source	Mean Length (in μ)	Length (in μ)			Number of Septa	
			Mean	Modal	Range	Mean	Range
01	Original material	121 ±0.91	94 ±0.70	92	81-107	10.0	7-11
02	Original material	123 ±0.78	103 ±0.56	105	92-118	9.6	5-11
	Culture	136 ±0.84	105 ±0.68	105	89-131	11.6	9-15
03	Original material	124 ±0.84	91 ±0.55	92	84-105	10.0	7-13
04	Pathogenicity Test - Oats	117 ±0.79	93 ±0.73	97	79-110	7.0	5-11
	Culture	125 ±0.67	102 ±0.44	102	92-113	7.9	5-11
06	Pathogenicity Test - Oats	115 ±0.73	96 ±0.76	92	81-118	6.9	5- 9
	Culture	128 ±0.58	99 ±0.49	97	92-113	8.0	5-11
W1	Original material	106 ±0.91	83 ±0.58	81	68- 94	7.0	5- 9
	Pathogenicity Test - Oats	108 ±0.80	85 ±0.52	85	73- 97	6.9	5- 9
	Culture	120 ±0.72	95 ±0.56	92	81-110	7.7	5-11
W2	Original material	113 ±0.91	91 ±0.60	92	79-105	6.9	5- 7
	Pathogenicity Test - Oats	114 ±0.91	87 ±0.54	84	76-102	7.0	5- 9
	Culture	118 ±0.65	99 ±0.53	100	89-113	7.0	5- 9
W3	Original material	114 ±0.69	92 ±0.57	92	81-105	7.0	5- 7
	Pathogenicity Test - Oats	114 ±0.74	88 ±0.85	84	71-110	7.0	5- 9
	Culture	120 ±0.76	97 ±0.80	92	79-115	7.0	5- 7

TABLE 23.

ASCUS AND ASCOSPORE MEASUREMENTS OF THREE WHEAT ISOLATES OF OPHIOBOLUS GRAMINIS.

Source of Isolate	Asci			Ascospore Measurements					
	Source	Mean Length (in µ)		Length (in µ)				Number of Septa	
				Mean		Modal	Range	Mean	Range
Glen Osmond, Sth. Aust.	Original material	106	±0.56	86	±0.56	89	73-100	6.9	5- 9
	Culture	122	±0.65	110	±0.73	110	89-123	7.1	7- 9
Glen Osmond, Sth. Aust.	Original material	113	±0.71	90	±0.53	92	79-100	7.0	5- 9
	Culture	119	±0.71	104	±0.55	105	86-115	7.0	5- 7
Swan Hill, Victoria	Original material	96	±0.73	76	±0.54	79	66- 86	6.4	3- 9
	Culture	109	±0.71	86	±0.53	84	76-100	6.3	3- 8

(e) Growth of Isolates from Wheat and Oats on Media Containing Oat Leaf Extract.

The growth of six isolates from oats was compared with that of three isolates from wheat on a liquid medium containing different concentrations of oat leaf extract. Three isolates from oats (O1, O2 and O3) were from Western Australia and the remainder (O4, O7, O8, W1, W2 and W3) from South Australia.

The aqueous oat leaf extract was prepared as described on page 29 and mixed with the basal medium of Lilly and Barnett (Appendix A) so as to give a series of nutrient solutions containing 0, 100, 150, 200, 300, 400, 500 and 1000 ml. extract per litre. Each of the series was dispensed in 5 ml. aliquots in 100 ml. flasks and sterilized by autoclaving at one atmosphere for seven minutes.

Three flasks of each concentration were each inoculated with a 3 mm. diameter disc from a fourteen day old culture of a test isolate and incubated at 25°C for ten days. Mycelial mats from the cultures were harvested on previously dried and weighed filter papers, washed thoroughly with distilled water and dried for 24 hours at 95°C. The mean dry weight for three replicates of each isolate at each concentration is given in Table 24 and Fig. 21.

The Western Australian isolates from oats were more tolerant than South Australian wheat isolates to increasing concentrations of oat extract. Janes (1947) reported similar results when comparing the growth of a South Australian

TABLE 24.

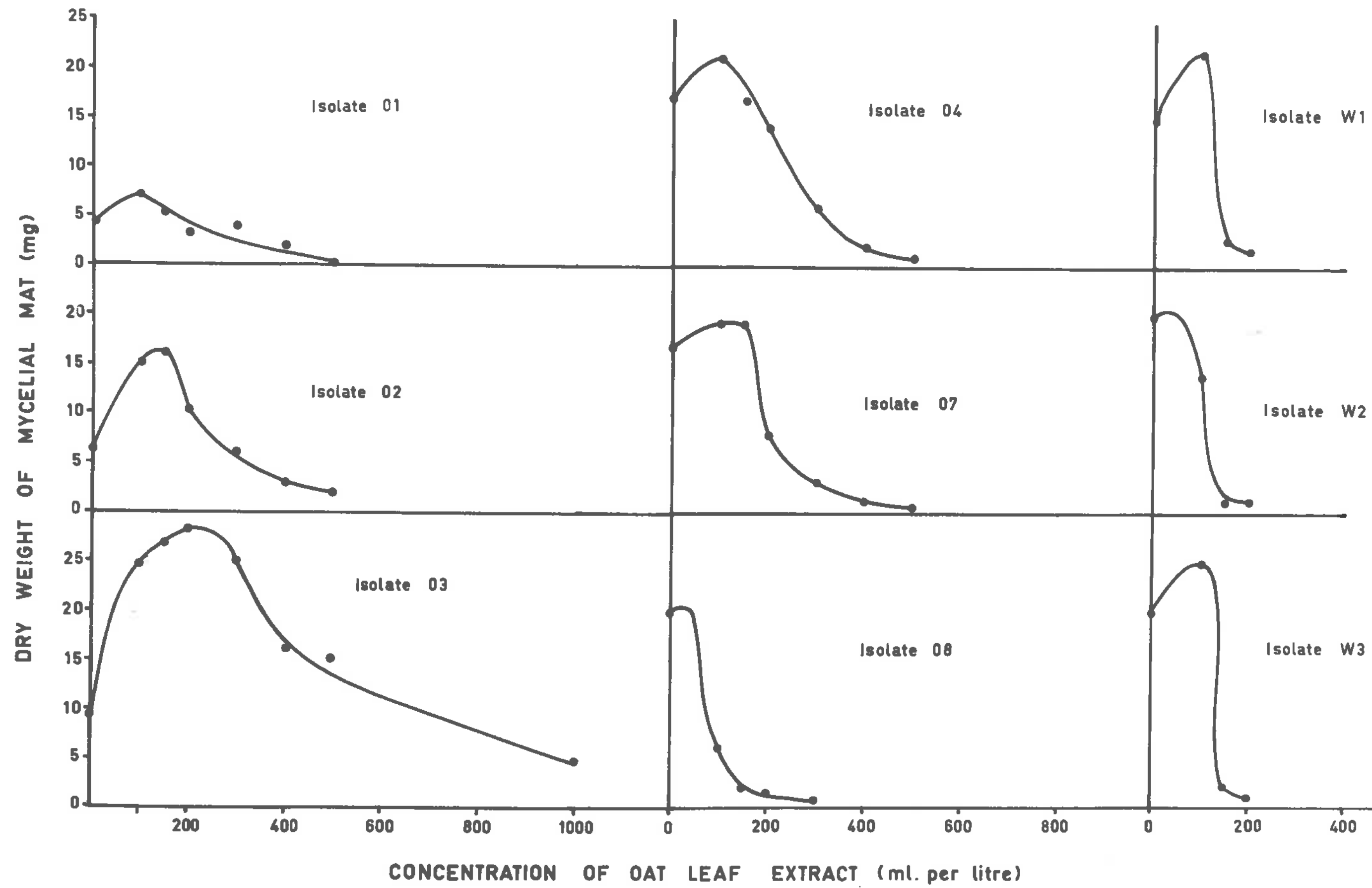
EFFECT OF OAT LEAF EXTRACT ON MYCELIAL DRY WEIGHT

OF OAT AND WHEAT ISOLATES OF OPHIOBOLUS GRAMINIS.

Concentration of Oat Extract (ml./litre medium)	Mycelial Dry Weight (in m.g.) of:								
	West Aust. Oat Isolates			Sth. Aust. Oat Isolates			Sth. Aust. Wheat Isolates		
	01	02	03	04	07	08	W1	W2	W3
0	4.7	6.7	9.3	17.3	17.0	19.7	15.0	20.0	20.0
100	7.0	15.0	24.7	21.3	18.7	6.3	22.3	14.0	25.0
150	5.3	16.0	26.7	16.7	18.7	2.0	2.7	1.3	2.3
200	3.3	10.3	28.0	13.7	7.7	1.7	1.7	1.3	1.3
300	3.7	5.7	24.7	6.0	3.3	0.7	0.0	0.0	0.0
400	1.7	2.7	15.7	2.3	1.3	0.0	0.0	0.0	0.0
500	0.3	1.7	14.7	1.0	0.7	0.0	0.0	0.0	0.0
1000	0.0	0.0	4.7	0.0	0.0	0.0	0.0	0.0	0.0

Fig. 21.

EFFECT OF VARIOUS CONCENTRATIONS OF OAT LEAF EXTRACT ON MYCELIAL DRY WEIGHT OF OAT AND WHEAT ISOLATES OF <u>O. GRAMINIS</u>



isolate from wheat with a British isolate of var. avenae as represented graphically in Fig. 22. This experiment provides further evidence of the similarity between the Western Australian isolates and var. avenae.

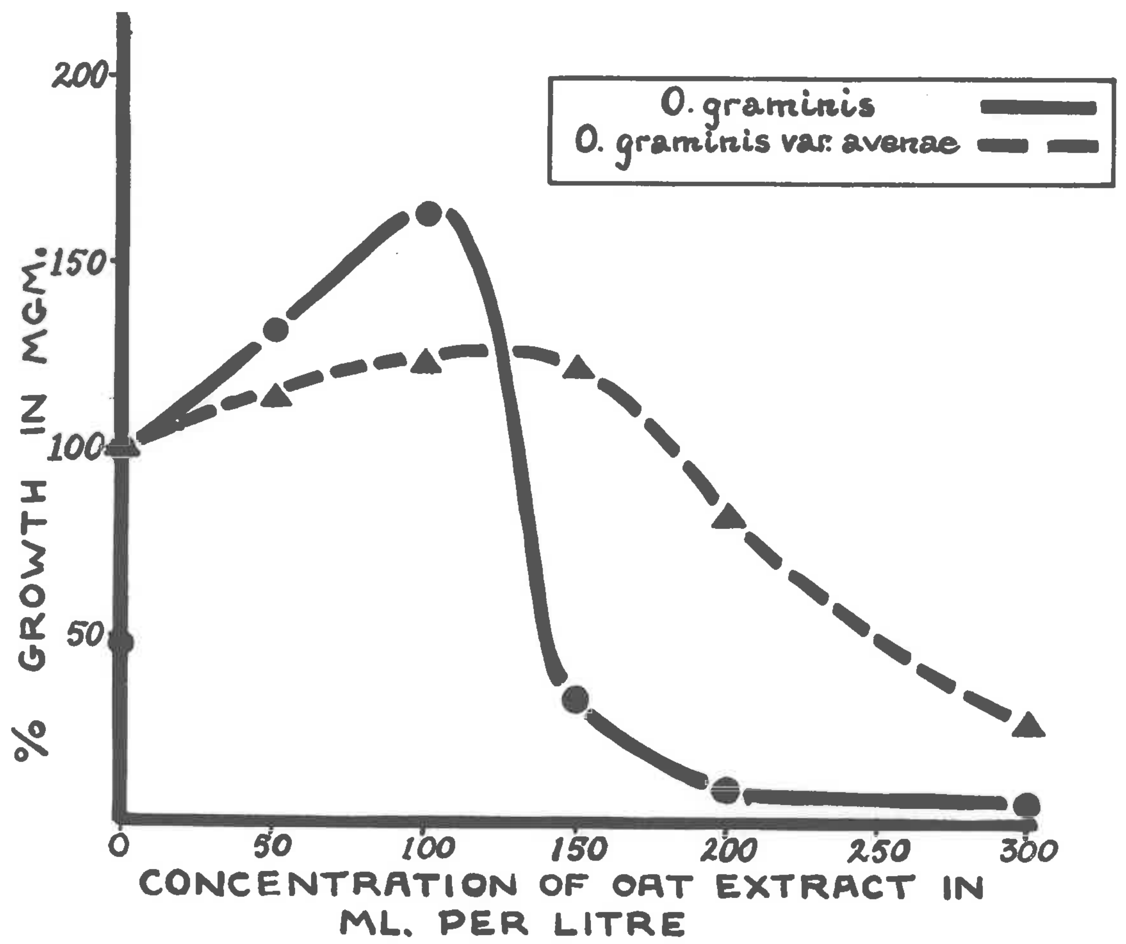
Results with South Australian isolates O4 and O7 show a variant which is intermediate between the type variety and var. avenae; both O4 and O7 reacted as the type variety in the pathogenicity test (Tables 20, 21) but were equally tolerant to oat extract as O2, the Western Australian isolate from oats (Fig. 21) and var. avenae isolate examined by Janes (Fig. 22).

(f) Effect of Cysteine and Cystine on Cultural Growth of Isolates from Wheat and Oats.

Turner (1957) reported that both cysteine and cystine retard the cultural growth of var. avenae but stimulate that of the type variety. Comparisons were therefore made between the mycelial dry weight of six isolates from oats (O1, O2, O3, O4, O7, and O8) and three isolates from wheat (W1, W2, and W3) on media containing either cysteine or cystine.

The basal medium was prepared in accordance with Turner's formula (Appendix A) and the initial quantity was divided into four equal volumes. To one aliquot, 0.05 percent (W/V) white soluble casein (Judex Chemicals) was added; two received additions of 0.04 M L-cystine and 0.04 M L-cysteine hydrochloride respectively; the fourth was left as a control.

Fig. 22. Reproduction of graph by Janes (1947) showing the differential effect of oat extract on the growth of the two varieties of _O. graminis_.


O. graminis
O. graminis var. avenae
% GROWTH IN MGM.
200
150
100
50
0
50
100
150
200
250
300
CONCENTRATION OF OAT EXTRACT IN
ML. PER LITRE

Each of the four media was dispensed in 3 ml. aliquots in 100 ml. flasks and autoclaved at one atmosphere for seven minutes. The glucose component of the medium was autoclaved separately in 2 ml. aliquots and afterwards added aseptically to the flasks.

Three flasks of each medium were inoculated with 3 mm. diameter discs from a fourteen day old culture of a test isolate. A series of cultures was thus prepared for each of the nine test isolates and incubated at 25°C for six days. Mycelial mats were then washed thoroughly, dried for 24 hours at 95°C and weighed. Mean dry weight for three replicates of each isolate is set out in Table 25. The unprocessed data (Table 25) suggested a general tendency for cystine and cysteine to increase the growth of isolates from wheat and to suppress that of the Western Australian isolates from oats. However, statistical analysis revealed that only some of these increases and decreases were significant so that the results were not as decisive as those reported by Turner (1957). In the case of the South Australian isolates from oats, the effects of the two amino acids were variable, thus providing no information for classifying them as either variety of *O. graminis*.

TABLE 25.

EFFECT OF CYSTINE, CYSTEINE AND SOLUBLE CASEIN ON MYCELIAL DRY WEIGHT

OF ISOLATES OF *OPHIOBOLUS GRAMINIS* FROM WHEAT AND OATS

Isolate	Dry Weight of Mycelium on: (mg.)				Analysis of Dry Weights (Using Transformation $Log_e(X+1)$)			
	Basal Medium (Control)	Basal Plus Cystine	Basal Plus Cysteine	Basal Plus Casein	Basal Medium (Control)	Basal Plus Cystine	Basal Plus Cysteine	Basal Plus Casein
01	6	5	2	18	1.9904	1.7824	1.1945	2.9227
02	10	7	2	14	2.3661	2.1135	1.0594	2.7268
03	9	7	4	16	2.3026	2.0966	1.6472	2.8130
04	13	12	16	35	2.6047	2.5570	2.8130	3.5825
07	12	17	0	29	2.5467	2.9025	0.0000	3.3884
08	16	13	11	35	2.8130	2.6589	2.5022	3.5924
W1	10	19	20	36	2.3867	3.0120	3.0557	3.6015
W2	15	19	18	37	2.7928	2.9778	2.9615	3.6273
W3	15	16	18	32	2.7713	2.8130	2.9615	3.4943

Differences for significance $P = 0.05$ 0.2101

$P = 0.01$ 0.2786

TABLE 26.

SIZE AND NUCLEAR NUMBER OF CELLS OF ISOLATES W1, 01 AND 09

Source of Cells	Isolate	Measurements (in μ)					Number of Nuclei per Cell	
		Length			Width		Mean	Range
		Mean		Range				
Peripheral Tip	W1	33.19	±1.57	10.6- 86.1	1.63	±0.04	1.1	1-2
	01	43.78	±1.88	14.2-101.4	1.54	±0.03	1.1	1-2
	09	63.79	±3.02	14.2-152.2	2.08	±0.03	4.1	1-11
Internal Tip	W1	16.32	±0.59	7.1- 37.8	1.76	±0.04	1.1	1-2
	01	23.62	±0.70	14.2- 41.3	1.77	±0.04	1.0	1-2
	09	25.63	±1.10	9.4- 64.9	2.29	±0.07	2.2	1-7
Single Hyphae	W1	17.56	±0.61	5.9- 61.4	2.17	±0.04	1.1	1-4
	01	24.15	±0.86	7.1- 97.9	2.05	±0.05	1.1	1-2
	09	25.45	±0.57	9.4- 68.4	2.06	±0.04	3.8	1-18
Hyaline Strand	W1	14.15	±0.32	7.1- 33.0	1.87	±0.03	1.2	1-2
	01	18.60	±0.73	10.6- 72/0	3.20	±0.05	1.4	1-4
	09	24.84	±0.53	11.8- 44.8	4.46	±0.06	2.1	1-5
Dark Strand	W1	37.32	±3.07	5.9-236.0	3.70	±0.08	1.9	1-10
	01	35.94	±1.90	10.6-206.5	4.02	±0.06	2.2	1-8
	09	36.03	±1.44	9.4-192.3	4.73	±0.06	4.8	1-24

(g) Compatibility Studies with Isolates from Wheat and Oats.

An important consideration arising from the variability exhibited by isolates in these comparative tests is whether variants can readily combine to produce further variants. This would be of particular significance in the case of the two recognized varieties of *O. graminis* as it could lead to the existence of a number of intermediate strains.

One mechanism whereby two variants may give rise to a third is by hyphal anastomosis, but no observations have been made on anastomosis between isolates of *O. graminis*. Furthermore, little information is available on the nuclear content of hyphae of *O. graminis*. Jones (1926) found the cells were uninucleate, but Garrett (1942) suggested Jones' data referred to var. *avenae* and that some of the structures he described were of another fungus. Thus it was necessary to study nuclear distribution in *O. graminis* before attempting to anastomose different isolates.

(i) Nuclear Distribution in Hyphae of *O. graminis*.

Segments of cultures containing both hyaline and dark coloured hyphae of isolates W1-W5 and 01-09 were stained and an examination was made of nuclear distribution. In all isolates except 09, hyaline cells were predominately uni-nucleate and dark coloured cells were generally bi- or multi-nucleate (Figs. 23, 24). The number of nuclei in dark coloured cells also appeared to be related to cell length; the longest cells contained most nuclei. Cells varied

Fig. 23. Uni-nucleate tip cells of isolate W1.

Mag. x1750

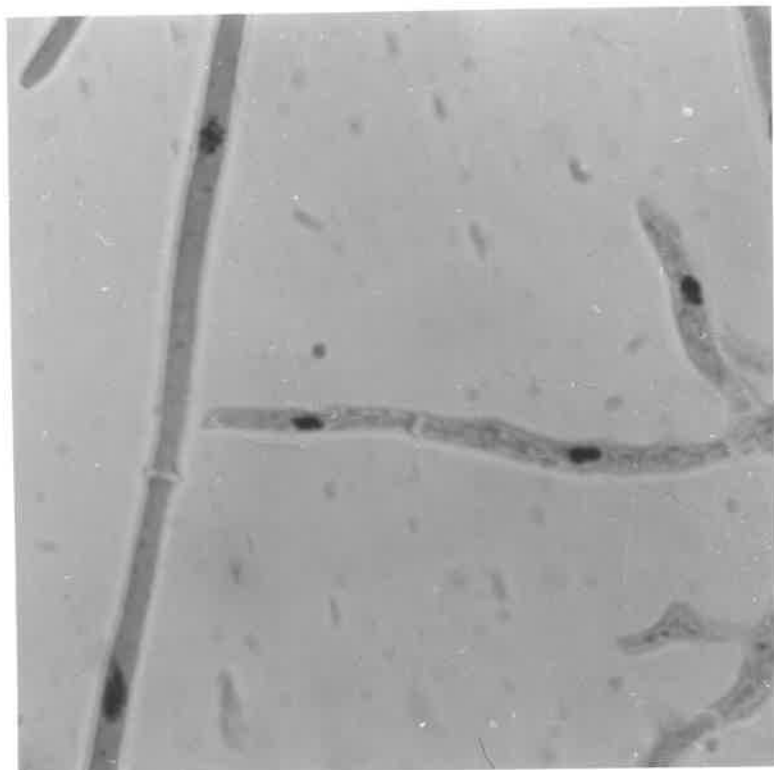


Fig. 24. Bi- and multi-nucleate cells of dark-coloured hyphal strands of isolate W1.

Mag. x1750.

considerably in size and in general dark-coloured cells and peripheral tip cells were larger than the remaining hyaline cells. Strands of two or more hyphae were present in all cultures and were usually composed of dark-coloured cells. Isolate 09 differed from all others in that the majority of its cells were multi-nucleate (Figs. 25, 26).

A more detailed examination was then made of 800 cells from each of isolates W1, 01 and 09. Nuclei were counted in equal numbers of cells of the four sources; hyphal tips, single hyaline hyphae, hyaline strands and dark-coloured hyphae. The hyphal tip cells were of two types, internal and peripheral, and only 100 cells of each were examined. All cells were then measured with a calibrated eyepiece micrometer and details of these counts and measurements are supplied in Table 26. Counts were also made of the number of nuclei in cells of 100 ascospores of isolates W1 and 02; all cells were uni-nucleate (Fig. 27).

The results in Table 26 indicate that there is little difference between the nuclear distribution in the mycelium of the oat-attacking isolate 01 and the wheat-attacking isolate W1. A point of interest is the difference in nuclear content of hyaline and dark-coloured cells, especially in view of the different functions of hyaline "infection" hyphae and dark "runner" hyphae in nature. The results also show that the nuclear content of isolate 09 is very different from that of 01 and W1. However, discussion

Fig. 25. Peripheral tip cell of isolate 09 with three nuclei.

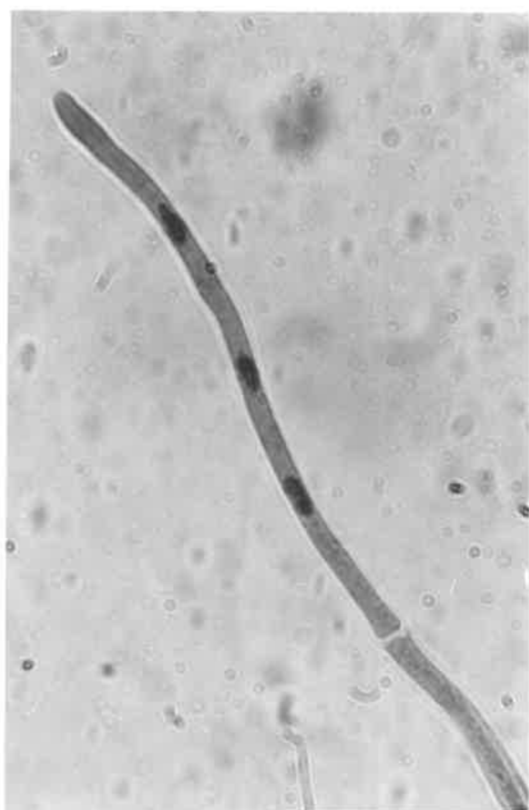
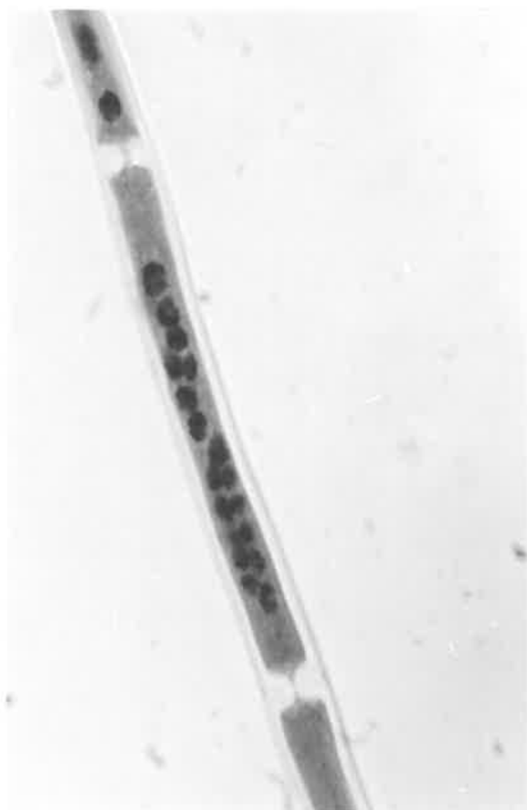
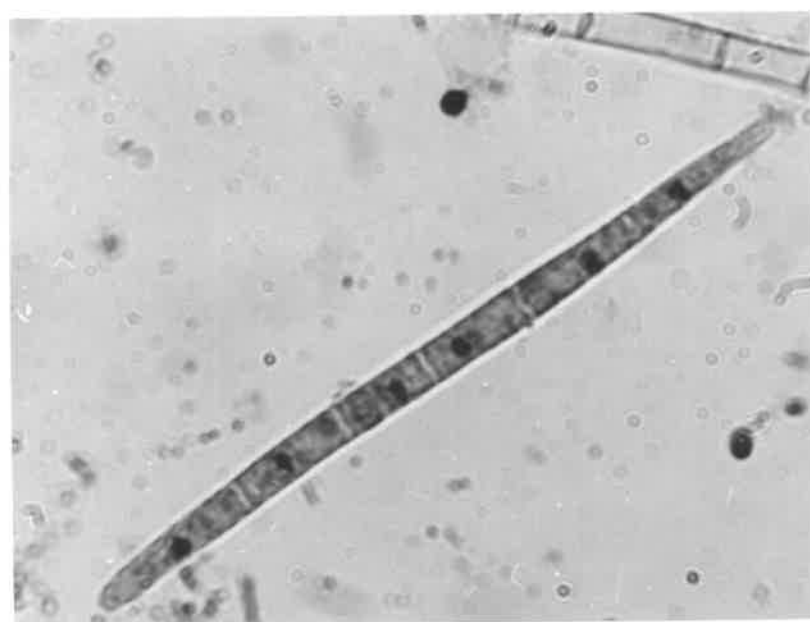
Mag. x1200

Fig. 26. Multi-nucleate cell of isolate 09.

Mag. x1200

Fig. 27. Ascospore of isolate W1, containing six uni-nucleate cells.

Mag. x1250

of this isolate will be deferred, as evidence which will be presented later suggests that 09 may not be an isolate of *O. graminis*.

(ii) Hyphal Anastomosis in Isolates from Wheat and Oats.

