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STUDIES ON THE INCIDENCE AND SURVIVAL OF *OPHIOBOLUS GRAMINIS* IN WHEAT-FIELD SOIL

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SUMMARY

The incidence and survival of *Ophiobolus graminis* Sacc. under a range of conditions was studied. Emphasis was placed on the use of natural 'undisturbed' soil; field soils being studied *in situ* and as soil cores in controlled environments.

Levels of viable *O. graminis* in soil were determined by growing wheat seedlings in soil cores for 4 weeks; an estimate of the *O. graminis* present was made by recording the percentage of roots infected per core. Viable *O. graminis* in wheat stubble was detected by growing wheat seedlings in contact with the remains of individual plants.

A method of using grain yield to estimate the incidence of *O. graminis* in the field was developed. It was found that a map based on yield alone under-estimated the real incidence. However when incidence and yield were coupled by the use of an 'incidence-yield' regression established on a few sites, a map showing estimated levels of *O. graminis* was obtained without destroying the experimental area. Bioassays of stubble or cores were used to establish incidence, but the former gave better results.

'Incidence-yield' regressions were used to study the incidence of *O. graminis* in consecutive crops of wheat at Turretfield and Ceduna. At both locations there were only small differences between the 2 years, although at Turretfield (third and fourth crops) there was a tendency for high incidence areas to contract, while at Ceduna (second and third crops) incidence appeared to increase.

Survival of O. graminis in the field was studied using the bioassay of stubble and of cores containing stubble. There were marked differences in survival between the 2 localities investigated. At Turretfield the percentage of sites with crowns containing viable O. graminis dropped from 96 in late January to 30 in late August, while at Ceduna the drop was from 90 in early February to 82 in mid November.

Cores were used to study the survival of O. graminis in naturally infested soil. There was only a small drop in the incidence of O. graminis in cores removed at regular intervals from a take-all patch at Ceduna over a period of nearly 1 year. When cores removed from a take-all patch at Ceduna in mid-summer were stored, there was no change in the incidence of O. graminis when the soil was maintained in a cool dry or cool moist condition. Even when maintained in a hot dry or cool wet condition there was considerable viable fungus remaining after 45 weeks of storage. As these periods of survival were generally longer than those reported using artificially colonized straws, a comparison was made using the latter buried in naturally infested soil. The fungus in the naturally infested soil generally survived better than the fungus in the straws.

A comparison was also made of the survival of O. graminis in artificially colonized straws stored in similar soils from the same area, but with different cropping histories. Generally, survival decreased with increasing numbers of consecutive crops. It is hypothesized that a 'factor' antagonistic to the saprophytic survival of O. graminis in artificially colonized straws is induced by continuous cropping to wheat. The importance of this 'factor' is unknown, but it may have no significance in the field.

STATEMENT

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University. To the best of my knowledge it contains no material previously published or written by another person, except where due reference is made in the text.

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INTRODUCTION

When reviewing the incidence and importance of cereal root rots in Australia, McKnight (1960) concluded that the important pathogens were *Ophiobolus graminis* Sacc., *Rhizoctonia solani* Kühn, *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, *O. graminis* appears to cause the greatest reduction in yield (Butler, 1961). The disease 'take-all', caused by *O. graminis*, has been recorded in all states of Australia (McAlpine, 1904; Musson, 1907; Sutton, 1920; Simmonds, 1953; Anon., 1959), but is of minor importance in Queensland (McKnight, 1960).

Take-all has been known to exist in South Australia since 1852 (Anon., 1868), but it was not until early in this century that McAlpine (1904) proved the causal organism to be *O. graminis*. Since that time various workers (Richardson, 1910; Gray, 1913; Osborn, 1919; Samuel, 1923, 1924; Griffiths, 1933; Samuel and Garrett, 1933; Garrett, 1934a, b, c; Banyer, 1966) have studied or reported on aspects of the disease and its incidence. Despite this (and the work of many others throughout the world), take-all is still a major problem in South Australia.

The most recent survey of the incidence of cereal diseases in South Australia was conducted by Banyer (Pers. Comm.) in 1964. He found that of 70 crops inspected in the Lower North region of the wheat belt, 37 per cent had 'severe' take-all (estimated 20% or more of field showing visual symptoms), a further 19 per cent were 'moderately' affected (10 to

20%), while the remainder had little or no take-all (less than 10%). This type of survey gives only a general impression of the extent of the problem. There is a need for more intensive studies to provide detailed information about losses caused by O. graminis.

A request was made to the Conference on root rots of wheat held at Wagga Wagga in 1960 (Anon, 1960) for limited but very detailed surveys to be conducted in selected wheat growing areas in consecutive years. It was suggested that "the emphasis on detail and thoroughness should not be lost by attempting to cover too wide an area in the surveys. In addition to providing accurate information on the incidence of disease and its cause, such surveys, if selectively carried through in detail, could give information on the effects of certain farming practices on the incidence of disease, and yield most helpful leads to control."

The need for intensive field studies was the starting point for the investigations reported in this dissertation. My aim has been to study the incidence and survival of O. graminis in the field or in natural soil removed from the field.

In the field, O. graminis is not evenly distributed. A number of attempts (usually with only limited success) have been made to overcome this by the introduction of artificial inoculum (Kirby, 1925; Fellows and Ficke, 1934, 1939; Cunningham, 1967). Unevenness of incidence is inevitable in the field where O. graminis survives in organic debris of various sizes, ranging from intact stem bases and straws to small root fragments (Hornby, 1969b). These infective units tend to be concentrated along the drill row (Hornby, 1969b) and not distributed randomly. Thus even if all

wild types present have similar virulence (Chambers, 1970), the varying distance of the inoculum from the host will lead to unevenness of infection (Fellows and Ficke, 1939). The patchiness of O. graminis incidence in the field and the problem of reliably estimating the level of inoculum present, have made research difficult. However, as crops are grown in the field, time spent on the study of the ecology and biology of O. graminis in that situation is justifiable, despite the difficulties. New techniques are required for this type of study; some of the work that follows describes my attempts to develop and use techniques for the study of O. graminis in the field.

A logical extension was to study survival in the field over a period of 12 months with the assessment of incidence being made under controlled conditions. The removal of intact soil cores from the field, and the study of both survival and incidence in such cores kept in controlled environments was a further development. Finally, a study was made of the survival of O. graminis in artificially colonized straws buried in naturally infested soil stored in controlled environments.

It was hoped that these investigations would contribute to an understanding of O. graminis in its natural environment, thereby leading to the possibility of more effective disease control.

LITERATURE REVIEW

A. Methods of assessing the effect of *O. graminis* on wheat

Nilsson (1969) has given a comprehensive review of methods of testing pathogenicity and measuring disease severity and host resistance. It seems sufficient here to review the more common techniques used.

The testing of the effect of *O. graminis* on wheat seedlings in test-tubes was first carried out by Waters (1920) and subsequently by many others. Due to limited size, test-tubes have usually been confined to seedling tests for specific purposes such as production of perithecia; infectivity of ascospores (Garrett, 1939); or the effect of aeration on infectivity (Garrett, 1937). Nilsson (1969) has used large tubes (2.5 to 7.5 cm diameter and up to 150 cm long) for host resistance investigations. Although in such tubes observation of root growth, disease symptoms and spread of *O. graminis* along roots can be made on plants from seedlings to maturity, considerable equipment is required to handle them. Most other seedling tests have been performed in various sized pots or boxes under glasshouse conditions or in controlled environments. Although seedling tests have the disadvantage of generally not reflecting the expected field results (Butler, 1961), they have found wide acceptance, especially as pathogenicity and virulence tests (Garrett, 1936).

Numerous tests for measuring the incidence of *O. graminis* in the field are mentioned in the literature. There appear to be 2 main approaches used. In the first, the host is grown in the field and then

attempts are made to detect the presence of O. graminis and to estimate its effect on the host (Kirby, 1925). Most investigators employ some type of random sampling to remove the host from the field for assessment of the selected parameters. The second method is to remove soil from the field and then examine it for the presence of O. graminis. Only a limited number of investigations have been reported. Warcup (1957) isolated O. graminis directly from wheat-field soil by 'hyphal isolation' and more recently Hornby (1969a, b, c) has developed methods of extracting and bioassaying organic debris for the presence of O. graminis.

To determine the effect of O. graminis on hosts, methods range from disease ratings or subjective types of assessment to objective measurement of specific parameters. In the former, scaled ratings, usually based on visual assessments of size, colour, amount of dead tissue, etc., are generally expressed as a composite disease index. Many indices are calculated by the formula devised by McKinney (1923):-

$$\frac{\text{Sum of all numeral ratings} \times 100}{\text{Total number of plants assessed} \times \text{highest rating}} = (\text{Disease rating})\%$$

Apart from the obvious disadvantages of subjective assessments (i.e. bias with time or between individual assessors), there are problems with analysis of the results. For instance, analysis of variance is based on the assumption that the population is part of a normal distribution; once a scale with distinct intervals has been introduced, the above assumption is destroyed and the results should not legitimately be analysed by analysis of variance.

Objective methods of measuring parameters avoid the pitfalls

of personal judgement and the biometrical disadvantage mentioned above. Most workers employ more than 1 parameter. Padwick (1936) used an 'injury index' based on leaf length, the index being the difference in height between the infected plant and the control, expressed as a percentage of the control. Turner (1940) used growth of runner hyphae along the 3 oldest roots, top weight, length of discoloured root and on some occasions the percentage of roots infected. Slagg and Fellows (1947) measured the length of the 3 oldest roots and the length of diseased root, expressing the latter as a percentage of the former. Chambers and Flentje (1967a) measured fresh leaf weight, and total length, length discoloured and length of runner hyphae along the 3 oldest seminal roots.

In the field, 1 of the most common parameters used to estimate the effect of O. graminis on wheat is to measure grain yield. This is usually associated with either a record of the percentage of plants, or parts of the plants, infected (Chambers, 1962) or a disease severity rating based on symptoms (Nilsson, 1969).

One form of measurement originally used by Garrett (1934a) is to record the length of runner hyphae along the root. Garrett considered that infection never lagged more than 1 or 2 cells behind the apices of the hyphae. Nilsson (1969), however, reported that: "very extensive internal spread of runner hyphae was observed in the vascular cylinder without any corresponding external runner hyphae spread along the outside of the roots." Also, Russell (1934), Turner (1940), and others have reported runner hyphae growing along roots of plants other than wheat or barley without any evidence of infection. It would appear, as admitted

by Garrett (1941), that measurement of external runner hyphae may not always be a good indication of the extent of infection.

Another common method of determining disease severity has been to measure the length of discoloured root tissue. The main disadvantage with this method is that it is difficult to determine whether discoloration is due to infection by O. graminis or to some other organism or factor; this is particularly so with mature plants. However, with practice, workers assessing seedling roots should be able to correctly interpret the cause of discoloration. Padwick (1936) used the amount of discoloration to make infection ratings and found the results generally agreed with injury indices based on plant height.

Garrett (1941) recorded the number of seminal roots infected; roots with 1 or more lesions causing typical vascular discoloration were held to be infected. No indication of actual severity of infection is given by this method, and there is always the possibility that recent infections without discoloured tissue may be missed. Despite these disadvantages, this form of assessment is suited to the purpose to which Garrett put it, viz. estimation of the relative amount of O. graminis inoculum in different soils.

Measurement of leaf length (Padwick, 1936) has the advantage that it can be assessed without destroying the host. But recording leaf length is time-consuming, especially in older plants, and some decision has to be made as to whether total length or length of the longest leaf is to be used. The main disadvantage is that there is no way of being sure the host is infected without removing the plant from the soil, thus

nullifying the advantage mentioned above.

Leaf weight has the advantage that leaves are easily cleaned and weighed, but the disadvantage of host destruction. There is also the fact that considerable leaf growth can be produced even after plants have sustained substantial root pruning (Simmonds and Sallans, 1930, 1933). However, if infection damages the tissue of the leaf base, as is the case with seedling tests using scutellar-node inoculation, then this latter objection is no longer valid.

Total root length is very difficult to measure but some investigators, using seedlings, have settled for the measurement of a representative number of roots (Turner, 1940; Chambers and Flentje, 1967a). Measurement of root weight, like root length, is destructive but has the advantage of ease of measurement provided the adhering soil can be removed. This is impossible in mature plants and very difficult in seedlings grown in undisturbed natural soil. With seedling tests in soil free of gross organic matter this should not be a problem. One advantage of recording root weight rather than root length is that the roots do not have to be untangled.

Measurement of grain yield is a simple matter in pot experiments with mature plants and in the field (where the only problem is deciding the sample size to be collected). Grain yield has the very real advantage that it is the parameter of most interest to the farmer. However, grain yield alone reveals nothing about the factors, pathogenic or otherwise, that may be present and influencing yield. For this reason it is usually necessary to associate yield with other parameters. The problem then

arises that the host needs to be removed for examination, thus destroying the host and the field situation. For this reason, yield often needs to be correlated with subsampling for the assessment of other parameters (Chambers, 1962). Suzuki et al. (1957) in field experiments, were able to show a close relationship between average yield per plant and infection ratings. Nilsson (1969) has shown that a very strong correlation exists between disease rating (based upon degree of root discoloration) and decrease in grain yield. Slope (1967) and Rosser and Chadburn (1968) have established significant regression coefficients for grain yield and percentage of plants infected.

B. Incidence of O. graminis in the field

Incidence of O. graminis in the field is influenced by 2 primary factors: the spread of the fungus during the parasitic phase, and survival during the saprophytic phase. Secondary factors, like climatic conditions, field management, etc., will influence the primary factors, which in turn will affect incidence and subsequently yield.

If conditions are favourable for both phases throughout 1 seasonal cycle, the incidence of O. graminis will increase from 1 crop to the next. Much early work concentrated on factors affecting increase in O. graminis but since the 1930's, interest has been in situations where conditions are unfavourable to both phases (or at least heavily biased against 1 phase) and there is a subsequent decline in O. graminis incidence (Fellows and Ficke, 1934; Glynne, 1935).

There is also a possibility of what might be called a 'state of

equilibrium'. Although this is difficult to define, it is probably achieved with the balancing out of the 2 phases mentioned above. Garrett (1942) drew attention to this state of equilibrium when pointing out that the amount of O. graminis in the soil at any time will be the result of "the multiplication and spread of the fungus mycelium on the underground parts of its host plant, and the gradual exhaustion and death of this mycelium in the absence of living host plants". Any factor which causes an upset in this equilibrium could result in an increase or decrease in incidence (Fellows and Ficke, 1939; Garrett, 1942).

While the investigations reported in this dissertation are concerned with the incidence of O. graminis in the field, emphasis has been placed mainly on the survival phase. Interest in the parasitic phase is confined to a study of the amount of, rather than the method of spread, however it is appropriate to briefly review the latter subject at this point.

(1) Spread of O. graminis in the field.

There are several ways the fungus can spread inter-field and intra-field. One possibility is aerial dissemination of ascospores (Samuel and Garrett, 1933; Garret, 1934c). Laboratory experiments conducted by Garrett (1939) and Padwick (1939) threw doubt on the ability of ascospores to cause infection of wheat in unsterilized soil. More recently Salt and Hirst (1956) obtained infection of wheat seedlings grown in unsterile soil by ascospores of O. graminis and Brooks (1964, 1965) demonstrated infection of exposed roots on the surface of unsterile soil by ascospores, while Gerlagh (1968) claims inter-pot infection of wheat by

ascospores in glasshouse trials.

Although Gregory and Stedman (1958) were able to trap ascospores in the field, doubt remained about the importance of ascospores, as Gregory and Henden (1967) were unable to demonstrate infection of exposed roots of seedlings in boxes placed in a wheat field containing sporulating perithecia. Gerlagh (1968) presents strong circumstancial evidence that air-borne ascospores are responsible for the infection by O. graminis of grasses on recently reclaimed polders.

Another form of spread is distribution of infected plant material. McAlpine (1904) suggested that O. graminis-infected material could be spread by wind and Samuel (1924) observed wind dispersal of infected pieces of grass. Fellow and Ficke (1939) found that any agency which could transport plant material or soil particles was likely to spread O. graminis, although they found the establishment of O. graminis in new areas was slow and uncertain. Butler (1948) warned against the cultivation of take-all patches in dry dusty soil; this being a possible form of dispersal of infested plant material.

Probably the most important form of spread is along the root systems of hosts (Garrett, 1934a), and in some instances non-hosts (Russell, 1934; Turner, 1940; Chambers, 1971a). It has been demonstrated by Padwick (1935) that O. graminis is unable to spread to any extent in bare unsterilized soil, although Warcup (1957) recovered O. graminis from field soil containing wheat plants by his 'hyphal isolation' method on a few occasions.

The spread of O. graminis along a root system is by the growth

of dark-coloured runner hyphae over the surface of the roots (Garrett, 1934a) and in some instances, in the vascular cylinder (Nilsson, 1969). The soil and climatic factors affecting the rate of extension of runner hyphae along the root have been reviewed by Garrett (1942).

Rates of growth equivalent to 3.2 to 3.7 mm per day have been reported by Garrett (1934a, 1936). Winter (1940) observed a growth rate equivalent to 6.4 mm per day on wheat seedlings grown in sterile humus-sand and 3.7 mm per day in unsterile soil. Nilsson (1969) reported an internal rate of extension equivalent to 8.6 mm per day over a 12 week period, while the external growth on the same root was equivalent to 1.8 mm per day.

With wheat plants growing in boxes of field soil kept in the glasshouse, Fellows and Ficke (1939) demonstrated that O. graminis could spread 25 to 30 cm from infested soil into non-infested soil during 1[illegible] season. In the field, Adam and Colquhoun (1936) were able to demonstrate that spread during a growing season was affected by soil type and plant spacing. They recorded spread of 51 cm along the row from the inoculum when the seedlings were 5 cm apart but no spread when they were 30.5 cm apart. Wehrle and Ogilvie (1956) found infected wheat plants up to 150 cm from the source of inoculum. They also found considerable spread across rows. Suzuki et al. (1957) found a spread along the rows of 60 cm, while they recorded a spread across the rows of 2 m or more. They suggested the latter spread may have been associated with the prevailing wind, however details of the type of propagules spread by the wind was not given in their English summary.

White (1945) studied the distribution of diseased seedlings in the field and concluded that there was a "locality factor" favouring the occurrence of diseased plants. He found that clustering of diseased seedlings along the rows was produced when the rows cut across these localities. He suggested that the locality factors were foci of inoculum.

White (1945) also mapped, in consecutive years, an area of crop containing take-all patches. He found tht although the patches did shift, there was a tendency for the areas to be associated in successive years. In the third consecutive crop, White was unable to map well-defined patches, because most plants were diseased. Although White did not suggest any particular method of dispersal, he considered the foci of _O. graminis_ inoculum had become dispersed over the entire area. The dispersal of foci was presumably by mechanical spread and root contact. However, spread by ascospores cannot be dismissed, as White found that the fungus on at least 64 per cent of the plants from the take-all patches had the potential to produce mature perithecia.

(2) Survival of _O. graminis_.

In his schema for soil fungi, Garrett (1956) described _O. graminis_ as a root inhabitant, because it is confined almost entirely to the host during both its parasitic and saprophytic phases. During the latter phase, the fungus appears to be nearly always confined to the host tissue it invaded during the parasitic phase; being unable to spread to other host residue, even when this debris is in contact with the occupied tissue (Lucas, 1955). However, Warcup (1957) has found _O. graminis_ hyphae in wheat field soil, while Brown and Hornby (1971) have established that in

some circumstances O. graminis can grow through soil. Although the fungus is usually confined to the tissue of its former host, it does not remain passive, but continues to grow slowly within the debris (Garrett, 1940).

Garrett (1970) has provided a synopsis of current thinking on saprophytic survival of 'root-inhabitants'. It will suffice here to discuss briefly the main factors affecting survival of O. graminis.

Although early work (Davis, 1925; Russell, 1934; Hynes, 1937) established the ability of O. graminis to survive for periods of up to 25 months, Garrett (1938, 1940 and 1944) was the first to supply critical data on survival in natural soil. Using artificially colonized straws, Garrett (1938) was able to establish that the fungus disappeared most quickly under soil conditions (such as good aeration, medium to high temperatures and adequate moisture) favouring general micro-biological activity. This was confirmed by Fellows (1941) using soil naturally infested with O. graminis. He found that O. graminis could survive for more than 2 years in either moist or dry soil stored in a warm glasshouse. The fungus survived best in cool, compact, moist soil.

Probably the most interesting discovery on survival made by Garrett (1938) was that the rate of decline in viability was reduced by the addition of nitrogen-rich material to the soil. Seeking to explain this, Garrett (1940) hypothesised that survival depended mainly upon the supply of available food in the straw. He thought that addition of nitrogen prolonged the viability of O. graminis in the colonized straw, by allowing the fungus to assimilate more of the undecomposed carbohydrate in the straw. Where nitrogen is limited, other organisms compete with

O. graminis depriving it of food reserves essential for its survival. This is in contrast to Garrett's (1938) first hypothesis, that limiting nitrogen allowed other soil organisms to actively decompose the *O. graminis* mycelium.

After studying the cellulose-decomposing ability of several cereal root-rotting fungi, Garrett (1963, 1966, 1967) further modified his hypothesis, giving other soil micro-organisms an even more subsidiary role than previously. He postulated that individual hyphae of *O. graminis* die of starvation as they exhaust their zone of enzymic erosion, but that adequate nitrogen allows the production of new hyphal branches.