Five isolates from oats and three from wheat were opposed in all possible combinations on cellophane overlying PMD to determine whether they would anastomose with each other. On meeting, opposing hyphae reacted in one of the following ways:

1. Intermingled freely, usually growing over and around each other (Fig. 28). Occasionally the opposing hyphae formed a relatively loose common strand, separating again after a short distance ... ... (Reaction -) (Table 27)
2. Formed many common strands (Fig. 29) which were similar to those in pure cultures ... ... (Reaction ST)
3. Formed peg-like outgrowths towards hyphae of the opposing isolate (Fig. 30) ... ... (Reaction P)
4. Anastomosed but the cells collapsed and died soon afterwards (Fig. 31) ... ... (Reaction K)
5. Anastomosed without any adverse effects upon the cells ... ... (Reaction +)

Although evidence of attempted anastomosis between isolates was relatively uncommon, self-anastomosis occurred in all preparations. It was most frequently observed between

Fig. 28. Hyphal tip of isolate W1 growing over and around hypha of isolate 01.

Mag. x1120.

Fig. 29. Common hyphal strand formed by dark-stained hyphae of isolate 05 (ascending) and light-stained hypha of isolate W1 (descending).

Mag. x1120.

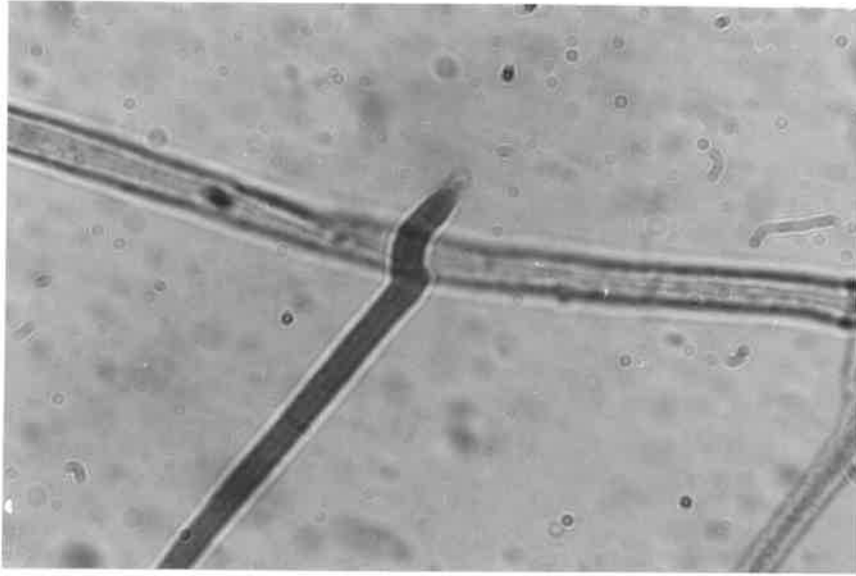
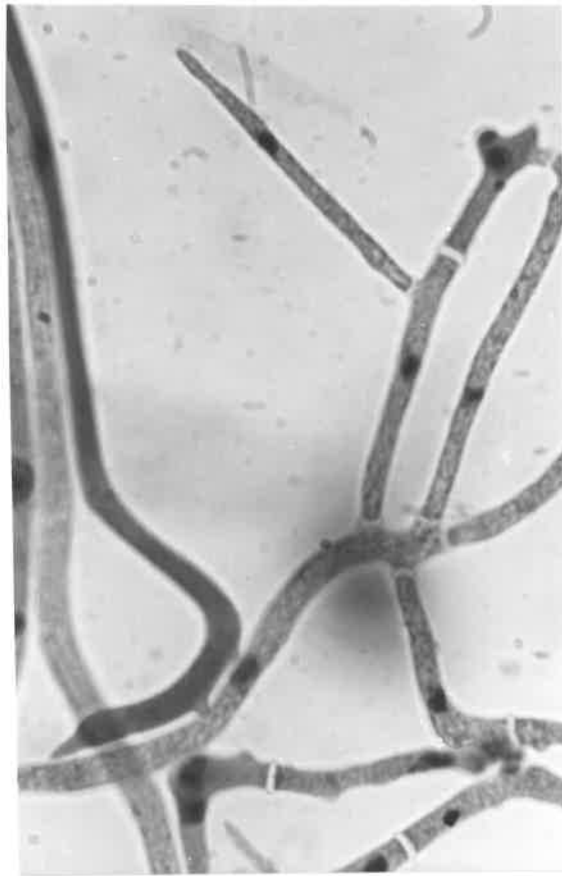


Fig. 30. Peg-like outgrowths from
dark-stained hypha of isolate O5
towards hypha of light-stained
isolate W1.
Mag. x1120

Fig. 31. Killing reaction following
anastomosis between hyphae of
isolates W1 and W3.
Mag. x1120



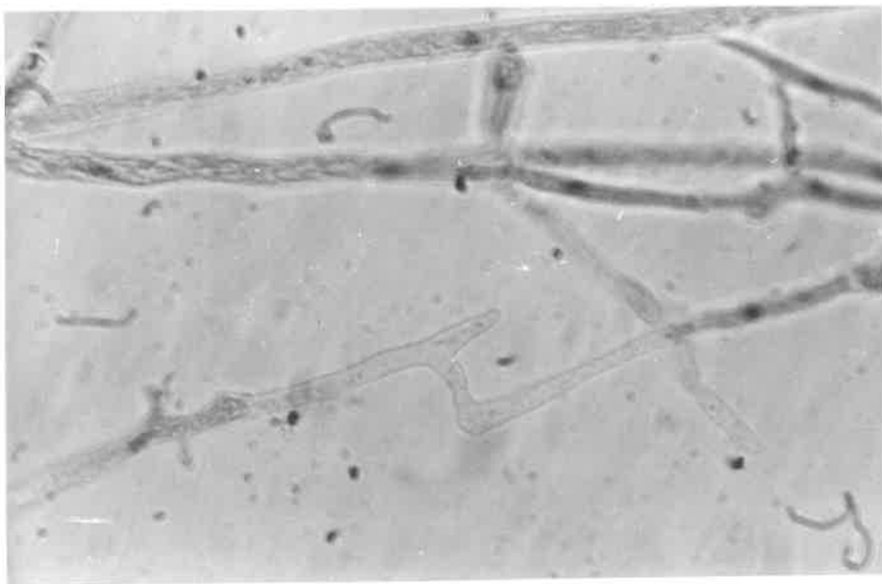


TABLE 27.

REACTIONS OF WHEAT AND OAT ISOLATES WHEN OPPOSED ON CELLOPHANE OVERLYING PMD IN PETRI DISHES

For explanation of symbols, see text page 86.

Isolate	01	02	04	07	08	W1	W2	W3
01	+	ST	-	-	-	P	ST	-
02		+	ST	ST	ST	K	-	-
04			+	+	ST	K-ST	ST	-
07				+	ST	K	-	-
08					+	K	P-ST	-
W1						+	K	K
W2							+	+
W3								+

hyphae near the advancing edge of the colony (Figs. 32, 33).

The reactions of the eight isolates towards each other are summarized in Table 27. These observations suggest the isolates from oats are incompatible with the wheat isolates. The Western Australian isolates from oats were also incompatible with those from South Australia. Compatibility of isolates, expressed as successful hyphal anastomosis, appears to be a relatively restricted phenomenon and was observed only between pathogenically similar isolates from the same locality. Furthermore, it occurred only occasionally in the two successful combinations, despite the large masses of intermingling hyphae. This suggests that the mechanism governing hyphal anastomosis is relatively complex and requires specific circumstances for it to occur and be successful. Nevertheless, it would be interesting to oppose isolates of the two varieties of _O. graminis_ if they could be obtained from the same crop.

(h) _Survival of Isolates from Wheat and Oats in Unsterilized Soil_.

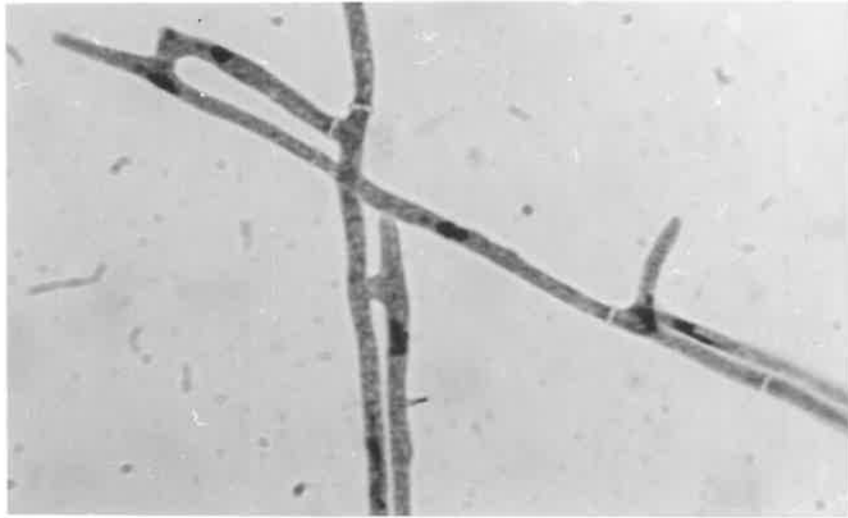
Perusal of the literature revealed that much information is available on the survival of the type variety of _O. graminis_ but little on the var. _avenae_. A comparison was therefore made of the survival of the oat-attacking isolate O1 with the wheat isolate W1 on artificially infected wheat straws.

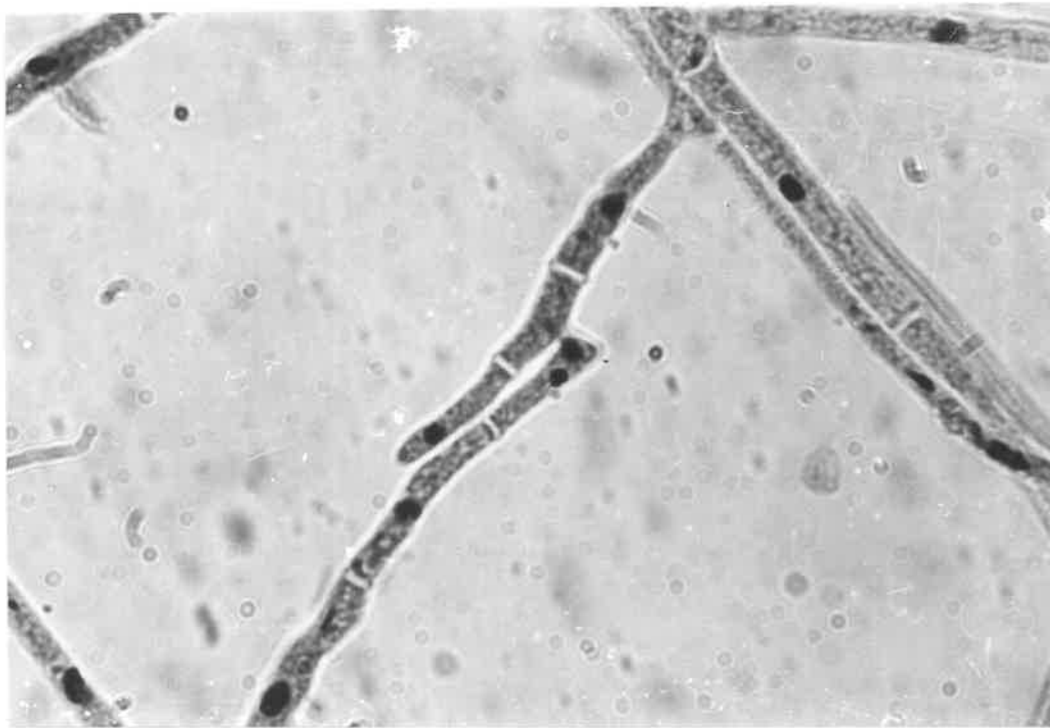
Fig. 32. Self anastomosis between hyphae of isolate W1.

Mag. x1120.

Fig. 33. Self anastomosis between two hyphae of isolate W1. The nucleus of one cell has moved across the isthmus into the second cell.

Mag. x1750.





The straws were buried in two series of jars, one containing nitrate-enriched and the other unamended Moonta soil. The initial moisture content was adjusted to the high level of 16 percent (pF 1.56) as isolate O1 was obtained from an area subject to water-logging. At intervals straws were tested for viable hyphae of O. graminis. The percentage of straws with viable hyphae is given in Table 28. Microscopic examination of infected straw fragments showed that mycelial development by the two isolates was similar at the time of burial. After burial, macrohyphae were produced in the tissue of the straws infected with W1, their development being greater in the presence of added nitrogen. However, no similar development of macrohyphae was observed in straws inoculated with O1. As previously, all straws still cohered after 24 weeks' burial and those in nitrate-enriched soil were darker and softer than those in unamended soil. In general, straws inoculated with W1 were slightly darker than those with O1. The effect of nitrate-enrichment of soil in promoting the survival of both isolates (Table 28) suggested that the hypothesis of Garrett (1944, 1966) for the type variety was also applicable to var. avenae.

Another point from the results was the rapid decline in viability of O1. It was considered that the poor survival of O1 may have been due to:

(1) the inability of an oat isolate to survive on wheat straw as the substrate.

(2) the inability of oat isolates to survive in some

TABLE 28.

PERCENTAGE OF WHEAT STRAW IN WHICH ISOLATES O1 AND W1 SURVIVED WHEN BURIED IN UNSTERILIZED "MOONTA" SOIL WITH (+N) AND WITHOUT (-N) ADDED NITROGEN.

Survival Period (Weeks)	Percentage of Straws Containing Viable Hyphae of:			
	Isolate O1		Isolate W1	
	+N	-N	+N	-N
0	100	100	100	100
4	26	7	100	100
8	2	0	100	78
12	0	0	99	61
24	0	0	77	7

soils. This possibility could account for its limited distribution in Australia.

(3) an inherent inability to survive in any soil because of mutations which may have occurred during the three years that the isolate had been in culture.

Because of the poor survival of the oat-attacking isolate 01 on wheat straw, a second smaller experiment was carried out with oat straw as the substrate. The straw was artificially infected with 01 and buried under conditions similar to those described in the first experiment. The percentages of straws containing viable hyphae of 01 after 4, 8, 12 and 24 weeks' burial are summarized in Table 29. Microscopic examination of straws indicated that no obvious development of dark hyphae had occurred in the tissue after burial. The straws still cohered after 24 weeks' burial and were much firmer than the wheat straws had been in the first experiment. These results (Table 29) provided further evidence that nitrate enrichment of soil promotes the survival of the oat-attacking isolate 01. However, the survival of 01 on oat straw was no better than on wheat straw and it is doubtful, therefore, that the use of wheat straw had contributed to its poor survival in the first experiment.

In order to determine whether the poor survival of 01 was due to a soil factor, the first experiment was repeated using soil from the field from which 01 had been isolated. Artificially infected wheat straws were buried in two series of jars, one containing nitrate-enriched and the other unamended

TABLE 29.

PERCENTAGE OF OAT STRAW IN WHICH ISOLATE 01 SURVIVED WHEN BURIED IN UNSTERILIZED "MOONTA" SOIL

Survival Period (weeks)	Percentage of Straws Containing Viable Hyphae in:	
	Nitrate Enriched Soil	Unamended Soil
0	100	100
4	40	3
8	7	0
12	0	0
24	0	0

soil from Forest Hill, Western Australia. The initial moisture content was adjusted to 18 percent (pF 2.92). The percentage of straws with viable hyphae of _O. graminis_ after various periods of burial is given in Table 30. Microscopic examination of the straws during the experiment showed a pattern of mycelial development similar to that noted during the first experiment. The appearance of the straws after 24 weeks' burial was also similar.

These results confirm the previous observations that nitrate-enrichment of soil promotes survival of the oat-attacking isolate O1. The poor survival of isolate O1 in the soil in which it originally occurred suggests that it is unable to survive well in any soil. At this stage the isolate O1 was noticeably less virulent than in earlier experiments. Previous observations using F_1 progeny of isolate W1 suggest that weakly pathogenic mutants may accumulate in laboratory culture and that these weakly pathogenic types survive poorly. It is suggested that isolate O1 through three years of laboratory culture had altered in this way and it would therefore be necessary to make fresh isolations direct from the field to get meaningful comparisons of survival ability.

(5) Studies with Isolate O9 from Oats.

The distribution of nuclei in the mycelium of O9 (reported in Table 26) indicated that the isolate was either

TABLE 30.

PERCENTAGE OF WHEAT STRAW IN WHICH ISOLATES O1 AND W1 SURVIVED WHEN BURIED IN UNSTERILIZED FOREST HILL SOIL WITH (+N) AND WITHOUT (-N) ADDED NITROGEN.

Survival Period (weeks)	Percentage of Straws Containing Viable Hyphae of:			
	Isolate O1		Isolate W1	
	+N	-N	+N	-N
0	100	100	100	100
4	84	79	100	100
8	33	7	91	98
12	1	0	92	93
24	0	0	77	43

a variant of O. graminis or a different species. Attempts were therefore made to induce the isolate to fruit so that it could be identified. At the same time, its reactions were compared with those of authentic isolates of O. graminis in a series of tests.

(a) Attempts to Induce Fructifications.

All efforts to induce fruiting in isolate 09 were unsuccessful. Initially, 09 cultures were placed in conditions conducive to perithecial formation by O. graminis. The control cultures of O. graminis formed perithecia but those of 09 did not.

As isolate 09 was somewhat similar to Wojnowicia graminis (McAlp.) Sacc. in cultural appearance, single spore isolates of W. graminis and cultures of 09 were exposed to near-ultra violet light (Philips TL40W/08 tube) at 20°C as suggested by Leach (1962). Within ten days, numerous pycnidia had formed in the cultures of W. graminis but cultures of 09 remained sterile even after three months' exposure.

Thereafter, the following substrates were used: autoclaved straws of barley grass, oats and wheat; autoclaved grains of barley, oats and wheat; water agar, oatmeal agar and PMD, both with and without supplements of ground-up oat roots. The cultures were placed in a variety of environments involving constant and variable temperatures, continuous light, intermittent light and continuous darkness. All cultures

remained sterile even when maintained in these environments for periods up to six months.

Wheat and oat seedlings, inoculated with 09, were kept for three to four months after their death as mature plants. Although the fungus was readily re-isolated from these plants, there was no evidence of any fructifications that could be associated with 09. The re-isolated fungus was used for repeating many of the previous attempts, but it could not be induced to fruit.

In the majority of attempts, small black sclerotia-like bodies were formed in the cultures within ten days, but these did not develop further.

(b) Pathogenicity.

The pathogenicity of isolate 09 was compared with that of two isolates (01 and W1) of O. graminis on wheat and oats and the results are given in Tables 31 and 32.

In wheat, root development was restricted by isolates 01 and W1 but not by isolate 09; in oats, root development was restricted only by isolate 01. However, isolate 09 caused a black discolouration (Fig. 34) to roots of both cereals. Microscopic examination revealed that this discolouration was caused by a loose but dense network of dark brown hyphae around the roots. Some hyphae were also on the surface of the roots and resembled runner hyphae of O. graminis. Sections of roots were stained with lactophenol cotton blue and examined for hyphal penetration. In wheat and oat roots infected with

TABLE 31.

PATHOGENICITY TEST WITH ISOLATES W1, O1 AND O9 ON WHEAT.

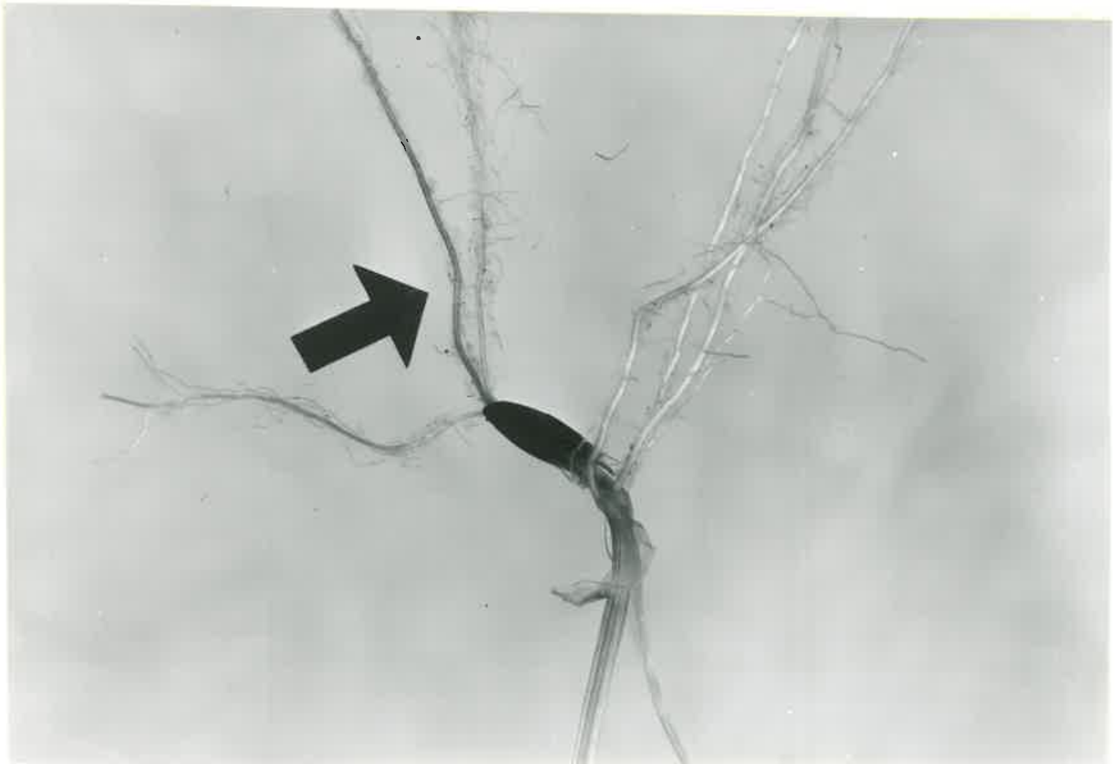
Isolate	Fresh Weight of Host (mg.)	Length (in mm.) of:			Analysis of Data			
					Using Transformations:			
		Discoloured Root	Runner Hyphae along Root	Whole Root	$\log_e X$. Fresh Weight of host	$\log_e (X+1)$ Length of Discoloured Root	$\log_e (X+1)$ Runner Hyphae along Root	$\log_e X$. Length of Whole Root
W1	188	23	33	99	5.2269	3.1633	3.5141	4.5932
O1	252	14	20	127	5.5262	2.6870	3.0186	4.8339
O9	581	23	47	227	6.3646	3.1545	3.8738	5.4220
Control	484			186	6.1731			5.2169
Differences for significance				$P = 0.05$	0.4300	0.3606	0.3355	0.2436
				$P = 0.01$	0.5812	0.4887	0.4546	0.3293

TABLE 32.

PATHOGENICITY TEST WITH ISOLATES W1, 01 AND 09 ON OATS.

Isolate	Fresh Weight of Host (mg.)	Length (in mm.) of:			Analysis of Data Using Transformations:			
		Discoloured Root	Runner Hyphae Along Root	Whole Root	Log_e X Fresh Weight of Host	Log_e (X+1) Length of Discoloured Root	Log_e (X+ 1) Runner Hyphae Along Root	Log_e X Length of Whole Root
W1	847	0	2	146	6.7405	0.0000	1.1945	4.9762
01	485	19	33	153	6.1822	2.9924	3.4950	5.0302
09	835	4	52	141	6.7166	1.5214	3.9765	4.9456
Control	798			158	6.6693			5.0633
Differences for Significance P = 0.05					0.1802	0.3075	0.6906	N.S.
P = 0.01					0.2435	0.4659	0.9358	

Fig. 34. Root system of an oat plant inoculated with isolate O9. Note the dark colour of the seminal root (arrow) which is covered by a dense network of dark brown hyphae. Compare this root system with those of oat plants inoculated with *O. graminis* and illustrated in Figs. 19 and 20.



isolate 09, hyaline hyphae were observed in the cortical cells but not in the vascular tissue. However, infection of the roots had no apparent ill-effects upon plant growth as these plants were similar in size to the controls (Figs. 35 and 36). In wheat roots inoculated with isolates of O. graminis, hyphae were present in both the cortical and vascular tissues and the plants were stunted. In oats, hyphae of both isolates of O. graminis were observed in root tissue, but only those of isolate 01 penetrated to the vascular tissue; infections by isolate W1 were few and restricted to the outer cortical cells. These observations suggest that the relationship between isolate 09 and the hosts may have been an ectotrophic one in contrast with the pathogenic relationship of O. graminis with the hosts.

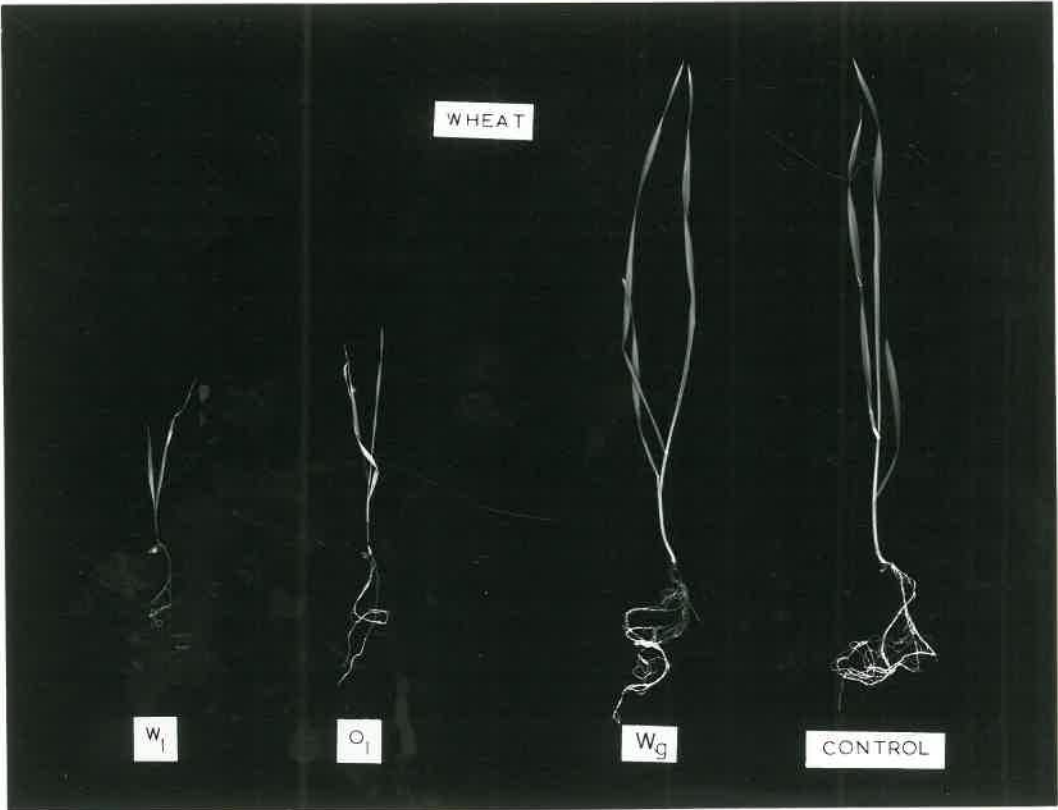
(c) Growth on Media Containing Oat-leaf Extract.

The growth of isolate 09 was compared with that of isolates 01 and W1 of O. graminis on media containing oat-leaf extract. The technique employed was that outlined on page 76. Mycelial dry weight of each isolate when grown on media containing various concentrations of oat extract is set out in Table 33 and Fig. 37.

These results suggest that isolate 09 is perhaps even more tolerant than 01 to increasing concentrations of oat extract. Nevertheless, it is doubtful whether this difference would be of any value for differentiating between the oat-attacking isolates of O. graminis and isolate 09. The results also confirm the earlier observations (Table 24)

Fig. 35. Appearance of wheat seedlings, four weeks after inoculation with *O. graminis* isolates W1 and O1 (left) and isolate O9 (labelled Wg). Note the normal size of the plant inoculated with isolate O9 in contrast with the stunted plants infected with *O. graminis*.