Other authors have confirmed the 'N effect' discovered by Garrett. Butler (1953, 1959) found this effect in nitrogen-amended soil and in naturally fertile soil. Chambers and Flentje (1967b) demonstrated that, although strongly pathogenic forms of *O. graminis* were able to survive longer than weakly pathogenic isolates, the 'N effect' still applied to both types of isolates. Chambers and Flentje (1968) have shown that nitrogen enrichment also promotes survival in gramineous species other than wheat. Decline of viability of *O. graminis* in irradiated soil is very slow (Chambers and Flentje, 1969), but even here, nitrogen amendment improves survival. Scott (1969) found that nitrogen added as an amendment to soil, or to straw prior to colonization, prolonged survival when compared with untreated straws in unamended soil.

Van der Watt (1965) found that the 'N effect' was operative in some South African soils only in the presence of *Rhizoctonia solani*. Chambers and Flentje (1968) demonstrated the 'N effect' on the survival

of *O. graminis* in South Australian Mallee soils where *R. solani* is a cereal pathogen. Recently, Chambers (1971a) has demonstrated the same effect in soil from the Victorian Mallee where the population of *R. solani* was negligible.

With the exception of the work by Davis (1925), Russell (1934) and Fellows (1941), all the survival studies discussed above were made using artificially colonized straws. Garrett (1938) established the use of this type of standardized survival unit because of convenience of preparation. Although this practice has lead to uniformity of medium, the work of Chambers and Flentje (1967b) has shown that the virulence of the isolate used in survival studies has a marked effect on the results. Chambers (1971b) also found that the method of straw sterilization will affect the survival of *O. graminis*.

(3) Changes in the incidence of *O. graminis* and Take-all in the field.

As mentioned previously, changes in the incidence of *O. graminis* in the field are governed by the relationship between the parasitic and saprophytic phases. It has been demonstrated that an equilibrium between these 2 phases may be reached with continuous wheat or barley culture (Buddin and Garrett, 1941).

Fellows and Ficke (1934) studied take-all patches in the field over a period of 5 years. They found that patches that appeared in the crop in the first year increased in size and take-all severity in the second year, decreased in size in the third year and often disappeared in the fourth year. However, wheat seedlings grown in soil removed from the area where a patch existed previously became infected with *O. graminis*.

Fellows and Ficke (1939) also reported another example where take-all was severe in 1924 but did not appear again during the next 10 years of continuous wheat.

Glynne (1935, 1965) also reported that continuous wheat rotation caused a decrease in take-all incidence. Glynne (1935) suggested that take-all 'decline' may be due to an 'auto-intoxication', or dying out of the O. graminis as it becomes more abundant. However, as her assessment method was based on the percentage of plants infected per unit length, Glynne suggested her results may have reflected the disappearance of plants severely infected at an early stage. Take-all 'decline' has been reported by a number of other authors (Slope, 1963; Slope and Cox, 1964; Slope, 1967; Lemaire and Coppenet, 1968; Rosser and Chadburn, 1968; Slope, Etheridge and Palmer, 1969).

In Australia, take-all was reported to have "practically 'died out' after the first four years" of continuous cropping on an area at the Waite Institute, S.A. (Anon, 1941). Chambers (1962) compared 1, 2 and 3 years of continuous wheat on 4 experimental stations in Western Australia: on 2 stations continuous wheat was accompanied by a decrease in the incidence of O. graminis while on the others, the number of crops had no effect on incidence. Price (1970) found that 4 consecutive crops of wheat "virtually eliminated O. graminis" from a field trial in Victoria. White (1945, 1947) however, found that both O. graminis and take-all increased with 4 consecutive wheat crops on an experimental plot in A.C.T. By the fourth crop about 75 per cent of the plants in the plot were infected and the entire area became part of a take-all patch.

Take-all 'decline' is now well documented. An equilibrium situation appears to be reached after the initial decline; *O. graminis* is present in the crop, but does not cause marked reduction in yield (Buddin and Garrett, 1941). However, sudden increases in take-all can occur in the field when conditions are favourable for the disease (Buddin and Garrett, 1941).

With the move towards intensive cropping attention has been directed to determining the cause of take-all 'decline'. Cox (1963) and Glynne (1965) suggest that some unknown inhibitory factor prevents establishment of new lesions after initial infection has taken place. Lemaire and Coppenet (1968) consider take-all 'decline' might be related to the production by other micro-organisms of substances antagonistic to *O. graminis*. Pugh and Van Emden (1969) consider that an increase of fungal antagonists on reclaimed land may cause a decline in *O. graminis*; the pathogen having become established in the early years of cultivation prior to the colonization of the new soil by antagonistic micro-organisms (Oort, 1965). Etheridge (1969b) was unable to identify micro-organisms inhibitory to *O. graminis* in either the rhizosphere or rhizoplane. A technique for studying the inhibition of the parasitic phase of *O. graminis* in field soil was developed by Lester and Shipton (1967). The test shows there is widespread inhibition of *O. graminis* in soils carrying continuous wheat or barley rotations, but gives no indication of what causes the inhibition. Lapierre, Lemaire, Jouan and Molin (1970) have proposed that take-all 'decline' may be associated with a loss in virulence of *O. graminis* caused by progressive contamination of the fungus

by a virus.

Gerlagh (1968) proposed 5 hypotheses that could explain take-all 'decline': induced biological antagonism; production of harmful by-products by *O. graminis*; loss of virulence; escape due to improved growth on nutrients left by previous poor crop; and reduction in the level of inoculum in soil due to decomposition of diseased plants. Gerlagh considered biological antagonism the most likely explanation for 'decline'. He found that the antagonistic capacity of the soil could be removed by heating (50°C for 30 minutes) or by application of some chemical fumigants. Antagonistic soil could be diluted (logarithmically) by the addition of non-antagonistic soil. The growth of *O. graminis in vivo*, but not *in vitro*, could be reduced by a sterilized aqueous-extract of the soil. Gerlagh, however, was unable to find any difference in the microflora of antagonistic and non-antagonistic soil.

Of particular interest to me is the discovery by Gerlagh (1968) that antagonistic soils affect both the parasitic and saprophytic phases. He found that the survival of *O. graminis* in colonized straws was reduced in soils with artificially induced antagonism. The only other reference to cropping history affecting survival is in Van der Watt (1965, Table 3), but there are insufficient data to draw firm conclusions.

GENERAL MATERIALS AND METHODS

A. Isolates of O. graminis

Ophiobolus graminis was isolated from infected root tissue by the method employed by Chambers and Flentje (1967b). Small pieces of root were immersed in 1 per cent silver nitrate for 30 seconds followed by 3 rinses in sterile distilled water. They were then placed (3 or 4 pieces per plate) on potato-"Marmite"-dextrose agar* (PMDA) containing 30 ppm Aureomycin hydro-chloride. After incubation at 20°C for 2 to 4 days, hyphal tips from typically whorled hyphae were transferred to glucose-asparagine medium (Lilly and Barnett, 1951). Of the isolates obtained, only 1 was selected for further use.

Details of the isolates used for experimental purposes are as follows: isolate 2C was a single ascospore culture originally obtained from isolate W1 and classed as strongly pathogenic (Chambers and Flentje, 1967b). This isolate was originally obtained from wheat stubble at Alford, 140 km north-east of Adelaide. Isolate 044 was obtained from a wheat seedling grown in soil taken from a wheat-stubble field at Ceduna in August 1969. It produced perithecia in glucose-asparagine cultures left in the light at room temperature (21 to 25°C). Ascospore size (83μ; standard error of mean, ±0.86μ; range, 70-92μ), pathogenicity and virulence to wheat seedlings identify this isolate as O. graminis.

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* Details of the composition of this and other media used in this investigation are given in Appendix A.

Stock cultures were maintained on glucose-asparagine medium. It has been shown (Russell, 1934, 1939; and Chambers, 1970) that continued maintenance on artificial media may result in loss of virulence for some O. graminis isolates. Tests carried out at various times showed that both isolates used retained their virulence throughout the period of these investigations.

B. Determination of physical and chemical properties of experimental soils

The moisture characteristic curves (drying curve boundaries) of the soils used in this investigation were determined as follows. Thoroughly mixed samples (composed of 4 randomly selected large cores, 9.8 cm x 10.5 cm deep) of each soil were sieved (2 mm), packed into brass rings (1 cm deep, 3.5 cm diameter) to a predetermined bulk density and then placed under various moisture tensions. Moisture content was determined on an oven-dry weight basis (Schofield, 1935). Three replications were used at each determination.

Moisture tensions were applied in the following manner:

(1) Tensions of 10 to 158 cm of water suction (pF 1.0 to 2.2) were applied to soils on a fine ceramic tile in the apparatus described by de Beer (1965). This equipment is a modification of the 'pressure plate' apparatus designed by Richards and Fireman (1943). Suction was applied to the soils for 5 to 7 days.

(2) Tensions equivalent to 300 to 600 cm of water suction (pF 2.47 to 2.78) were obtained by applying pressure to the soil samples on a fine ceramic tile. Pressure was applied for 8 to 10 days by the use of a

bubble tower (Richards and Fireman, 1943) connected to a compressed air supply.

(3) Tensions equivalent to 3,000 to 15,000 cm of water suction (pF 3.48 to 4.18) were obtained with pressure membrane apparatus (Richards, 1941). The soil was placed on a high pressure "Visking" membrane and pressure applied from a compressed air cylinder for 5 to 7 days.

(4) Tensions equivalent to 230,000 cm and 1,000,000 cm of water suction (pF 5.36 and 6.00) were applied by the use of saturated salt solutions (Griffin, 1963). Soils were allowed to equilibrate for 3 months at 25°C above a saturated solution of KCl (relative humidity, 85%) or a saturated solution of $Ca(NO_3)_2.4H_2O$ (relative humidity, 50.5%) (Winston and Bates, 1960).

The soil moisture in undisturbed cores held at 100 cm tension (pF2) was determined using method (1) above. Cores were collected and transferred to bottomless cans prior to placement on the ceramic plate. Although it is difficult to establish soil moisture equilibrium with large cores (Danfors, Pers. Comm.), provided the soils are physically similar and received the same suction for the same time, they would have approximately equal moisture tensions.

Mechanical analysis of the experimental soils was made by the hydrometer method (Piper, 1950). Hydrogen ion concentration, conductivity, and soluble salts were determined according to the methods described by Piper (1950). A soil-water ratio of 1:5 was used to prepare suspensions for the required determinations. Total nitrogen, total phosphorus and total carbon were determined by Staff of the South Australian Department

of Agriculture. Soil colours were defined using the revised Standard Soil Charts of Oyama and Takehora (1967).

C. Experimental areas in the field

Three locations representing a range of soil types, were chosen for field experiments (Fig. 1). One area was located on the property of Mr. R. Wilsdon, 8 km south-east of Keith. The soil is a sandy, alkaline grey soil (Northcote, 1960). Details of some of the physical and chemical properties of the soil from the area are given in Table 1, while the moisture characteristic (drying boundary) curve is shown in Figure 2. The experimental area is close to the 51 cm mean annual rainfall isohyet (Anon, 1965).

The field containing the experimental area was sown to wheat in 1964 and 1968, with pasture in the intervening years. The experimental area was sown with wheat in 1969, but work at this location was subsequently abandoned because the root-attacking strain of *R. solani* was prevalent.

The second experimental area was located on the Turretfield Research Station of the South Australian Department of Agriculture, 10 km north-east of Gawler. The soils at this location are classified as hard-setting loamy soils with red clay subsoils (Northcote, 1960). The soil in the experimental area is a dull reddish brown loam, subject to cracking when dry. Further details of the soil properties are given in Table 1 and Figure 2. Turretfield is close to the 51 cm mean annual rainfall isohyet (Anon, 1965).

The experimental area was part of a field that contained lucerne from 1957 to 1966. It was fallowed in August 1966 and sown to

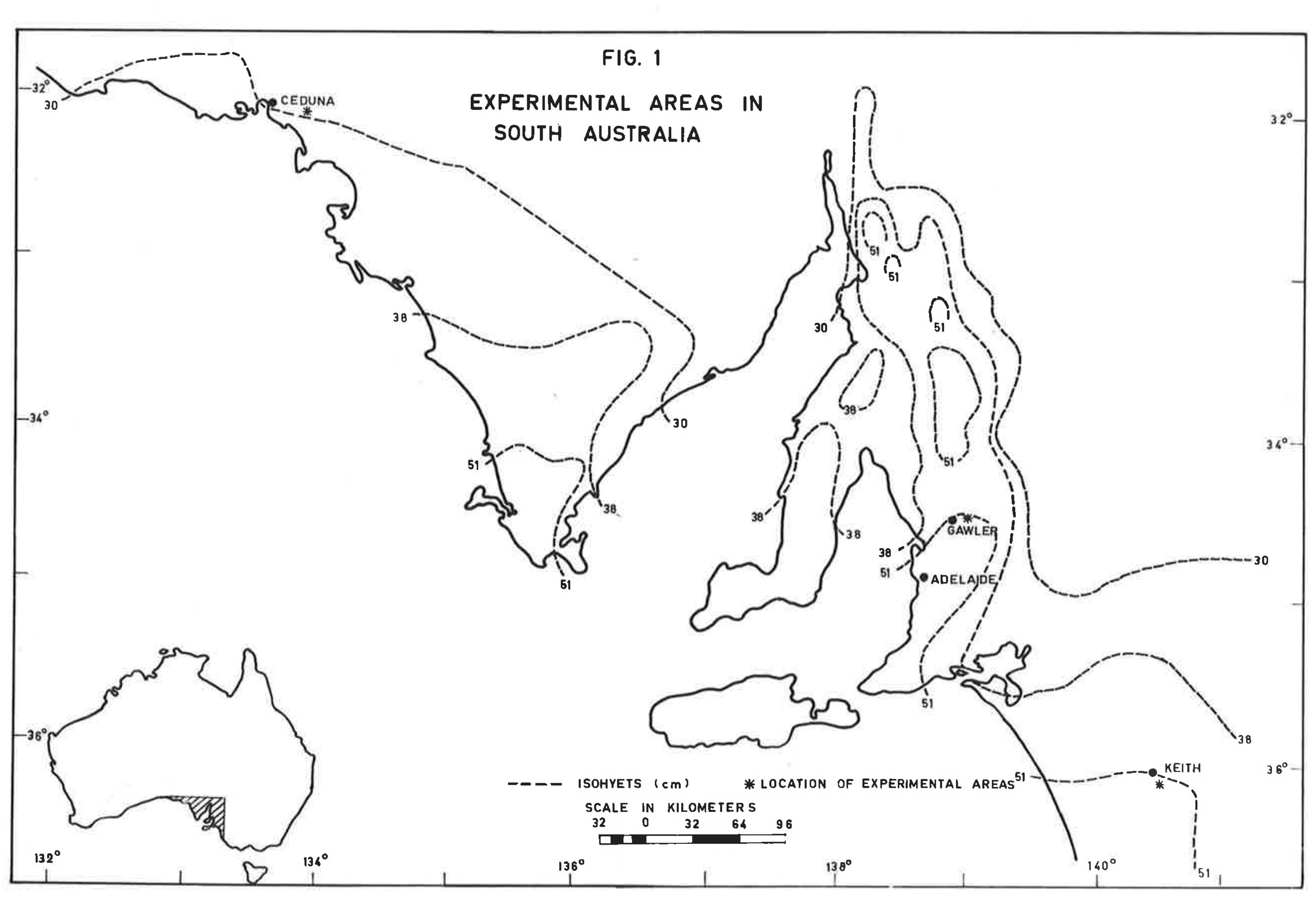


FIG. 1
EXPERIMENTAL AREAS IN SOUTH AUSTRALIA
CEDUNA
GAWLER
ADELAIDE
KEITH
ISOHYETS (cm)
LOCATION OF EXPERIMENTAL AREAS
SCALE IN KILOMETERS
32 0 32 64 96
132°
134°
136°
138°
140°
32°
34°
36°
30
38
51

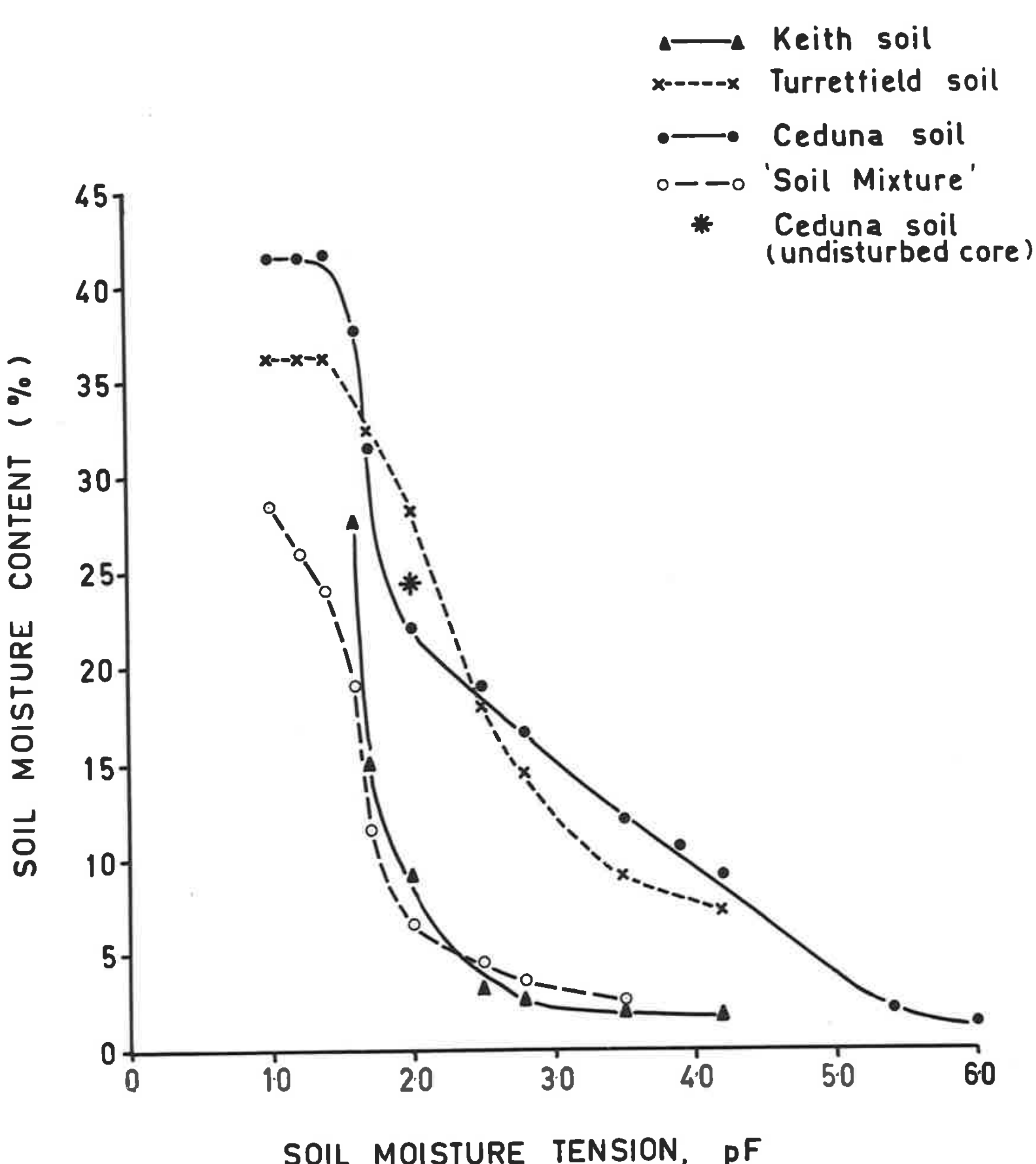
TABLE 1

Physical and chemical properties of field and experimental soils

Name	Particle size distribution			pH	Colour	Hue
	<2μ (Clay) %	2-20μ (Silt) %	>20μ (Sand) %			
Keith	3	2	95	6.9	grayish yellow brown	10YR (6/2)
Turretfield	22	18	60	7.0	dull reddish brown	5 YR (4/4)
Ceduna	17	8	75	8.5	dull brown	7.5YR (5/4)
'soil mixture'	7	4	89	8.0	dull brown	7.5YR (5/4)
Golden Grove Sand	4	1	95	7.2	dull yellow orange	10YR (7/4)
Gawler loam	14	6	80	8.0	dark brown	7.5YR (3/4)

FIG. 2

DRYING BOUNDARY CURVES FOR FOUR EXPERIMENTAL SOILS



wheat in 1967 and 1968; in the latter year take-all patches appeared. The experimental area was sown to wheat in June 1969. Take-all patches were again observed in this crop (Fig. 3). Details of air temperature (screen) and rainfall at Turretfield during the period of experiments are given in Table 2.

The remaining experimental area was on the property of Mr. E. Miller, approximately 22 km east of Ceduna. Soils in the area have been classified as brown calcareous earths (Northcote, 1960). The experimental area has a dull brown sandy loam soil containing scattered lime nodules; further details are given in Table 1 and Figure 2. The experimental area is close to the 30 cm mean annual rainfall isohyet (Anon, 1965).

The field containing the experimental area was pasture in 1965 and 1966 and prepared for sowing to wheat in 1967, but due to drought it was not planted that year. Wheat was grown in 1968 and 1969; in both years the crop contained considerable _O. graminis_, with severe take-all in 1969 (Fig. 3). Details of screen air temperature, rainfall and soil moisture are given in Table 2. (Soil moisture was calculated on the top 9 to 10 cm of soil).

The cores used in experiments studying the survival of _O. graminis_ in stored natural soil were all removed from the Ceduna area. Chemical properties other than those described in Table 1 are as follows: total nitrogen, 0.1%; total phosphorus, 248 ppm; total carbon, 0.99%; C:N ratio, 9.9; NaCl equivalent, 0.6%.

D. Standard soil mixture

The soil used in all tests requiring standardized conditions has

FIG. 3

TAKE-ALL IN EXPERIMENTAL AREAS, 1969

(Shaded portions of blocks were take-all patches)

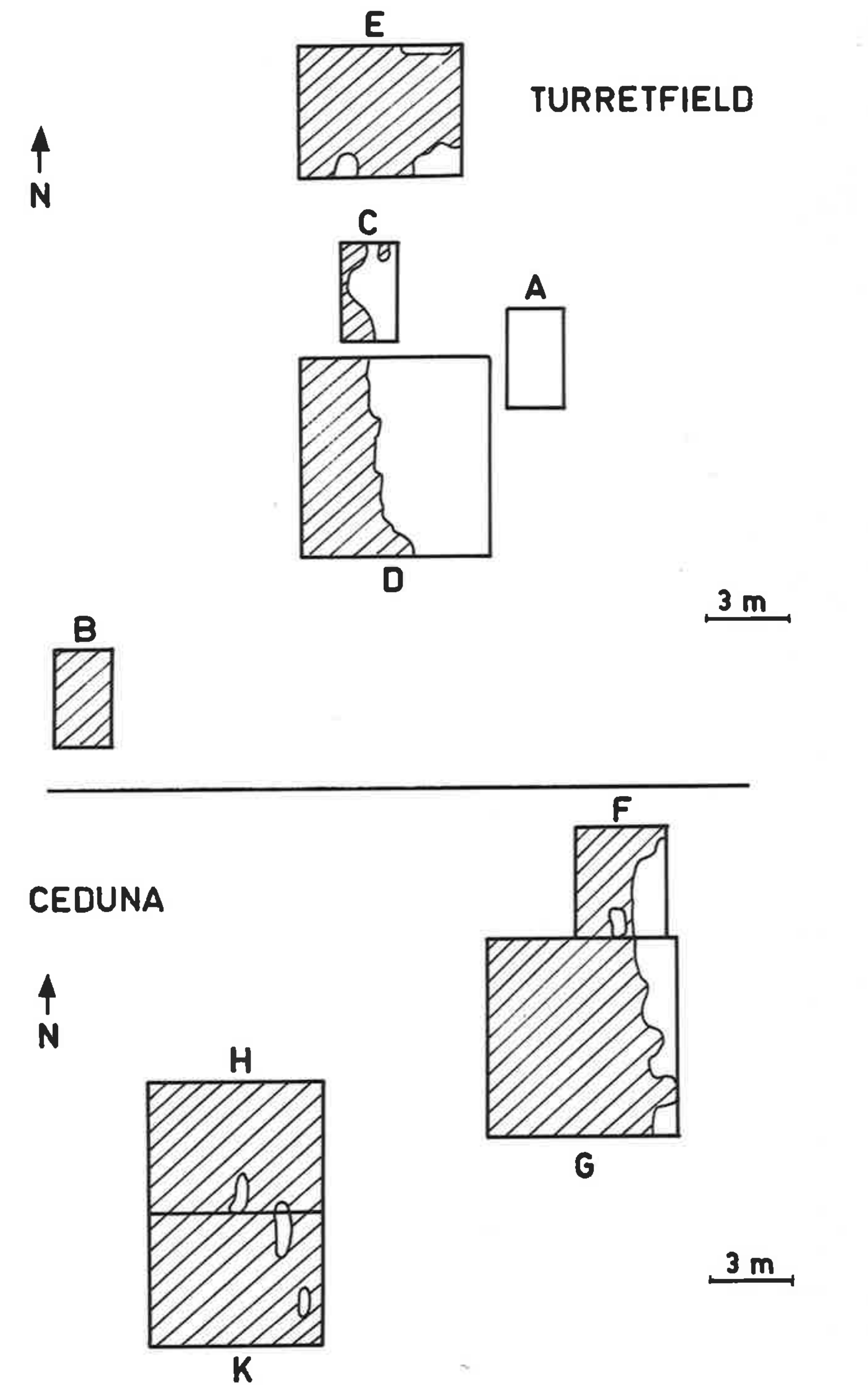


TABLE 2

Monthly mean maximum and mean minimum screen air temperatures and rainfall for Turretfield and Ceduna. Soil moisture figures for Ceduna.

Month	Turretfield			Ceduna			
	Temperature Mean monthly maximum °C	Temperature Mean monthly minimum °C	Rainfall (cm)	Temperature Mean monthly maximum °C	Temperature Mean monthly minimum °C	Rainfall (cm)	Soil* moisture %
1969							
September	15.6	4.5	5.44	19.2	7.5	0.76	
October	23.3	8.6	0.13	28.2	13.5	2.57	
November	24.8	10.6	2.21	27.4	14.3	2.06	
December	24.9	11.3	3.86	30.8	16.2	0	
1970							
January	27.5	13.1	2.74	32.3	17.2	0.05	
February	31.8	14.6	0	35.8	20.5	0	1.5(6/2)
March	25.9	11.4	0.38	29.4	16.0	0.03	1.5(11/3)
April	23.4	11.5	6.15	25.8	12.7	0.18	1.5(17/4)
May	16.9	8.0	4.88	19.6	9.2	0.56	9.3(14/5)
June	15.9	7.5	5.18	19.0	6.9	0.53	7.5(24/6)
July	14.7	4.7	4.11	17.6	4.7	0.05	6.3(29/7)
August	14.1	4.1	9.68	17.5	5.6	2.18	
September	15.9	6.2	5.74	19.8	8.1	2.62	9.8(1/9)
October	21.4	6.6	0.58	26.8	11.9	0.89	4.2(5/10)
November	25.5	11.2	3.18	29.1	14.7	0.10	1.4(10/11)

* pF4.2 = 8.8% soil moisture, date of field sampling in brackets.

been called 'soil mixture'. This mixture was made from equal parts (v/v) of Gawler loam and Golden Grove sand. Enough 'soil mixture' to last at least 2 years was made as follows: the 2 ingredient soils were thoroughly mixed, spread on the floor to dry and then sieved (4.5 mm mesh), prior to being stored in covered bins. Details of some of the physical and chemical properties of the 'soil mixture' and the ingredient soils are given in Table 1. The moisture characteristic (drying boundary) curve for the 'soil mixture' is shown in Figure 2.

E. Seeds used in investigation

Wheat seed of the variety 'Gabo' was used in all laboratory experiments. Seed from the previous season was replaced annually with a new sample obtained about 3 months after harvest. All discoloured or shrivelled seeds were discarded.

In all laboratory experiments unsterilized seeds were used. Preliminary tests with mercuric chloride showed that residues, inhibitory to fungal growth, could be detected on the seed even after thorough washing. Sterilization with sodium hypochlorite soaks was excluded because of possible loss of seed exudates. Schroth, Toussoun and Synder (1963), Flentje and Saksena (1964) and Kerr (1964) have shown that seed exudates can stimulate fungal growth in the vicinity of the seed, and Brown and Hornby (1969) have reported that "new mycelium growth (of *O. graminis*) was stimulated by germinating wheat seeds".

In field experiments 'Heron' wheat (67 kg per h) was sown at Turretfield while 'Saba' wheat (59 kg per h) was used at Ceduna.

F. Statistical analyses employed during investigation

Analysis of variance and regression analysis were performed by

using the C.S.I.R.O.-Waite statistical program system GENSTAT (Wilkinson, 1970) and executed on the C.S.I.R.O. Control Data 3200 Computer. Another program used was the Waite Biometry section program for chi-square analysis. I also developed several FORTRAN programs, including 1 to calculate regression coefficients for paired sets of values, and 2 to calculate and tabulate the results from the core bioassay and crown bioassay. The latter 2 programs were coupled to a plotting and mapping program developed by Mr. R. Lamacraft of the Waite Biometry section. All these programs were executed on the University of Adelaide Control Data 6400 computer.

EXPERIMENTAL

PART I

ANALYSIS OF INCIDENCE OF *OPHIOBOLUS GRAMINIS* IN THE FIELD

A. Bioassay of soil for the presence of O. graminis

If intensive field studies of the incidence of *O. graminis* are to be made, ways of measuring its distribution and persistence are needed. Workers at Rothamsted have recently reported 2 approaches to this problem. The first (Slope, Henden and Etheridge, 1969), uses an assay that measures the intensity of attack on plants in terms of an 'infection index'. The second (Hornby, 1969c) studies the form and distribution of surviving inoculum, estimating the number of 'infective units' per unit volume of soil.

For the 'infection index' soil cores (5 cm x 15 cm deep) were taken at random from the field and assayed for the presence of *O. graminis*. It is not specifically stated that the soil is removed from the core, but as the standard test had 16 wheat seeds sown in 350 cm^3 of test soil in a pot, it seems likely that the soil was removed and presumably mixed. Sixteen pregerminated seeds were sown and the seedlings harvested 35 days later. After washing, the roots were trimmed to 6 cm below the seed and then scored. The index is equivalent to the percentage of the first 5 seminal roots infected.

Slope, Henden and Etheridge (1969) and Anon (1969) suggested that the above approach to the study of *O. graminis* in field soil was successful, but Hornby (1969b) mentioned that it was considered unreliable for advisory purposes because of lack of correlation with the incidence of

O. graminis in crops. When I commenced this investigation early in 1969, I was unaware of the work of Slope, Henden and Etheridge (1969) and the problems they were encountering.

The second approach (Hornby, 1968, 1969a, b, c) uses a series of volumetric dilutions (1969c) of either whole soil or of organic debris removed from soil by a sedimentation and sieving procedure (1969b). By growing wheat seedlings in the series of soil dilutions, it is possible to estimate the number of infective units of *O. graminis* in the soil being examined.

In the investigations reported in this dissertation the technique for detecting *O. graminis* in field soils is similar to that of Slope, Henden and Etheridge (1969). Investigations leading to the development of the method of bioassaying soil for the presence of *O. graminis* are described below.

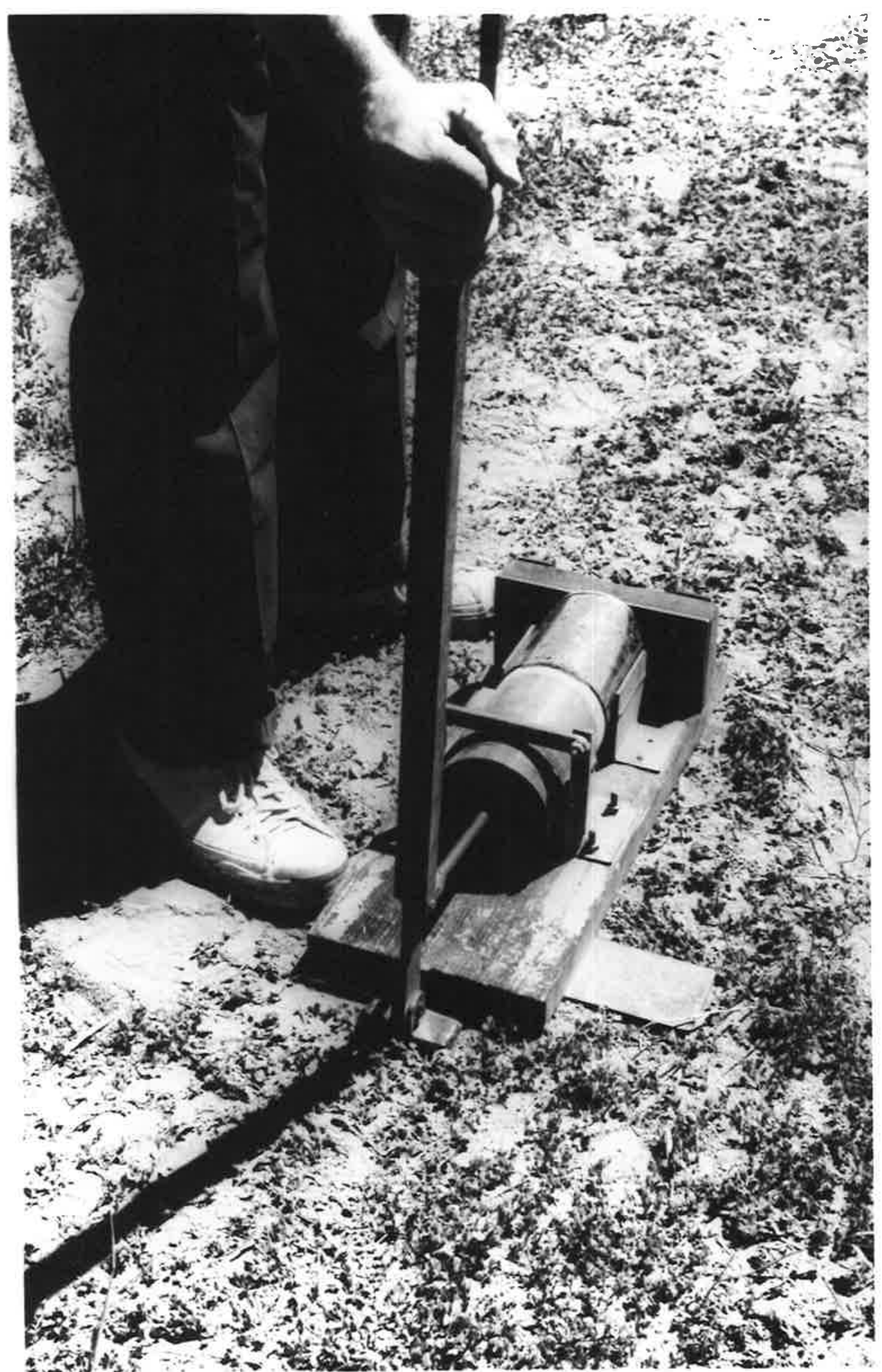
(1) Collection and use of soil cores.

Undisturbed soil cores were removed from the field as follows. A steel cylinder (9.8 cm internal diameter and 14.5 cm long) was driven into the soil to a depth of approximately 10.5 cm. Removal of a small quantity of soil from 1 side allowed the soil column to be broken at the base of the cylinder and the intact soil core to be removed. The core was then pushed by a plunger (Fig. 4) into a can. The cans (internal diameter 9.9 cm and 12.4 cm deep) were previously painted on the inside with "Black Bituminous Paint" (British Paint Ltd.) to prevent rusting.

On return to the laboratory all plants present in the cores were clipped to surface level and any surface trash removed. The cores

FIGURE 4

Core ejector used for pushing soil core into container. The handle operates a detachable plunger which is slightly smaller in diameter than the steel cylinder and container.



were then watered with distilled water to a moisture content equivalent to pF2.8. (Distilled water was used in all experiments where watering was required because of the high salt content in the untreated water supply). Except in the initial experiments, 7 wheat seeds were sown at a depth of 2.5 cm in each core (1 central hole surrounded by 6 holes all placed so that every seed was 3.2 cm from its neighbours). Cores were then placed in a controlled environment (16 hours of fluorescent light 17,200-18,300 lm/m^2 (1600-1700 lm/sq.ft.) and 15°C constant temperature) and brought to constant weight with water every second day. After 4 weeks, the seedlings were washed free of soil and the roots examined for the presence of O. graminis. Examination was made in water with a white background (Garrett, 1941)

(2) Selection of parameters.

Initially, the aim was to see whether undisturbed cores could be used to estimate the level of O. graminis in field soil. If the result was affirmative, a secondary aim was to select the best parameters to assess the level. To carry out the primary aim, a transect was made at the experimental area at Keith, in such a way as to cross a take-all patch observed in the previous spring.

At each site on the transect, 4 cores were taken side by side and at right angles to the direction of the transect. Site 1 was on a bare, graded fire break. Site 2 was on a small, uncultivated area under a hoarding, and contained several species of grass (Danthonia sp. and Vulpia sp.) and Erodium botrys (Cav.) Bentol. Cereals had not been planted on this site for at least 4 years and possibly 9 years. The

remainder of the transect cores were taken at 3 m intervals across the stubble (which had recently been lightly cultivated). Sites 7, 8 and 9 were considered to be within the patch recorded in the previous season.

In this initial experiment with cores, 9 seeds were sown per core and the soil moisture content was 9 per cent or pF2. From the 4 cores from each site, 2 were harvested after 4 weeks of growth and the following parameters were recorded per core: emergence; number of seedlings infected; total leaf length; total number of seminal roots; total number of seminal roots infected; total length of discoloured seminal roots.

The results are shown in Table 3. (Site 5 was eliminated because some of the seedlings were infected by the root-attacking strain of Rhizoctonia solani). The presence of O. graminis was established, but before the selection of parameters could be made, it was necessary to establish that the levels of O. graminis within the cores represented an expected result. It was assumed that the number of seminal roots infected was likely to be a reliable measure of the presence of O. graminis and as the total number of seminal roots per site was not significantly different, the various sites could be compared.

There was no O. graminis present at Site 1. The bare nature of the site, being a graded fire break and thus grass free, would mean this was the anticipated result. The number of roots infected at Site 2 was significantly higher than at Sites 3, 4, 6, 8, 10 and 11. It was found that the species of Danthonia and Vulpia growing at Site 2, when the cores were collected, were infected with O. graminis. This would account for

TABLE 3

Parameters recorded from wheat seedlings grown in cores taken from a transect across an *O. graminis* infested field at Keith (mean of 2 replications)

Site	Vegetation	Emergence	Number of seedlings infected	Total leaf length (cm)	Total number of seminal roots	Total number of seminal roots infected	Total length of seminal root discoloured (cm)
1	Bare	8.0	0	231	30.0	0*	0*
2	Grasses & *Erodium*	9.0	8.5	220	39.5	30.0	79.0
3	1968 Wheat stubble	8.5	7.0	244	33.0	13.0	26.0
4	"	8.5	6.5	243	34.5	12.0	18.5
6	"	8.0	6.5	303	32.5	11.0	18.5
7	"	8.5	9.0	258	38.0	30.0	35.5
8	"	8.5	5.5	254	34.5	9.5	12.5
9	"	9.0	9.0	252	39.5	33.5	40.5
10	"	8.0	7.0	244	30.0	13.0	15.5
11	"	8.5	7.0	273	36.0	20.0	25.0
Standard error				37.9	5.1	3.6	8.6
LSD p = 0.05				ns	ns	8.3	19.8
= 0.01						11.2	29.3

* not included in analysis of variance

the high incidence of *O. graminis* at Site 2. Sites 3, 4, 6, 10 and 11 had moderate levels of infection which were not significantly different. Although these sites were outside the take-all patch recorded in the previous season, there was obviously considerable *O. graminis* present in the crop debris surrounding this patch.

Sites 7 and 9, which were within the patch, had significantly more roots infected than the surrounding stubble sites, while Site 8 was not significantly different from the sites outside the patch. The reason for the low reading for Site 8 is unknown.

The bioassay of the cores from the transect gave, with the exception of Site 8, a reasonable indication of the anticipated levels of *O. graminis* in the transect area.

The parameters chosen for the bioassay of cores need to indicate the relative amount of *O. graminis* present. As the roots will be exploring the soil mass and are likely to come into contact with scattered inoculum, the number of lesions on the root system would appear to be the best method of estimating the number of propagules. However, the possibility of lesions coalescing with time (Slope, Henden and Etheridge, 1969) makes it difficult to assess this parameter if enough time is given for the roots to explore a considerable volume of soil. For this reason, the use of the length of discoloured root is possibly a reasonable compromise. Unfortunately the length of discoloured root has the disadvantage of being very tedious to measure, since areas of discoloured tissue may be scattered over the entire root system. In a similar situation, Garrett (1941) recorded the number of seminal roots infected. This is more con-

venient and as there is a highly significant correlation (Fig. 5) between the number of roots infected and the length of root discoloured, I decided to use this as the main parameter. In later experiments, it became obvious that there was some variability in emergence and it was thus more accurate to use percentage of roots infected.

The only other parameter recorded in all later experiments was percentage of seedlings infected per core. This result gives an indication of the possible result at maturity. If it is assumed that any infection on a seedling less than 4 weeks old is likely to cause severe take-all later in the growing period (and evidence is produced later to support this assumption), then this parameter is of interest, although of limited value, because of the small number of results possible from only 7 seeds.

In the transect there was no significant difference between leaf length of seedlings grown in cores from any of the sites (Table 3). In other experiments, there were obvious relationships between leaf growth and incidence of _O. graminis_, but even in the example shown in Figure 6 (taken from a later experiment) the vigorous plant growth in Core 68 gave no indication of the extent of infection on the roots. Leaf growth is affected significantly only in those cores where there is a severe _O. graminis_ infection. Leaf weight and root weight were thus recorded only in those experiments where the treatments were likely to affect plant growth as well as the incidence of _O. graminis_.

(3) Variability between cores from the same site.