Fig. 36. Appearance of oat plants, eight weeks after inoculation with *O. graminis* isolates W1 and O1 (left) and isolate O9 (labelled Wg). Note the dark root system on "Wg" in contrast with that of the control plant.


WHEAT
W1
O1
Wg
CONTROL

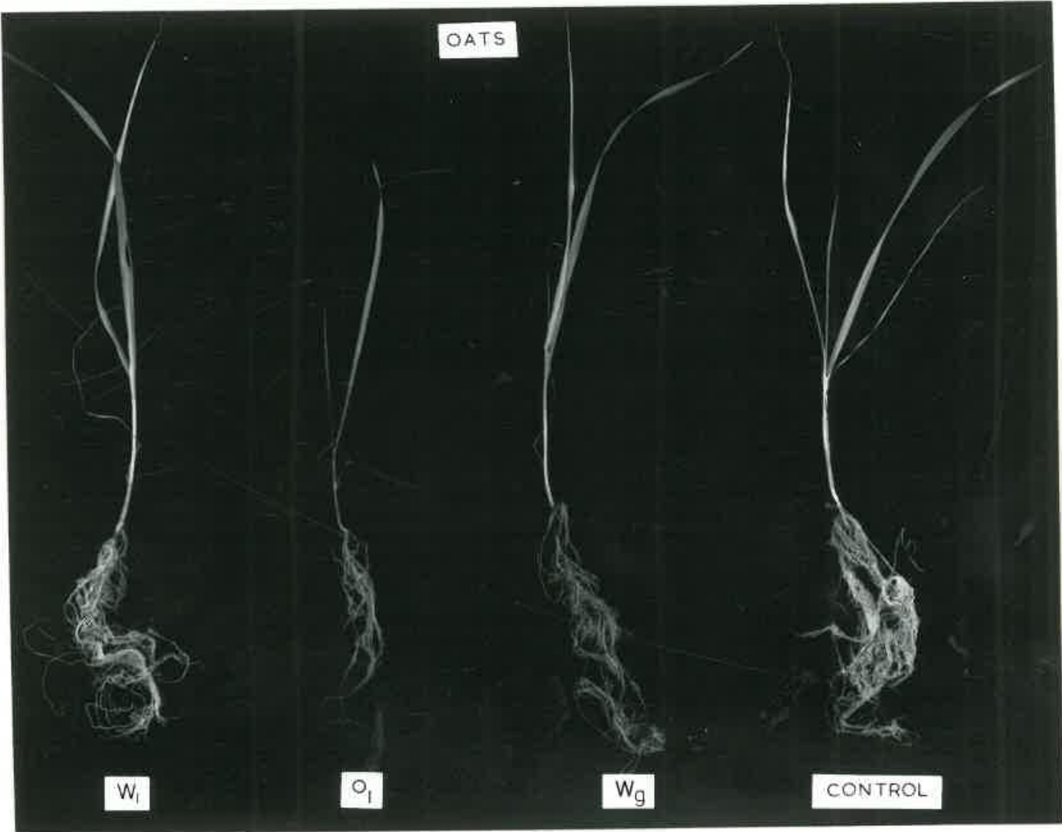

OATS
W1
O1
Wg
CONTROL

TABLE 33.

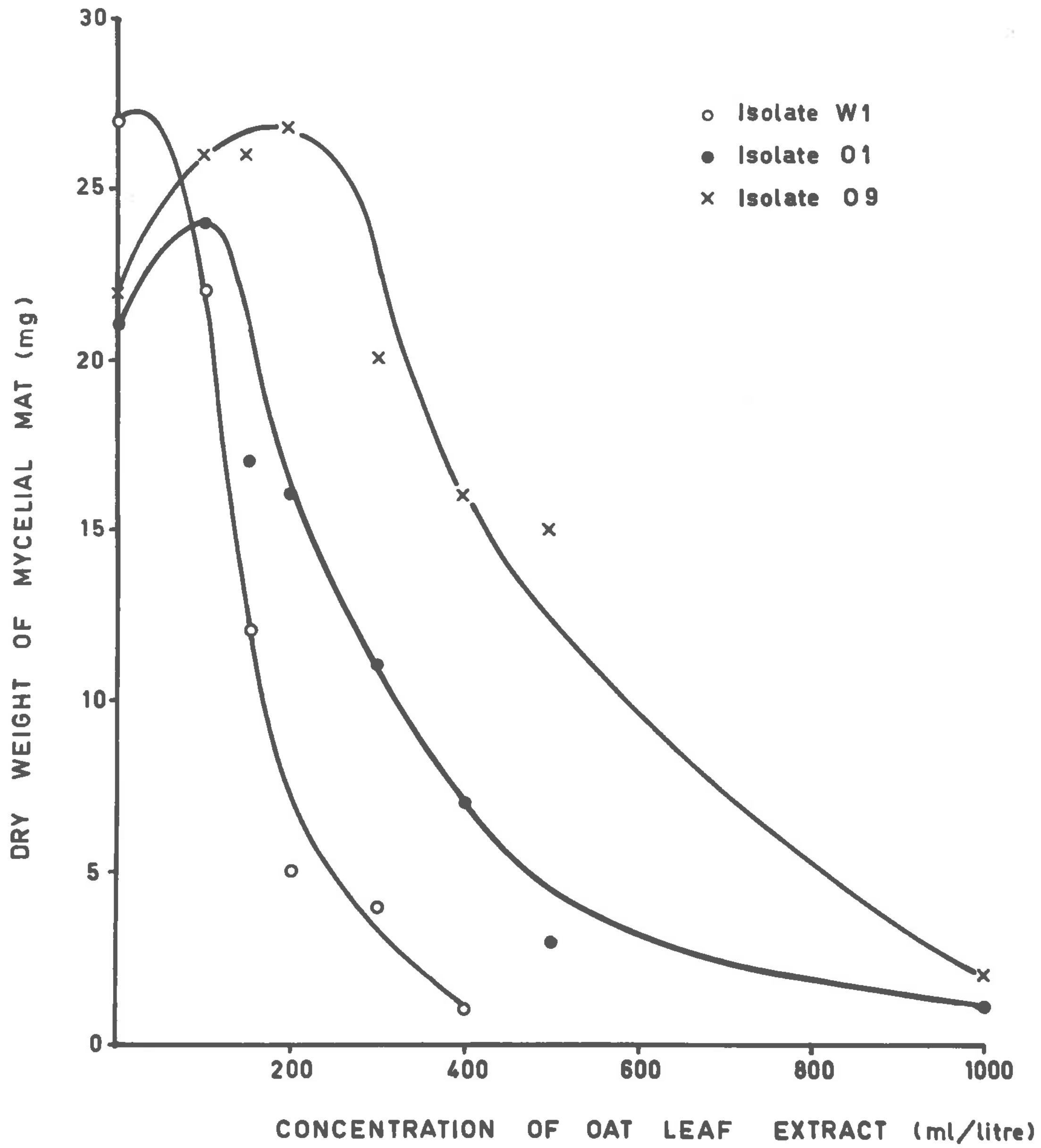
EFFECT OF OAT LEAF EXTRACT ON MYCELIAL DRY WEIGHT

OF ISOLATES W1, O1 AND O9.

Concentration of Oat Extract (ml./litre medium)	Mycelial Dry Weight (mg.)		
	Ophiobolus graminis		Isolate O9 Oats Sth. Aust.
	W1 Wheat Sth. Aust.	O1 Oats West Aust.	
0	27	21	22
100	22	24	26
150	12	17	26
200	5	16	27
300	4	11	20
400	1	7	16
500	0	3	15
1000	0	1	2

Fig. 37.

EFFECT OF OAT LEAF EXTRACT ON MYCELIAL DRY WEIGHT OF ISOLATES <u>W1</u>, <u>01</u> AND <u>09</u>



that the oat-attacking isolate 01 is more tolerant of oat extract than the wheat isolate W1.

(d) Effect of Cystine and Cysteine on Cultural Growth.

The growth of isolate 09 was also compared with that of isolates 01 and W1 of *O. graminis* on a basal medium containing supplements of either casein, cystine or cysteine. The method was identical with that described previously (page 78). The effect of the three supplements on the mycelial dry weight of each isolate is summarized in Table 34.

Supplements of cystine and cysteine significantly reduced the mycelial dry weight of both 01 and 09. This, together with the observed tolerance of both isolates to oat extract (Table 30), suggests a metabolic similarity between the two isolates.

The effect of the two amino acids on the dry weights of 01 and W1 was more definite than that reported previously (Table 25). In this test, the growth of 01 was significantly reduced and that of W1 significantly increased by supplements of cystine and cysteine. Thus the results are in accordance with the observations of Turner (1957).

(e) Incompatibility of 09 with Isolates of O. graminis.

Isolate 09 was opposed to each of four isolates (01, 05, W1 and W5) of *O. graminis* on cellophane overlying PMD to study the reaction between the two fungi. In three of the combinations, the opposing hyphae on meeting anastomosed

TABLE 34.

EFFECT OF CYSTINE, CYSTEINE AND SOLUBLE CASEIN ON MYCELIAL DRY WEIGHTS

OF ISOLATES W1, O1 AND O9.

Isolate	Mycelial Dry Weight (mg.) on:				Analysis of Dry Weights Using Transformation $Log_e X$			
	Basal Medium (Control)	Basal Medium Plus:			Basal Medium (control)	Basal Medium Plus:		
		Cystine	Cysteine	Casein		Cystine	Cysteine	Casein
W1	15	21	20	32	2.6897	3.0178	2.9823	3.4711
O1	10	4	3	24	2.3001	1.4421	0.9973	3.1504
O9	20	12	11	53	2.9811	2.4614	2.3482	3.9624

Differences for Significance P = 0.05 0.1411

P = 0.01 0.1931

but the cells collapsed and died soon afterwards (Fig. 38). Isolate 09 appeared to be the more sensitive and usually several of its cells died on either side of the one which anastomosed. However, re-growth invariably occurred along the lumen of the old hyphae (Fig. 39). This reaction was characteristic of the combinations 01, W1 and W5 with 09. In the combination of 05 with 09, the peripheral growth of the 05 colony was obviously retarded as 09 grew towards it. On meeting, the hyphae of 09 attempted to form common strands with 05, but the hyphae of 05 died as 09 grew along them (Fig. 40). Thus, in all four combinations, isolate 09 was obviously incompatible with the isolates of O. graminis. However, self-anastomosis was observed in all preparations of 09 and occurred between hyphae near the advancing edge of the colony (Figs. 41, 42).

Although the sterility of 09 prevented its positive identification, studies on the number of nuclei of its hyphae and also on its pathogenicity, suggest 09 may not be an isolate of O. graminis. However, its original acceptance as such emphasizes the need for further studies on the identity of species which form "Ophiobolus-like" runner hyphae on cereal roots. Warcup (pers. comm.) recorded "Ophiobolus-like" runner hyphae on 86 percent of wheat plants in a field experiment at the Waite Agricultural Research Institute. According to him, these isolates formed a range of types, some of which were O. graminis, whereas others were obviously different.

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Fig. 38. Death of cells following anastomosis between uni-nucleate tip cell of W1 and multi-nucleate cell of 09. Note the empty swollen tip of W1 overlying the empty cell of 09. Regrowth is occurring along the lumen of each hypha.

Mag. x1050

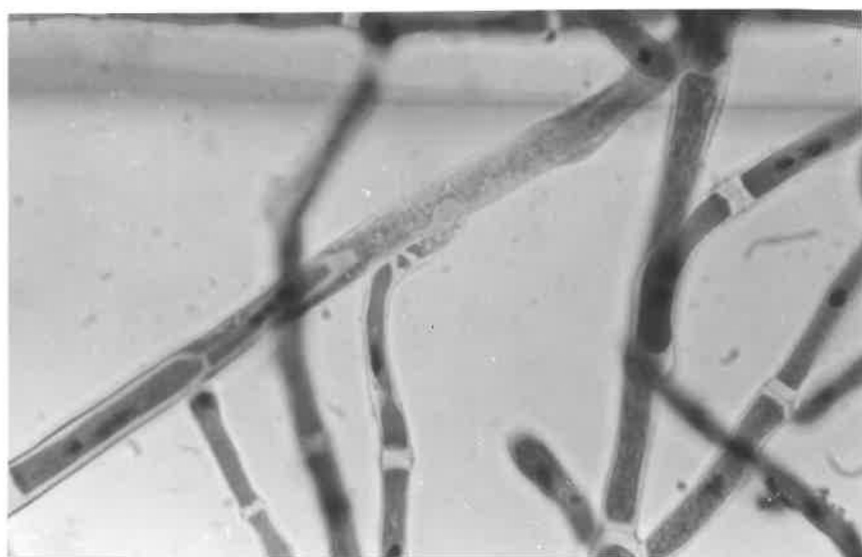


Fig. 39. Extensive re-growth of multi-nucleate cells in lumen of old hypha of O9.(dark coloured hypha). The light coloured uni-nucleate cells around it are cells of W1.

Mag. x1050

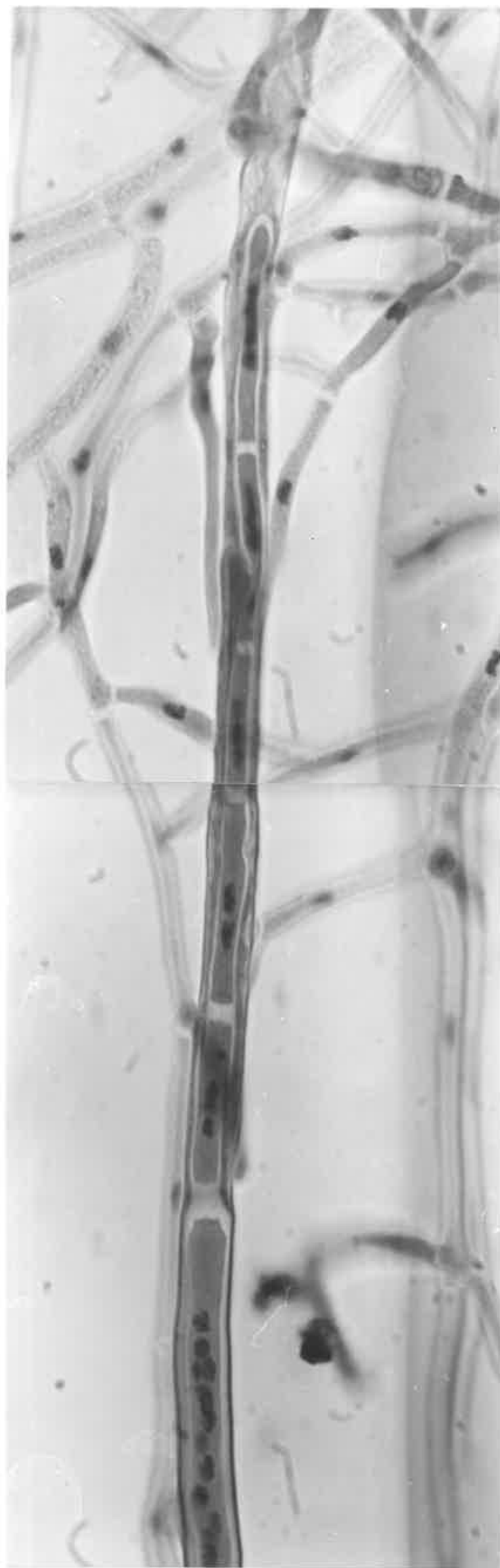


Fig. 40. Multi-nucleate strand cells of 09 overlying empty strand cells of 05.

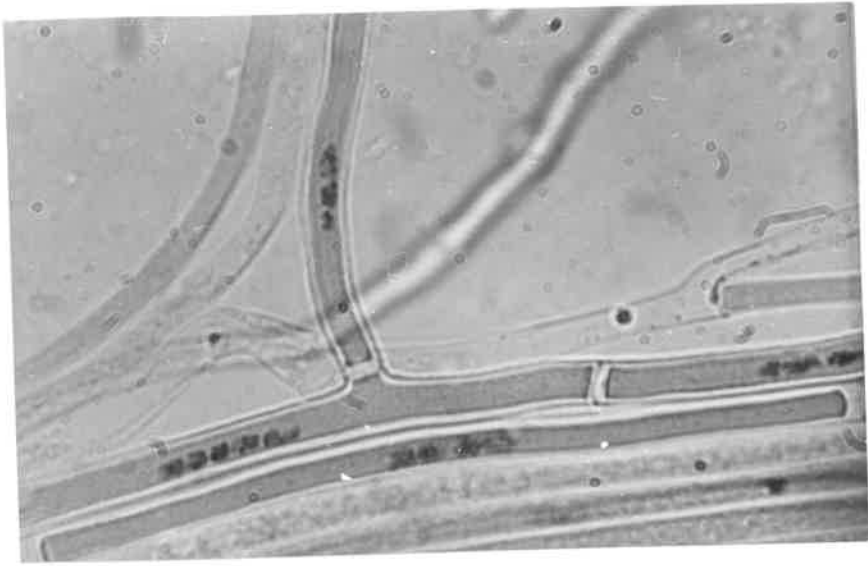
Mag. x1050.

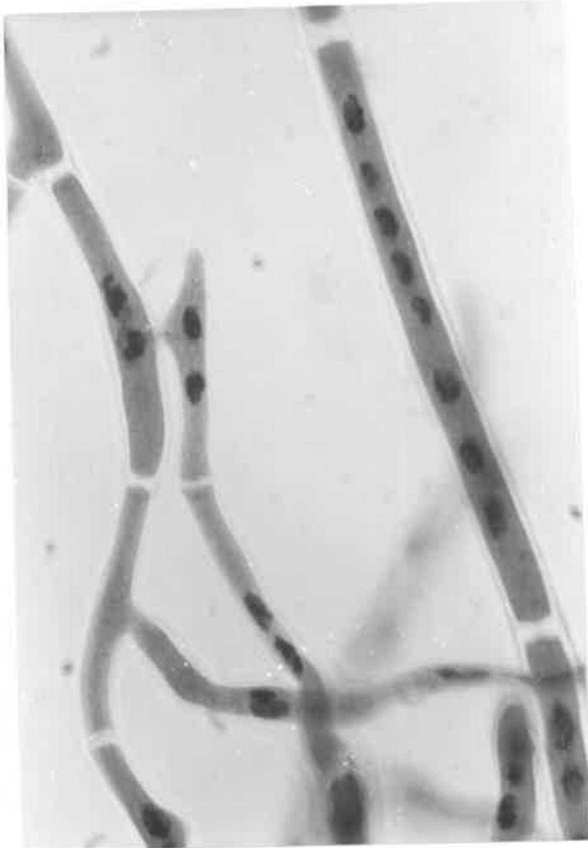
Fig. 41. Self-anastomosis between hyphae of isolate 09.

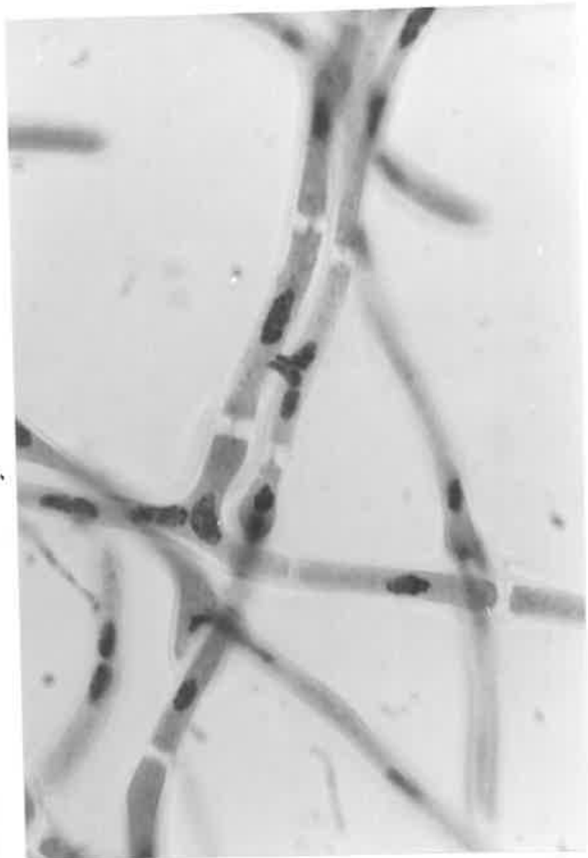
Mag. x1100

Fig. 42. Self-anastomosis between two hyphae of 09. Two nuclei have moved into the isthmus connecting the two cells.

Mag. x1100







DISCUSSION

The increased survival of *O. graminis* in nitrate amended soil in all experiments was in accordance with previous work (Garrett, 1938, 1940, 1944; Butler, 1953a, 1959; Macer, 1961). Data from irradiated soil studies indicated that nitrate enrichment of soil outweighed any unfavourable effects of other soil organisms on the survival of *O. graminis*. This was in accordance with the hypothesis of Garrett (1966).

In these survival studies, artificially infected straws initially contained many hyaline hyphae which were replaced by dark-coloured hyphae after burial. A few naturally infected wheat straws, collected from stubble in late summer, were subsequently examined and found to contain only dark-coloured hyphae. The significance of the type of hyphae was not considered in this study but it may be a profitable subject for further investigation. Should *O. graminis* survive only as dark-coloured hyphae in nature, the presence of hyaline hyphae in artificially infected straws at burial may complicate the interpretation of results. For example, nitrogen enrichment of soil may influence the relative numbers of hyaline hyphae which die or are replaced by dark-coloured hyphae, quite apart from any effect of nitrogen upon survival of dark-coloured hyphae in the straw at burial.

Isolates of common origin differed in their ability to survive saprophytically and the apparent linkage between survival and virulence emphasized the need for detailed studies on physiological variations which affect survival. The inability of weakly pathogenic isolates to survive in straw suggests they have a limited role, if any, in nature. At present there is no available information on the occurrence of weakly pathogenic variants in the field. It would therefore be interesting to obtain monosporous isolates at intervals from the earliest appearance of perithecia until late autumn and determine the percentages of weakly pathogenic ascospores occurring before and after harvest.

There was evidence that virulence of monosporous isolates can be influenced possibly by the relationship of the parent with the substrate or by nutrition at the time of ascosporogenesis. White (1942) deduced that his eight monosporous isolates were derived from a diploid primary ascus nucleus which was heterozygous for the pair of genes determining degree of virulence. Segregation of the two alleles occurred during the reduction division and resulted in four strongly- and four weakly-pathogenic ascospores. The same explanation seems applicable to the results of the pathogenicity test with monosporous isolates derived from a perithecium formed in culture. However, all monosporous isolates proved to be strongly pathogenic when obtained from perithecia formed on living oat plants. This suggested that the diploid primary ascus was homozygous for virulence

and also that the plant may have selected the virulent allelomorph. It is not known whether the fungus is a parasite or a saprophyte when all or most perithecia are formed in nature and this would be a profitable avenue for further study.

Differences in the relative importance of hosts as perpetuators of O. graminis were clearly demonstrated by the field experiment at Esperance. The results with Vulpia myuros, however, indicate that perpetuation of the pathogen by a host is not determined by susceptibility alone. Adam (1951) attributed a reduction in take-all to the quick decomposition of Vulpia spp., the main weeds during a short fallow of a rotation experiment. It would be interesting to compare decomposition of infected and un-inoculated roots of hosts differing in susceptibility under field conditions. Macer (1961) noted that wheat straw infected with Cercosporella herpotrichoides decomposed more slowly in the field than un-infected ones. It would be profitable also to study the role of insects in the decomposition of straw.

Comparative studies with isolates of O. graminis from wheat and oats have shown that an oat-attacking variety similar to var. avenae occurs in Western Australia. The South Australian isolates from oats were similar in pathogenicity to the type variety, but some differed in that they were more tolerant of oat extract. This suggested that some of the South Australian isolates may be intermediate

between the two recognised varieties. Pathogenicity was the most reliable of the four differential criteria suggested by Turner (1940, 1940a, 1957). Measurements of asci and ascospores was the least reliable as there was evidence that substrates can influence their sizes. Nuclear distribution was similar in the mycelium of a South Australian type variety isolate and a Western Australian var. avenae isolate, but there was no evidence of compatibility between the two varieties. Hyphal anastomosis was rare between compatible isolates, but self-anastomosis occurred in all isolates. Self-anastomosis is another mechanism whereby variants may arise vegetatively should the mycelium be heterocaryotic. It may therefore be a profitable line of study to isolate anastomosed cells and determine the number which give rise to recognizable variants. As the fungus is homothallic, self-anastomosis is also necessary for the change from the monocaryotic state to the dicaryotic state during ascosporogenesis. It would therefore be interesting to compare the relative frequencies of vegetative self-anastomosis in isolates which do and do not form perithecia in culture. There was evidence of a linkage between virulence and perithecial formation, both in culture and on the host. No explanation can be offered for this.

The studies with isolate 09 suggest it is not an isolate of O. graminis, but its original acceptance as such emphasises the need for further studies on the identity of

species which form "Ophiobolus-like" runner hyphae on cereal roots.

PART 2.

STUDIES ON A ROOT-ATTACKING STRAIN OF RHIZOCTONIA SOLANI KÜHN.

REVIEW OF LITERATURE.

(1) Distribution and Types of a Cereal Root-Attacking Strain.

Rhizoctonia solani was first identified as causing "bare patch" of cereals in South Australia (Samuel and Garrett, 1932) and subsequently in New South Wales (Hynes, 1933), England (Dillon Weston and Garrett, 1943) and Canada (Benedict and Mountain, 1956). As several pathogenic strains of *R. solani* have since been recorded in South Australian cereal fields (Kerr, 1955; de Beer, 1965), the one causing bare patch was designated the root strain by Kerr; de Beer further separated the root strain into Types 1 and 2 after consistently isolating two distinctly different cultural types from cereal fields at Moonta. According to de Beer, cultures of the two types differed in colour and in ability to produce sclerotia, to fruit, and to colonize substrates in the soil. The two types also differed in the patterns of proteins separated by starch-gel electrophoresis from their mycelia. De Beer induced isolates of both types to fruit on soil and identified them as *Thanatephorus cucumeris* (Frank) Donk (Talbot, 1965). Previously, the root strain had been reported as *Pellicularia filamentosa* (Pat.) Rogers by Kerr (1955),

Flentje (1956) and Flentje and Saksena (1957).

(2) The Root Strain in Culture.

The vegetative features of the root strain in culture were described by Flentje (1950) for English isolates, and by Saksena (1952) for Australian isolates. Comparison of their data suggests that English and Australian isolates are identical in vegetative morphology. De Beer (1965) described Type 1 isolates as being darker in colour than Type 2 isolates. Sclerotia are formed in culture (Samuel and Garrett, 1932; Hynes, 1937a; Flentje, 1950; Saksena, 1952), but fewer by Type 1 than Type 2 (de Beer, 1965). In general, field isolates from the same area are very similar in cultural appearance, but quite different from other strains of the same geographical origin (Flentje and Saksena, 1957; de Beer, 1965); ninety-five percent of the root strain isolates from Moonta were identified as Type 1 by de Beer. However, field isolates of the root strain from different localities may differ considerably in cultural appearance (Flentje and Saksena, 1957). Further-more, single basidiospore progeny of one field isolate may exhibit a wide range of different cultural characters.

The root strain is believed to be homothallic, but this has not yet been established conclusively (McKenzie, 1966). The morphology of the perfect stage was described by Flentje (1956) who obtained it on soil under laboratory conditions but not on agar or plant tissue. He reported significant

variations in measurements of basidia and spores of isolates from different areas and also from the same locality. An unusual synnema-like fruiting structure of a root strain was obtained by Stretton, McKenzie, Baker and Flentje (1964).