Although there is some variability between cores taken together

FIG. 5

RELATIONSHIP BETWEEN NUMBER OF ROOTS INFECTED (x) AND LENGTH OF ROOTS DISCOLOURED (y) FOR SEEDLINGS GROWN IN CORES FROM A TRANSECT AT KEITH

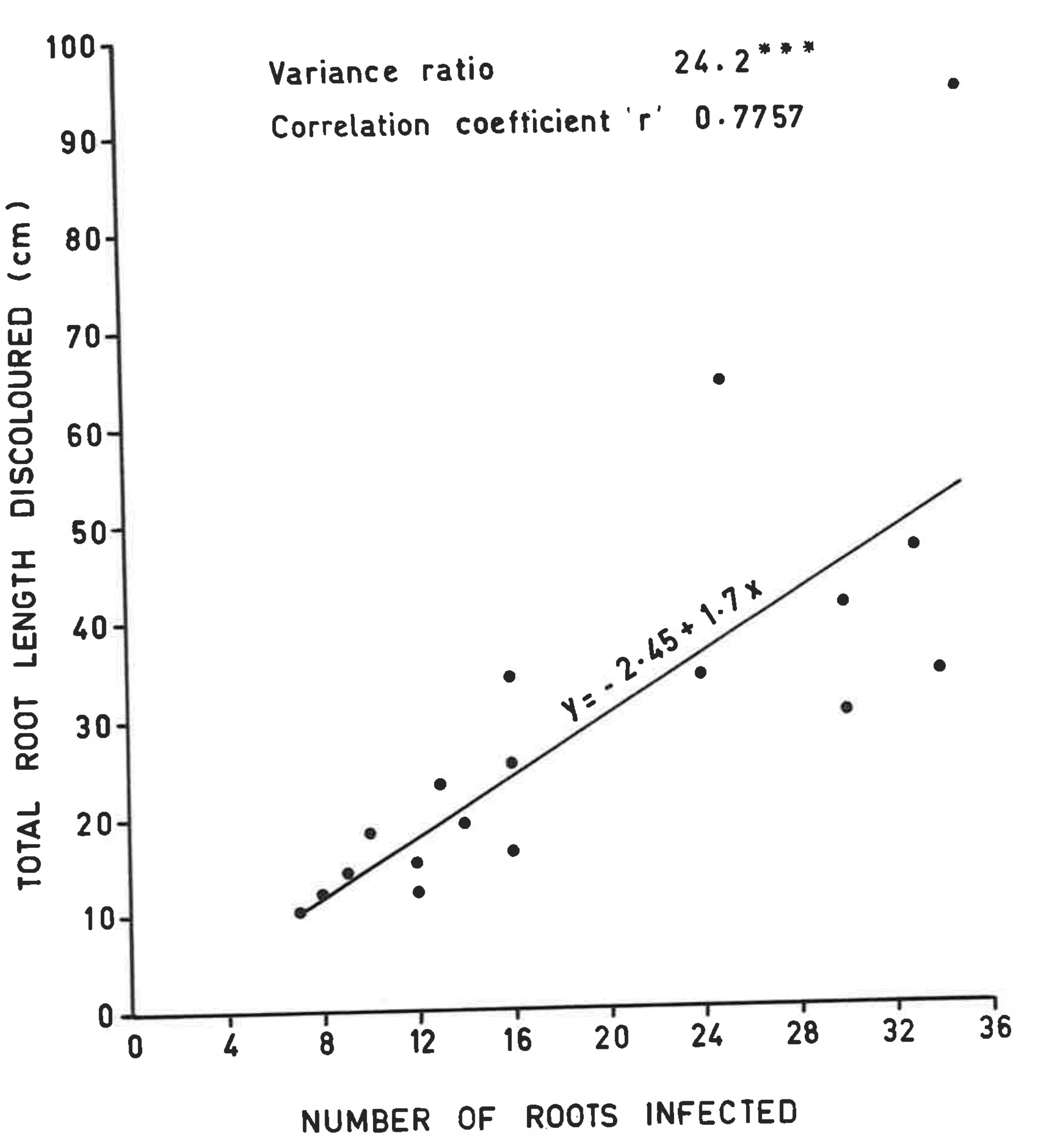


FIGURE 6

Wheat seedlings, grown in undisturbed cores, showing a range of effects of _O. graminis_ on plant height. Although seedlings in Core 68 were large and vigorous, most were infected (see Table below)

	Core Number			
	56	20	72	68
Percentage of seedlings infected	100	100	100	71
Percentage of roots infected	100	100	89	33
Average top fresh weight (g)	0.16	0.21	0.27	0.33

56
20
72
68

in the transect conducted at Keith (Table 4), the differences are not large enough to make the between-site results non-significant (Table 3). However, at Ceduna, another preliminary transect was taken at 3 m intervals across an area which had been generally infested with O. graminis in the previous season. It can be seen (Table 5) that there was considerable variability between the triplicate cores in this instance. Therefore, although the transect established the presence of O. graminis, it seems that a large number of cores is necessary to establish the possible incidence in the following crop.

If cores are to be used for the study of survival of O. graminis in natural soil under various environmental conditions, a collection of cores with much less variability than obtained in the Ceduna transect would be necessary. In an attempt to overcome this problem a series of cores was taken along a row containing plant remains in a take-all patch at Ceduna. Fifteen cores were selected at random and planted with seed. The results are given in Table 6 and show that there is considerable variability, both in percentage of infected seedlings (57 to 100%) and percentage of infected roots (29 to 100%). To calculate the estimated magnitude of differences between treatments necessary to obtain a significant difference at $p = 0.05$, the following general formula was employed:

$$\text{Diff.} = t \times \sqrt{\frac{2 \times \left[\dfrac{\Sigma x^2 - \dfrac{(\Sigma x)^2}{n}}{n - 1}\right]}{n}}$$

where n = the number of replications. The available controlled environ-

TABLE 4

Comparison of number of infected roots, length of discoloured root and number of infected seedlings in duplicate cores from a transect made at Keith

Site	Number of infected roots per core		Length (cm) of discoloured root per core		Number of infected seedlings per core	
	a	b	a	b	a	b
1	0	0	0	0	0	0
2	25	35	64	94	8	9
3	16	10	34	18	9	5
4	8	16	12	25	5	8
6	9	13	14	23	5	8
7	30	30	30	41	8	9
8	7	12	10	15	4	7
9	34	33	34	47	9	9
10	12	14	12	19	6	8
11	16	24	16	34	6	8

TABLE 5

Comparison of number of infected roots, length of discoloured root and number of infected seedlings in triplicate cores from a transect made at Ceduna.

Site	Number of infected roots per core			Length (cm) of discoloured root. per core			Number of infected seedlings per core		
	a	b	c	a	b	c	a	b	c
1	0	20	13	0	36	24	0	7	8
2	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
4	0	5	4	0	10	6	0	4	4
5	2	2	4	2	3	5	2	2	2
6	0	0	0	0	0	0	0	0	0
7	1	0	4	2	0	6	1	0	4
8	0	0	2	0	0	4	0	0	2

TABLE 6

Comparison of the incidence of *O. graminis* in wheat planted in cores taken along a drill row in a take-all patch at Ceduna.

Core	Percentage of seedlings infected		Percentage of roots infected	
		Arcsine (degrees)		Arcsine (degrees)
1	57	49.1	52	45.9
2	100	90.0	100	90.0
3	100	90.0	59	50.2
4	100	90.0	96	78.2
5	100	90.0	100	90.0
6	71	57.7	52	46.2
7	80	63.4	57	48.2
8	67	54.8	29	32.3
9	80	63.4	59	50.1
10	100	90.0	73	58.7
11	100	90.0	97	79.4
12	83	65.9	77	61.3
13	100	90.0	94	76.3
14	100	90.0	79	62.4
15	100	90.0	83	65.5
Mean	89	77.6	74	62.3
Diff.*	17.1	18.7	24.5	19.9

* Difference necessary for significance with 7 replications (see text)

ment space dictated a maximum of 7 replications. It can be seen (Table 6) that a difference of 17 per cent in percentage of infected seedlings and 25 per cent in percentage of infected roots would be necessary for a significant difference at p = 0.05 with 7 replications. In a later experiment it was found that variability between cores could be reduced (Table 7) by making sure each core was taken over a plant or its remains.

Although variability between cores was a problem, the evidence suggested that cores could be used to study the incidence of _O. graminis_ in natural soil.

(4) Selection of conditions for bioassay.

(a) Effect of mixing or sieving the soil.

The core bioassay is based on the assumption that the roots are exploring the soil mass sufficiently for a unit of inoculum to have an opportunity to infect a root. If this assumption is correct, mixing the soil in the core should not affect the percentage of roots infected, although severity of attack (especially in the seed region) may be reduced because of the dilution of the inoculum from the surface soil.

The effect of mixing and sieving on the incidence of _O. graminis_ was investigated in cores taken from 2 sites in the experimental area at Ceduna. At 1 site all plants exhibited take-all, while at the other there were no symptoms of take-all. Twentyfive cores were taken at each site, consisting of 5 cores, side by side, taken in 5 consecutive drill rows to form a latin square. The treatments were undisturbed cores, thoroughly mixed cores and 3 degrees of sieving: 5.0 mm, 2.0 mm or 1.0 mm. Cores were then bioassayed for the presence of _O. graminis_. Dry

TABLE 7

Comparison of the incidence of *O. graminis* in wheat planted in 7 cores taken over plant remains in a take-all patch at Ceduna

Core	Percentage of:	
	seedlings infected	roots infected
1	83	36
2	100	75
3	100	89
4	100	89
5	100	69
6	100	76
7	100	100
Mean	98	76
SE	2.4	7.8

weights of tops and roots were also recorded.

Mixing the soil from the take-all patch had no effect on the percentage of seedlings infected (Table 8), but did reduce (p = 0.05) the percentage of roots infected. This result suggests that where inoculum and seed are close together, as in undisturbed cores, some lesions may extend over more than 1 root. Thus the use of undisturbed cores may overestimate the amount of O. graminis present, but as the difference was only small, it was considered unnecessary to go to the extra trouble of mixing cores.

As expected, sieving the soil from a core reduced the amount of inoculum (Table 8). The use of a 5.0 mm mesh sieve caused a significant reduction (p = 0.05) in the percentage of infected seedlings, but not infected roots, when compared with the mixed soil. The reduction in mesh size to 2.0 and 1.0 mm significantly reduced the percentage of infected seedlings and of infected roots, but did not eliminate infection. Similarly, Hornby (1969b) found that a 0.42 mm sieve removed most of the organic debris containing O. graminis, but even at that mesh size, the fungus remained on some occasions. It is apparent, therefore, that the removal of large trash including crowns still leaves a small reservoir of O. graminis in the soil, and as will be shown later, this inoculum can have a considerable effect on the mature plant.

Results from the experiment using soil from the site where there were no symptoms of take-all, again raised the problem of variability (Table 9). There was too much variability between replications for significant differences between treatments. All treatments, except sieved

TABLE 8

The effect of mixing and sieving on the incidence of O. graminis, and on top and root weight of seedlings grown in cores from an area at Ceduna with high incidence of take-all (mean of 5 replications as a latin-square)

Core Treatment	Percentage of: seedlings infected	Arcsine (degrees)	roots infected	Arcsine (degrees)	Dry weight per seedling (mg) top	roots
Undisturbed	100	90.0	95	78.5	30	17
Mixed	100	90.0	85	67.8	31	24
Sieved(5.0mm)	91	79.1	76	61.5	35	31
Sieved(2.0mm)	67	58.3	35	35.9	43	38
Sieved(1.0mm)	50	45.2	16	23.4	43	42
Standard error		4.8		4.0	2.4	3.2
LSD p=0.05		10.4		8.8	5.2	6.9
=0.01		14.6		12.3	7.2	9.7

TABLE 9

The effect of mixing and sieving on the incidence of *O. graminis*, and on the weight of seedlings grown in cores taken from an area at Ceduna with no symptoms of take-all (mean of 5 replications as a latin-square)

Core Treatment	Percentage of: seedlings infected	Arcsine (degrees)	roots infected	Arcsine (degrees)	Dry weight per seedling (mg) top	roots
Undisturbed	21	18.6	10	12.0	36	33
Mixed	43	40.4	40	36.0	35	29
Sieved(5.0mm)	3	4.4	1	2.1	39	38
Sieved(2.0mm)	23	20.0	9	10.5	44	38
Sieved(1.0mm)	0	0	0	0	43	33
Standard error		11.9		10.0	2.2	2.4
LSD p=0.05		ns		ns	4.9	ns

1.0 mm, had a few cores containing heavily infected seedlings while the remaining cores contained no infected seedlings.

The results of these 2 experiments show that the removal of cores from a take-all patch can give reliable results in experiments where the cores are subject to different treatments. However, in the apparently healthy area, the presence of scattered infected plants makes it difficult to obtain reliable replication of either cores free of O. graminis or cores with low levels of O. graminis.

The significant reduction in top and root growth appears to be correlated with disease incidence (Table 8) rather than the treatments (Table 9).

(b) Effect of soil moisture.

Two experiments were conducted; the first being designed to indicate the likely water loss from cores during a bioassay test, the second to determine whether changes in moisture content were likely to affect the amount of O. graminis detected.

For the first experiment, 16 cores likely to include soil both infested with and free from O. graminis were obtained from the Ceduna experimental area. All cores were treated as previously described except that water loss was recorded. Of the 16 cores, 6 contained seedlings free of O. graminis, 6 contained severely diseased seedlings, and the remainder contained seedlings with intermediate levels of infection. There was no difference in moisture loss between cores with diseased and healthy seedlings. During the 48 hour period between watering, the moisture content fell from 16 per cent (pF2.8) to about 13 per cent (pF3.35).

In the second experiment, cores taken from a take-all patch at Ceduna were bioassayed as usual except that 4 soil moisture regimes (19%, 16%, 13% and 10%) were used. To minimize variability in moisture content the cores were watered every 24 hours rather than every 48 hours.

The results in Table 10 show that soil moisture in the range 13 to 19 per cent had no effect on the percentage of infected seedlings or on the percentage of infected roots. At 10 per cent soil moisture, emergence was affected by crusting of the soil surface. The poor germination at 10 per cent reduced the number of roots available to explore the soil which may account for the reduction in the percentage of infected seedlings and roots; on the other hand, the reduction may be a real soil moisture effect. Without further investigation it is impossible to say whether the reduction in incidence at 10 per cent is due to lack of exploration of soil or to an effect associated with soil moisture, or both.

Soil moisture had no effect on average root growth, but a reduction in top growth was associated with a reduction in soil moisture. It is apparent that this is an effect of soil moisture on the host rather than on the pathogen.

This experiment provides evidence to show that 16 per cent moisture (pF2.8) is sufficient to give good plant growth and adequate levels of infection to determine *O. graminis* incidence. The results suggest watering should be performed regularly enough (i.e. every 2 days) to prevent the soil moisture dropping below about 13 per cent (pF3.35).

(c) Effect of period of plant growth.

Experiments were designed to find out if there was a correlation

TABLE 10

Effect of soil moisture on the incidence of *O. graminis*, and on weight of seedlings grown in cores from a take-all patch at Ceduna (mean of 7 replications)

Soil moisture %	pF	Emergence	Percentage of: seedlings infected	Arcsine (radians)	roots infected	Arcsine (radians)	Dry weight per seedling (mg) top	root
19	2.30	5.9	100	1.57	90	1.36	46	15
16	2.80	6.4	100	1.57	85	1.21	38	18
13	3.35	5.3	97	1.50	77	1.15	25	17
10	3.95	3.7	47	0.76	25	0.49	20	15
Standard error				0.09		0.10	2.4	1.8
LSD p=0.05				0.18		0.21	5	ns
=0.01				0.25		0.28	7	

between O. graminis incidence on seedlings in cores at 4 weeks and plant yield at maturity. In the first instance, an observational trial was conducted with the remaining cores from the transect at Keith (page 37). After 4 weeks in the controlled environment these cores were transferred to an open-sided glasshouse. Each core was removed from its can and placed in an 11.5 litre plastic bucket containing 'soil mixture'. All buckets were watered to 17 per cent (approximately pF1.7) soil moisture once a week. The plants were placed in the glasshouse in June and reached maturity in late November.

During the period of growth, considerable variation in plant height developed between plants within buckets, between replications and between sites. In some instances, 1 or 2 plants dominated, tillering and growing vigorously; a similar result was reported by Gerlagh (1968). By late September and early November, white-heads began to appear amongst the plants.

Although there were considerable differences in plant height between replications and sites, excluding Site 1, examination of yield (Table 11) shows that differences in production of plump grain were small. Although it was possible to differentiate between different sites on the transect by the bioassay, at maturity only Site 1 was different from the remainder.

The following season another experiment was conducted with what was hoped would be a wide range of inoculum levels. Thus undisturbed cores from a take-all patch and cores with 3 levels of sieving (5.0 mm, 1.0 mm and 0.5 mm) were used. Two series of all treatments were grown

TABLE 11

Comparison of number of plants with heads, number of plants with white-heads, and yield from wheat plants grown to maturity in cores transferred to large containers. Two cores per site from a transect made at Keith.

Site	Number* of plants with heads		Number of white-heads (no grain)		Total grain yield (g)		Total plump grain (g)	
	c	d	c	d	c	d	c	d
1	9	9	0	0	7.5	7.2	4.9	5.2
2	0	8	-	0	0	1.7	0	0.5
3	9	4	1	0	1.4	1.3	0	0.5
4	3	8	1	1	0.8	1.0	0.1	0
6	9	5	0	1	3.9	2.0	0.6	1.1
7	7	5	0	1	2.7	1.8	1.1	0.9
8	5	7	1	3	1.6	2.4	0.7	0.7
9	4	6	2	1	2.1	1.5	1.4	0.4
10	6	4	3	1	0.6	0.4	0.1	0
11	7	5	3	3	0.6	0.3	0.3	0

* All treatments had 9 plants except Site 2, d (8 plants) and Site 9, c (7 plants). All plants except those from Site 1 were infected.

simultaneously for 4 weeks, then 1 series was harvested and the other transferred to the open-sided glasshouse. At the time of transfer, the only parameters that could be measured for both series were leaf length (longest leaf only) and number of 'healthy' seedlings (i.e. those showing no yellowing or less than 0.5 cm of leaf tip yellowing). Comparison of these parameters between the 2 series is shown in Table 12 along with the infection percentages for the harvested series. As there were only 2 parameters for comparison, analysis of variance was performed on the data for the number of 'healthy' seedlings. It can be seen that for the comparable parameters, only the seedlings in undisturbed cores were significantly different from those of other treatments. The similarity of the standard error for both series (in both parameters) suggests that the bioassay results for percentage of infected seedlings and percentage of infected roots may be similar in both series. Further, the range of infected roots (3-86%) show that a wide range of initial infection has been achieved.

In the remaining cores the number of plants was reduced to 6 to remove some variation in emergence. The growth patterns were similar to those observed in the previous season with 1 or 2 plants dominating the bucket in some instances. At the time of harvesting all plants were rated for severity of disease on the following scale:

Very low - Plants large and vigorous; no obvious O. graminis infection of roots, but presence later detected by growing wheat seedlings in contact with roots.

TABLE 12

Comparison of average leaf length and number of 'healthy' seedlings after 4 weeks growth for undisturbed and sieved cores harvested for bioassay or grown to maturity. Also incidence of O. graminis on seedlings from the harvested cores (mean of 7 replications).

Core treatment	Average leaf length (cm)		Number of 'healthy' seedlings		Harvested series of cores			
					Percentage of:			
					seedlings infected		roots infected	
	A*	B*	A	B		Arcsine (radians)		Arcsine (radians)
Undisturbed	14.9	12.2	3.1	3.7	98	1.51	86	1.29
Sieved(5.0mm)	16.8	15.3	5.1	5.4	90	1.36	66	0.99
Sieved(1.0mm)	17.3	16.8	6.0	6.6	55	0.88	26	0.50
Sieved(0.5mm)	18.0	16.7	6.1	6.4	7	0.18	3	0.10
Standard error	0.8	0.7	0.5	0.5		0.10		0.08
LSD p = 0.05	1.7	1.5	1.0	1.0		0.21		0.17
= 0.01	2.3	ns	1.4	1.4		0.29		0.23

* A harvested at 4 weeks

B grown to maturity

Moderate - Plants large and vigorous; *O. graminis* infection on roots easily observed.

Severe - Reduction in plant size; stem blackening; head formed.

Very Severe - Plant very reduced in size; considerable stem blackening; no head formed.

The results of this assessment are shown in Table 13. The results for the undisturbed cores and the 0.5 mm sieved cores (lowest level of inoculum) are of particular interest. As anticipated, most of the plants in the former treatment were severely infected, but an unexpectedly high number of plants (72%) from 0.5 mm sieved cores were in the severe category. To a lesser extent, the same is true of the 1.0 mm sieved core results. Examination of plant growth and yield (Table 14) indicates that the number of tillers produced followed the pattern expected, but the yield (especially the yield of plump grain) was quite different from that expected. Although the differences in yield are large, they are not significantly different because of the variability of the replications (Table 15) caused by scattered vigorous plants. However, the over-all impression was that disease severity in the sieved cores was opposite to that expected. Without further investigations it is impossible to determine whether the infection in the 0.5 mm sieved soil was due to all cores having at least 1 root infected at the 4 week stage, or whether with extra time the roots were able to explore more soil and thus come into contact with more inoculum. The possibility of cross contamination in the glasshouse, as noted by Gerlagh (1968) can be discounted as check plants grown in 'soil mixture' as part of the replicated trial were all disease-free.

TABLE 13

Number of plants out of 42 in 4 disease rating categories. Plants grown to maturity in undisturbed and sieved cores which were transferred to large containers.

Core treatment	Disease rating			
	Very low	Moderate	Severe	Very severe
Undisturbed	0	4	4	34
Sieved (5.0mm)	7	16	18	1
Sieved (1.0mm)	4	11	27	0
Sieved (0.5mm)	0	12	30	0

TABLE 14

Number of tillers and grain production for plants grown to maturity in undisturbed and sieved cores transferred to large containers (mean of 7 replications).

Core treatment	Number of tillers per bucket (6 plants)	Total grain yield (g)	Total yield plump grain (g)
Undisturbed	8.3	1.8	1.3
Sieved (5.0mm)	13.3	7.6	6.7
Sieved (1.0mm)	16.3	7.5	4.4
Sieved (0.5mm)	16.0	5.6	2.6
Standard error	0.7	1.5	1.6
LSD p = 0.05	1.5	ns	ns
= 0.01	2.0		

TABLE 15

Comparison of yield per replication for plants grown to maturity in undisturbed and sieved cores transferred to large containers

Core Treatment	Yield of grain per replication (g)							Mean yield of grain (g)
	1	2	3	4	5	6	7	
Undisturbed	0	3.9 (1)	1.8 (2)	0	0	3.5 (2)	3.7 (2)	1.8
Sieved(5.0mm)	10.4	13.8	3.1	13.9	10.1	0.1	2.2	7.6
Sieved(1.0mm)	9.7	7.3	8.6	4.2	2.4	12.3	8.1	7.5
Sieved(0.5mm)	2.7	1.4	6.2	12.5	6.5 (3)	7.1	2.8	5.6

(1) 3.5g from 1 plant

(2) All from 1 plant

(3) 3.0g from 1 plant

It is possible that sieving affected soil texture and consequently aeration. Garrett (1936, 1937) demonstrated that improved aeration encouraged hyphal growth along the root surface, and it is known that take-all is favoured by light textured soils (Griffiths, 1933; Garrett, 1934a). On the other hand, while sieved soil is initially more 'fluffy', after continued watering, it compacts more than undisturbed soil, and this would reduce aeration. Without further investigation it is impossible to determine why early levels of infection could not be correlated with yield at maturity.

B. Bioassay of wheat stubble for the presence of O. graminis.

Many authors have assessed the incidence of *O. graminis* in the field by the number, or percentage, of plants infected (White, 1945; Chambers, 1962; Chambers and Flentje, 1968; Etheridge, 1969a; Ebbels, 1969). These assessments are generally made by visual examination of the root or foot region of the plant and some have been confirmed by production of perithecia (White, 1945) or by isolation (Chambers, 1962).

Although symptoms of *O. graminis* infection may be obvious on immature or recently mature plants, they are difficult to detect on old wheat stubble. Visual examination of stubble also gives no indication of whether viable *O. graminis* is still present. As isolation of *O. graminis* from old wheat stubble is difficult (White, 1945), a bioassay to detect the presence of *O. graminis* was developed. The bioassay is based on a test developed by Garrett (1938) and is similar to that used

by Hornby (1969b).

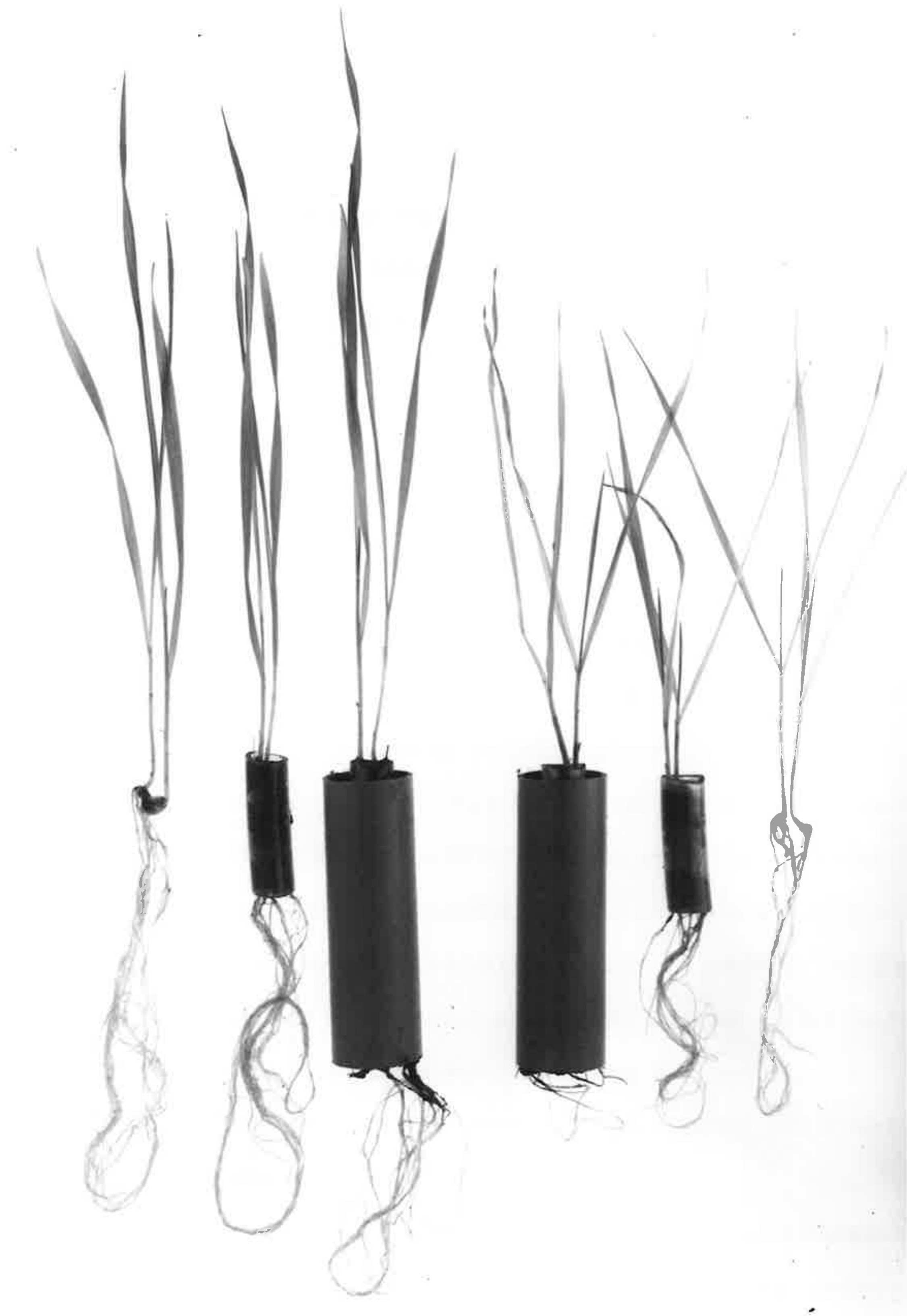
After collection of the stubble, adhering soil was removed from each crown (taken to include attached roots), which was then placed inside an 'assay tube' ("Camelec" polyvinyl chloride, internal diameter 1 cm, and length 4 cm). Each was threaded through the 'assay tube' and the roots packed down against the base of the crown. Any stem straw extending beyond the bottom of the tube was removed. If the crown was so large that it was difficult to insert in the tube, some tillers were broken off and discarded. If the crown was very small, it was rolled into a ball and inserted into the tube.

Two wheat seeds were placed on top of the crown in the 'assay tube', covered with 'soil mixture' and grown for 4 weeks. A small preliminary trial demonstrated that growing of a wheat seedling on top of infected material could detect the presence of O. graminis. This applied to stubble from the current and previous season. It was noticed that the confined nature of the system sometimes caused the leaf of the seedling to grow down the tube. For this reason, and to minimize germination failures, 2 seeds were sown per 'assay tube'.

'Growth cylinders' ("Clipsal" Class A Rigid 1 inch polyvinyl chloride Conduit, internal diameter 2.25 cm and length 8 cm) were packed into a box and filled with 'soil mixture' to about 1 cm from the top. The 'assay tube' was inserted in the 'growth cylinder' (Fig. 7). After watering, the box was covered with a plastic sheet and placed in the controlled environment described previously. The plastic sheet was removed as soon as seedlings started to emerge. The box was watered daily to

FIGURE 7

Method used to detect the presence of *O. graminis* in wheat stubble. Two wheat seedlings are grown on crowns inserted in 'assay tubes'. These tubes are placed in soil inside 'growth cylinders'. Seedlings on left disease free. Seedlings on right infected from crowns containing viable *O. graminis*.



saturation and allowed to drain freely. After 4 weeks the seedlings were removed and assessed for O. graminis infection. The number of seedlings showing vascular discoloration of 1 or more roots was recorded.

To determine the time needed for seedlings to become infected when grown above an infected crown, 20 sets of seedlings were assessed at 2, 3 or 4 weeks. Treatments in the 'assay tubes' were (1) obviously infected crowns, (2) doubtfully infected crowns from the same crop and (3) no plant material present. Half the 'assay tubes' were placed in 'soil mixture' in cups (35 cm^3) and the remainder in soil in a seed box to assess the likelihood of cross-infection. Within the boxes the time treatments were separated, but other treatments were inserted randomly in the soil to a depth of about 2 cm at 2.5 cm centres on a square grid. Separating 'growth cylinders' were not used in this experiment. All other conditions for the experiment were as described above.

The results are shown in Table 16. It can be seen that 4 weeks were necessary for 80 per cent or more of the test seedlings to become infected by O. graminis from those crowns visually assessed as infected; the discrepancy between the crowns visually assessed as positive and the bioassay may have been due to incorrect assessment, lack of viable fungus, or the possibility that more than 4 weeks is needed for seedlings to become infected. These possibilities were not investigated, but 4 weeks for seedling growth was adopted as standard practice.

It can be seen (Table 16) that there was some cross-infection amongst the seedlings within the box. In some instances where seedlings were infected from a source external to the material in the 'assay tube',

TABLE 16

Effect of harvest interval on the number of wheat seedlings infected with *O. graminis*. Seedlings grown in 'assay tubes', with or without infected plant material.

Treatment	Number of seedlings infected from:	Plant material visually assessed as:						No plant material (max 20)		
		infected (max 20)			doubtfully infected (max 20)					
		weeks			weeks			weeks		
		2	3	4	2	3	4	2	3	4
'Assay tubes' in cups	material in 'assay tube'	3	12	19	2	3	8	-	-	-
'Assay tubes' in seed boxes	material in 'assay tube'	7	12	16	6	7	10	-	-	-
	source outside 'assay tube'	0	0	2	0	0	0	0	0	4

discoloration of roots extended up into the tube. To minimize the possibility of incorrect assessment (without the extra trouble of using individual cups), 'growth cylinders' were employed to keep roots separate until they reached the bottom of the seed box.

Of the group of crowns assessed as doubtfully infected, about half contained viable O. graminis (Table 16). This demonstrates the difficulty of determining the presence of O. graminis in stubble by visual assessment. This problem was further investigated. A large number of mature wheat plants were collected from the experimental area at Ceduna. These were assessed for the presence of O. graminis, both visually and by the bioassay.

Table 17 shows that of the crowns visually assessed as infected, less than 4 per cent were incorrectly assigned, but of those considered to be free of infection, 41 per cent were incorrectly assigned. This discrepancy could be due to my inability to detect all lesions present on the crowns or the presence of O. graminis in a form difficult to detect macroscopically. It is also possible that the bioassay would detect O. graminis which was present on the stubble as runner hyphae (Warcup, Pers. Comm.), but which had not invaded the plant. The detection of O. graminis that had not invaded the plant would not affect the studies envisaged, as I am interested in the survival of the fungus, rather than detecting where it is surviving on the plant remains or debris.

C. Relationship of incidence of O. graminis and grain yield

For intensive studies of a limited area in the field over a long

TABLE 17

Comparison of visual assessment and bioassay for the detection of *O. graminis* in mature wheat stubble.

Visual assessment	Bioassay	Number of crowns	Percentage of crowns in visual categories	Percentage of total number of crowns
+	+	339	96.6	53.3
+	-	12	3.4	1.9
-	-	168	59.0	26.4
-	+	117	41.0	18.4

period of time, it is necessary to develop methods of assessing the incidence of *O. graminis* with minimum disturbance to the location. Methods based on an above ground part of the host, that is those parts (e.g. grain yield, plant height, straw weight, etc.) which may portray the level of take-all would be advantageous. The problems are, however, to correlate take-all with indices measuring the incidence of *O. graminis* (e.g. number of plants infected, length of discoloured root tissue), and secondly, to be sure that differences in growth are a reflection of *O. graminis* alone and not some other factor.

Grain yield, which is more convenient to record than plant height or straw yield, has been shown to be negatively correlated with *O. graminis* incidence. Suzuki *et al.* (1957) demonstrated a significant correlation between average grain yield per plant and infection based on an incidence scale ranging from 0 to 4. Slope (1967) has shown that for each 1 per cent increase in the number of tillers infected there was an 0.3 cwt decrease in grain per acre (or approximately 0.6%). Rosser and Chadburn (1968) found, over a period of 3 years, that the regression equation was:

$$y = 36.55 - 0.127x$$

where y = yield in cwt per acre and x = per cent of tillers infected with *O. graminis*. Nilsson (1969) demonstrated that a relationship between yield and O. graminis incidence for the spring wheat variety Kärn II could be expressed by the equation:

$$y = 36.51 - 0.346x$$

In this expression y = the yield (1000-kernels dry weight in g), while

x = the mean disease index composed of the disease index of the seminal roots (%), the disease index of the crown roots (%) and the disease index of the straw base (%). In this study 1,800 plants were examined. He found similar equations when measuring the same parameters for a smaller number of plants (300) for 2 other spring wheats. Unfortunately the disease indices used by Nilsson (1969) are based on a complicated system of visual assessment.

The investigations reported below were designed to study the possibility of using yield to estimate *O. graminis* incidence. The aim was to correlate yield with incidence, and to develop a method of finding the latter with the minimum of work and disturbance to the field location.

(1) At Turretfield.

In the first experiment, at the Turretfield experimental area, 3 blocks (A, B & C in Fig. 3, page 28) within the 1969 crop were chosen at the end of the growing season for detailed investigation. The only apparent differences between the blocks was the incidence of take-all and some soil compaction where take-all was severe due to insufficient ground cover to protect the soil surface from rain.

Block A was chosen to represent a location without take-all. Nearly all plants were vigorous, being even in height and producing grain-filled heads. Block B was situated in a take-all patch. All plants were small compared to those in Block A and many had failed to produce heads. Block C contained both vigorous and small plants, a take-all patch extended down most of the western side and there also appeared to be a small patch in the north-east corner.

Each block contained 12 drill rows 3.6 m long and divided into 12 sites 30 cm in length. The heads from each site were collected, threshed and the grain weighed. Detailed results are shown in Appendix B. Within each block, the plants were removed from 12 sites (1 site selected at random per drill row) and the crowns bioassayed for the presence of *O. graminis*. These results are given in Tables 18, 19 and 20.

The aim was to determine whether the yield results from Block A and B could be used to predict the incidence of *O. graminis* in Block C. Two approaches were employed. They were, firstly, to examine the variability of yield within Blocks A and B to determine the sample size necessary to differentiate between A and B; and then to map the *O. graminis* incidence in C based on the yields in A and B. In the second approach the regression of the incidence of *O. graminis* and yield was established, and then used to predict the yield at selected levels of incidence. The maps obtained by this method and the former method were then compared.

To examine the variability in yield, it was assumed that A was a uniformly high yield, low incidence block, while B was a uniformly low yield, high incidence block. If this assumption was correct it would be possible to divide C into parts similar to both A and B, and possibly some sections intermediate to both. However, examination of the detailed yield results in Appendix B reveals that there is considerable variability of yield between sites within both A and B.

The range of the distribution within which the yield from 95 per cent of the sites were expected to fall, was calculated for each block. The 95 per cent range was estimated by the following equation:

TABLE 18

Number of plants, yield and level of infection from 12 random sites in Block A at Turretfield.

Row	Site	Number of plants	Total yield (g)	Average yield (g)	Percentage of infected crowns
1	6	23	19	0.83	30
2	12	10	14	1.40	20
3	1	13	21	1.62	15
4	9	8	10	1.25	38
5	10	20	17	0.85	10
6	1	11	8	0.73	64
7	10	8	15	1.88	13
8	7	12	9	0.75	8
9	12	11	9	0.82	36
10	9	19	13	0.68	10
11	10	9	11	1.22	33
12	4	17	13	0.76	24
Mean		13.4	13.3	1.07	25.1
Standard error		1.7			

TABLE 19

Number of plants, yield and level of infection from 12 random sites in Block B at Turretfield.

Row	Site	Number of plants	Total yield (g)	Average yield (g)	Percentage of infected crowns
1	4	10	3	0.30	60
2	10	14	0	0	100
3	7	10	1	0.10	90
4	12	8	1	0.13	100
5	12	9	4	0.44	100
6	7	9	1	0.11	100
7	8	7	2	0.29	100
8	5	9	0	0	100
9	12	12	0	0	100
10	7	6	4	0.67	100
11	8	8	1	0.13	100
12	9	14	1	0.07	93
Mean		9.7	1.5	0.19	95.3
Standard error		0.7			

TABLE 20

Number of plants, yield and level of infection from 12 random sites in Block C at Turretfield.

Row	Site	Number of plants	Total yield (g)	Average yield (g)	Percentage of infected crowns
1	6	18	7	0.38	78
2	8	13	0	0	100
3	10	10	4	0.40	100
4	8	23	2	0.09	100
5	5	12	16	1.33	92
6	10	10	2	0.20	100
7	1	9	14	1.56	67
8	8	11	8	0.73	55
9	11	12	13	1.08	58
10	7	9	8	0.89	56
11	1	11	0	0	100
12	8	15	12	0.80	93
Mean		12.8	7.3	0.62	83.3
Standard error		1.3			

$$95\% \text{ range} = \bar{x} \pm \text{S.D.} \times t$$

where $\bar{x}$ = the mean for the total yield for all sites in each block, S.D. = the standard deviation of the mean and t = "t" value at p = 0.05 for the number of sites (n). The standard deviation was determined by the formula:

$$\text{S.D.} = \sqrt{\frac{1}{n-1}\left(\Sigma x^2 - \frac{(\Sigma x)^2}{n}\right)}$$

The results of various steps in the calculation are included in Table 21. The results for 144 sites (12 x 12) show that the limits for the 95 per cent ranges for A and B overlap (i.e. 4.5 to 20.1 and 0 to 6.6). This means that the use of sites of this size (i.e. 1 drill row x 30 cm) to establish *O. graminis* incidence limits for Block C is impractical, as the sites within the so-called uniform Blocks A and B were too variable.

However, if 4 sites are combined to make 1 macro-site (2 drill rows x 60 cm long) and the yield results are remapped (Table 22), it can be seen that variability is reduced. This is confirmed by examination of the results for the 36 macro-sites (6 x 6) in Table 21. The range for A and B in which the yield from 95 per cent of the macro-sites is expected to fall, do not overlap (34.1 to 64.1 and 0.6 to 16.8 respectively). All macro-sites with a yield of 17g or less are considered to be similar to Block B (based on the upper limits of the 95 per cent distribution range) and therefore have a high *O. graminis* incidence. Sites with yields greater than 34 g are considered to be similar to A (based on the lower limit).

If the above results are applied to Block A (Table 22) it can

TABLE 21

The range of the distribution within which the yield (g) from 95% of the sites are expected to fall for 144 sites (12 x 12) and 36 macro-sites (6 x 6) for Blocks A, B and C at Turretfield. Also corresponding means and standard deviations x "t"

		AREA A	AREA B	AREA C
Mean ($\overline{x}$)	yield (12 x 12)	12.3	2.2	8.1
	yield (6 x 6)	49.1	8.7	32.6
Standard deviation x "t"	yield (12 x 12)	7.8	4.4	9.4
	yield (6 x 6)	15.0	8.1	27.0
95% range ($\overline{x}$±SDx"t")	yield (12 x 12)	4.5 - 20.1	0 - 6.6	0 - 17.5
	yield (6 x 6)	34.1- 64.1	0.6 -16.8	5.6- 59.6

TABLE 22

Grain yield (g) from each of 36 macro-sites for Blocks A and B at Turretfield

BLOCK A

58	51	31	54	60	46
53	60	61	52	35	52
61	48	45	53	57	52
56	37	50	43	42	42
50	40	49	47	42	43
58	50	51	51	38	49

BLOCK B

6	8	11	11	3	10
16	8	12	5	3	6
11	12	7	5	14	19
14	6	5	11	11	8
7	14	2	9	8	3
13	2	8	7	11	6

be seen that 1 macro-site would be placed in the intermediate incidence division and the remaining 35 in the low incidence type. One macro-site in Block B (Table 22) is classed as intermediate, while the remainder are classed as high incidence macro-sites.

With this information it was possible to again examine Block C. The macro-site yields for C (6 x 6) are shown in Figure 8a. Superimposed are 3 levels of O. graminis incidence. The map produced is similar to the visual assessment of the situation made prior to harvest. However, a large number of macro-sites are classed as low incidence areas and, as can be seen from Table 20, this is probably not the case. The inference is therefore that mapping O. graminis by yield alone is underestimating the true incidence. The map based on yield alone possibly gives an indication of early levels of O. graminis rather than incidence at the end of the season.

In the second approach the correlation of O. graminis and yield was studied. Correlation coefficients and analyses of variance for the regressions comparing percentage of infected crowns with number of plants, total yield and average yield per site were computed (Table 23). In no instance was incidence correlated with the number of plants per site and only in Block C was incidence and average yield correlated, with a significant variance ratio at $p = 0.05$.