The vegetative cells of the root strain are multinucleate (Flentje, Stretton and Hawn, 1963) but nuclear numbers vary among the cells because of aberrations during and following mitosis. Numbers of nuclei are also reduced in older cells by secondary septa being formed without nuclear division. Basidiospores are mainly uni-nucleate but a small percentage are bi-nucleate because of malfunction during the reproductive phase. In explanation of the variability, Flentje and Stretton (1964) have shown that it may occur through mutation, heterocaryon formation and meiosis.

Nutritional studies by McKenzie (1966) indicated that potassium, phosphorus, magnesium and trace elements were essential for satisfactory growth by the root strain. None of the test isolates showed a vitamin requirement. However, single basidiospore cultures derived from one field isolate differed in their ability to utilize various forms of carbon and nitrogen. Nevertheless, in general, growth was better on peptone, casein or ammonium nitrate as a source of nitrogen than on potassium nitrate or ammonium sulphate. Hynes (1937a) reported that the addition of 0.25 percent ammonium sulphate to "glucose agar" reduced the growth of the root strain.

Samuel and Garrett (1932) determined the minimum, optimum and maximum temperatures for growth as 4°C, 23°-26°C,

and 32°C respectively; Hynes (1937a) reported optima of 20°C and 20°-25°C for oat and wheat isolates respectively. Samuel and Garrett also noted that the optimum reaction for growth in culture was pH 6.0-6.5, although the fungus grew over the whole test range of pH 3.5-9.0. Blair (1943) studied the effect of atmospheric carbon dioxide on growth of the root strain; he found that growth was significantly reduced by a content of one percent carbon dioxide in the atmosphere.

(3) Life Cycle of the Root Strain.

The root strain of *R. solani* differs from *Ophiobolus graminis* in that it is able to grow freely through soil (Blair, 1943). The saprophytic phase of its life is therefore not restricted to the period between crops, as in the case of *O. graminis*, but may also continue during the cropping period. Thus, some hyphae of the root strain may be actively saprophytic at the same time as other hyphae are parasitic.

(a) Parasitic Phase.

Infections by the root strain are confined to immature root tissues and invariably result in death of root tips (Samuel and Garrett, 1932). The process of infection has been studied in detail by Flentje (1957), who noted that hyphae formed "infection cushions" on the root surface just ahead of the root hair zone. Actual infection was by

"infection pegs", which developed from the cushion and penetrated the root surface directly beneath them. After entering the cortex, the hyphae grew up and down the axis of the root. The root rotted back rapidly to leave a short stump which was not invaded by the fungus. Kerr (1955), Flentje, Dodman and Kerr (1963), and Dodman (1965) have presented considerable evidence that infection cushions are formed in response to exudates from the host root. Samuel and Garrett (1932) noted that hyphae in the cortical cells were initially hyaline, but were replaced by thinner brown hyphae when the tissue began to rot. They postulated that the brown hyphae grew through soil to cause fresh infections of other roots and therefore referred to them as "distributive" hyphae.

Essentially similar descriptions have been given of the plant and field symptoms of the bare patch disease in South Australia (Samuel, 1928; Samuel and Garrett, 1932; Kerr, 1955); New South Wales (Hynes, 1933, 1937a); England (Dillon Weston and Garrett, 1943; Moore and Moore, 1945) and Canada (Benedict and Mountain, 1956). The disease is also known as "purple patch" in New South Wales, because affected plants usually have a purplish tint (Hynes, 1933). In Canada and South Australia, the root lesion nematode *Pratylenchus neglectus* (Rensch) Chitwood and Oteifa (syn. *P. minyus* Sher and Allen) is frequently found in association with the root strain in bare patches (Benedict and Mountain;

de Beer, 1965). The cereal eelworm Heterodera avenae Wollenweber has also been recorded as causing bare patches in cereal fields of South Australia (Samuel, 1928; Samuel and Garrett, 1932; Fisher, 1960).

The natural host range of the root strain is extensive and in South Australia it has been recorded on the following species:
Avena sativa L. (oats), Hordeum vulgare L. (barley) and Triticum vulgare Vill. (wheat) by Samuel and Garrett (1932); Bromus spp., Cryptostemma calendula (L.) Druce (Cape weed), Erodium spp., Hordeum leporinum L. (barley grass), Leonotodon hirtus L., Medicago sativa L. (lucerne), M. tribuloides Desr. (barrel medic), Oenothera stricta Ledeb. (evening primrose), Phalaris tuberosa L. (phalaris), Pisum sativum L. (garden pea), Trifolium arvense L. (hare's foot clover) and T. subterraneum L. (subterranean clover) by Ludbrook, Brockwell and Riceman (1953). Hynes (1933) has also recorded it on Avena fatua L. (black oats) in New South Wales.

Pathogenicity tests indicate that South Australian isolates may attack the roots of many more species and according to Flentje and Saksena (1957), there is no evidence of host specialization. In pathogenicity tests the roots of the following additional species were shown to be susceptible: Solanum tuberosum L. (potato) by Samuel and Garrett (1932); Brassica oleraceae L. var. capitata (cabbage) by Saksena (1952);

Bromus mollis L. (soft brome), *Ehrhata calycina* Sm. (perennial veldt grass), *Lolium rigidum* Gaud. (Wimmera rye grass), *Medicago hispida* Gaertn. var. *denticulata* Urb. (burr medic), *M. hispida* var. *confinis* Burnat. (spineless burr medic), *M. minima* (L.) Grufb. (small woolly burr medic), *M. rugosa* Desr., *M. scutellata* All. (snail medic), *Schismus barbatus* (L.) Juel (Arabian grass) and *Secale cereale* L. (rye) by Kerr (1955); *Beta vulgaris* L. (sugar beet), *Lactuca sativa* L. (lettuce), *Lycopersicum esculentum* Mill. (tomato) by Flentje and Saksena (1957); *Raphanus sativus* L. (radish) by Flentje, Dodman and Kerr (1963).

Hynes (1937a) has shown that *Secale cereale* is also susceptible to the root strain in New South Wales. In contrast with the non-specificity of South Australian isolates, Flentje (1950) found that English isolates were specific to wheat and would not attack the root of beet, cabbage, lettuce or tomato.

Infection of host plants is favoured by low soil temperatures (Samuel and Garrett, 1932; Hynes, 1937a), the range being 12°-18°C (Samuel and Garrett, 1932). This is considerably less than the optimum temperature of 23°-26°C for growth in culture (Samuel and Garrett). By way of explanation, Garrett (1956) has suggested that higher soil temperatures increase the growth and maturation of cereal roots proportionately more than the growth and infectivity of the pathogen. Hynes (1937a) also noted that soil moisture was not a limiting factor to infection at low temperatures but at high temperatures infection was favoured by low levels of

soil moisture.

Soil reaction appears to have little influence on the incidence of the pathogen in cereal crops. For example, the disease is most prevalent on light alkaline sandy soils of pH 8.0-9.0 in South Australia and on lime-deficient soils of pH 6.0-6.3 in New South Wales (Samuel and Garrett, 1932; Adam, 1935; Anon., 1954; Hynes, 1937a). Soil type, however, may influence the prevalence of bare patch as de Beer (1965) recorded a higher incidence in the light than in the heavier soil of the same field. The incidence of bare patch was reduced by the application of ammonium sulphate at seeding (Hynes, 1937a; de Beer, 1965), but not by increased rates of superphosphate (Hynes).

(b) Saprophytic Phase.

Active saprophytic growth by the root and other strains of *R. solani* through unsterilized soil was first demonstrated by Blair (1943). He found that growth proceeded apparently indefinitely through soil, whereas in quartz sand it was limited by the nutrient reserves of the agar inoculum. Although Blair considered that cellulose may have been the nutrient source in the soil for the fungus, he was unable to resolve this point. Garrett (1962) has since demonstrated that other strains can colonize and decompose cellulose in the soil. He has therefore suggested that cellulose is the nutrient source for the saprophytic growth of *R. solani* in soils devoid of living roots or fresh organic material.

De Beer (1965) has shown that the root strain is one of the primary colonizers of plant residues, but is only active in the residue for a limited period. During colonization the fungus forms morphologically distinct structures, some of which are concerned with nutrient absorption and others with the growth of the fungus to other substrates.

The effect of soil moisture on the growth of the root strain was studied by Blair (1943) and de Beer (1965). Using various English soils, Blair noted that saprophytic growth was most rapid at the lowest moisture level tested, viz., 30 percent saturation, and was further increased by forced aeration. Similar results were obtained in two "Moonta" soils by de Beer, who found that optimum growth occurred when approximately 50 percent of the pore space was drained. According to de Beer, growth was inhibited at high soil moisture levels and in soils where the distribution of moisture was discontinuous. Blair and de Beer also determined that the optimum soil reaction for saprophytic growth by English and Australian isolates was pH 7.0 and pH 6.0-7.0 respectively.

Soil amendments of fresh grass meal were reported to have a depressing effect upon the growth of the root and other strains of R. solani by Blair (1943). He attributed this effect to the activities of cellulose-decomposing micro-organisms in the soil. By way of counter explanation, Garrett (1962) has suggested that the amendments would contain free sugars and simple carbon compounds. These

materials would cause a sudden, but temporary, increase in the soil population of saprophytic sugar fungi with a consequent depression, or temporary inhibition, of growth of R. solani. More recently, de Beer (1965) has determined that the root strain is tolerant of high carbon-nitrogen ratios in the soil. However, he found that its ability to colonize substrates was impaired when nitrogen alone was added to unsterilized soil under laboratory conditions.

The extent of growth by the root strain in a horizontal plane in the soil was compared with that in the vertical plane by Blair (1942). Using an adaptation of the Rossi and Cholodny glass slide technique, Blair was able to demonstrate that the rate of growth was greater radially than vertically. At the same time, Blair also measured the radial growth of two other strains at various depths and found that growth rate decreased with depth. De Beer (1965) determined the vertical distribution of the root strain in bare patches and detected hyphae at depths of up to 15 inches. However, the greatest density of population was in the surface three inches.

Kerr (1955) examined the population of the root strain in a cereal field showing the bare patch disease, and reported a higher population inside than outside the patches. De Beer (1965) obtained similar results, although he noted an exception in which the population was greater at 6-18 inches outside the patch than at its centre. De Beer

considered that the population may have been greater in the centre of the patch when the plants were seedlings. However, as the season advanced, the fungus grew outwards but at the same time the plants outside the patch became more deeply rooted, thereby escaping serious damage to their root tips. According to Kerr (1955), patches observed in one year appear in exactly the same position the following year. Furthermore, such patches may not increase in size, but if they do so, it is seldom by more than one to three feet.

Although the root strain is non-specific in its host range and is also able to grow saprophytically through the soil, there is evidence that it cannot survive in all cereal soils. For example, Kerr (1955) and Flentje and Saksena (1957) deliberately introduced it into cereal fields at the Waite Institute, but it failed to persist from one season to the next. De Beer (1965) has studied its mode of survival and concluded that it persists in soil as hyphae or resistant cells in organic matter. However, he found no evidence that it formed sclerotia in nature and considered that it differed from other strains in this respect. De Beer also noted that its survival was impaired in laboratory experiments by unfavourable moisture levels and by soil amendments of organic and inorganic nitrogen. Under unfavourable soil moisture conditions, the hyphae of the root strain were killed and decomposed by bacteria and actinomycetes.

MATERIALS AND METHODS.

(1) Isolates.

The type, pathogenicity and source of the root strain isolates are given in Table 35. All had been originally isolated by J. F. de Beer and designated by him as either a Type 1 or 2 root strain isolate of *Thanatephorus cucumeris* on the basis of cultural characters, pathogenicity and fructifications. The Type 1 and Type 2 isolates used by de Beer (1965) for his studies on the ecology of the root strain in South Australian cereal soils were stock cultures 91 and 98 respectively. Isolate 91 was used by the writer for initial cultural studies but it became unstable and was replaced by isolate 92 as the main Type 1 isolate for subsequent studies. Stock cultures were maintained on soil agar (Appendix A) at 5°C.

(2) Temperature Studies.

Growth rates of isolates were determined on PMD in Petri dishes over a range of temperatures by measuring the daily increase in the colony diameters.

Cultures were also incubated at temperatures above the maxima for growth to assess the sensitivity of isolates to excessive heat. The inoculated plates were then transferred to 25°C and examined daily for three weeks to determine whether

TABLE 35.

ORIGIN, PATHOGENICITY AND TYPE OF ROOT STRAIN ISOLATES OF THANATEPHORUS CUCUMERIS

Stock Culture number	Origin	Pathogenicity	Cultural Type
90	Soil, Moonta, S.A.	Roots, not specialized	1
91	" Murray Bridge, S.A	" "	1
92	" Moonta, S.A.	" "	1
93	" " "	" "	1
97	" " "	" "	2
98	" " "	" "	2
100	" " "	" "	2

the treatment was lethal or only inhibitory.

In all temperature experiments, the freshly poured plates were placed at the selected temperature overnight before being inoculated with an eight mm. agar disc from the periphery of the test isolate. Each determination was based on at least three and usually four replications.

(3)
Nutrition Studies.

Growth of isolates was assessed on a series of defined media which varied only in the sources of equivalent amounts of nitrogen and carbon. The "control" basal medium, containing glucose and ammonium nitrate as the carbon and nitrogen sources respectively, was of the following composition:

KH_2PO_4	0.005 M	Fe	0.80 p.p.m.
K_2HPO_4	0.005 M	Zn	0.40 p.p.m.
$MgSO_4.7H_2O$	0.005 M	Cu	0.05 p.p.m.
NH_4NO_3	0.020 M	Mn	0.10 p.p.m.
Glucose	0.050 M	Mo	0.04 p.p.m.
Distilled water up to 1 litre		Ca	4.00 p.p.m.

The trace elements were added as chlorides and the pH adjusted to 6.8. The media were dispensed in 50 ml. aliquots into 250 ml. flasks and autoclaved at one atmosphere for seven minutes. The inoculum was held at the surface of the medium by a glass rod with a bent and flattened tip, the rod being held in position by the cotton wool plug of the flask. Inocula consisted of seven mm. diameter discs cut from the periphery of colonies growing on the control basal medium,

solidified by agar.

All cultures were incubated at 25°C for five days. Mycelial mats were harvested on to previously dried and weighed filter papers, washed thoroughly with distilled water and dried for 24 hours at 95°C. Dry weights were based on either three or four replications.

(4) Soil.

One soil was used, viz., the calcareous sandy soil from Moonta (Table 2, Fig. 1).

(5) Inoculating Soil with the Root Strain.

The technique was similar to that of de Beer (1965), using ground-up washed mycelial mats. The mats were obtained by inoculating 50 mls. of synthetic liquid medium (Appendix A) in a 250 ml. flask with a seven mm⁄ PMD disc of the test isolate and incubating at 25°C for one month. Each mat was washed thoroughly, dried with clean blotting paper and ground with 50 gms. of soil in a mortar. The total quantity of mycelium-soil mixture was blended thoroughly before use. Air-dry soil was inoculated at the rate of half a mat per Kg. soil for studies on the colonization of filter paper and at twice this rate for studies involving population counts.

(6) Soil Amendments.

In all soil experiments, carbon or nitrogen amendments were added as solutions to air-dry soil immediately after

inoculation with the test isolate. The concentration of the amendment was calculated so that sufficient water was added to the soil to achieve the experimental soil moisture level. The procedure was to add the amendment to 750 g. soil; after thorough mixing, 250 g. were weighed into each of three jars and compressed to a bulk density of 1.3. The surfaces were covered with a thin polythene film before sealing the jars with screw caps.

An unamended "control" series of the same moisture content was included in all such experiments.

(7) Colonization of Filter Paper (de Beer, 1965).

Whatman No. 3 filter paper was cut into 0.5 $cm.^2$ pieces. These were introduced vertically into soil of six percent moisture content and incubated at 20°C for four days to allow colonization by soil organisms. The papers were sieved from the soil and transferred to jars (50 per jar) containing inoculated soil. Sterile filter papers were introduced into an identical series of inoculated soils as control.

After an incubation period of 40 hours at 20°C (except for temperature experiments), the papers were sieved from the soil, washed in running tap water and plated on to water agar containing 50 p.p.m. streptomycin sulphate and 33 p.p.m. Aureomycin hydrochloride. After a further 24 hours' incubation at 25°C, the papers were examined under a dissecting microscope (x50) and the number with mycelial growth of

Rhizoctonia was recorded.

As a further control, 50 colonized papers taken directly from the original un-inoculated soil were also plated on to water agar containing antibiotics. *Rhizoctonia* did not grow from any and the same soil was used thereafter for the initial colonization of filter papers with organisms other than *Rhizoctonia*.

(8) *Estimating Soil Populations* (de Beer, 1965).

Fifty gram samples were taken from test soils after ten and 35 days' incubation and suspended in a 600 ml. beaker by a jet of fast flowing water. The heavier particles were allowed to settle and the supernatant containing hyphae was poured through a sieve (0.0021 mesh); the procedure was repeated five times; the debris on the sieve was washed into a blender, suspended in 250 ml. water and macerated for 30 seconds to break and disperse the hyphal clumps. The macerated material was poured onto the sieve, washed into a graduated cylinder and suspended in 100 ml. water. The cylinder was inverted twice and the contents allowed to settle for ten seconds. Ten ml. were then pipetted from immediately below the surface into a Petri dish marked into quadrants. The suspension was examined under a dissecting microscope (x50) and the numbers of *Rhizoctonia* fragments counted in each of 100 microscopic fields (25 per quadrant). Knowing the area of one microscopic field, the area of the dish and the dilution factor, an estimate was made of the hyphal fragments

per gram of soil. The viability of the fragments was determined by incubating the suspension overnight at 15°C and counting the number of hyphae in 100 showing new growth. The percentage so obtained was used to estimate the number of viable hyphae from the total number per gram of soil.

This method differed from that of de Beer in the period of blending (30 seconds instead of 15 seconds). The effect of varying the blending period and the initial quantity of inoculum on hyphal counts is shown in Tables 36 and 37. Increasing the blending period significantly increased the total number of fragments. An increase from 15 to 30 seconds significantly reduced the percentage of viable fragments, but a further increase from 30 to 60 seconds did not. Doubling the quantity of inoculum more than doubled the total number of fragments without significantly affecting the percentage of viable fragments. These counts were made immediately after adding the inoculum to the soil and the percentages of viable hyphae (63-71) suggest that grinding the mycelial mats may have killed many fragments.

(9) Irradiated Soil Studies.

Soil in polythene bags (50 g. per bag) was sterilized with a dosage of five megarads gamma irradiation by the Australian Atomic Energy Commission, Lucas Heights, New South Wales.

The soil amendment (or moisture) for 50 g. soil was sterilized as a 5 c.c. solution in an 8" x 1-1/8" tube which

TABLE 36.

EVALUATION OF DE BEER'S TECHNIQUE FOR ESTIMATING THE POPULATION OF RHIZOCTONIA SOLANI IN SOIL.

Factors Varied		Population Estimates		Analysis of Population Estimates (Using Transformation $\log_e$)	
Quantity of Inoculum Added to soil (No. Mats/Kg.soil)	Maceration time for material extracted from soil (secs.)	Number of Hyphal Fragments of *R. solani* per gram of soil	Percentage of Viable Hyphal Fragments	Number of Hyphal fragments per gram of soil	Percentage of Viable Hyphal Fragments
1.0	15	138.1	71	4.9266	4.2673
1.0	30	220.0	67	5.3929	4.1991
1.0	60	354.4	63	5.8702	4.1425
1.5	15	268.5	70	5.5930	4.2484
1.5	30	443.7	67	6.0951	4.1994
1.5	60	558.7	67	6.3255	4.2089
2.0	15	459.5	71	6.1299	4.2626
2.0	30	716.3	66	6.5740	4.1891
2.0	60	861.6	65	6.7586	4.1792
Differences for Significance		P = 0.05		0.0512	N.S.
		P = 0.01		0.0702	N.S.

TABLE 37.

EVALUATION OF DE BEER'S TECHNIQUE FOR ESTIMATING THE POPULATION OF *RHIZOCTONIA SOLANI* IN SOIL.

(i) Effect of Quantity of Inoculum.

Number of Mats per Kg. soil	Population Estimates		Analysis of Population Estimates (Using Transformation Log_e)	
	Number of Hyphal Fragments of *R. solani* per gram of soil	Percentage of Viable Hyphal Fragments	Number of Hyphal Fragments per gram of soil	Percentage of Viable Hyphal Fragments
1.0	237.5	67	5.3966	4.2030
1.5	423.6	68	6.0045	4.2189
2.0	679.1	67	6.4875	4.2103
Differences for Significance P = 0.05			0.0296	N.S.
P = 0.01			0.0405	

(ii) Effect of Maceration Time for Material Extracted from Soil.

Period of Maceration (secs.)	Population Estimates		Analysis of Population Estimates (Using Transformation Log_e)	
	Number of Hyphal Fragments of *R. solani* per gram of soil	Percentage of Viable Hyphal Fragments	Number of Hyphal Fragments per gram of soil	Percentage of Viable Hyphal Fragments
15	288.7	71	5.5498	4.2595
30	460.0	67	6.0206	4.1959
60	591.6	65	6.3181	4.1769
Differences for Significance P = 0.05			0.0296	0.0328
P = 0.01			0.0405	0.0450

had a metal stirring rod through the cotton wool plug. It was autoclaved for seven minutes. Upon cooling, the soil was added to the amendment (or water) and mixed thoroughly.

Inoculum was obtained by allowing colonies on PMD in Petri dishes to overgrow 10 mm. cellophane discs placed in a ring around the original inoculum. The mycelium on a disc was carefully scraped into a ball and introduced into the moist irradiated soil.

After eleven days' incubation at 20°C (except for temperature studies) the total and viable numbers of hyphal fragments were estimated as described above for unsterilized soils.

(10) Survival on Straw.

The method was identical with that described for *Ophiobolus graminis* (page 27).

(11) Isolation from Tissue.

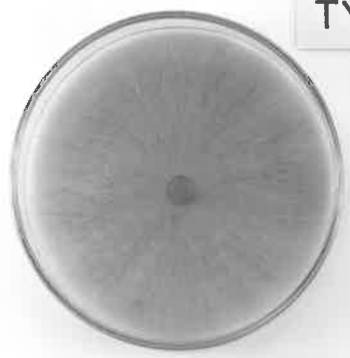
Washed material bearing hyphae was placed on water agar containing streptomycin and Aureomycin. No surface sterilant was used. Hyphal tips were transferred to PMD slopes within 24-48 hours.

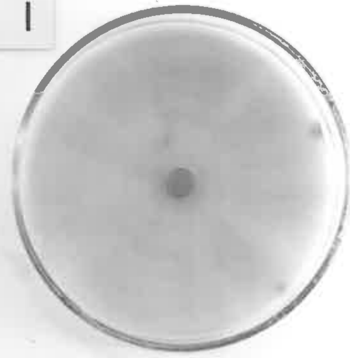
EXPERIMENTAL.

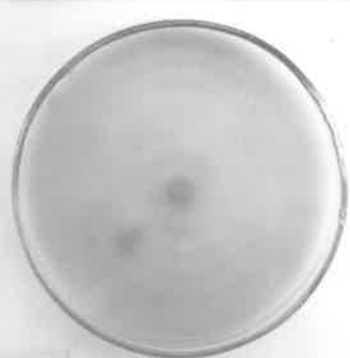
(1) A Comparison of Two Root Strain Types.

De Beer (1965) differentiated Moonta isolates of the root strain into two cultural types. He described colonies of the commoner Type 1, representing 95 percent of the isolates, as being darker in colour. However, this proved a variable and unreliable criterion as Type 1 colonies were frequently lighter than Type 2 when grown under identical conditions (Fig. 43). Furthermore, colony colour was affected by light and cultures incubated in continuous light of 600 foot candles were paler than those in darkness at the same temperature (Fig. 43). De Beer also observed that Type 1 colonized filter papers more readily than Type 2 in unsterilized soil. Confirmatory results were obtained (page 144) but differences between isolates of the same type were also apparent. No observations were made on the remaining three differential criteria, viz., ability to produce sclerotia; ability to fruit; protein patterns obtained by starch-gel electrophoresis. However, a comparison was made of their growth response to different sources of carbon and nitrogen, to different temperatures and also their sensitivity to above-maximum temperatures. A comparison was also made of their growth and survival in soil amended with urea, but this will be discussed later.

Fig. 43. Appearance of cultures of 92 (top) and 97 (bottom) maintained at 20°C for one month in darkness (left) and a continuous light intensity of 600 foot candles (right).




TYPE 1
GROWN IN DARK
GROWN IN LIGHT


TYPE 2

(a) Temperature Studies.

Growth of isolates 91 and 97 was compared over the range 3°-37°C on PMD, the colony diameters being measured daily for four days. The results (Fig. 44) indicate optima of 27°C and 25°C for 91 (Type 1) and 97 (Type 2) respectively.

Peripheral growth by both isolates was regular before and at optimum temperatures, but irregular at 33°C and 30°C respectively (Fig. 45). Microscopic examination showed that regular peripheral growth was due to the dominance of the tip cells of peripheral hyphae (Fig. 46). However, at near-maximum temperatures these tip cells were often killed (Fig. 47) and the consequent loss of apical dominance was followed by development of side branches to give the colony an irregular appearance (Fig. 48). Associated with the checked marginal growth was an increase in the frequency of anastomosis between hyphae near the margin of the colony (Fig. 49). The mean number of anastomoses per microscopic field (x1000) were 0.08 and 0.21 for culture 97 at 20°C and 30°C respectively. This was based on an examination of 500 fields for each temperature. Thus there is an increased opportunity for redistribution of nuclei at the margin of a colony in a stress environment, and if nuclear ratios are important, the anastomosis may be a mechanism for countering conditions which are adverse to the advance of the organism.

The effect of near-maximum temperatures on cultural appearance was further studied in a series of successive

Fig. 44.

EFFECT OF TEMPERATURE ON GROWTH OF RHIZOCTONIA SOLANI IN CULTURE

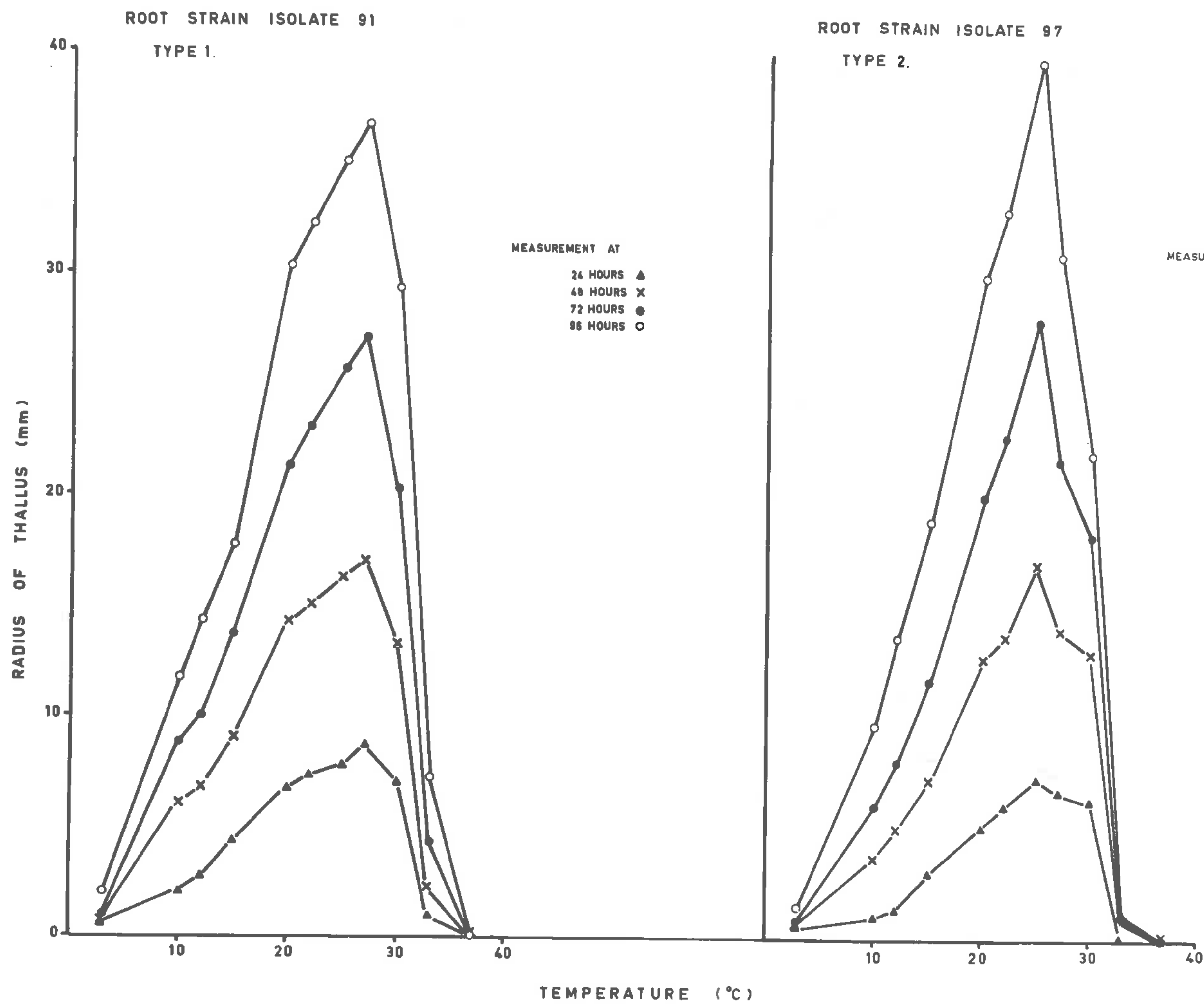
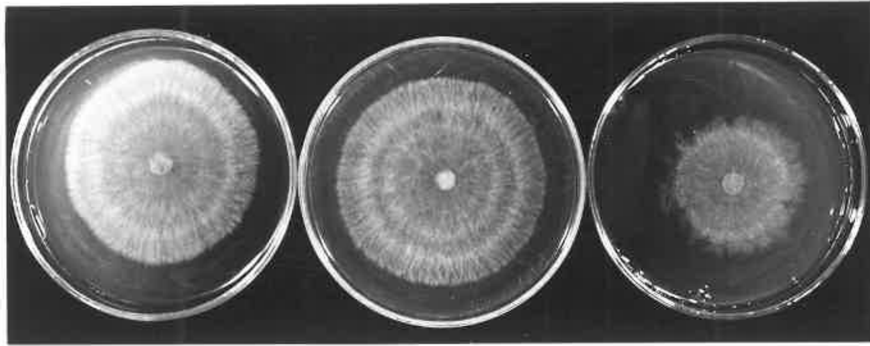


Fig. 45. Appearance of colonies of 97 after four days' incubation at 20°C (left), 27°C (centre) and 30°C (right).
Note the irregular peripheral growth of the colony at 30°C.

Fig. 46. Dominance of tip cell in peripheral hypha of colony of isolate 97 when grown at 20°C.

Mag. x140



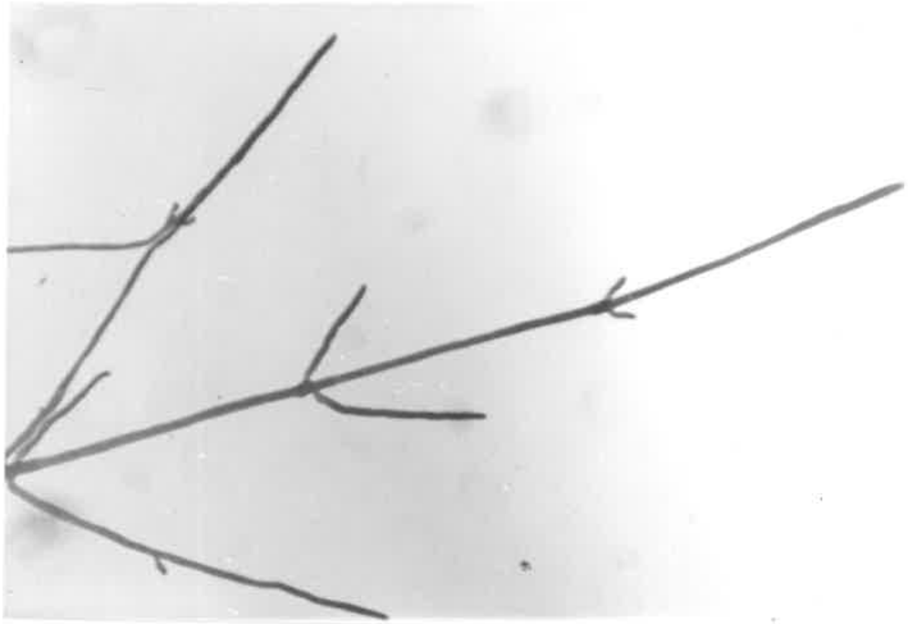


Fig. 47. Dead tip cells of peripheral hyphae of 97 when incubated at 30°C. Note regrowth in lumen of old hyphae.

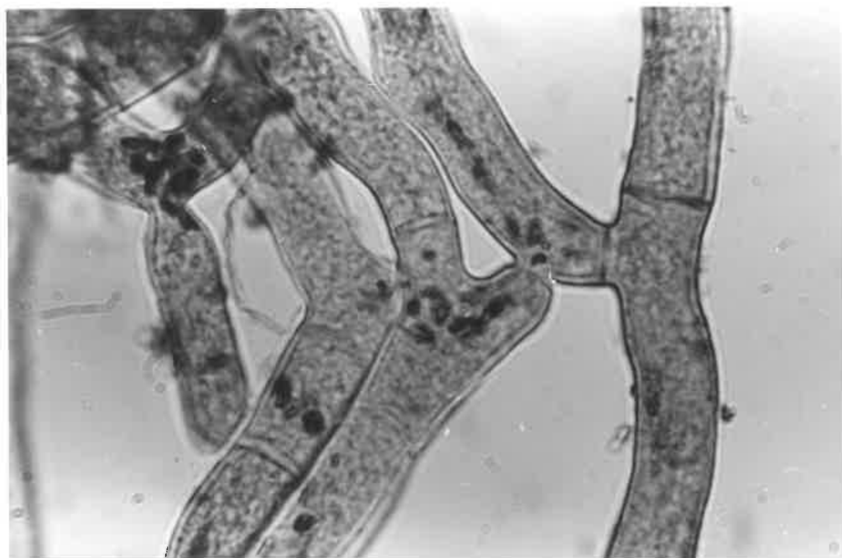
Mag. x484

Fig. 48. Development of side branches in peripheral hyphae of isolate 97 when incubated at 30°C.

Mag. x112

Fig. 49. Frequent anastomosis between hyphae in colony of 97 incubated at 30°C. Stained with HCl-Giemsa.

Mag. x1120



sub-cultures on PMD in Petri dishes. Commencing with a four-day-old dense regular colony of 97 grown at 20°C, two series of three sub-cultures were taken from it and incubated at 20°C and 30°C respectively. After four days' incubation, one culture of each series was sub-cultured six times, three of the new sub-cultures being incubated at 20°C and the other three at 30°C. The procedure was repeated again with the new sub-cultures after four days' incubation. Just before sub-culturing, the appearance of the colony was recorded and the diameter of each colony was measured (Fig. 50). The final series of sub-cultures is illustrated in Fig. 51.

Colonies were dense and irregular when incubated at 30°C, but sub-cultures from these generally reverted to a dense regular appearance when incubated at 20°C. Three cultures with a sub-culturing history of 30°C-30°C-20°C did not immediately revert to dense regular colonies but produced only sparse irregular growth. When these three were again sub-cultured and placed at 20°C, one failed to grow but the other two reverted to dense regular colonies. Thus changes in cultural appearance by exposure to 30°C are generally reversible, although the experience may occasionally be lethal. Colony diameter was reduced in the second and third successive sub-cultures at 30°C; in one instance the second sub-culture at 30°C failed to grow.

A comparison was made of the sensitivity of isolates 91 and 97 to above-maximum temperatures such as frequently

Fig. 50.

EFFECT OF TEMPERATURE AND SUBCULTURING HISTORY ON APPEARANCE AND SIZE OF ISOLATE 97 COLONIES

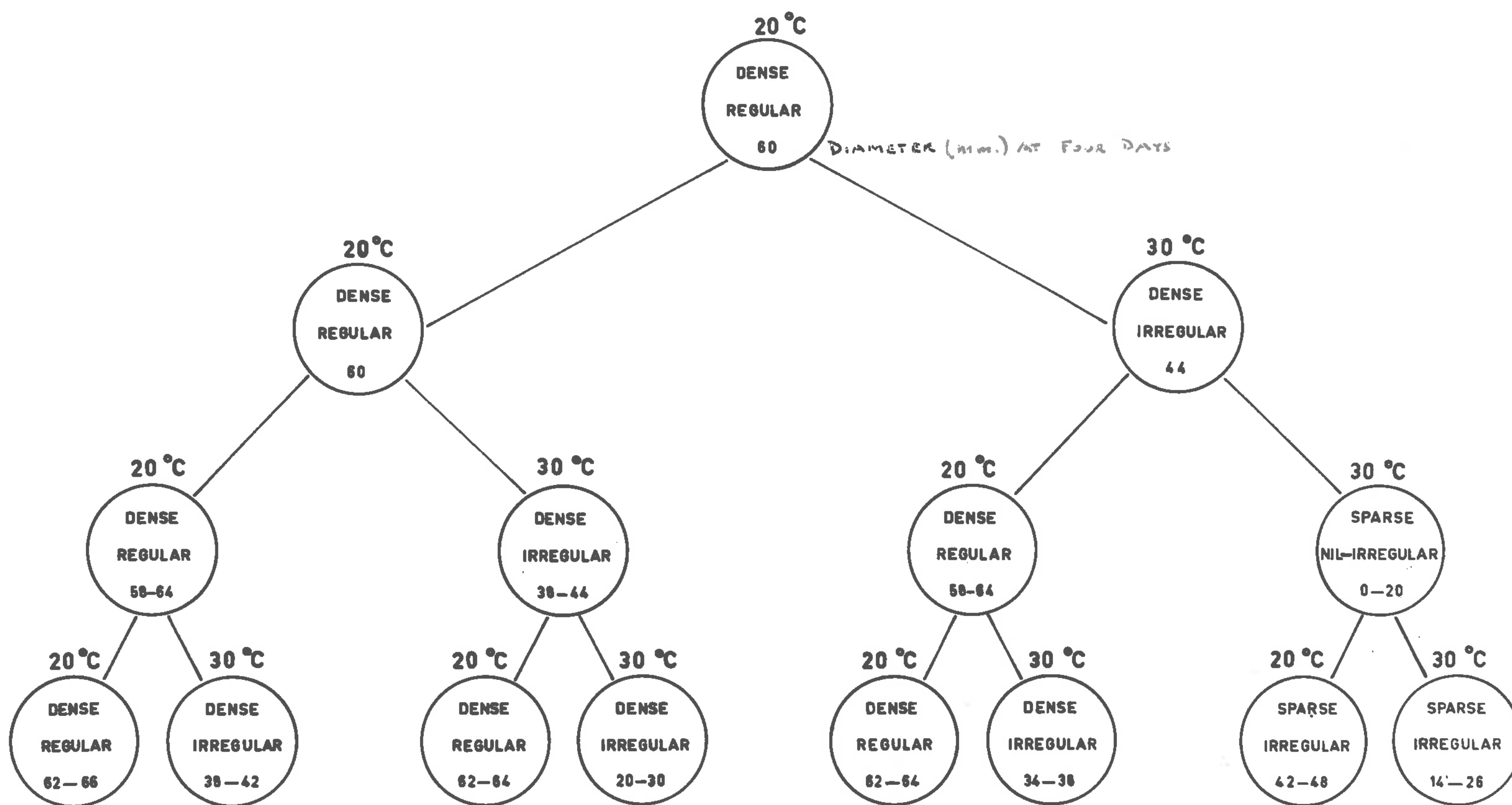
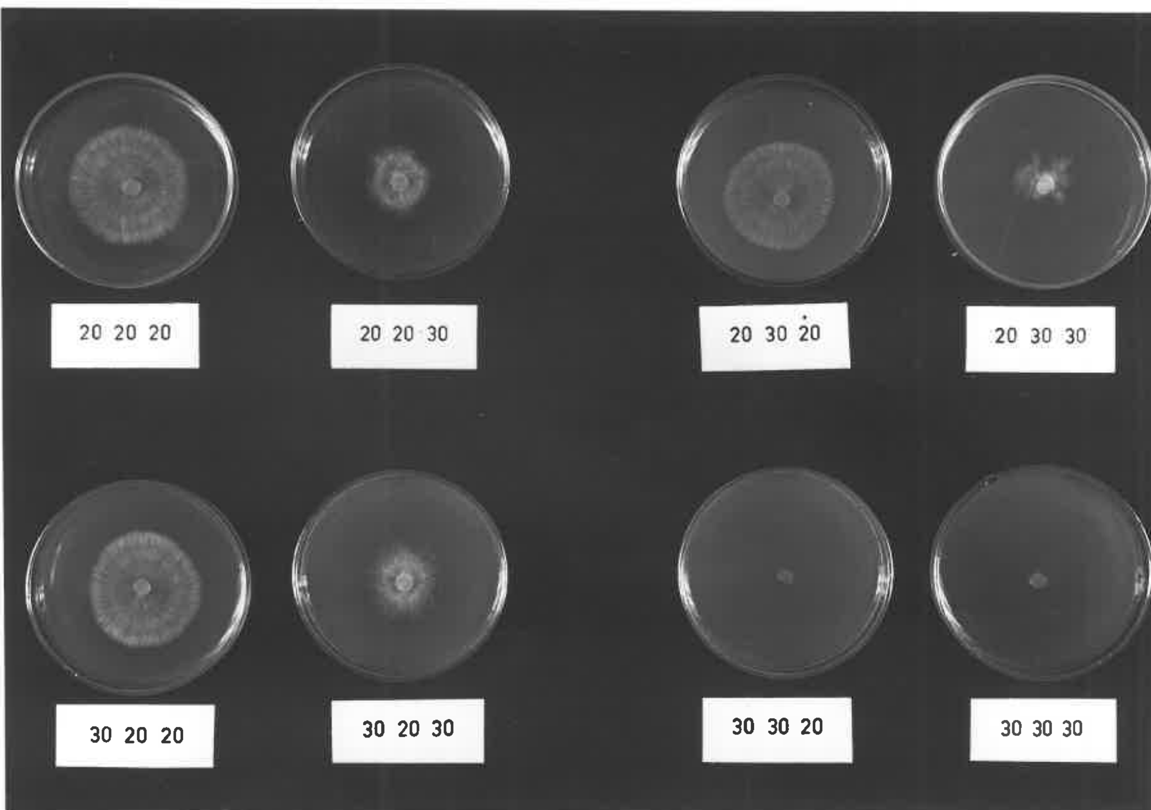


Fig. 51. Effect of temperature and sub-culturing history on the cultural appearance of isolate 97. The three numbers below each plate indicate the temperatures at which three successive sub-cultures were incubated, the final temperature referring to the present culture.


20 20 20
20 20 30
20 30 20
20 30 30
30 20 20
30 20 30
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30 30 30

occur in the field during summer, when the maximum soil temperature at one inch is within the range 40°-55°C. Exposure periods which cause death of the two isolates over the temperature range 40°-55°C are given in Table 38. These results indicate that isolate 91 is slightly more sensitive than 97 to above-maximum temperatures, although 91 has the higher optimum temperature.

(b) Nutritional Studies.

The mycelial dry weights of isolates 91 and 97 were determined on two series of liquid media. The first series contained ammonium nitrate as the source of nitrogen, but each medium of the series differed in the source of carbon; in the second series, twenty-one sources of nitrogen were used, but glucose was the only carbohydrate used as a source of carbon. However, as most of these nitrogen compounds contained carbon also, a parallel series omitting glucose was included in the second experiment. The mycelial dry weights of the two isolates after five days' incubation at 25°C on the various media are given in Tables 39 and 40. These results indicate differences in the ability of the two isolates to utilize various forms of carbon and nitrogen. The mycelial dry weight of the more common Type 1 isolate (91) was significantly greater than, equal to, and significantly less than that of the Type 2 isolate (97) on a total of 14, 14 and 3 media respectively. Thus the Type 1 isolate made better growth than the

TABLE 38.

THERMAL DEATH POINTS OF ISOLATES 91 AND 97.

Temperature (°C)	Lethal Exposure Period *	
	Isolate 91	Isolate 97
40	48-64 hours	64-72 hours
44	90-105 mins.	105-120 mins.
47	60- 75 mins.	75- 90 mins.
49	45- 60 mins.	45- 60 mins.
55	0- 5 mins.	0- 5 mins.

* Some growth after the first period, but none after the second.

TABLE 39.

EFFECT OF DIFFERENT SOURCES OF CARBON ON MYCELIAL DRY WEIGHT OF RHIZOCTONIA SOLANI

(i) Using Ammonium Nitrate as a Source of Nitrogen

Source of Carbon	Mycelial Dry Weight (mg.) of R. solani		Analysis of Dry Weights Using Transformation $Log_e X$	
	Type 1 Isolate	Type 2 Isolate	Type 1 Isolate	Type 2 Isolate
L-Arabinose	21	19	3.0283	2.9435
D-Xylose	27	27	3.2804	3.3061
D-Fructose	78	40	4.3604	3.6792
D-Galactose	47	35	3.8522	3.5398
D-Glucose	68	42	4.2144	3.7296
D-Mannose	68	37	4.2237	3.6013
Lactose	6	12	1.7824	2.4559
Maltose	53	50	3.9694	3.9092
Sucrose	65	44	4.1734	3.7728
Inulin	8	10	2.1187	2.3282
Starch	58	38	4.0529	3.6359
Nil	3	3	1.1945	1.0594

DIFFERENCES FOR SIGNIFICANCE		
	P = 0.05	0.1918
	P = 0.01	0.2560

TABLE 40.

MYCELIAL DRY WEIGHT OF RHIZOCTONIA SOLANI WHEN GROWN ON MEDIA CONTAINING DIFFERENT SOURCES OF NITROGEN AND WITH OR WITHOUT GLUCOSE AS THE SOURCE OF CARBON

Source of Nitrogen	Mycelial Dry Weight (in mg.) of R. solani				Analysis of "Plus Glucose" Dry Weights (Using Transformation $\log_e X$)	
	Type 1 Isolate		Type 2 Isolate			
	+ Glucose	- Glucose	+ Glucose	- Glucose	Type 1 Isolate	Type 2 Isolate
L-Alanine	24	2	12	1	3.1893	2.4508
L-Arginine HCl	45	1	20	1	3.8087	2.9737
L-Asparagine	121	1	42	2	4.7980	3.7273
L-Aspartic Acid	17	1	15	2	2.8299	2.7268
L-Cysteine HCl	3	2	2	2	0.9635	0.4621
L-Cystine	21	0	27	0	3.0538	3.2940
L-Glutamic Acid	27	1	13	0	3.3026	2.5303
Glycine	12	0	9	1	2.4826	2.2323
L-Histidine HCl	7	1	8	0	1.9228	2.0742
L-Isoleucine	11	0	10	1	2.4218	2.2992
L-Leucine	12	0	10	1	2.5022	2.2992
L-Lysine HCl	5	0	7	0	1.4999	1.9904
L-Methionine	6	0	5	1	1.8431	1.5607
L-PhenylAlanine	8	1	8	1	2.1093	1.9175
L-Proline	9	2	6	1	2.1931	1.8431
L-Serine	26	0	11	0	3.2545	2.4026
L-Threonine	8	1	5	0	2.0364	1.4769
L-Tryptophane	6	0	8	0	1.7661	2.0621
L-Tyrosine	7	0	6	0	1.9390	1.7824
L-Valine	12	0	9	0	2.4465	2.2323
Nil - Inoculum only	3	0	1	0	0.9635	0.2311
Ammonium Sulphate (Control)	67	0	41	0	4.2092	3.7197
DIFFERENCE FOR SIGNIFICANCE $P = 0.05$					0.3292	
$P = 0.01$					0.4361	

Type 2 isolate on more media. Metabolic differences may partly account for Type 1 being more prevalent in the field than Type 2. Neither isolate was able to utilize the carbon in the organic nitrogen compounds.

(2) Effects of Nitrogenous Soil Amendments on the Activity and Survival of Root Strain Isolate 92.

De Beer (1965) reported that survival of the root strain and its ability to colonize substrates were impaired by soil amendments of peptone and ammonium nitrate under laboratory conditions. A series of laboratory experiments was therefore carried out to examine the effects of several other nitrogenous soil amendments on activity and survival of isolate 92 of the root strain.

(a) Survival in Wheat Straw in Unsterilized Soil.

Wheat straws were artificially infected with isolate 92 and buried in unsterilized soil in three series of jars; one series was not amended but the other two received sodium nitrate and ammonium sulphate respectively at the rate of 10 mg. per 100 g. soil. Soil moisture was adjusted to 7.1 percent (pF 1.9). The number of viable hyphae of R. solani occurring naturally in the soil at commencement was determined as 1.6 fragments per gram. After 3, 4, 8 and 12 weeks' burial, straws were tested for viable hyphae of R. solani by the wheat seedling test; the total and viable

number of hyphal fragments per gram of soil were also determined. The results are given in Tables 41a and 41b.

Throughout the experiment, straws were examined for mycelium of R. solani. At burial, almost all hyphae were brown and composed of either short irregular-shaped cells or longer narrower "runner hyphae" cells. Many hyphae were in the lumen of the straw or associated with the leaf sheath tissue. After burial, a slight increase occurred in the quantity of dark brown hyphae and by twelve weeks noticeably more hyphae were associated with straws in unamended soils than with those in nitrogen-enriched soil. By twelve weeks, a large proportion of hyphal cells were swollen, empty or collapsed.

Because the majority of hyphae were in the lumen of straws rather than in the tissue, the seedling test must be regarded as determining the number of straws with associated viable hyphae. Thus the results (Table 41a) indicate that nitrogen enrichment of soil reduced the number of viable hyphae associated with the decomposing straw. The effect was similar with both the nitrate and ammonium forms of nitrogen. All straws were firm and coherent after twelve weeks' burial, although those in nitrogen-enriched soil were darker and softer than those in unamended soil. Severe root-tip necrosis of test seedlings occurred at the beginning of the experiment, but as R. solani declined in viability in the straws, symptoms became milder. Symptoms were generally milder with straws from nitrogen-enriched soil than straws from unamended soil.

Determinations of numbers of R. solani hyphae in the

TABLE 41a.

PERCENTAGE OF WHEAT STRAWS IN WHICH R. SOLANI (ROOT STRAIN) SURVIVED WHEN BURIED IN UNSTERILIZED SOIL WITH OR WITHOUT ADDED NITROGEN.

Survival (weeks)	Percentage of Straws Containing Viable Hyphae of *R. solani* in:		
	Un-amended Soil	Nitrate-Enriched Soil	Ammonium-Enriched Soil
0	100	100	100
3	100	99	99
4	100	100	98
8	97	88	88
12	52	39	31

TABLE 41b.

EFFECT OF NITROGENOUS SOIL AMENDMENTS ON GROWTH OF THE ROOT STRAIN OF R. SOLANI

INTO UNSTERILIZED SOIL.

Time of Estimate	Soil Amendment (100mg. N per Kg. Soil)	Numbers of Hyphal Fragments per gram of Soil		Analysis of Population Estimates (Using Transformation $Log_e X$)	
		Viable	Total	Viable	Total
At 3 weeks	Nil	71.7	144.8	4.2708	4.9745
	$(NH4)_2SO_4$	25.7	100.6	3.2396	4.6100
	$NaNO_3$	45.2	113.6	3.8060	4.7324
At 4 weeks	Nil	72.1	156.4	4.2742	5.0513
	$(NH4)_2SO_4$	28.8	103.0	3.3590	4.6339
	$NaNO_3$	46.5	119.4	3.8384	4.7812
At 8 weeks	Nil	138.8	427.4	4.9312	6.0566
	$(NH4)_2SO_4$	59.2	229.4	4.0680	5.4347
	$NaNO_3$	76.4	273.0	4.3289	5.6069
At 12 weeks	Nil	69.7	661.0	4.2330	6.4926
	$(NH4)_2SO_4$	22.2	382.0	3.0764	5.9424
	$NaNO_3$	34.0	485.0	3.5196	6.1839
			Differences for Significance $P = 0.05$	0.2474	0.7415
			$P = 0.01$	0.3301	0.9892

soil indicated a general increase in viable hyphae up to eight weeks after burial of straw; increase was greatest in unamended and least in ammonium-amended soil (Table 41b). Thereafter the number of viable fragments decreased although total number increased. It is interesting to note that the number of viable fragments associated with the straws was decreasing whilst the number in the soil was still increasing. As the straws were still quite firm and coherent, this suggests that much of the straw was unavailable as food material to the fungus.

(b) Colonization of Filter Paper.

A comparison was made of ammonium sulphate, L-alanine, L-asparagine and L-glutamic acid as soil amendments (100mg.N/Kg. soil) on colonization of sterile filter paper by isolate 92. Unsterilized soil was inoculated with 1.2 mycelial mats of isolate 92 per Kg. soil (de Beer,1965) and soil moisture was adjusted to four percent(pF3.0). In contrast with de Beer's observations, no amendment reduced the number of filter papers colonized by 92 and all counts were near maximum(Table 42). However, there was much less growth on to water agar from filter paper pieces which had been in amended soil.

The experiment was therefore repeated but the amount of inoculum was reduced from 1.2 to 0.5 mat per Kg. soil. In this and all subsequent experiments, filter papers pre-colonized by other organisms were used as well as sterile filter papers. The results (Table43) show that the number of filter papers colonized by 92 was reduced by all amendments and most by ammonium sulphate. Pre-colonization of filter

TABLE 42.

EFFECT OF VARIOUS NITROGENOUS SOIL AMENDMENTS ON COLONIZATION OF FILTER PAPER BY RHIZOCTONIA SOLANI

SOIL AMENDMENT 100 ppm Nitrogen Added as:	NUMBER OF STERILE FILTER PAPERS COLONIZED (Maximum Number = 50)	ANALYSIS OF DATA Using Transformation Log_e X
L-Alanine	49.7	3.9053
Ammonium Sulphate	46.3	3.8352
L-Asparagine	49.3	3.8986
L-Glutamic Acid	48.7	3.8848
Nil - Control	50.0	3.9120

DIFFERENCES ... ... Not significant

TABLE 43a

EFFECTS OF VARIOUS NITROGENOUS SOIL AMENDMENTS ON COLONIZATION OF FILTER PAPER BY <u>RHIZOCTONIA SOLANI</u>

SOIL AMENDMENT 100 ppm Nitrogen added as:	* Number of Pieces Colonized When using Sterile Paper	* Number of Pieces Colonized When using Paper Pre-colonized by Other Organisms	Mean Number	Analysis of Mean Using transformation Log_e X
L-Alanine	44.3	18.7	31.5	3.3871
Ammonium Sulphate	32.7	13.7	23.2	3.0970
L-Asparagine	42.0	17.0	29.5	3.3225
L-Glutamic Acid	41.7	20.3	31.0	3.4038
Nil - Control	49.7	39.0	44.4	3.8050
Differences for Significance			$P = 0.05$	0.3728
			$P = 0.01$	0.5050

* Possible number = 50

TABLE 43b

EFFECT OF PRE-COLONIZATION OF SUBSTRATE BY OTHER ORGANISMS ON SUBSEQUENT COLONIZATION BY <u>R. SOLANI</u>

State of Filter Papers When Placed in Soil	Mean Number of Filter Papers Colonized (Maximum No. = 50)	Analysis of Colonization Using Transformation Log_e X+1
Pre-colonized by Other Organisms	42.1	3.7527
Sterile	21.7	3.0535
	Level of Significance	$P = 0.01$

papers by other organisms further reduced the number colonized by isolate 92. These results were in accordance with de Beer's observations. Later work (page 144) also demonstrated that isolate 92 colonized filter paper more readily than isolate 91 (used by de Beer) and this could partly explain the differences between our results.