As all blocks are located in the same vicinity, and as there are no obvious differences in soil type, and no pathogens of importance other than O. graminis, examination of the regression using the combined data from all sites can be undertaken. Table 23 shows that the percentage

FIGURE 8

MAPS SHOWING GRAIN YIELD FOR MACRO-SITES WITHIN BLOCK C AT TURRETFIELD. SUPERIMPOSED ARE MAPS SHOWING THE ESTIMATED INCIDENCE OF *O. GRAMINIS*

(a) Incidence map based on yield results from adjacent take-all and non-take-all blocks.

High incidence : less than 17 g

Intermediate incidence : 18 to 33 g

Low incidence : more than 34 g

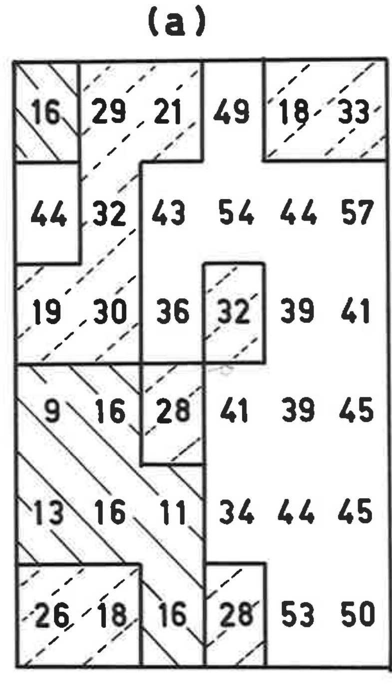
(b) Incidence map based on the regression of infected crowns and yield.

High incidence : less than 30 g

Intermediate incidence : 31 to 60 g

FIG. 8

(a)

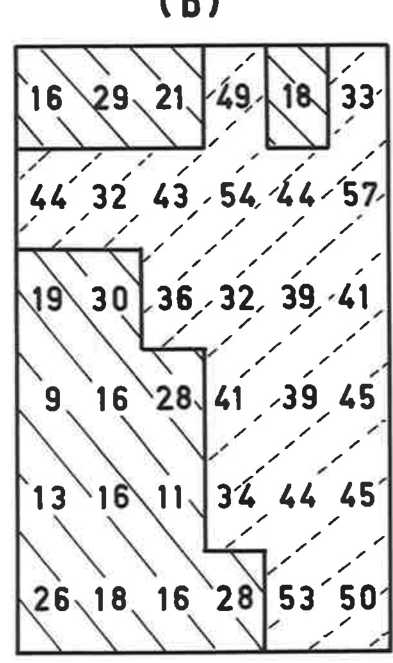


High incidence

Intermediate incidence

Low incidence

(b)



High incidence

Intermediate incidence

Scale ⟷ 30 cm

TABLE 23

Results of the regression analysis for the comparison of percentage of crowns infected with the number of plants per site, and yield per site for Blocks A, B and C (individually and combined) at Turretfield.

Parameters	Block	Variance ratio	Correlation coefficient 'r'
Percentage infected crowns vs Number of plants	A	0.8	0.2805
	B	0.2	0.1454
	C	1.1	0.3137
	Combined	2.6	0.2665
Percentage infected crowns vs Total yield	A	3.2	0.4909
	B	0.9	0.2793
	C	4.2	0.5428
	Combined	56.0***	0.7888
Percentage infected crowns vs Average yield	A	0.5	0.2187
	B	0.1	0.1094
	C	6.1*	0.6169
	Combined	34.2***	0.7081

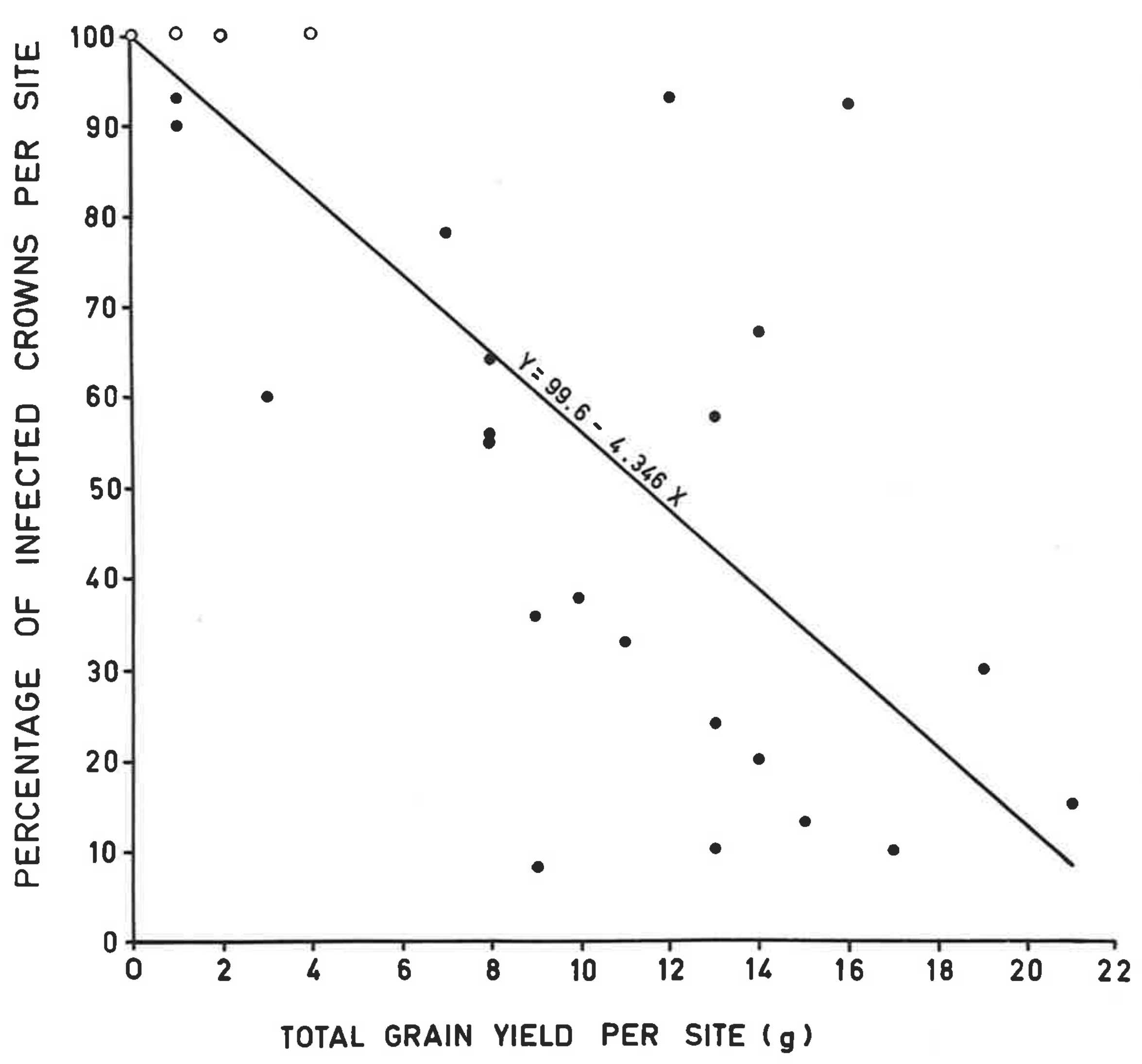
of infected crowns is significantly ($p = 0.001$) correlated with both total yield and average yield. The relationship between infection and total yield is shown in Figure 9. It is of interest that the correlation coefficient for the percentage of infected crowns is higher with total yield than with average yield (Table 23). Calculation of average yield is a problem because of the difficulty in counting the number of plants per site (i.e. determining which are plants and which are tillers) and the work involved in making and recording the count. It is possible that tillering removes gaps caused by variation in number of plants, and this may be the reason why total yield is better than average yield for making comparisons with incidence.

From the regression equation established (Fig. 9), I was able to calculate the expected yield for any level of incidence of O. graminis. From this it was possible to again map Block C. To delimit boundaries for incidence categories, 2 arbitrary levels were selected. All sites with 67 per cent or more of the crowns infected were called high incidence sites, while those with 33 per cent or less were called low. If these points are applied to the regression in Figure 9, it can be calculated that high incidence macro-sites would have a yield of 30 g or less (7.5 x 4 to bring site yield to macro-site equivalent). Similarly those macro-sites with a yield of 61g or more would be in the low incidence category.

Using the above categories all of Block A, except 2 macro-sites, would be mapped as intermediate incidence (Table 22). The 2 exceptions would be low incidence macro-sites. Although visually, Block A was

FIG. 9

RELATIONSHIP BETWEEN PERCENTAGE INFECTED CROWNS AND TOTAL YIELD FOR 36 SITES AT TURRETFIELD 1969



apparently disease-free, the bioassay of crowns suggests that about 25 per cent of the plants were infected (Table 18). All of Block B would be in the high incidence category.

When the above incidence levels are superimposed on the 'yield' map for Block C (Fig. 8b), only high and intermediate incidence macro-sites are obtained. Examination of results for Block C (Table 20) suggests that, although the yield mean is intermediate to A and B, the percentage of infected crowns is heavily biased towards B. This in turn indicates that all of C should be in either high or intermediate categories. The map (Fig. 8b) based on the arbitrary categories for levels of infection appears to best portray this condition.

In conclusion, it seems that mapping by yield (sample size, 2 drill rows x 60 cm) could be used to obtain a map showing the estimated incidence of O. graminis but it needs to be associated with actual incidence by obtaining an 'incidence-yield' regression.

(2) At Ceduna.

In the experiment at Turretfield only a small number of sites were used to determine the percentage of infected crowns. In an experiment, conducted at the Ceduna experimental area, the regression of incidence and yield was established using a large number of samples. The experimental block (F on Fig. 3, page 28) contained part of a poorly defined take-all patch and a small section in the south-east corner with relatively vigorous plants (Fig. 10). The block had 16 drill rows each 4.8 m long, which were divided into 16 sites 30 cm long. From each site the grain yield was recorded. From every second site in the drill row

FIGURE 10

General view of experimental area at Ceduna showing *O. graminis* infected wheat crop. Block F pegged out (looking south).

(even numbers in row 1, odd numbers in row 2 and so on), all the plants were removed and the crowns bioassayed for the presence of O. graminis. From all the remaining sites a soil core (9.8 cm) was removed from the centre of the 30 cm length of row and bioassayed for the presence of O. graminis. The detailed results for this experiment are given in Appendix C.

Details of the regression analysis for the comparison of the percentage of infected crowns with number of plants, total yield and average yield per site are given in Table 24. The percentage of infected crowns per site was not influenced by the number of plants per site. As previously, (Table 23) the correlation between the percentage of infected crowns and total yield was superior to that for the percentage of infected crowns and average yield. This again suggests that total yield removes some of the variability caused by the differences in numbers of plants per sample site. The regression details for the comparison of the percentage of infected seedlings and the percentage of infected roots per core with total yield are given in Table 24. There is a highly significant correlation between yield and both these parameters, with the percentage of infected roots being the better.

With these regression equations established, a series of maps were prepared based on (1) the relationship of incidence and yield, and (2) incidence alone. These maps were then compared.

If the arbitrary points selected previously (i.e. 33% and 67%) are used in that part of the experiment employing the percentage of infected crowns (per site), it can be calculated from the regression curve in

TABLE 24

Results of the regression analysis for comparison of (1) the percentage of infected crowns per site with number of plants and yield from every second site; (2) the percentage of infected seedlings or roots per core with yield from the alternative sites. From Block F at Ceduna.

Type	y	x	Variance Ratio	Correlation coefficient 'r'	Regression equation
Plants	% crowns infected	Number of plants per site	3.7	0.1766	-
	"	Total yield per site (g)	139.7***	0.7377	y = 100.69-9.95x
	"	Average yield per site (g)	79.8***	0.6368	y = 97.45-41.90x
Cores	% seedlings infected	Total yield per site (g)	37.3***	0.4642	y = 94.59-6.33x
	% roots infected	Total yield per site (g)	53.4***	0.5470	y = 78.61-7.51x

Figure 11a, that low incidence macro-sites would have a yield of 27g or more while high incidence macro-sites would have a yield of 14g or less. If these categories are superimposed on the 'yield' map shown in Figure 12a, a map predicting the incidence of O. graminis is obtained. This type of map will be called an 'incidence-yield' map. If the actual 'incidence' map based on the percentage of infected crowns per site is drawn (Fig. 12b) with the same delimiting points (33% and 67%) it can be seen that the maps are very similar. The 'incidence-yield' map has under-estimated the parts shown as low incidence in Figure 12b, but otherwise Figures 12a and 12b show a good correlation.

The regression for the percentage of infected roots per core and total yield per site intercepts the y axis at 78.6 per cent (Fig. 11b). If the arbitrary points of delimitation are again selected to represent 3 equal portions of the y axis, the upper limit for low incidence would be 26.2 per cent and the lower limit for high incidence would be 52.4 per cent. As these points are difficult to fit into the computer mapping program used, the limiting points were chosen as 25 and 50 per cent respectively. If these points are applied to the regression (Fig. 11b), the upper yield limit for the high incidence macro-sites would be 15g and the lower limit for the low incidence macro-sites would be 28g. When these limits are used to superimpose the estimated O. graminis incidence on the yield results (Fig. 13a), the resulting 'incidence-yield' map is similar to the map shown in Figure 12a. When the actual 'incidence' map is drawn (Fig. 13b), it is similar to its 'incidence-yield' counterpart Figure 13a. However the 'incidence' map shown in Figure 13b is more patchy than that

FIGURE 11

'INCIDENCE-YIELD' REGRESSIONS FOR BLOCK F AT CEDUNA, 1969

(a) Relationship between percentage of infected crowns and total grain yield per site.

(b) Relationship between percentage of infected roots per core and total grain yield per site.

FIG. 11

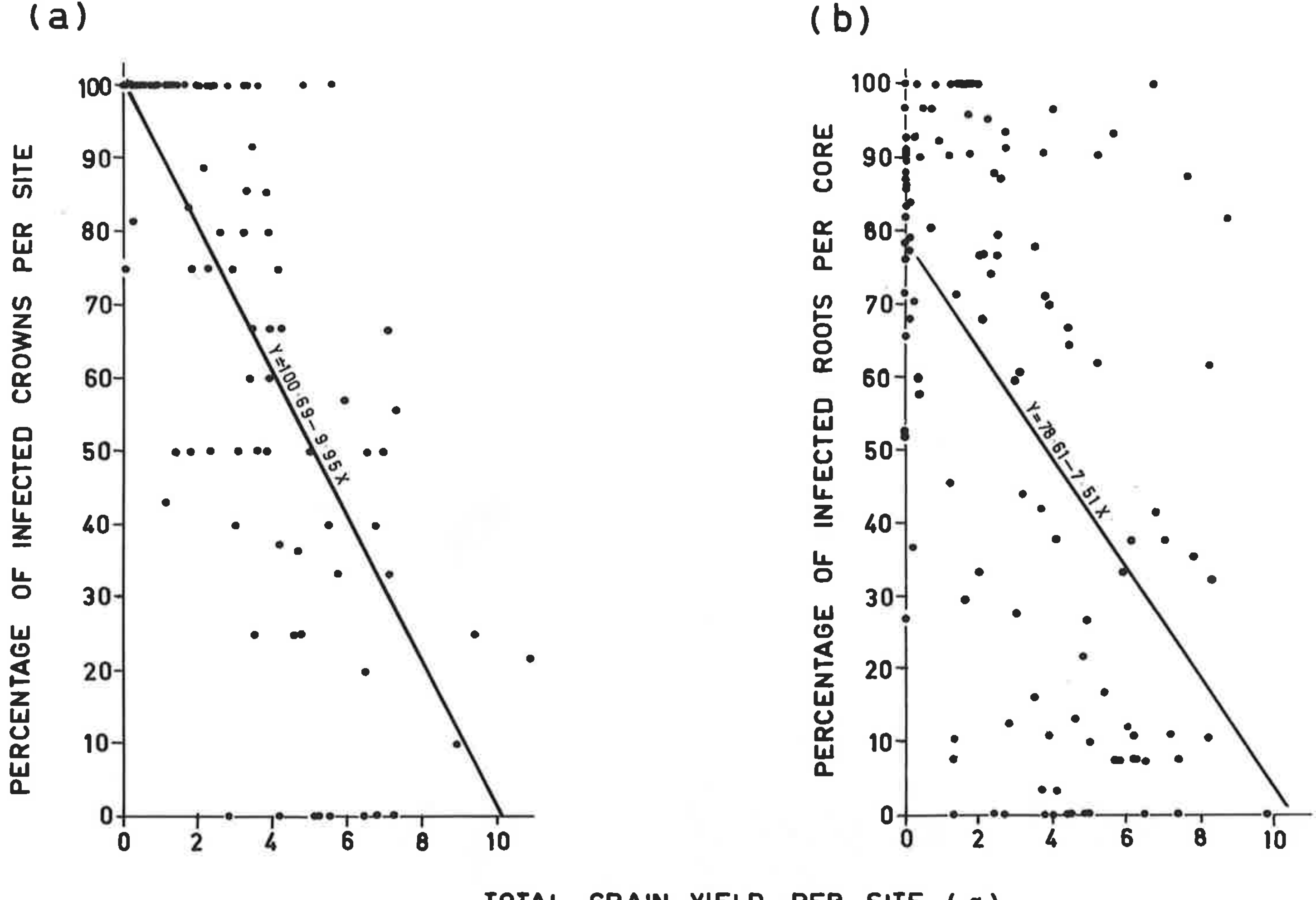


FIGURE 12

(a) 'INCIDENCE-YIELD' MAP CEDUNA, 1969

Map showing grain yield per macro-site. Superimposed is an incidence map based on regression of percentage of infected crowns per site and yield per site.

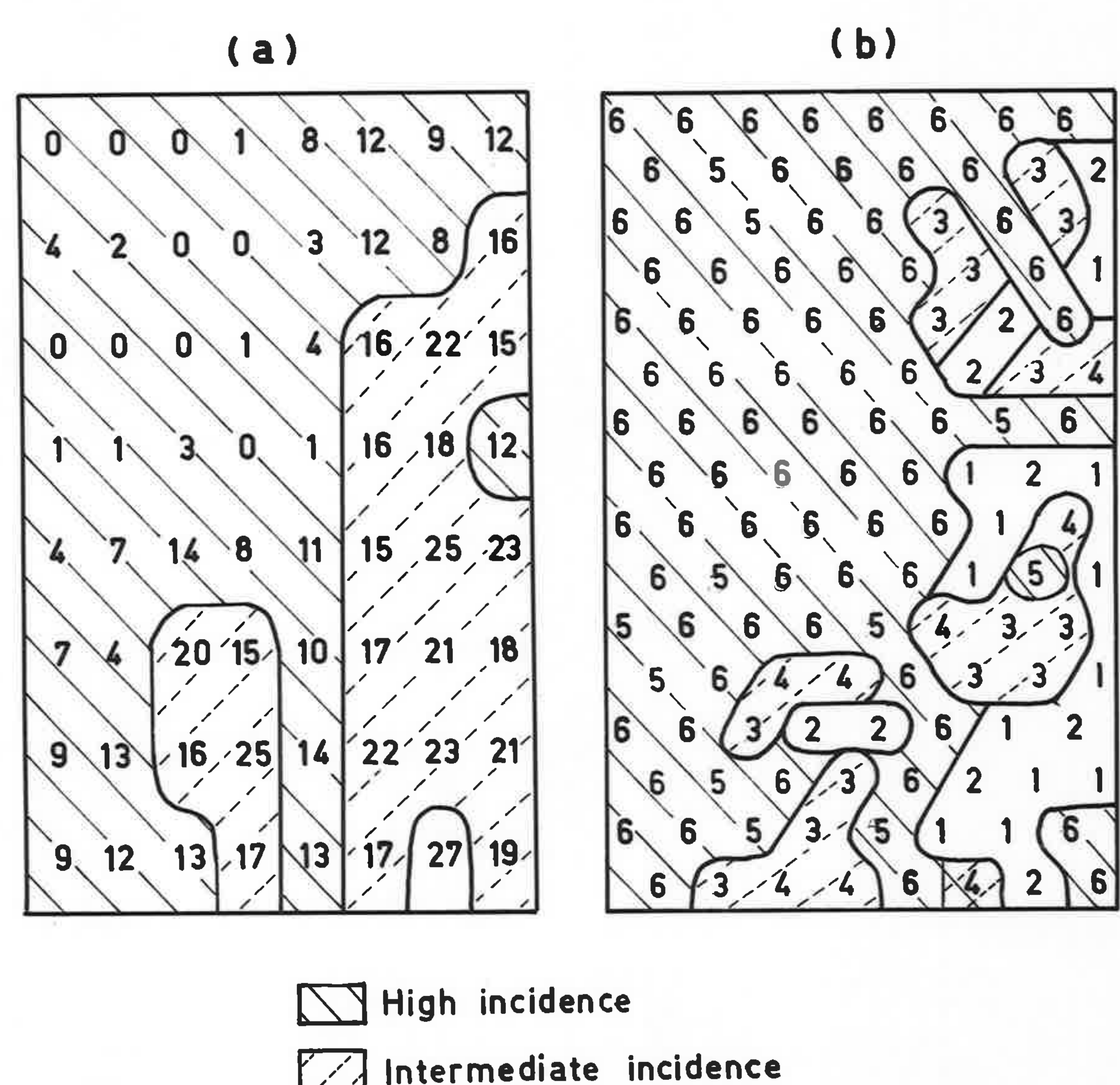
High incidence	:	less than 14g
Intermediate incidence	:	15 to 26g
Low incidence	:	more than 27g

(b) 'INCIDENCE' MAP CEDUNA, 1969

Map showing incidence (percentage of infected crowns per site) based on every second site.

High incidence	:	(5 = 68 to 83%, 6 = 84 to 100%)
Intermediate incidence	:	(3 = 34 to 50%, 4 = 51 to 67%)
Low incidence	:	(1 = 0 to 16%, 2 = 17 to 33%)

FIG. 12



Scale ⟼ 30 cm

FIGURE 13

(a) 'INCIDENCE-YIELD' MAP CEDUNA, 1969

Map showing grain yield per macro-site. Superimposed is an incidence map based on regression of percentage infected roots per core and yield per site.

High incidence : less than 15g

Intermediate incidence : 16 to 27g

(b) 'INCIDENCE' MAP CEDUNA, 1969

Map showing incidence (percentage of infected roots per core) based on 1 core taken from the centre of every second site.

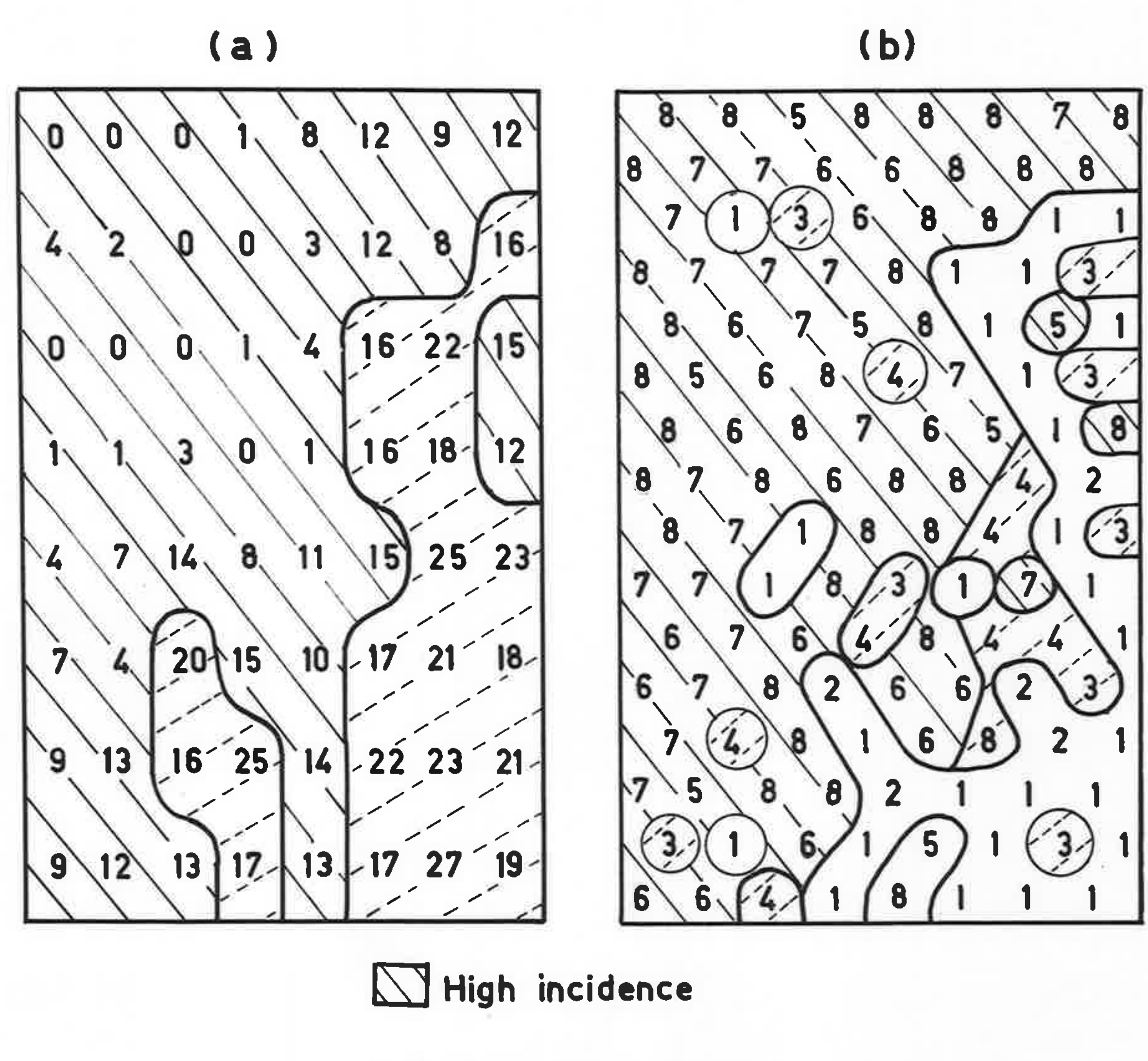
High incidence : (7 = 76 to 87%, 8 = 88 to 100%)

(5 = 51 to 62%, 6 = 63 to 75%)

Intermediate incidence : (3 = 26 to 37%, 4 = 38 to 50%)

Low incidence : (1 = 0 to 12%, 2 = 13 to 25%)

FIG. 13



shown in Figure 12b. This indicates that 1 or both methods are giving an incorrect estimate of the incidence of O. graminis. As exactly half the entire area (30cm in every 60cm) was sampled using the percentage of infected crowns, while slightly less than a sixth of the entire area (9.8cm in every 60cm) was sampled with the cores, it could be expected that the crowns would give a better indication of incidence than the cores. The 'incidence' map based on the percentage of infected roots per core has probably underestimated true incidence.

In conclusion, it can be seen that the percentage of infected crowns per site and to a lesser extent, the percentage of infected roots per core show a good correlation with yield when intensive sampling of a limited area is undertaken. Such sampling destroys the area. For this reason there was a need to know the number of samples required to give a consistent regression equation.

(3) Determination of 'incidence-yield' regression.

A series of regression analyses was performed on the results of the Ceduna experiment. For both the bioassay of crowns and cores, a random selection of 1, 2, 3 or 4 samples per row (4.6 m) was compared with the yield from the same sites. The regression equations obtained were then compared with the original regression equation based on the maximum number of sites per row (8). A regression analysis was also performed on 8 samples from the take-all patch and 8 from that part of the block with vigorous plants. The sites chosen were the 8 most north-west and the 8 most south-east for both types of assays. The results are shown in Table 25 and Figure 14. At least 2 sites per 4.6 m row are needed for

TABLE 25

Results of regression analysis comparing incidence and total grain yield per site for 1, 2, 3, 4 or 8 samples per row in a take-all area at Ceduna. Also regression analysis based on 8 take-all and 8 non take-all sites.

Relationship	Number of samples	Variance ratio	Correlation coefficient 'r'	Regression equation
Percentage of crowns infected per site (y) vs total yield per site (x)	1 sample per row (16)	5.4*	0.5423	y = 100.27 - 6.76x
	2 samples per row(32)	45.5***	0.7977	y = 98.50 - 10.56x
	3 " " " (48)	62.7***	0.7666	y = 101.81 - 10.18x
	4 " " " (64)	73.3***	0.7530	y = 99.44 - 10.40x
	8 " " " (128)	139.7***	0.7377	y = 100.69 - 9.95x
	8 take-all and 8 non take-all sites (16)	17.9***	0.7873	y = 100.50 - 9.03x
Percentage of roots infected per core (y) vs total yield per site (x)	1 core per row (16)	13.4**	0.7001	y = 81.11 - 11.24x
	2 cores per row (32)	13.4***	0.5561	y = 84.02 - 8.39x
	3 " " " (48)	21.6***	0.5693	y = 78.92 - 6.72x
	4 " " " (64)	21.4***	0.5068	y = 78.12 - 7.53x
	8 " " " (128)	53.4***	0.5470	y = 78.61 - 7.51x
	8 take-all and 8 non take-all sites (16)	25.7***	0.8150	y = 81.04 - 10.55x

FIGURE 14

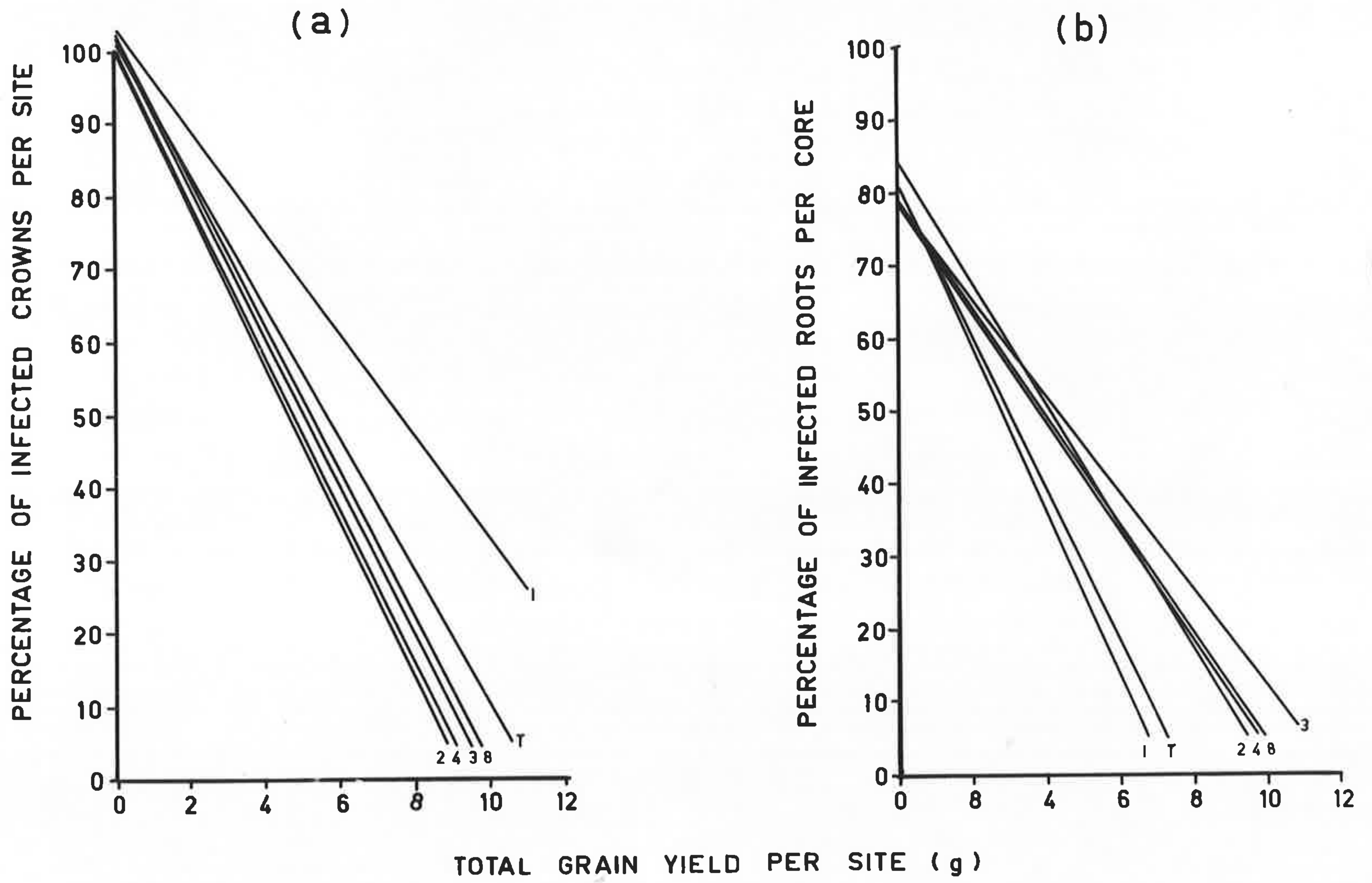
EFFECT OF NUMBER OF SAMPLES PER ROW ON 'INCIDENCE-YIELD' REGRESSION.

(a) Relationship between percentage of infected crowns per site and total grain yield per site.

(b) Relationship between percentage of infected roots per core and total grain yield per site.

Note: Figures equal number of samples per row. 'T' equals 8 take-all and 8 non-take-all samples.

FIG. 14



both types of assay to obtain a regression similar to that obtained from 8 sites per row. The take-all and non-take-all sites gave a regression similar to the 8 sites per row for the percentage of infected crowns, but an unsatisfactory regression with the percentage of roots infected per core.

These observations would indicate that the number of samples taken in the Turretfield experiment (1 per 3 m of row) may have been insufficient to establish the correct regression. However, the use of sample sites from take-all and non-take-all blocks at Turretfield would have compensated for the low number of samples per row.

These results suggest that mapping the incidence of _O. graminis_ by yield can be undertaken provided 2 important criteria are observed. Firstly, close observation of the experimental area must be maintained to establish that no other major pathogens are influencing yield. Secondly, areas within or adjacent to the trial, must be kept and used to establish the 'incidence-yield' regression on each occasion that the experiment is planted.

A difficulty with this mapping technique, however, could be the establishment of the 'incidence-yield' regression when all the area is relatively healthy or uniformly diseased. Also if the equilibrium situation reported by Fellows and Ficke (1934, 1939) and Buddin and Garrett (1941) is established, there could be the problem of obtaining a meaningful 'incidence-yield' regression (i.e. incidence may be relatively high with little effect on yield).

D. Incidence of *O. graminis* and take-all in consecutive wheat crops

It is important to study the incidence of *O. graminis* in consecutive crops and follow the spread or contraction of take-all patches with continuous cropping. Ideally these studies should be carried out over a period of several years with the idea of recording increases in *O. graminis* incidence and take-all during the early years and the possible take-all 'decline' with continued cropping. Although time allowed the examination of consecutive crops in only 2 seasons, 2 experiments were conducted using the mapping technique described above. It was also hoped that aerial photography could be used to follow changes in take-all incidence during the growing period.

(1) At Turretfield.

Block D (Fig. 3, page 28) was chosen for this experiment. A large part of the block contained plants showing take-all. The experimental block (40 drill rows, 7.2 m long) was composed of 240 macro-sites, which were harvested at the end of the 1969 growing season. The yield results for the 1969 crop are shown in Figure 15. If the regression obtained previously (Fig. 9), is applied to these results, the estimated *O. graminis* incidence can be superimposed on the 'yield' map, to give the 'incidence-yield' map shown in Figure 15.

The block was burnt at the end of summer 1969-1970 and sown in early June 1970. As the position of another experiment prevented a straight

FIGURE 15

O. graminis 'INCIDENCE-YIELD' MAP TURRETFIELD,

1969

Map showing yield per macro-site. Superimposed is an incidence map based on the regression of percentage of infected crowns and yield.

High incidence : less than 30g

Intermediate incidence : 31 to 60g

FIG. 15

16 7 29 46 44 32 27 41 14 23 24 24 46 33 49 47 40 42 35 36
16 9 34 39 24 33 26 34 37 23 40 45 37 32 44 42 53 38 33 34
8 15 29 41 17 16 31 22 34 27 41 45 39 53 51 51 44 55 39 37
13 21 34 37 15 18 27 30 35 46 43 40 49 53 40 36 51 50 43 45
7 40 33 50 17 16 41 22 17 22 37 35 39 47 43 44 35 39 31 54
5 15 13 35 11 15 24 14 27 31 37 44 50 31 41 45 30 33 27 38
9 14 27 23 12 11 42 25 30 49 31 38 41 32 24 42 25 41 29 44
2 5 36 28 6 28 31 16 21 27 36 43 40 30 37 42 30 46 48 47
2 3 31 16 8 26 14 27 11 31 42 33 38 38 31 33 26 35 32 45
7 7 24 19 4 11 6 11 15 24 21 32 33 39 36 36 43 44 36 36
1 9 41 7 7 21 1 33 22 16 30 38 45 33 31 26 27 35 35 37
2 6 19 14 4 25 8 30 11 25 13 19 28 38 31 33 35 43 49 51

High incidence

Intermediate incidence

Scale 30 cm

run with a big drill, the block was sown with a 9 row drill. Unfortunately a gap was left on either side of the run, second from the eastern side of the plot. This meant 1 drill row was missing from the tenth, eleventh and sixteenth macro-rows (Fig. 16).

Aerial photographs were taken of the block on September 30 and October 15. On the first occasion, technical problems with the equipment prevented the taking of a satisfactory photograph which included all of the experimental block. Areas of infected plants were obvious, but no attempt was made to develop a composite photograph. On the second occasion, a photograph taken from a height of 18 m gave a successful print (Fig. 16). Take-all areas appear a green colour in the print and areas with vigorous plants appear red. From the photograph, it appears that the eastern half of the plot is relatively healthy, while most of the remainder contains mainly stunted plants.

The yield results for the 1970 crop are shown in Figure 17. Because macro-rows 10, 11 and 16 had only one drill row each, they have been eliminated from the map. As the block was not going to be used again, the 'incidence-yield' regression for 1970 was obtained from within the block. Previously, it was established that 2 sites (each 1 drill row x 30 cm) per 4.6 m of drill row was necessary to establish an 'incidence-yield' regression. On this occasion the plants from a random selection of 2 macro-sites per macro-row were bioassayed for the presence of <u>O. graminis</u>. This is an assessment of the plants from one-sixth of every 7.2m drill row, a higher portion of the row than was assessed by the above method. Although the portion assessed was greater, the scatter of

FIGURE 16

Aerial photograph of Block D (4 white markers) at Turretfield. Red coloured areas relatively healthy wheat plants and green coloured areas take-all patch. Area within markers is free of all weeds. Photograph was taken using a Pentax Spotmatic 35 mm camera suspended from 3 hydrogen-filled 800 g 10 PCS Weather Balloons. Kodak Infra-red Aero 8443 film was used with a Hoya Y (K2) yellow filter.

FIGURE 17

O. graminis 'INCIDENCE-YIELD' MAP TURRETFIELD, 1970

Map showing yield per macro-site. Superimposed is an incidence map based on the regression of percentage of infected crowns and yield.

High incidence : less than 23 g

Intermediate incidence : 24 to 48 g

FIG. 17

40	16	15	40	30	15	22	31	22	27	36	36	28	35	39	27	30
23	14	20	22	24	26	31	34	33	29	34	39	56	32	32	26	28
9	10	12	34	20	33	26	29	31	30	36	38	34	35	28	30	19
19	12	22	34	28	16	35	30	43	32	37	41	41	39	31	31	33
10	4	14	23	13	16	18	33	19	37	31	32	39	26	28	37	19
7	6	15	20	21	18	19	23	42	41	42	33	50	31	54	38	27
3	6	12	25	22	18	15	18	45	38	33	37	50	46	49	43	42
10	5	16	14	24	25	21	25	40	41	40	36	35	38	50	35	39
19	8	13	16	34	17	14	15	31	28	33	35	48	36	41	37	39
23	16	10	26	28	9	19	18	23	43	30	42	45	38	48	42	47
38	14	14	41	29	22	25	22	24	27	34	30	32	39	40	42	39
19	15	7	29	33	21	31	35	24	38	33	33	50	33	30	35	36

High incidence

Intermediate incidence

Scale 30 cm

samples was reduced. This may have reduced the chance of obtaining extremes of incidence and yield due to a reduction in the variability that could be expected from smaller sites. The number of plants, total yield, average yield and percentage of infected crowns per macro-site are given in Table 26.

Details of the analysis of variance of the regression comparing the percentage of infected crowns with number of plants, total yield and average yield per macro-site are given in Table 27. In contrast to the previous experiments, there was a significant ($p = 0.05$) relationship between incidence and the number of plants per site. Once again the correlation of incidence and total yield per site was superior to average yield per site.

The 'incidence-yield' regression is shown in Figure 18. As previously, the portion below the line on the y axis was divided into 3 equal parts, thus the delimiting points for the 1970 season were 27 per cent or below for the low incidence category and 54 per cent and above for the high incidence category. High incidence areas have a yield of 23 g or less per macro-site while intermediate areas have a yield of 24 to 48 g per macro-site. The estimated incidence of _O. graminis_ based on this calculation is superimposed on the 'yield' map to give the 'incidence-yield' map shown in Figure 17.

Comparison of the 'yield' or 'incidence-yield' maps for 1969 and 1970 (Figs 15 and 17) reveal that the same general pattern of take-all and _O. graminis_ applied in both seasons. As shown in the aerial photograph the western half of the block still had a high level of take-all

TABLE 26

Details of number of plants, yield and percentage of crowns infected per macro-site from Block D at Turretfield

Macro-row	Macro-site	Number of plants per site	Total yield 1970 (g)	Average yield 1970 (g)	Percentage of crowns infected	Total yield 1969 (g)
1	3	23	9	0.39	83	8
1	8	28	10	0.36	86	2
2	1	37	16	0.43	78	7
2	5	25	4	0.16	83	40
3	7	28	12	0.43	43	27
3	10	27	10	0.37	86	24
4	2	30	22	0.73	50	39
4	10	34	26	0.76	56	19
5	3	31	20	0.65	38	17
5	7	28	22	0.77	14	12
6	9	27	17	0.63	57	26
6	12	32	21	0.66	38	25
7	2	41	31	0.76	50	26
7	8	24	21	0.88	67	31
8	2	41	34	0.83	10	34
8	4	32	30	0.94	75	30

TABLE 26 (cont.)