As colonization of filter paper by isolate 92 was reduced by all the above amendments, a wider range of nitrogen compounds was examined. Soil amendments of ammonium nitrate, ammonium sulphate, L-alanine, L-asparagine, L-glutamic acid, sodium nitrate and urea were used at a concentration of 200 mg. nitrogen per Kg. soil. The soil was inoculated with half a mat per Kg. soil and the moisture content was adjusted to 6.9 percent (pF1.95). As previously, colonization by isolate 92 was reduced more by ammonium sulphate than by alanine, asparagine and glutamic acid, but it was restricted even more by sodium nitrate and urea (Table 44). Thus there is evidence that nitrogen enrichment of soil will cause an initial reduction in the activity of the root strain and that the effect will vary quantitatively according to the material used. These results, however, do not indicate the mechanism whereby each compound restricts the activity of this strain nor the duration of the effect.

Pre-colonization of papers by other organisms also reduced subsequent colonization by isolate 92; the restrictive effect of pre- colonization was greater than that of glutamic acid but considerably less than that of urea and the inorganic

TABLE 44

EFFECT OF VARIOUS NITROGENOUS SOIL AMENDMENTS ON COLONIZATION OF FILTER PAPER BY RHIZOCTONIA SOLANI

SOIL AMENDMENT 200 mg. Nitrogen per Kg. Soil Applied as:	Number of Pieces Colonized When Using		Analysis of Colonization (Using Transformation Logit 2X+1	
	Sterile Paper	Paper Pre-colonized by Other Organisms (Possible Max. No. =50)	Sterile Paper	Pre-colonized Paper
Nil	47 *	32 *	+2.7224	+0.5921
L-Asparagine	41	21	+1.4906	-0.2995
L-Alanine	34	16	+0.7135	-0.7091
L-Glutamic Acid	32	16	+0.5685	-0.7120
Ammonium Sulphate	25	14	-0.0005	-0.9286
Ammonium Nitrate	20	11	-0.3721	-1.1999
Sodium Nitrate	12	7	-1.0905	-1.8144
Urea	6	2	-2.0056	-2.9324

Difference for Significance P = 0.05 0.5844
P = 0.01 0.7859

* Possible Number = 50

compounds.

(c) In Unsterilized Soil.

The effects of the above soil amendments on the viable and total numbers of isolate 92 hyphae were studied in artificially inoculated soil; the amendments were applied at the same rate as previously and soil moisture was adjusted to 5.3 percent (pF 2.3). Determinations at ten and 35 days indicated that both viable and total numbers of hyphal fragments of isolate 92 were significantly reduced by all amendments (Table 45). Thus nitrogen enrichment of soil may reduce the viability and mycelial density of the root strain for at least 35 days under laboratory conditions. A similar reduction under field conditions would be of considerable value as nitrogenous fertilizers applied at seeding could restrict activity of the root strain during the most susceptible period of crop growth.

The amendments differed in their relative effects on the number of viable hyphae of isolate 92 and urea caused the greatest reduction during the first ten days. However, viability continued to fall over the test period in the two nitrate-amended soils; by 35 days, the number of viable hyphae was not significantly different in soil amended with urea or ammonium nitrate.

(d) In Gamma-Irradiated Soil.

The above amendments were further studied in gamma-

TABLE 45.

EFFECTS OF VARIOUS NITROGENOUS SOIL AMENDMENTS ON NUMBERS OF R. SOLANI HYPHAE IN UNSTERILIZED SOIL

Time of Estimate	Soil Amendment	Hyphal fragments per gram of soil		Analysis of Population Estimates (Using Transformation Log$_e$ X)	
		Total Number	Number Viable	Total Numbers	Viable Numbers
At 10 days	Nil	216.0	93.8	5.3736	4.5090
	L-Asparagine	189.1	50.5	5.2386	3.8873
	L-Alanine	186.1	49.1	5.2261	3.8897
	L-Glutamic Acid	179.2	37.6	5.1877	3.6271
	Ammonium Sulphate	187.2	47.8	5.2309	3.8420
	Ammonium Nitrate	176.3	38.8	5.1717	3.6688
	Sodium Nitrate	184.3	51.4	5.2147	3.9158
	Urea	158.4	26.6	5.0651	3.2511
At 35 days	Nil	266.4	79.8	5.5847	4.3765
	L-Asparagine	201.6	42.8	5.3028	3.7319
	L-Alanine	194.9	38.4	5.2723	3.6459
	L-Glutamic Acid	199.2	35.9	5.2941	3.5748
	Ammonium Sulphate	209.9	35.0	5.3463	3.5482
	Ammonium Nitrate	190.1	26.0	5.2473	3.2549
	Sodium Nitrate	212.3	33.7	5.3564	3.4963
	Urea	166.7	23.4	5.1158	3.1482

DIFFERENCE FOR SIGNIFICANCE		Total Numbers	Viable Numbers
	$P = 0.05$	0.0916	0.3297
	$P = 0.01$	0.1239	0.4434

irradiated soil to determine whether any were having a direct inhibitory effect on isolate 92. Each was applied as a concentration of 200 mg. Nitrogen/Kg. soil and soil moisture was initially adjusted to 9.4 percent (pF 1.75). Hyphal counts at eleven days suggested that only urea had a direct inhibitory effect on isolate 92 (Table 46). Therefore the remaining amendments must indirectly reduce the viability and mycelial density of the root strain by increasing the competitive and antagonistic activity of other soil organisms. McLaren, Luse and Skujins (1962) have shown that enzymes, including urease, continue to function in soil which has been sterilized by irradiation. It is not known whether such urease activity contributed to the observed results with urea in this experiment.

A point of interest is the magnitude of the mycelial density of the root strain in unamended irradiated soil compared with that previously recorded in unamended, unsterilized soil (Table 45). This difference gives some measure of the restrictive biological forces which normally operate in unsterilized soil, although more food material would be available in sterilized soil.

TABLE 46.

EFFECT OF VARIOUS SOIL AMENDMENTS ON NUMBERS OF R. SOLANI HYPHAE IN GAMMA-IRRADIATED SOIL

SOIL AMENDMENT 200 ug. Nitrogen per g. soil applied as:	NUMBERS OF HYPHAL FRAGMENTS PER GRAM OF SOIL		ANALYSES OF TOTAL NUMBERS
	VIABLE	TOTAL	(Using Transformation $\log_e X$)
Nil	1041	1052	6.9581
L-Asparagine	1291	1308	7.1688
L-Alanine	1285	1311	7.1773
L-Glutamic Acid	1289	1301	7.1643
Ammonium Sulphate	1196	1217	7.1024
Ammonium Nitrate	1049	1073	6.9772
Sodium Nitrate	1127	1155	7.0506
Urea	205	208	5.2533

DIFFERENCE FOR SIGNIFICANCE	P = 0.05	0.3590
	P = 0.01	0.4946

(3) Further Studies with Urea.

As urea restricted the activity and survival of isolate 92 more than any other amendment, a further series of laboratory experiments was carried out with this compound. Unless otherwise stated, urea was applied to the soil at the rate of 200 mg. per Kg. soil; for all studies on filter paper colonization, soil was inoculated with half a mycelial mat per Kg. soil.

(a) Reactions of Other Root Strain Isolates to Urea.

The effect of urea on filter paper colonization by isolates 90, 91, 92, 93, 97, 98 and 100 was compared on soil adjusted to a moisture content of 5.4 percent (pF 2.3). Urea almost completely inhibited filter paper colonization by all isolates (Table 47) thereby establishing that it is effective in restricting the activity of both types of root strain isolates.

In unamended soil, Type 1 isolates colonized more filter papers than Type 2 isolates, thereby confirming previous observations (de Beer, 1965). However, Type 1 isolates differed considerably in their activity and isolate 92 colonized significantly more filter papers than any other isolate. Thus the results provide an explanation of why the amount of inoculum had to be reduced from 1.2 mycelial mats (as used by de Beer for isolate 91) to half a mat for studies with isolate 92. Pre-colonization of filter papers by other organisms also restricted subsequent colonization by root

TABLE 47.

EFFECT OF UREA ON COLONIZATION OF FILTER PAPER BY DIFFERENT ISOLATES OF THE ROOT STRAIN OF *R. SOLANI*.

Isolate	Soil Amendment	* Number of Pieces Colonized When Using:		Analysis of Colonization (Using Transformation $Log_e(X+1)$)	
		Sterile Paper	Paper Pre-colonized by other organisms	Sterile Paper	Pre-colonized Paper
90	Nil	24	18	3.2269	2.9615
	Urea	1	0	0.3662	0.2311
91	Nil	18	9	2.9435	2.2890
	Urea	0	0	0.2311	0.2311
92	Nil	47	33	3.8640	3.5252
	Urea	7	2	2.0134	0.7675
93	Nil	23	15	3.1741	2.7903
	Urea	3	1	1.4607	0.5973
97	Nil	8	1	2.2249	0.4621
	Urea	1	0	0.5973	0.2311
98	Nil	8	2	2.1931	1.0594
	Urea	2	1	1.1945	0.4621
100	Nil	8	1	2.1411	0.4621
	Urea	1	0	0.5973	0.2311
	Differences for Significance		$P = 0.05$	0.5549	0.6714
			$P = 0.01$	0.7486	0.9058

* Maximum number = 50.

strain isolates; the inhibitory effect of pre-colonization was less than the effect of urea for Type 1 but equal to it for Type 2.

At the moisture level of this experiment (5.4 percent) urea again reduced the viable and total numbers of hyphae of the same isolates in unsterilized soil (Figs. 52 and 53), an effect which continued as in previous experiments for at least 35 days under laboratory conditions. In contrast with the filter paper colonization results, there were no significant differences between the viability and mycelial density of any isolates in unamended soils. The inhibitory effect of urea on these isolates was also demonstrated in gamma-irradiated soil of 9.0 percent (pF 1.76) moisture content (Tables 48 and 49). Again there were no significant differences between the viability and mycelial density in unamended soils.

In view of these results and their possible application for the control of bare patch disease, it would be of value to determine whether urea also has a suppressive effect upon the activities and survival of other cereal-attacking strains of *R. solani*.

(b) Effects of Urea at Different Levels of Soil Moisture.

The effect of urea on the viability and mycelial density of isolate 92 was studied in unsterilized soil at moisture levels of 1.7, 4.7 and 9.0 percent (pF > 4.2, 2.6, and 1.76 respectively). These moisture levels were chosen because samples of the experimental soil collected at various

Fig. 52.

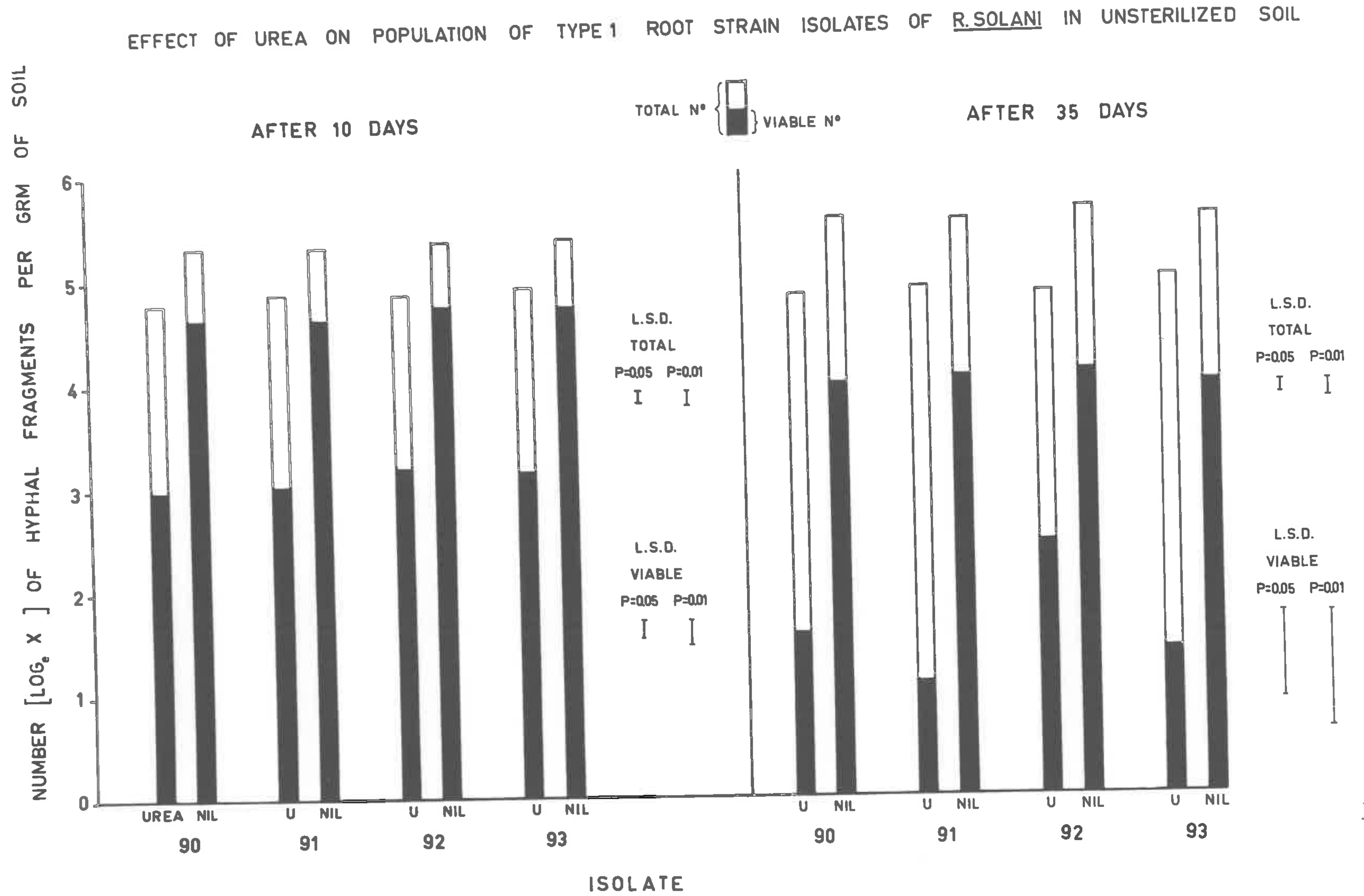


Fig. 53.

EFFECT OF UREA ON POPULATION OF TYPE 2 ROOT STRAIN ISOLATES OF R.SOLANI IN UNSTERILIZED SOIL

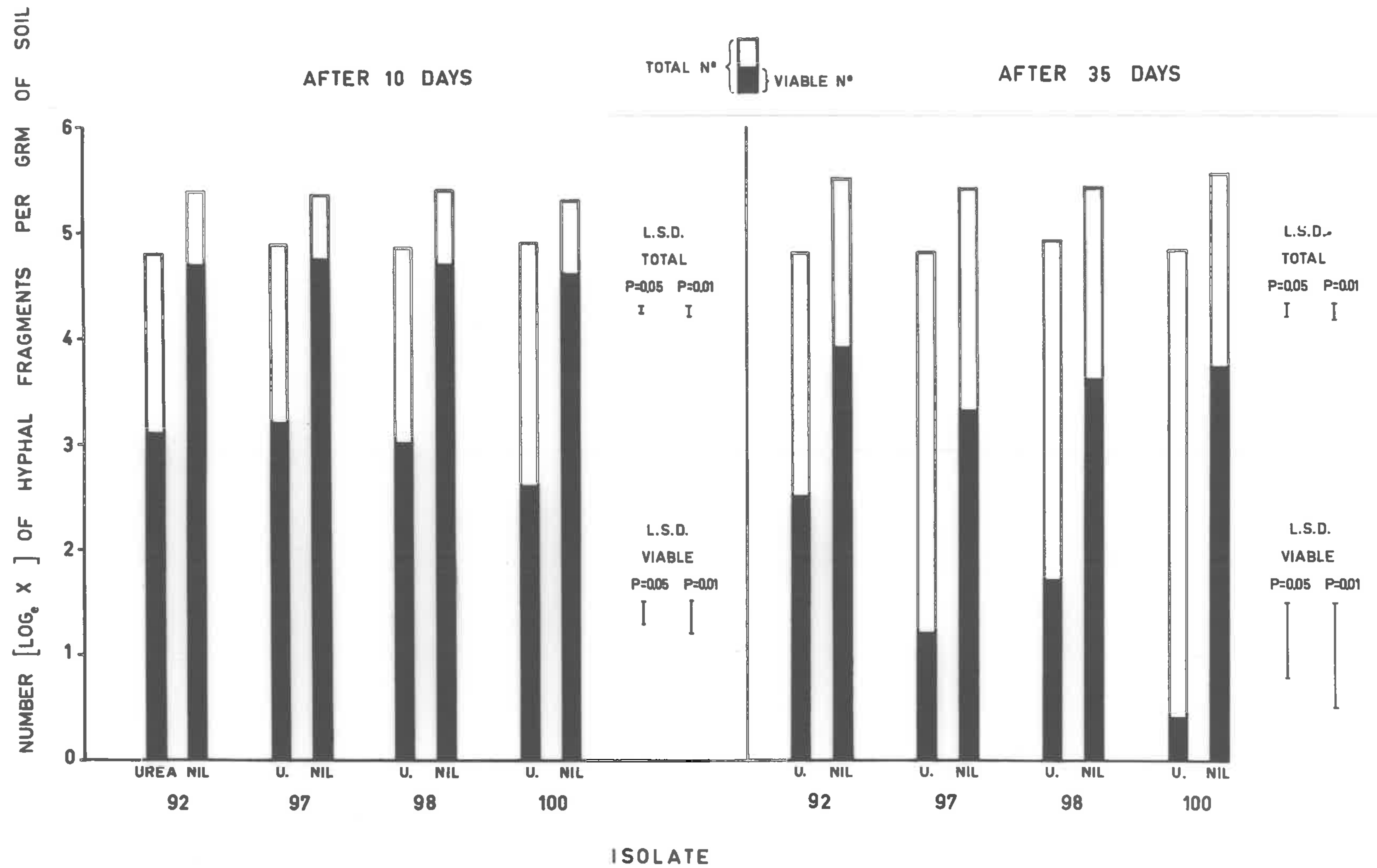


TABLE 48.

EFFECT OF UREA ON POPULATION OF "TYPE 1" ROOT STRAIN ISOLATES OF R. SOLANI IN IRRADIATED SOIL.

Isolate	Soil Amendment	Number of Hyphal Fragments per gram of soil		Analysis of Total Numbers (Using Transformation $\log_e X$)
		Viable	Total	
90	Nil	1559.0	1570.7	7.3564
	Urea	0.0	2.4	1.4982
91	Nil	1594.7	1600.0	7.3777
	Urea	0.5	5.6	1.8669
92	Nil	1438.3	1453.3	7.2790
	Urea	0.0	1.1	0.7104
93	Nil	1515.7	1526.7	7.3297
	Urea	1.1	3.7	1.4830
		Difference for Significance	P = 0.05	0.5004
			P = 0.01	0.6894

TABLE 49.

EFFECT OF UREA ON POPULATION OF "TYPE 2" ROOT STRAIN ISOLATES OF R. SOLANI IN IRRADIATED SOIL.

Isolate	Soil Amendment	Number of Hyphal Fragments per gram of soil		Analysis of Total Numbers (Using Transformation $\log_e X$)
		Viable	Total	
97	Nil	1117.7	1140.0	7.0395
	Urea	0.3	0.8	0.5878
98	Nil	1156.7	1184.0	7.0766
	Urea	0.5	1.6	0.9224
100	Nil	1125.3	1133.3	7.0332
	Urea	0.3	1.1	0.7104
92(Type1)	Nil	1305.3	1332.0	7.1936
	Urea	2.1	4.5	1.6058
		Differences for Significance	P = 0.05	0.4344
			P = 0.01	0.5986

times of the year ranged from 1.8 to 10.4 percent in moisture content. The lowest and highest recordings were from soils collected during dry summer and wet winter periods respectively. The results (Fig. 54) show that within ten days urea caused similar significant reductions in the viability and mycelial density of isolate 92 at moisture levels of 4.7 and 9.Opercent; the inhibitory effect of urea was still evident in soils of these moisture contents at 35 days. In urea-amended soil of 1.7 percent moisture, the majority of hyphae were killed within ten days, but this appeared to be due to desiccation rather than any lethal effect of urea (Fig. 55a). The wilting point of the soil was 3.4 percent.

In unamended soils, the total numbers of hyphal fragments of isolate 92 were greatest and least at the highest and lowest moisture levels respectively. These results are in accordance with those of de Beer (1965). However, the numbers of viable hyphae of isolate 92 were not significantly different at moisture levels of 4.7 and 9.0 percent (Fig. 55b).

(c) Effect of Urea at Different Soil Temperatures.

The effect of urea on filter paper colonization by isolate 92 was studied over the soil temperature range 15°-$30^{\circ}C$ in unsterilized soil of 5.3 percent moisture content (pF 2.3).

At temperatures of 15°, 20° and $25^{\circ}C$, filter paper colonization was reduced by urea amendment of soil, but the reduction was significantly less at $25^{\circ}C$ than at 15° or $20^{\circ}C$ (Table 50). At $30^{\circ}C$ no filter papers were colonized by the

Fig. 54.

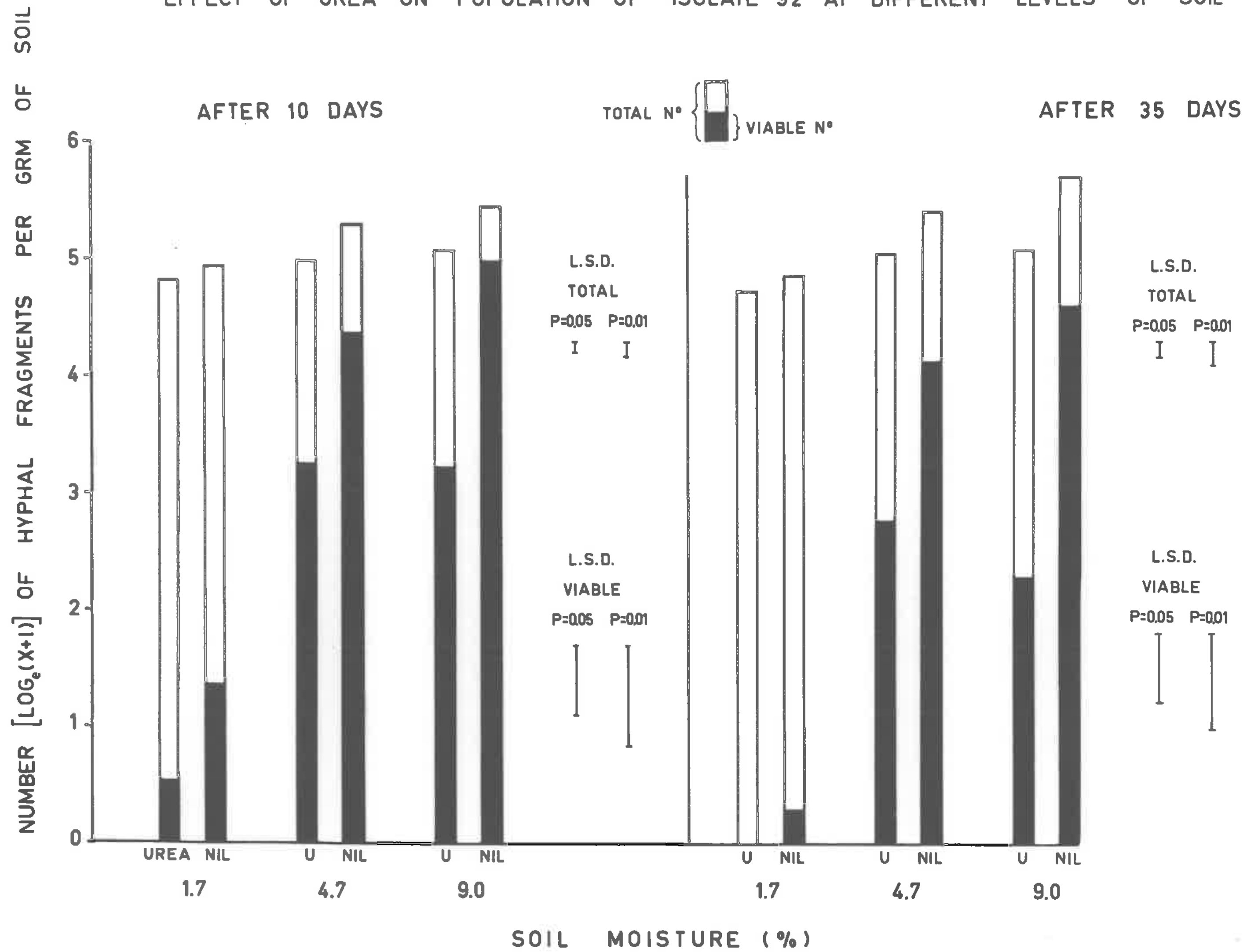
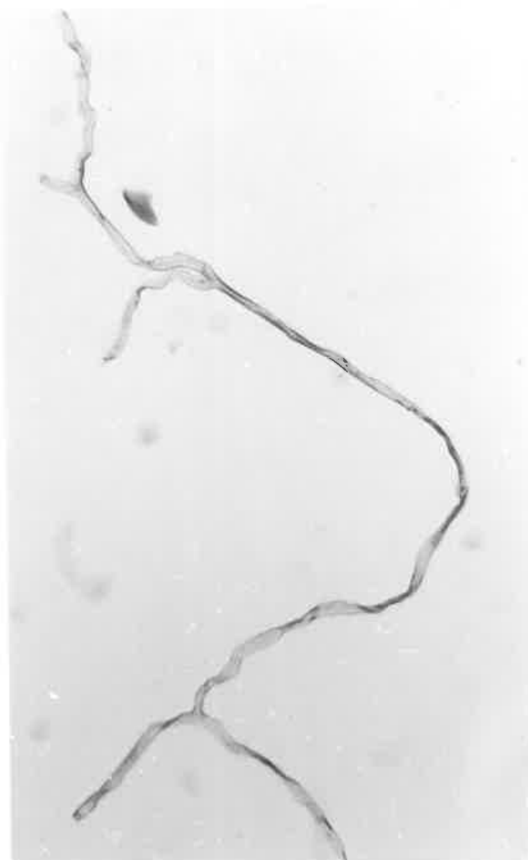


Fig. 55a. Twisted dead hyphal fragment of isolate 92.
Mag. x112

Fig. 55b. Viable fragment of isolate 92.
Note the new hyaline hyphal tip emerging from the old brown cell.
Mag. x450



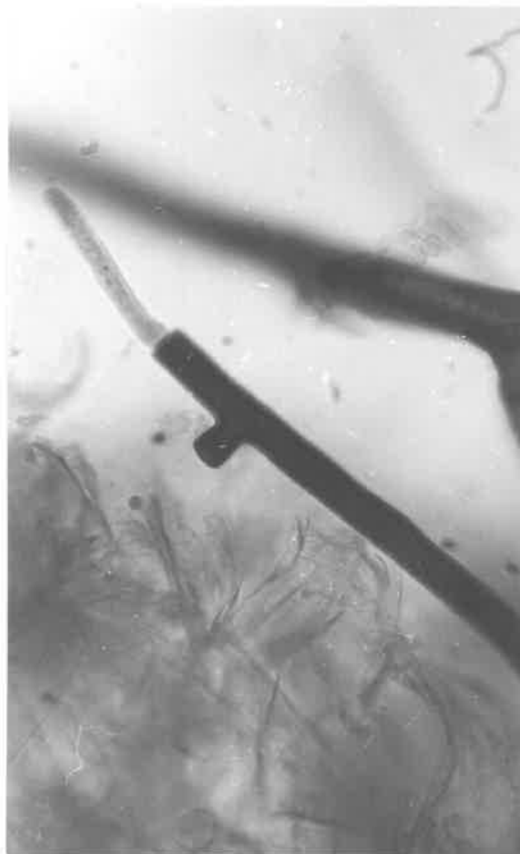


TABLE 50.

EFFECT OF UREA ON COLONIZATION OF FILTER PAPER BY *R. SOLANI* AT VARIOUS SOIL TEMPERATURES.

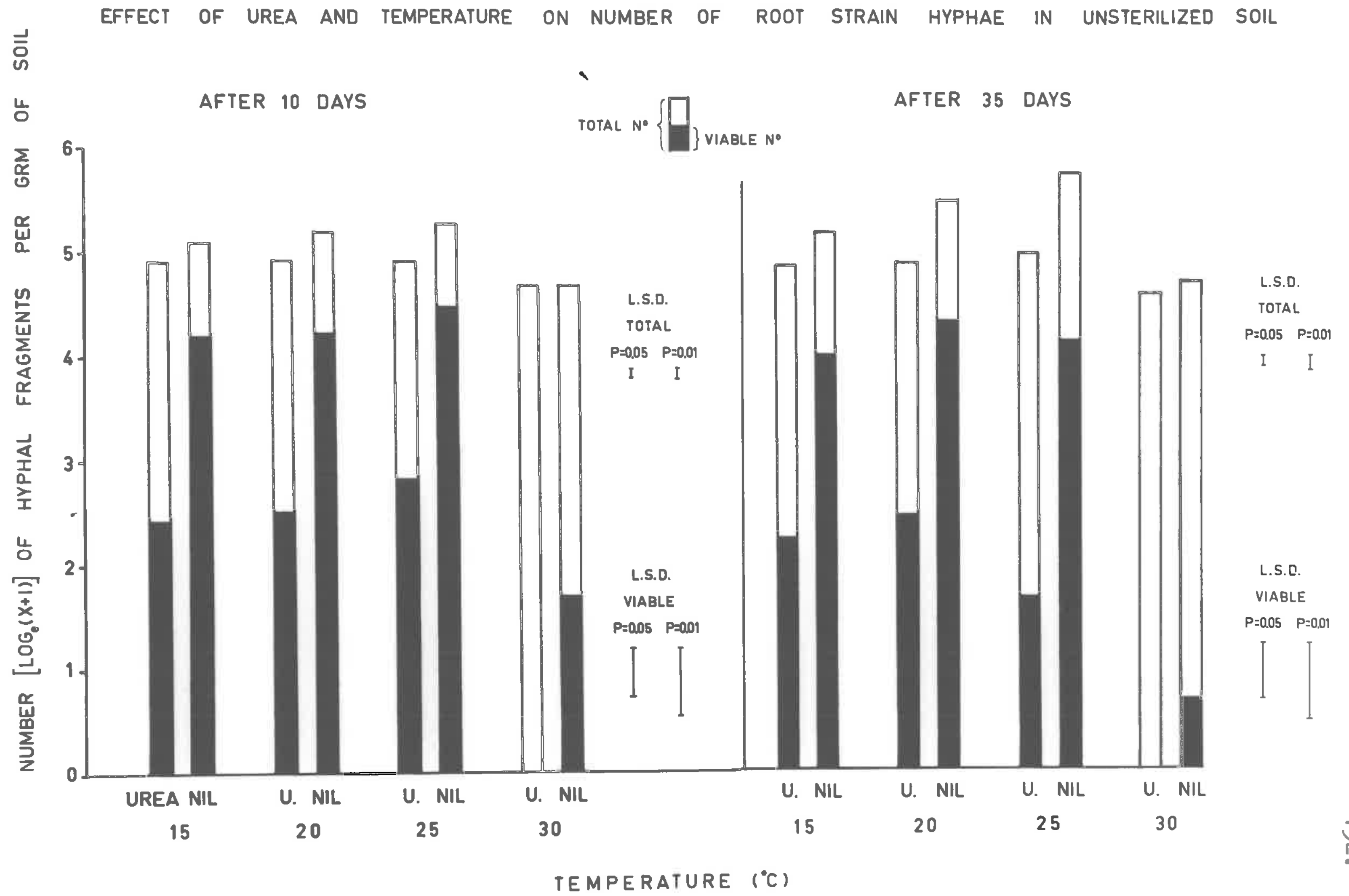
Soil Temperature (°C)	Soil Amendment	* Number of Pieces Colonized When Using: Sterile Paper	Paper Pre-colonized by Other Organisms	Analysis of Colonization (Using Transformation Logit 2X) Sterile Paper	Pre-colonized Paper
15	Nil	44	33	+1.9504	+0.6860
	Urea	12	2	-1.1397	-3.1095
20	Nil	49	44	+3.5152	+1.9112
	Urea	10	1	-1.4305	-3.3194
25	Nil	50	45	+4.6151	+2.0499
	Urea	27	6	+0.1576	-1.9562
30	Nil	0	0	-	-
	Urea	0	0	-	-
		Differences for Significance	P = 0.05	0.9016	0.7059
			P = 0.01	1.2640	0.9897

* Maximum Number = 50.

fungus even in unamended soil. As the fungus will grow in culture at 30°C, it is probable that colonization was inhibited by competitive and antagonistic activities of other soil organisms. In unamended soil, optimum colonization by isolate 92 occurred at 20°-25°C and was significantly less at 15°C. Pre-colonization of filter papers by other organisms further reduced subsequent colonization by R. solani. However, the activity of isolate 92 was restricted more by urea amendment of soil than by the pre-colonization of filter papers by other organisms.

The effect of urea on the viability and mycelial density of isolate 92 was also studied over the same temperature range in unsterilized soil of 4.9 percent moisture content (pF 2.5). The results (Fig. 56) indicated that urea reduced viability and mycelial density of the fungus in unsterilized soil at 15°, 20° and 25°C for at least 35 days. No viable hyphae were observed in urea-amended soil at 30°C, but viability was also very low in unamended soil at this temperature. It is probable that the antagonism of other soil organisms was responsible for the low viability at this temperature. In unamended soil there were no significant differences between the numbers of viable hyphae over the temperature range 15° - 25°C; mycelial density, however, was least and greatest at 15° and 25°C respectively. These results suggest viability is independent of mycelial density and is probably determined more by

Fig.56.



availability of food material than by any change of temperature within the range 15°-25°C.

The effect of urea on the mycelial density of isolate 92 was also examined over the same temperature range in gamma-irradiated soil of 9.0 percent moisture content (pF1.76). The results (Table 51) showed that urea reduced mycelial density at all temperatures, but significantly less at 20°-25°C than at 15°C or 30°C. In contrast with the previous experimental results, a soil temperature of 30°C did not inhibit growth in unamended soil and the mycelial density was not significantly different over the range 20°-30°C. Growth in irradiated soil at 30°C further supports the hypothesis that activity of the fungus was inhibited in unsterilized soil at the same temperature by antagonistic soil organisms.

It is interesting to note that the effect of temperature on mycelial density in unamended irradiated soil is similar to its effect on colony diameter in culture (Fig. 44). These results are similar to those of Henry (1932) concerning the influence of soil temperature and soil sterilization on the activity of Ophiobolus graminis.

(d) Effect of Urea at Different Concentrations.

The effect of different concentrations of urea on filter paper colonization by isolate 92 was examined in unsterilized soil of 6.3 percent moisture content (pF 2.05). Urea was applied at concentrations equivalent to 0, 40, 80,

TABLE 51.

EFFECT OF UREA ON POPULATION OF ISOLATE 92 IN IRRADIATED SOIL AT VARIOUS TEMPERATURES.

Soil Temperature (°C)	Soil Amendment	Number of Hyphal Fragments per gram of soil		Analysis of Total Numbers (Using Transformation $Log_e X$)
		Viable	Total	
15	Nil	1013	1037	6.9440
	Urea	44	46	3.7051
20	Nil	1214	1243	7.1248
	Urea	103	109	4.6905
25	Nil	1584	1605	7.3807
	Urea	148	159	5.0655
30	Nil	1065	1109	7.0112
	Urea	24	40	3.6308

Difference for Significance $P = 0.05$ 0.4887

$P = 0.01$ 0.6733

120, 160 and 200 mg. nitrogen per Kg. soil. Filter paper colonization by isolate 92 decreased with increasing concentrations of urea, the greatest reduction occurring at 80-120 mg. nitrogen (Table 52). Pre-colonization of filter papers by other organisms also caused a significant reduction in subsequent colonization by isolate 92; the effect of pre-colonization in unamended soil was approximately equivalent to applying urea at a concentration of 80-120 mg. nitrogen per Kg. soil.

The effects of the same concentrations of urea on viability and mycelial density of the isolate were studied in unsterilized soil of 5.0 percent moisture content (pF 2.45). Both viability and mycelial density were reduced with increasing quantities of urea, the effect being still evident after 35 days (Fig. 57). In irradiated soil of 9.5 percent moisture content (pF 1.73), increasing concentrations of urea correspondingly reduced the mycelial density of isolate 92 (Table 53) thereby indicating its direct inhibitory effect upon the fungus.

The effect of these urea concentrations on the severity of Rhizoctonia root-rot symptoms in wheat was examined in unsterilized soil inoculated with one mycelial mat of isolate 92 per Kg. soil. The following method was used: Wheat seeds (five per pot) were sown in a series of pots each containing 250 mg. of inoculated urea-amended soil adjusted to a moisture content of ten percent (pF 1.72). The pots were watered daily thereafter to a constant weight and seedling

TABLE 52.

EFFECT OF UREA ON COLONIZATION OF FILTER PAPER BY ISOLATE 92.

Concentration of Urea mg. N/Kg. soil	*Number of Pieces Colonized When Using:		Analysis of Colonization (Using Transformation $Log_e(X+1)$)	
	Sterile Paper	Paper Pre-colonized by Other Organisms.	Sterile Paper	Pre-colonized Paper
0	49	19	3.9119	2.9753
40	41	10	3.7292	2.3867
80	30	7	3.4300	2.0297
120	10	3	2.3900	1.2296
160	6	2	1.8877	0.9635
200	2	0	0.9635	0.2311

Difference for Significance P = 0.05 0.4330

P = 0.01 0.5868

* Maximum Number = 50.

Fig. 57

EFFECT OF DIFFERENT CONCENTRATIONS OF UREA ON POPULATION OF ISOLATE 92 IN UNSTERILIZED SOIL

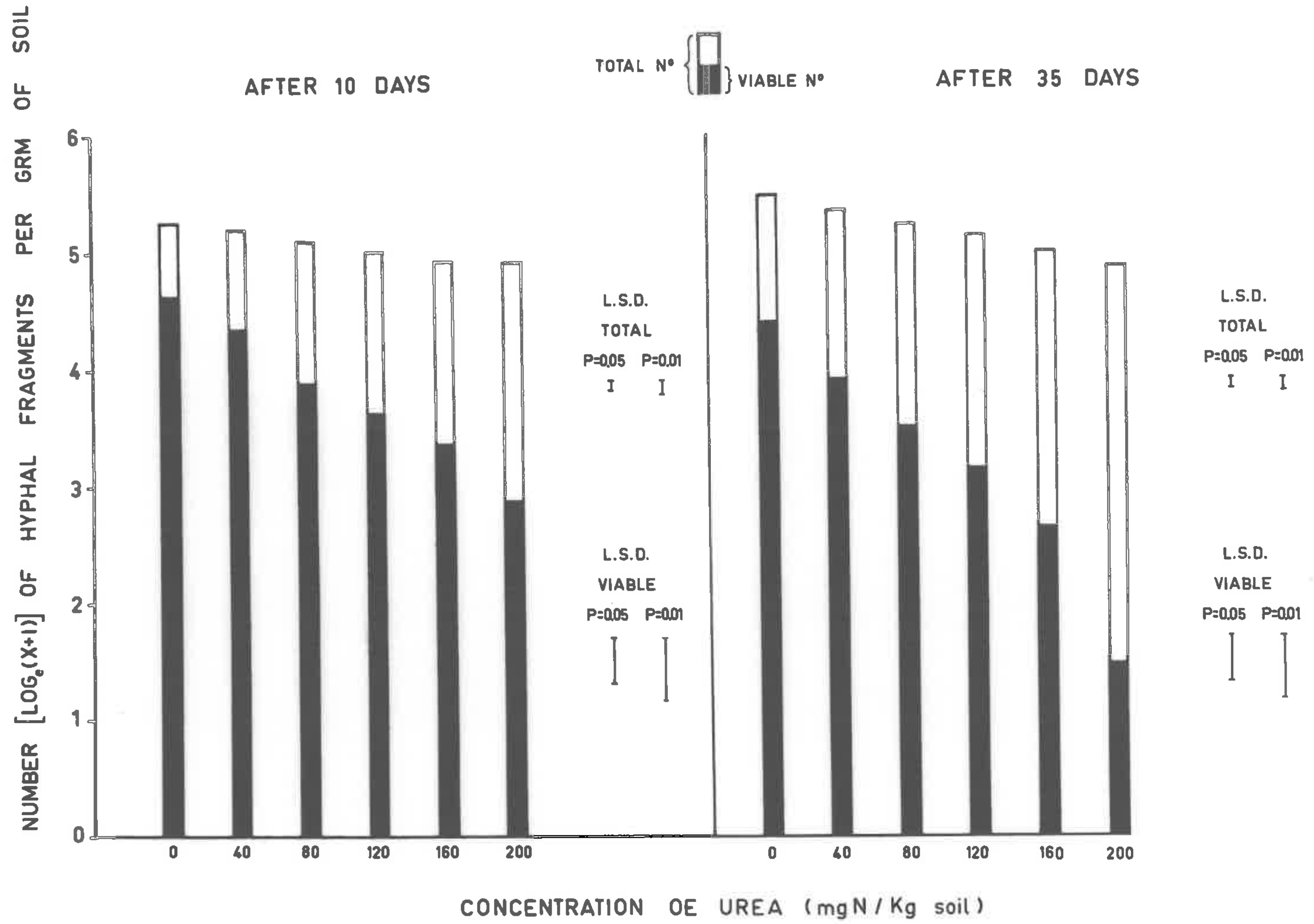


TABLE 53.

EFFECT OF DIFFERENT CONCENTRATIONS OF UREA ON POPULATION OF ISOLATE 92 IN IRRADIATED SOIL

Concentration of Urea (mg. N/Kg. soil)	Number of Hyphal Fragments per Gram of Soil		Analysis of Total Numbers (Using Transformation Log_e X)
	Viable	Total	
0	1588.0	1593.0	7.3718
40	1201.0	1201.0	7.0906
80	844.7	848.0	6.7389
120	237.0	244.0	5.4949
160	115.5	116.8	4.7521
200	22.2	23.2	3.0057
		Difference for Significance P = 0.05	0.4940
		P = 0.01	0.6926

numbers were reduced to four per pot after emergence. Three replications of the treatments were kept in a glasshouse with a temperature range of 20°-30°C for the test period of 35 days. The plants were then removed from the soil and the tops cut off and weighed. The three oldest roots were examined under a dissecting microscope for symptoms of <u>Rhizoctonia</u> root rot and for injuries by urea. Measurements were taken of the length of each root, the length discoloured by infection, the distance of hyphal growth along the root and the length killed by urea. The viable and total numbers of <u>R. solani</u> hyphae per gram of soil were determined for each pot. A second similar series of plants were grown in urea-amended but uninoculated soil. The results are given in Tables 54 and 55 and illustrated in Figs. 58 and 59.

As the root strain causes roots to rot back to stumps, the length of the whole root gives a better measure of the severity of symptoms than does the amount of root discolouration or the hyphal growth along the roots (Table 54). Thus the results show a decrease in the severity of <u>Rhizoctonia</u> root rot in artificially infested soil with increasing concentrations of urea; this was also reflected in the greater fresh weights of tops at the higher urea concentrations. As noted previously, increasing concentrations of urea reduced viability and mycelial density of isolate 92 (Table 55). However, even at the highest urea concentrations there were still viable hyphae which were able to attack the roots of

TABLE 54.

EFFECT OF UREA AS A SOIL AMENDMENT ON SEVERITY OF RHIZOCTONIA ROOT ROT OF WHEAT.

Concentration of Urea (mg. N/ Kg. Soil)	Fresh Weight of Tops (mg.)	Root Measurements Length (in mm.) of:				Analysis of Data (Using Transformation $Log_e X$)				
		Root Killed by Urea	Root Discolored by *R. solani*	Hyphal Growth Along Root	Whole Root	Fresh Weight of Tops	Root Killed by Urea	Root Discolored by *R. solani*	Hyphal Growth Along Root	Whole Root
Soil Uninoculated										
0	254	0	0	0	237	5.5287				5.4552
40	272	0	0	0	185	5.5767				5.2150
80	293	0	0	0	171	5.6383				5.1383
120	230	0	0	0	123	5.4254				4.8087
160	284	2	0	0	98	5.6419	0.6932			4.5845
200	220	8	0	0	64	5.3765	1.9844			4.1507
Soil Inoculated with One Mycelial Mat per Kg. Soil										
0	87	0	4	4	11	4.4517		1.2459	1.2296	2.0579
40	105	0	7	5	14	4.6568		1.7824	1.6121	2.4985
80	84	0	6	4	15	4.4258		1.7918	1.4392	2.6573
120	141	0	8	5	44	4.9500		2.0742	1.5513	3.7718
160	172	2	16	11	50	5.1446	0.4621	2.7409	2.2992	3.8926
200	189	10	5	8	64	5.2409	2.2607	1.4769	1.9938	4.1515
Differences for Significance P=0.05						0.3163	1.1142	0.7348	N.S.	0.6770
P=0.01						0.4286	1.6210	-		0.9175

TABLE 55.

EFFECT OF DIFFERENT CONCENTRATIONS OF UREA

ON POPULATION OF R. SOLANI IN ARTIFICIALLY INFESTED SOIL

Concentration of Urea (mg. N/Kg. soil)	Numbers of Hyphal Fragments		Analysis of Population Estimates (Using Transformation Log_e X)	
	Viable	Total	Viable	Total
0	105.3	258.9	4.6537	5.5541
40	86.6	240.5	4.4589	5.4825
80	71.0	209.3	4.2631	5.3432
120	51.4	177.1	3.9365	5.1759
160	46.4	180.0	3.8286	5.1906
200	34.0	176.3	3.5226	5.1717

Differences for Significance

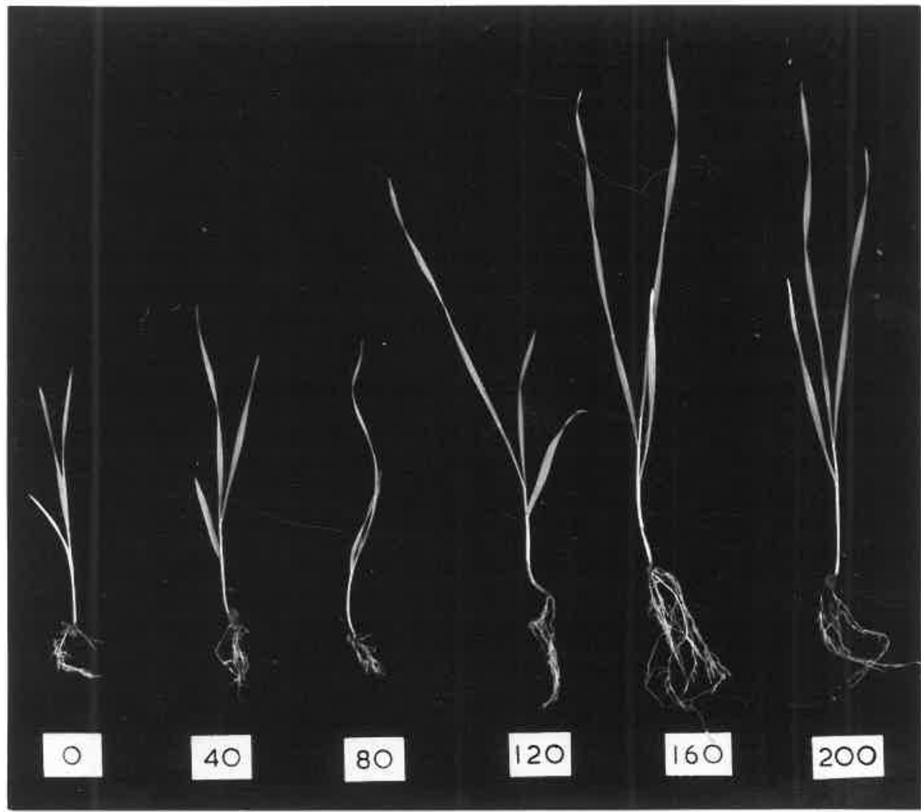
		Viable	Total
P = 0.05		0.1863	0.1039
P = 0.01		0.2613	0.1456

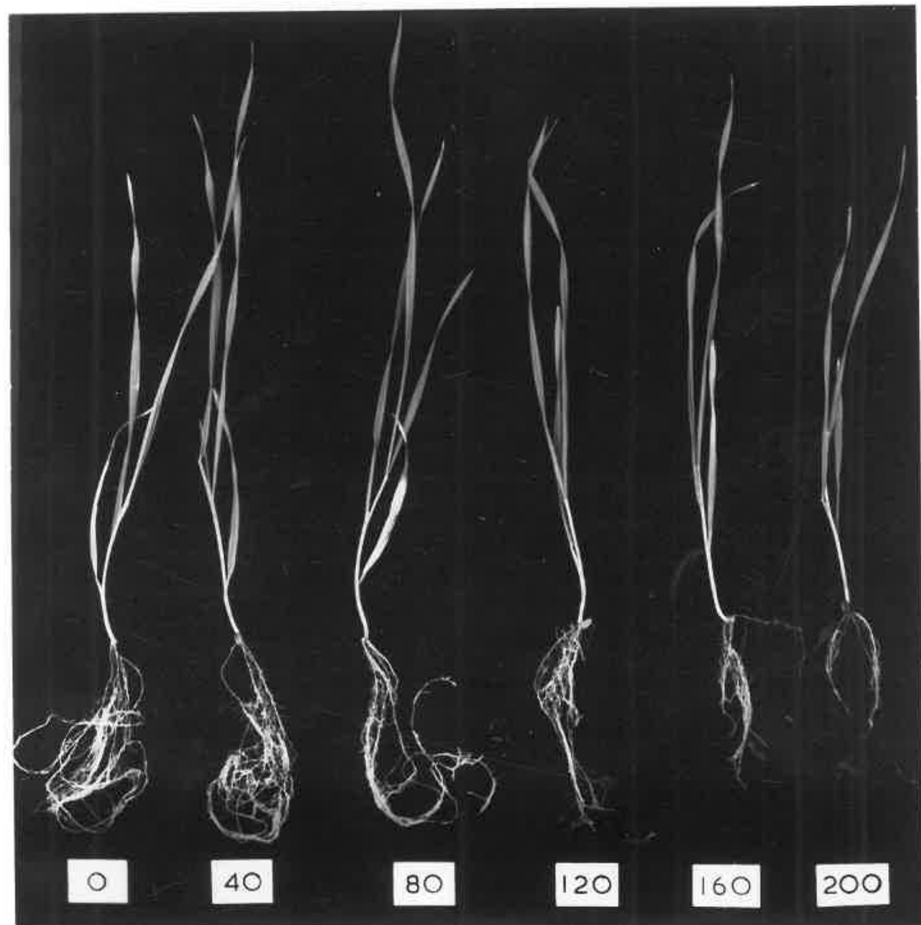
Fig. 58. Appearance of wheat seedlings grown in soil inoculated with isolate 92 of R. solani and amended with urea at concentrations of 0, 40, 80, 120, 160 and 200 mg. nitrogen per Kg. soil.

Mag. x1/3 nat. size.

Fig. 59. Appearance of wheat seedlings grown in uninoculated soil amended with urea at concentrations of 0, 40, 80, 120, 160 and 200 mg. nitrogen per Kg. soil.

Mag. x1/3 nat. size.


0
40
80
120
160
200


0
40
80
120
160
200

wheat seedlings.

In uninoculated soil the mycelial density of R. solani was determined as 1.6-2.9 hyphal fragments per gram and viability considerably less than one fragment per gram. As no root infections were caused by this natural population of R. solani (Table 54), it was possible to study the effect of urea on root systems without complications from infection by the pathogen. The first evidence of any effect of urea was a slight off-white discolouration of seminal roots at a concentration of 80 mg. nitrogen per Kg. soil. At 120 mg. nitrogen, the seminal roots were slightly yellow and the tips were discoloured more deeply than the proximal parts; the secondary roots were unaffected. At 160 mg. nitrogen, seminal roots were yellow and their tips dead; most secondary roots were unaffected but the tips of some were yellow. At 200 mg. nitrogen, the seminal roots were golden brown in colour and their distal parts were dead for several millimeters; secondary roots were yellow and their branches were shortened and thickened. Increasing concentrations of urea also caused a decrease in root length; fresh weights, however, were not reduced significantly (Table 54). Thus it is evident that increasing concentrations of urea not only reduce the soil population of the root strain but also cause damage to wheat seedlings. Both consequences should therefore be considered in any application of urea to a crop.

(e) Cultural Studies with Urea.

In order to study any morphological changes caused by urea, isolate 92 was grown on PMD amended with urea at concentrations of 0, 40, 80, 120, 160, 200 and 400 mg. nitrogen per litre of medium. The procedure was to autoclave an aqueous urea solution for seven minutes at 120°C and then to add the required concentration to melted agar cooled to 50°C. The media (pH 7.0) was poured into Petri dishes, inoculated and then incubated at 20°C for five days; colonies were examined daily.

Cultural appearance was not affected by increasing concentrations of urea (Fig. 60) and no morphological abnormalities were detected by microscopic examination of mycelium. Colony diameters were not significantly affected by urea amendment of medium.

Because of these results, the effect of urea was further studied by growing isolate 92 on defined media (page 121), differing in carbon source but having 0.04 M urea as the sole nitrogen source. This represented a concentrat-ion of 1120.6 mg. nitrogen per litre of medium. Mycelial dry weights were determined after five days' incubation at 25°C and indicated that the fungus could utilize urea (Table 56). Of particular interest, was the significantly greater dry weight with fructose than with any other carbon source. However, chemical changes had probably occurred in the "fructose" medium during autoclaving, as it had developed a

Fig. 60. Colonies of isolate 92 grown on PMD amended with urea at concentrations of 0, 40, 80, 120, 160, 200 and 400 mg. nitrogen per litre of PMD.

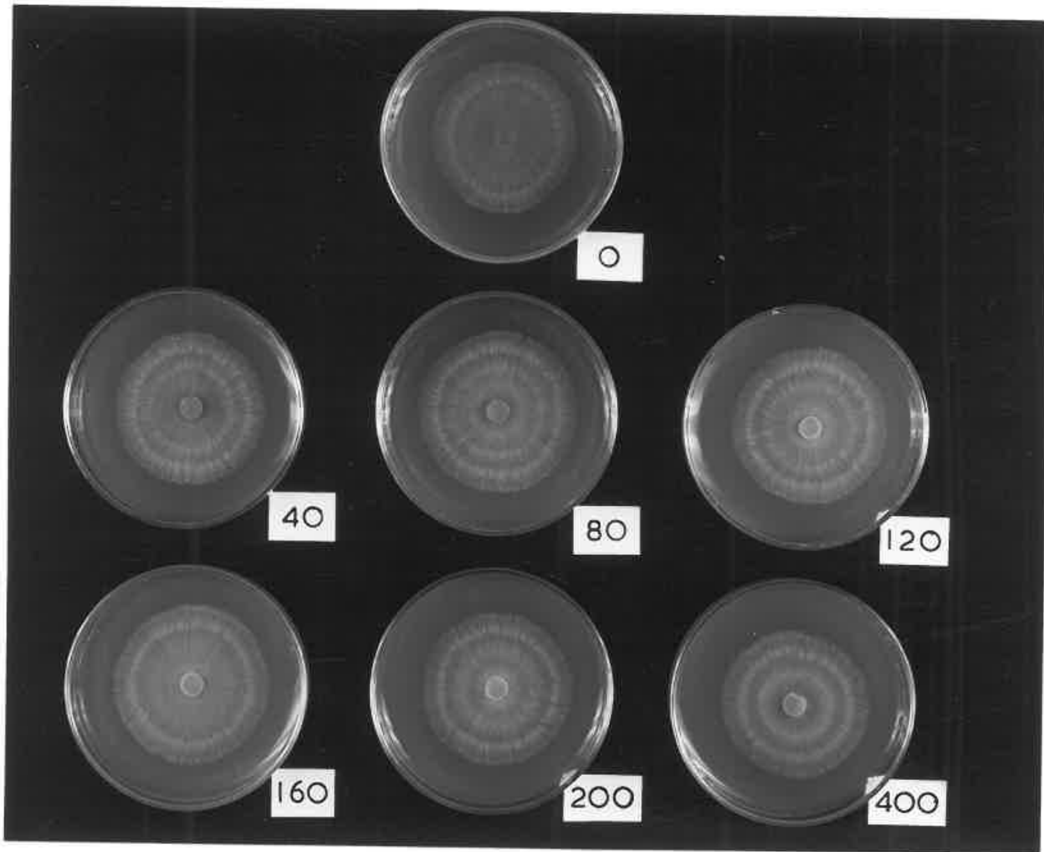

0
40
80
120
160
200
400

TABLE 56.

MYCELIAL DRY WEIGHT OF ISOLATE 92 GROWN ON MEDIA HAVING UREA AS THE NITROGEN SOURCE BUT DIFFERING IN CARBON SOURCE.

Carbon Source	Mycelial Dry Weight (mg.)	Analysis of Dry Weights (Using Transformation $Log_e X$)
L-Arabinose	14	2.6621
D-Xylose	24	3.1615
D-Fructose	107	4.6714
D-galactose	19	2.9573
D-Glucose	44	3.7826
D-Mannose	42	3.7221
Lactose	12	2.4508
Maltose	46	3.8170
Sucrose	24	3.1864
Inulin	10	2.2675
Starch	28	3.3399
Nil - (inoculum only)	3	1.1945

Difference for Significance $P = 0.05$ 0.1670

$P = 0.01$ 0.2228

brown colour.

The mycelial dry weight of isolate 92 was therefore determined on two "fructose-urea" defined media which differed only in the method of preparation. One was prepared as above by autoclaving all the ingredients in one mixture; the second, by autoclaving each of the fructose and urea components separately from the other ingredients and mixing the three sterile solutions after cooling. The pH was determined for both as 7.1. As the pH of the experimental soil used for urea studies was 8.5, half the flasks of each medium were adjusted to this pH with N/10 sodium hydroxide before autoclaving. The alkali was added to the "other ingredients" component of the second medium. During autoclaving, the first medium became brown in colour and pH fell to 7.4 in those flasks previously adjusted to 8.5; these flasks were discarded. No apparent change occurred in the components of the second medium and pH was not affected by autoclaving. Eight inoculated flasks of each medium were incubated at 25°C for six days.

The results (Table 57) demonstrated that isolate 92 was able to utilize urea as a nitrogen source in culture, irrespective of the method used for preparing the media. Excellent growth occurred at pH 7.1 but it was considerably reduced at pH 8.5. It is not known whether this was due to pH alone or due in part to some inhibitory effect of urea. Microscopic examination did not show any morphological abnormality of peripheral hyphae in colonies grown at pH 8.5.

TABLE 57.

MYCELIAL DRY WEIGHTS OF ISOLATE 92 WHEN GROWN ON THREE "FRUCTOSE - UREA" MEDIA.

Preparation of Medium	pH of Medium		Mycelial Dry Weight (mg.)	ANALYSIS OF DRY WEIGHTS (Using Transformation Log_e X)
	Initial	Final		
Ingredients mixed and autoclaved	7.10	7.05	174	5.1522
Fructose, Urea autoclaved separately	7.10	7.15	120	4.7689
Fructose, Urea autoclaved separately	8.50	8.00	22	3.0462
Difference for Significance			P = 0.05	0.2213
			P = 0.01	0.3012

Previously Samuel and Garrett (1932) found that colony diameter was considerably reduced on potato-dextrose-agar by any increase in pH over the range 7.0-9.0.

(f) Effects of Fructose and Urea Soil Amendments.

Because of the above results, the mycelial density of isolate 92 was studied in irradiated soil amended with either fructose, urea or both. Fructose and urea were applied at concentrations of 100 mg. carbon and 200 mg. nitrogen respectively per Kg. soil of 9.0 percent moisture content (pF 1.76). The amendments were sterilized either by autoclaving at 120°C for seven minutes or by filtering through sintered glass funnels of 0.0017 mm. porosity. Similar results were obtained by both sterilization methods, the inhibitory effect of urea on isolate 92 being reduced by fructose amendment of soil (Table 58). Thus a significantly greater mycelial density of isolate 92 occurred in soil amended with fructose and urea than in soil amended with urea alone.

The combined effect of fructose and urea on isolate 92 was further studied in irradiated soil amended with urea at concentrations equivalent to 0, 40, 80, 120, 160 and 200 mg. nitrogen per Kg. soil. Fructose was used at the same concentration as above and the soil moisture content was 9.2 percent (pF 1.75). As previously, the inhibitory effect of urea (200 mg. N/Kg. soil) on isolate 92 was reduced by

TABLE 58.

EFFECTS OF FRUCTOSE AND UREA ON POPULATION OF ISOLATE 92 IN IRRADIATED SOIL

Soil Amendment	Method of Sterilizing	Number of Hyphal Fragments per gram of soil		Analysis of Total Numbers (Using Transformation $Log_e X$)
		Viable	Total	
Nil		974.0	977.0	6.8844
Fructose	Filtered	1264.0	1277.0	7.1518
Fructose	Autoclaved	1325.0	1360.0	7.2123
Fructose & Urea	Filtered	81.6	85.1	4.4322
Fructose & Urea	Autoclaved	84.4	86.7	4.4610
Urea	Filtered	2.9	4.0	1.3497
Urea	Autoclaved	0.8	1.3	0.1431

Difference for Significance	$P = 0.05$	0.4935
	$P = 0.01$	0.6849

fructose amendment of soil (Table 59). Furthermore, at a concentration of 80 mg. nitrogen, urea had no significant effect on isolate 92 when fructose was added to the soil. This contrasted with an earlier result (Table 53) when the same urea concentration, in the absence of fructose amendment, caused a significant reduction in the total number of hyphal fragments of the fungus. Thus it appears that fructose may nullify any inhibitory effects of the lower urea concentrations on the mycelial density of the root strain. It is possible that other organic compounds may also influence the restrictive effect of urea and their presence or absence in a test soil could contribute to experimental variability. When soil was amended with fructose and urea (40 mg. N/Kg. soil), a significant increase occurred in the mycelial density of isolate 92. It is doubtful whether the increase was due to any utilization of urea as a nitrogen source as a similar increase occurred in soil amended with fructose alone.

The effects of the above concentrations of fructose and urea on the viability and mycelial density of isolate 92 were also examined in unsterilized soil of 7.4 percent moisture content (pF 1.89). The results (Fig. 61) were complicated by the reactions of other soil organisms to the amendments and were therefore more difficult to interpret than those of the irradiated soil experiments. For example, during the first ten days, viability and mycelial density of isolate 92 was reduced less by amendments of fructose and urea

TABLE 59.

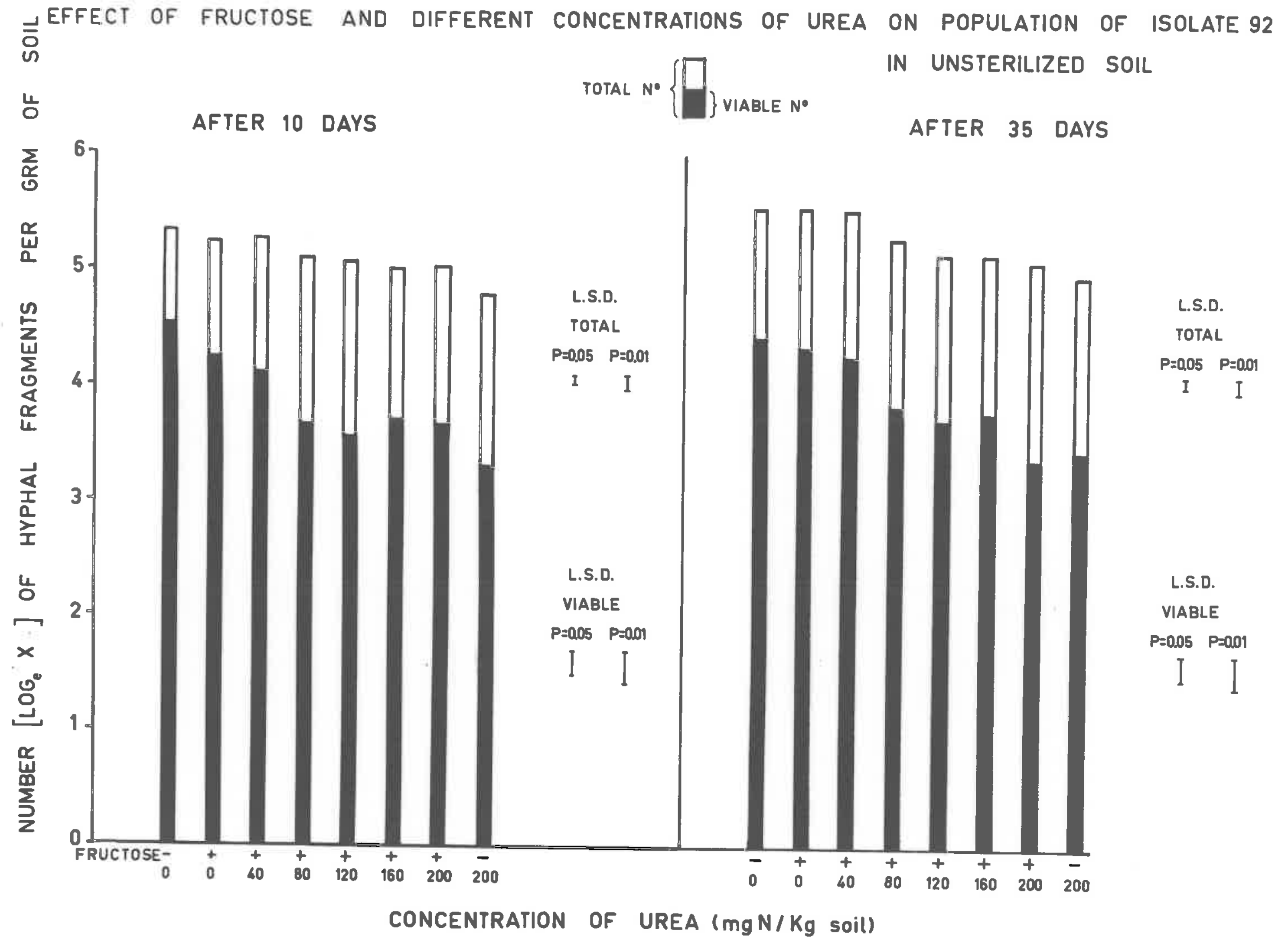
EFFECTS OF FRUCTOSE AND DIFFERENT CONCENTRATIONS OF UREA

ON POPULATION OF ISOLATE 92 IN IRRADIATED SOIL.

Soil Amendment (mg. Carbon (C) as Fructose and mg. Nitrogen (N) as Urea per Kg. soil)	Numbers of Hyphal Fragments per Gram of Soil		Analysis of Total Numbers (Using Transformation $Log_e X$)
	Viable	Total	
Nil	1441.0	1456.0	7.2833
100C	1835.3	1866.7	7.5318
100C + 40N	1934.3	1973.3	7.5869
100C + 80N	1555.3	1592.0	7.3719
100C +120N	962.0	978.7	6.8861
100C +160N	600.7	610.7	6.4130
100C +200N	442.3	448.0	6.1017
200N	261.0	266.7	5.5844

Difference for Significance	P = 0.05	0.0951
	P = 0.01	0.1310

Fig. 61



(200 mg. N/Kg. soil) than by urea alone. However, by 35 days viability was similar in both treatments, although mycelial density was still less in soil amended with urea alone. On the other hand, an amendment of fructose caused an initial reduction in viability and mycelial density, but by 35 days the viable and total number of fragments were not significantly different from those in unamended soil. The most marked reduction in viability occurred at a urea concentration of 80 mg. nitrogen/Kg. soil, but little change occurred when the concentration was increased from 80 to 160 mg. nitrogen. This contrasts with previous results when viability continued to fall over the same range of urea concentration in the absence of fructose amendments (Fig. 57).

(g) Survival of *Rhizoctonia solani* and *Ophiobolus graminis* in Urea-Amended Soil.

Wheat straws were artificially infected with *R. solani* (isolate 92) and *O. graminis* (isolate W1) and buried in unsterilized soil in two series of jars; urea was added to one series and the other was not amended. Soil moisture was adjusted to 7.1 percent (pF 1.9). The natural population of *R. solani* in the soil at commencement was 1.6 viable hyphal fragments per gram. At intervals, straws were unearthed and tested for viable hyphae of the fungi; the total and viable population of *R. solani* per gram of soil was also determined.

Urea increased the survival of *O. graminis* on straw

(Table 60) thereby indicating that it has no inhibitory effect upon the fungus. Thus the results are in accordance with Garrett's (1940) observations that all forms of nitrogen increase the longevity of O. graminis in straw. However, urea reduced the root strain's survival on straw and also its viable population in the surrounding soil (Tables 60 and 61). In view of these and previous results, it is probable that improving soil fertility may reduce the incidence of the root strain of R. solani in cereal crops, but simultaneously may increase the prevalence of O. graminis.

(h) Effect of Urea on the Incidence of Bare Patch in the Field.

De Beer (1965) noted that the application of ammonium sulphate (37 lb./acre) reduced the number of bare patches in a barley crop at Moonta. The effect of urea was therefore compared with that of ammonium sulphate in a further field experiment at Moonta.

In May 1966, part of a two year old "oats-feed mixture" pasture was divided into 18 plots, each measuring 50 metres by 4.5 metres and cultivated to a depth of four inches. On June 11, the plots were sown with Maltworthy barley, fertilizer being applied to give three randomized treatments in each of six replications. Urea and ammonium sulphate were each applied to six plots at the rate of 17 lb. nitrogen per acre, whilst the remaining six plots received no nitrogen. All plots were given a superphosphate amendment

TABLE 60.

PERCENTAGE OF WHEAT STRAWS IN WHICH <u>R. SOLANI</u> (ROOT STRAIN) AND <u>O. GRAMINIS</u> SURVIVED WHEN BURIED IN UNSTERILIZED SOIL WITH OR WITHOUT UREA.

Survival Period (weeks)	Percentage of Straws Containing Viable Hyphae of:			
	Ophiobolus graminis		*Rhizoctonia solani*	
	+ Urea	- Urea	+ Urea	- Urea
0	100	100	100	100
4	100	100	97	100
8	-	-	86	97
12	94	64	34	52
24	64	7	-	-

TABLE 61.

EFFECT OF UREA ON SOIL POPULATION OF *R. SOLANI* ORIGINATING FROM BURIED WHEAT STRAW.

Time of Estimate	Soil Amendment	Hyphal Fragments per gram of Soil		Analysis of Population Estimates (Using Transformation Log_e X)	
		Viable	Total	Viable	Total
At 4 weeks	Nil	72.1	156.4	4.2742	5.0513
	Urea	14.9	59.4	2.6904	4.0817
At 8 weeks	Nil	138.8	427.4	4.9312	6.0566
	Urea	28.1	120.4	3.3269	4.7902
At 12 weeks	Nil	69.7	661.0	4.2330	6.4926
	Urea	4.4	247.0	1.4128	5.4989
		Differences for Significance	P = 0.05	0.2474	0.7415
			P = 0.01	0.3301	0.9892

amendment at the rate of 80 lb./acre. Seven weeks later, the number of bare patches (Fig. 62) was counted in each plot. The results (Table 62) show that numbers of bare patches were reduced significantly by both urea and ammonium sulphate treatments, but there was no significant difference between the two nitrogenous treatments. It is not known whether the observed effect of nitrogenous fertilizer was due to increased growth of the barley plants or to a suppressive effect on the fungus. Some normal-sized plants outside bare patches were found to have one or more rotted, stumpy seminal roots bearing *R. solani* hyphae. Plants which received ammonium sulphate were slightly taller than those dressed with urea. **

** This experiment was carried out jointly with Mr. A. Dube, Honours Student, Department of Plant Pathology, Waite Agricultural Research Institute.

Fig. 62. "Bare Patch" in experimental barley plots at Moonta, August, 1966.

TABLE 62.

EFFECT OF NITROGENOUS FERTILIZERS ON INCIDENCE OF BARE PATCH IN A BARLEY FIELD AT MOONTA, STH. AUST.

Fertilizer	Mean Number of Patches per Plot
Urea (17 lb. N/acre)	3.83
Ammonium Sulphate (17 lb. N/acre)	4.67
Nil	12.67

Difference for Significance

$P = 0.05$ 4.26

DISCUSSION.

The activity and survival of the root strain of *Rhizoctonia solani* was reduced in unsterilized soil under laboratory conditions by a variety of nitrogenous amendments. This suggested that improvements in soil fertility may reduce the root strain population in the field and also that the population may vary within a field with variations in soil fertility. Some supporting evidence for this view was provided by de Beer (1965), who compared the root strain populations in sandy and heavy soils of the same field and recorded a lower population in the heavier but more fertile soil.

Studies on the root strain population in gamma-irradiated soil suggested that urea was the only test amendment which had a direct inhibitory effect; the other nitrogen amendments were therefore assumed to indirectly curb the activity of the root strain by stimulating the competitive and antagonistic activity of other soil organisms. Cultural studies, however, demonstrated that the root strain was able to utilize urea as a source of nitrogen; whereas the fungus was intolerant of urea (200 mg. N/Kg. soil) in irradiated soil, it grew well on a defined "fructose" medium containing urea as the sole source of nitrogen (1120 mg. N/litre medium). Nevertheless, growth in culture was reduced considerably by increasing the pH from 7.1 to 8.5, the pH of irradiated Moonta soil. Haenseler and Moyer (1936) noted that,

depending on soil reaction and soil type, amendments of calcium cyanamide could have a stimulative or depressive effect upon specific soil organisms. This influence of soil reaction was explained by Crowther and Richardson (1932), who observed variability in decomposition products of calcium cyanamide depending on hydrogen-ion concentration. Thus, aqueous solutions of calcium cyanamide formed free cyanamide which polymerized to dicyanodiamide in alkaline solutions, but hydrolysed to urea in acid conditions. It is possible that soil reaction may likewise influence the decomposition of urea in unsterilized soil and that urea may have an inhibitory effect on the root strain in alkaline but not in acid soils. This could partly explain the contrasting results obtained with urea in culture and in irradiated soil, but in any case it will be profitable to further examine the effect of urea in a variety of irradiated soils of pH 7.0 or less.

Although almost all soil fungi are eliminated by 0.5 megarads gamma-irradiation (Davis, Sheldon and Auerbach, 1956; Newbould and Lucas, 1959; Stotsky and Mortensen, 1959; McLaren, Luse and Skujins, 1962) and practically all soil organisms by 2.5 megarads (McLaren, Reshekto and Huber, 1957; Bowen and Rovira, 1961; Popenoe and Eno, 1962; McLaren, Luse and Skujins, 1962), urease activity may continue to function in such soils (McLaren, Reshekto and Huber, 1957; McLaren, Luse and Skujins, 1962). It

is not known whether urease activity influenced the observed inhibitory effect of urea on the root strain in irradiated soil, but it is a factor which should be considered in any future work. Fructose amendments reduced the inhibitory effect of urea and it is probable that a number of other organic compounds likewise influence the restrictive effects of urea; thus the presence or absence of such compounds in the test soil could contribute to experimental variability.

Urea exercised a restrictive effect on the activity and survival of the root strain over the soil moisture range 4.7 - 9.4 percent and the temperature range 15°-25°C; at 30°C the inhibitory effect of urea was masked by the competitive and antagonistic activities of other organisms in unsterilized soil but it was apparent in irradiated soil. The effect of urea on filter paper colonization was influenced by temperature, fewer papers being colonized by the root strain at 15°-20°C than at 25°C. Pre-colonization of filter papers by other organisms also reduced subsequent colonization by the root strain; this restrictive effect in one experiment was equivalent to a urea concentration of 80 - 120 mg. nitrogen/ Kg. soil. However, the influence of other soil organisms on the root strain will vary depending on the organisms concerned, their prevalence and activity and the prevailing environmental conditions. For example, experimental evidence suggested that antagonistic

organisms considerably reduced root strain activity in unamended soil at 30°C but their influence was not obvious at lower temperatures.

Increasing concentrations of urea correspondingly reduced the activity and viability of the root strain, but higher urea concentrations also caused root injury to wheat seedlings. Both of these consequences should therefore be considered in any application of urea to a crop. In the field trial at Moonta, barley plants were slightly smaller in the urea-amended plots than in the ammonium sulphate plots. This difference was probably due to greater growth response to ammonium sulphate rather than to urea injury, as urea-treated plants were not inferior to the control plants.

The use of nitrogenous fertilizers for the control of bare patch has distinct possibilities, especially as laboratory studies indicate that nitrogenous soil amendments may exercise a restrictive effect for at least 35 days. Thus the application of a suitable nitrogenous fertilizer at seeding could restrict the activity of the root strain during the most susceptible period of crop growth. Should soil reaction affect the decomposition of some fertilizers (calcium cyanamide and possibly urea), it may result in different fertilizers being recommended for different districts. For example, urea may prove the most satisfactory fertilizer in South Australia, as most soils in

which bare patch occurs have a pH in the range 8.0 - 9.0; in New South Wales, however, it is possible that another fertilizer may be more suitable as Hynes (1937a) reported bare patch mostly in soils with a pH in the range 6.0 - 6.3.

Although these studies have been concerned with nitrogenous soil amendments, soil fertility may also be improved by sowing legume pastures. Ludbrook, Brockwell and Riceman (1953) and Kerr (1955) reported that a large number of pasture legumes were susceptible to the fungus. Thus the establishment of the pasture may be difficult, but the application of a suitable nitrogenous fertilizer at seeding may restrict the activity of the root strain sufficiently to overcome this problem. Subsequent increased stocking of the pasture with sheep will increase the return of nitrogen to the soil mainly as urea in the urine of the animals.

These laboratory studies have supported de Beer's (1965) differentiation of root strain isolates from Moonta into two cultural types; the observed differences in metabolism and colonizing ability may partly account for Type 1 being the dominant "wild type" in Moonta cereal fields. However, field isolates from different localities may differ considerably in cultural characteristics (Flentje and Saksena, 1957). Thus it will be essential to study a wider range of field isolates from different localities in order to determine whether all are restricted in activity and survival by nitrogen

enrichment of soil. Should any isolates be unaffected by nitrogenous soil amendments, such variants would gradually become the dominant "wild type" of an area as soil fertility was improved. Single spore progeny of one field isolate may also exhibit a wide range of cultural characters (Flentje and Saksena) and it is also possible they may vary in their reactions to nitrogen enrichment of soil. Therefore, by detailed studies with the single spore progeny derived from a "wild type" it may be possible to anticipate the development of variants following improvement in soil fertility.

GENERAL DISCUSSION.

Changes in the relative importance of root pathogens are occurring in Australia because legume pastures are being increasingly used in many cereal areas to improve soil fertility. Butler (1959) described the changes which have occurred in southern New South Wales over the past thirty years. In order to anticipate the consequences of improved fertility, it is essential to study the effect of high levels of soil nitrogen on the saprophytic survival of root pathogens. The data presented here show that increasing soil nitrogen impairs the activity and survival of the root-attacking strain of <u>Rhizoctonia solani</u>, but enhances the survival of <u>Ophiobolus graminis</u>. However, these are only two of the important cereal root pathogens in South Australia and similar data are required for other strains of <u>R. solani</u> and the nematodes <u>Heterodera avenae</u> and <u>Pratylenchus neglectus</u>. It has also been shown that isolates of the same pathogen sometimes differ in activity and survival; it will therefore be necessary to obtain specific information for the "wild types" which occur in each district so that the effects of increasing fertility can be evaluated precisely. To date, legume nitrogen has been the most important source for improving soil fertility, but increasing interest is being shown in the supplementary use of fertilizer nitrogen. It would therefore be worthwhile examining the effects of various

nitrogenous fertilizers on survival of root pathogens in any further studies.

APPENDIX A.

Culture Media Used in This Investigation.

AN Basal Medium (Turner, 1957)

5 g.	NH_4NO_3
1 g.	KH_2PO_4
2 g.	$MgSO_4.7H_2O$
3 g.	Na_2HPO_4
20 g.	glucose
1 mg.	biotin
1 mg.	thiamine

Autoclave glucose separately and afterwards add aseptically to other constituents.

Basal Medium for *O. graminis* (Lilly and Barnett, 1951)

As a Liquid.

2 g.	asparagine
10 g.	glucose
1 g.	KH_2PO_4
0.5 g.	$MgSO_4.7H_2O$
0.2 mg.	Fe^{++} (as citrate)
0.2 mg.	Zn^{++} (as sulphate)
0.1 mg.	Mn^{++} (as sulphate)
5 µg.	biotin
100 µg.	Thiamine
1000 cc.	distilled water

As a Solid.

20 g.	agar.

Maize-Meal Sand (Butler, 1953)

100 g.	sand
3 g.	ground maize meal
13 cc.	distilled water

Potato "Marmite" Dextrose Agar (PMD)

22 g.	"Deb" instant mashed potatoes ("Deb" Brand, Rosella Foods Pty. Ltd., Melbourne)
20 g.	dextrose
15 g.	agar (Davis)
1 g.	"Marmite" (concentrated yeast extract, Sanitarium Health Food Co., Aust.)
1000 cc.	distilled water

Autoclave potato in one litre of water at one atmosphere for ten minutes and filter through macerated filter paper. Melt agar in filtrate, add dextrose, marmite and more distilled water to make up volume to one litre. Dispense and autoclave at one atmosphere for twenty minutes.

Soil Agar.

2 g.	KH_2PO_4
1 g.	Yeast extract (Difco)
1 g.	dextrose
15 g.	agar (Davis)
	Filtrate of 1000 g. air dried Waite soil in 1000 ml. distilled water, obtained by autoclaving for one hour, and filtering after allowing it to settle overnight.
to 1000 mls.	distilled water

Synthetic Liquid Medium for *R. solani* (de Beer, 1965)

0.6 g.	KH_2PO_4
0.87 g.	K_2HPO_4
0.133 g.	$MgSO_4.7H_2O$
17.0 g.	sucrose
7.0 g.	peptone
1 litre	distilled water

Dispense 50 ml. aliquots in 250 ml. flasks. Autoclave at one atmosphere for twenty minutes.

Water Agar.

15 g.	agar
1000 cc.	distilled water.

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