Macro-row	Macro-site	Number of plants per site	Total yield 1970 (g)	Average yield 1970 (g)	Percentage of crowns infected	Total yield 1969 (g)
9	4	29	43	1.48	71	35
9	11	28	24	0.86	43	22
12	9	24	28	1.17	50	33
12	10	38	43	1.13	33	32
13	4	33	37	1.12	38	49
13	10	29	30	1.03	43	33
14	5	34	32	0.94	22	47
14	9	32	35	1.09	25	38
15	3	34	34	1.00	56	44
15	12	34	50	1.47	56	31
17	2	33	32	0.97	11	53
17	6	36	31	0.86	75	30
18	3	41	28	0.68	30	38
18	4	38	31	0.82	60	50
19	8	32	35	1.09	13	48
19	10	40	42	1.05	40	36
20	5	28	19	0.68	100	54
20	11	30	39	1.30	13	37

TABLE 27

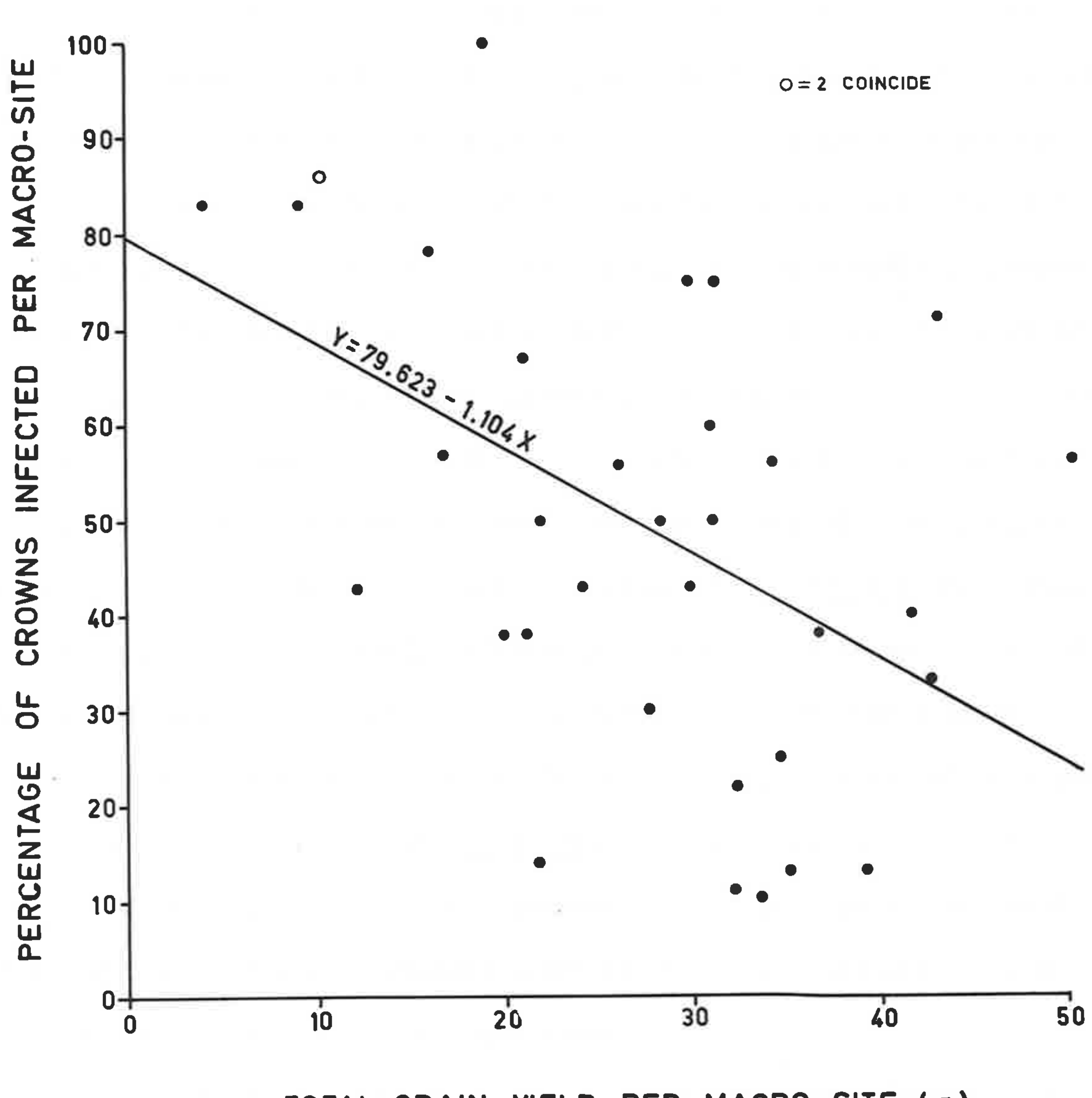
Results of regression analysis for comparison of *O. graminis* incidence and yield per macro-site for the 1970 crops at Turretfield and Ceduna. Also comparisons of 1969 and 1970 yield at Turretfield.

Location	y	x	Variance ratio	Correlation coefficient	Regression equation
Turret-field	Percentage of infected crowns 1970	Number of plants 1970	4.62*	0.3551	y = 104.81 - 1.73x
	"	Total grain yield 1970 (g)	9.98**	0.4876	y = 79.62 - 1.10x
	"	Average grain yield 1970 (g)	7.22*	0.4291	y = 78.30 - 34.00x
	Total grain yield 1969 (g)	Total grain yield 1970 (g)	8.95**	0.4675	y = 16.70 + 0.56x
Ceduna	Percentage of infected plants 1970	Total dry top weight 1970 (g)	ns	0.1579	-
	"	Average dry top weight 1970 (g)	26.06**	0.3141	y = 105.90 - 62.80x

FIG. 18

'INCIDENCE YIELD' REGRESSION

Relationship between infection and total yield at TURRETFIELD 1970



(low yield), while in the remainder the plants were relatively vigorous. The 'incidence-yield' map shows that there was an estimated high incidence of O. graminis in the western part of the plot, while the remainder was mainly in the intermediate category. However, examination of the 'incidence-yield' maps for both years shows that there was a reduction (46% to 34%) in the number of macro-sites considered to be in the high incidence category. As the 1970 sowing was the fourth consecutive wheat crop, there is the possibility that the take-all area was contracting.

To check the above possibility the 'incidence-yield' map for 1970 was redrawn in Figure 19 with the actual incidence reading (percentage of infected crowns) from random macro-sites included. A number of incidence figures are not in the appropriate categories (i.e. low incidence areas 27 per cent or less and high incidence areas 54 per cent or more). This indicates that the 'incidence-yield' method of mapping is reducing the variability of actual occurrence of O. graminis and allowing scattered pockets of severely infected plants within intermediate incidence areas (and vice-versa) to go undetected. However, the general pattern of incidence suggests that the classification of the whole block as either high or intermediate O. graminis macro-sites is a correct estimate of the situation. The experiments would need to be continued to determine whether the apparent contraction in take-all areas was a real effect or due to seasonal conditions.

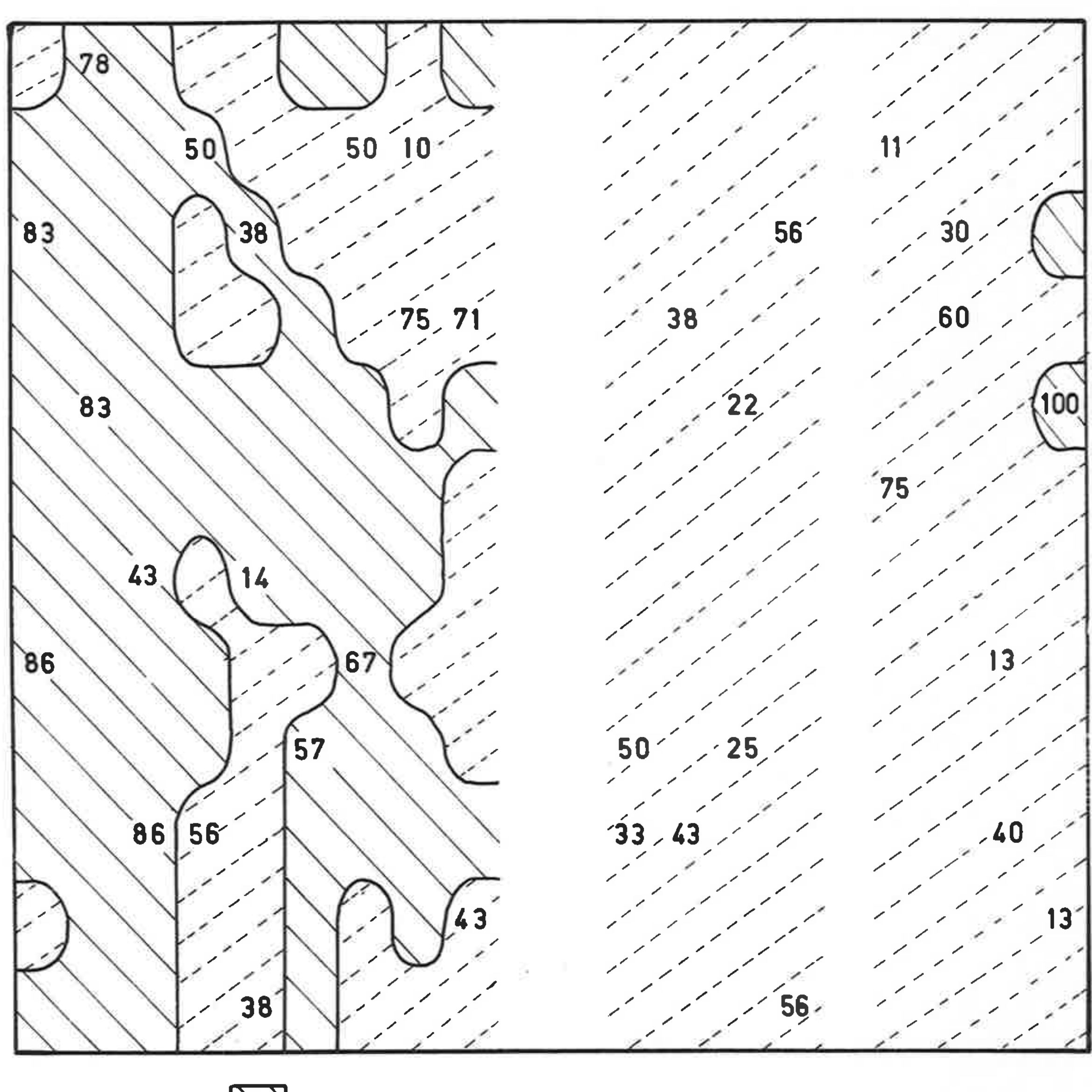
Total grain yield per macro-site for 1969 and 1970 were significantly correlated, confirming that there were no marked shifts in the position of take-all areas between the 2 seasons (Table 27). White

FIGURE 19

O. graminis 'INCIDENCE-YIELD' MAP TURRETFIELD, 1970

'Incidence-yield' map redrawn from Figure 17. Superimposed on selected macro-sites are the percentage of infected crowns per macro-site.

FIG. 19



(1945) also reported that take-all patches tend to be associated in consecutive years.

(2) At Ceduna.

Most of the block (G in Fig. 3, page 28) chosen for this experiment was part of a take-all patch. The experimental block was the same size and contained the same number of macro-sites as that used at Turretfield. The yield results for 1969 are shown in Figure 20. Using the regression obtained previously (Fig. 11a), the estimated incidence of O. graminis was superimposed on the 'yield' map to give the 'incidence-yield' map shown in Figure 20. Although this was only the second consecutive year of wheat, a large part of the experimental block was placed in the high incidence category.

The experimental block was fire harrowed at the end of summer and later prepared for sowing. As drought conditions prevailed throughout the first 6 months of the year (Table 2), the block was not sown until August 4, 1970. The seed germinated, but plant growth was very slow. By October 5, the plants were still too small to show in an aerial photograph. The area was examined again on November 10. Most plants still had some green tissue, but were in very poor condition; the maximum height reached was about 25 cm. The poor growth was probably due to late planting, low rainfall and the presence of O. graminis. As the experiment could not be continued the following season, and as the plants were unlikely to reach maturity, they were removed, placed in plastic bags and returned to the laboratory where they were stored at 5°C until they could be assessed.

FIGURE 20

<u>O. GRAMINIS</u> 'INCIDENCE-YIELD' MAP CEDUNA, 1969

Map showing yield per macro-site. Superimposed is an incidence map based on the regression of percentage of infected crowns and yield.

High incidence	:	less than 14 g
Intermediate incidence	:	15 to 26 g
Low incidence	:	more than 27 g

FIG. 20

5	1	3	1	1	0	1	0	6	0	1	3	8	6	12	24	18	19	24	16
6	1	4	7	3	5	0	0	2	1	3	2	7	6	12	15	17	16	16	28
4	1	6	0	1	3	2	1	1	1	3	5	10	5	10	13	16	13	14	16
1	2	0	0	1	2	1	0	1	0	5	1	13	12	7	10	11	14	17	13
0	0	0	4	0	1	3	1	6	2	3	4	11	21	19	9	22	17	22	19
0	0	0	0	0	0	3	1	9	5	9	5	17	0	8	19	11	14	13	19
0	0	0	0	0	3	1	0	2	2	8	5	18	11	5	7	6	0	10	14
2	1	2	0	0	0	0	0	3	1	6	6	14	6	7	12	10	16	17	24
1	3	2	2	0	2	0	0	0	1	2	4	8	3	2	0	13	13	9	15
1	1	1	1	1	5	4	1	2	2	10	10	11	1	8	7	4	7	8	7
7	2	2	3	0	4	5	1	1	0	14	14	11	6	3	4	5	14	13	13
2	0	1	0	0	3	6	0	1	0	9	7	11	6	0	0	3	14	11	17

High incidence

Intermediate incidence

Low incidence

Scale —— 30 cm

Plants from each macro-site were washed and their roots examined under water over a white background. As the plants were immature, this method was chosen rather than the crown bioassay. The percentage of infected plants, total and average top dry weight per macro-site are shown in Appendix D.

The 'incidence' map based on the percentage of infected plants is shown in Figure 21. When this map is compared with the 1969 'incidence-yield' map (Fig. 20), it is evident that there is an increase in the area in the high incidence category. However, the smallness of the intermediate incidence area in 1969 and the lack of a grain yield map for 1970 means that no definite conclusions can be drawn about changes in the incidence of O. graminis or take-all.

The percentage of infected plants per macro-site was not correlated with the total top dry weight per macro-site, but was correlated with average top dry weight (Table 27). In previous experiments at both Ceduna and Turretfield, where mature plants were examined, the percentage of infected crowns correlated better with total grain yield per site than average yield per site. In this experiment, growth was so poor that most plants failed to produce tillers and filling out of plants to take up gaps caused during seeding did not occur.

* * * * * * *

In Part I, methods of assessing the level of O. graminis in soil and the incidence of O. graminis in wheat stubble have been described. Both techniques have been employed to study the correlation of the incid-

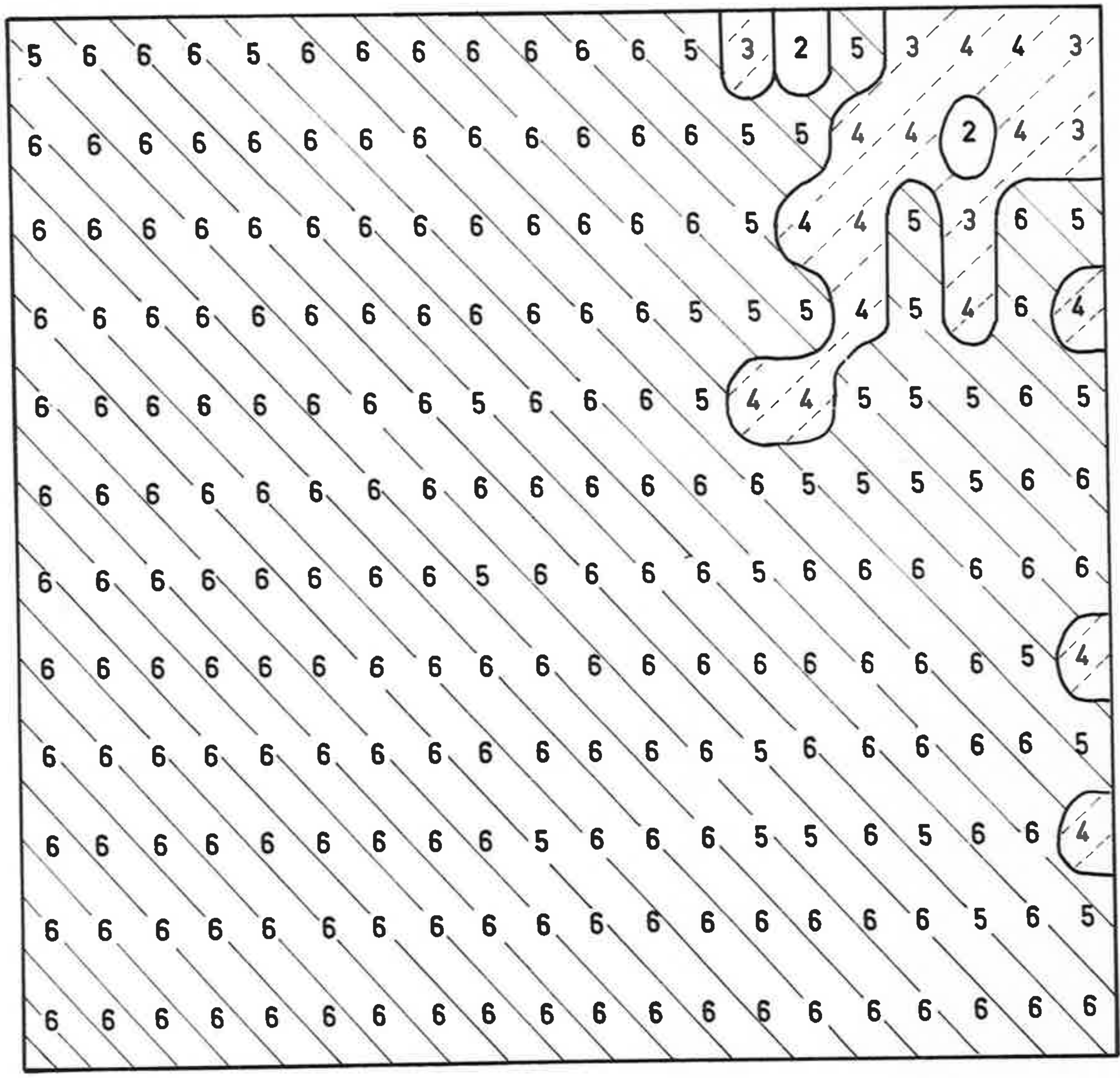
FIGURE 21

O. graminis 'INCIDENCE' MAP CEDUNA, 1970

Map showing incidence based on percentage of infected plants per macro-site.

High incidence	(5 = 68 to 83%, 6 = 84 to 100%)
Intermediate incidence	(3 = 34 to 50%, 4 = 51-67%)
Low incidence	(1 = 0 to 16%, 2 = 17 to 33%)

FIG. 21



High incidence

Intermediate incidence

Low incidence

Scale —— 30 cm

ence of _O. graminis_ with take-all and yield. Using the methods developed in the correlation studies, an investigation of the incidence of _O. graminis_ and take-all in consecutive wheat crops was undertaken. In Part II, the techniques developed in Part I are again employed to study the survival of _O. graminis_ in wheat-field soil.

PART II

SURVIVAL OF *OPHIOBOLUS GRAMINIS* IN FIELD SOIL

A. Survival under field conditions

The next step in this series of investigations was to study the survival of *O. graminis* in the field, assessing the level of viable fungus under controlled conditions. Both crown and core bioassays were employed for this purpose. Change in the proportion of stubble containing viable *O. graminis* would be detected by the former, while the latter would detect change in the level of viable *O. graminis* in soil. With time, exhaustion of nutrients within stubble or soil and the possibility of competition from other organisms, the amount of *O. graminis* could be expected to decline. However, the presence of grasses and self-sown wheat could lead to further growth of the fungus during the winter months. The experiments described below are designed to investigate these possibilities.

(1) Survival of *O. graminis* in wheat stubble at Turretfield.

A block (E in Fig. 3, page 28) within part of a take-all patch was chosen for this experiment. With the exception of 3 small parts, where the plants were relatively vigorous, the experimental block contained mainly stunted plants. The block was composed of 36 drill rows each 4.8 m long and was divided into 96 macro-sites. For this and the 2 following experiments the macro-site size was increased to 3 drill rows x 60 cms, thus each macro-site contained 6 sites each 1 drill row 30 cm long. The 1969 yield results for the macro-sites are given in Appendix E.

On 4 occasions in 1970 (January 28, March 19, May 28 and August 20), all wheat stubble from 1 site per macro-site was removed and bioassayed for the presence of O. graminis. As the previous crop was harvested in the third week of December 1969, the above dates are about 6, 13, 23 and 35 weeks from that time.

The results for the frequency distribution of the percentage of crowns containing viable O. graminis at each time of removal are given in Table 28. (The contribution of each element to the chi-square is shown in Appendix E). There was a reduction in the number of crowns containing viable O. graminis throughout the period of the experiment; the rate of reduction accelerating between May 28 and August 20. By August 20, crowns in 70 per cent of the sites did not contain viable O. graminis.

'Survival' maps for January 28 and March 19 are shown in Figure 22, and for May 28 and August 20 in Figure 23. In the 'survival' maps, areas with similar levels of surviving O. graminis have been surrounded with a free-hand line. For ease of observation, categories 1 and 2, 3 and 4, 5 and 6 have been combined. Occasionally a few sites contained no stubble or only 1 crown. These sites have been eliminated from the calculations and replaced on the map with a black spot.

From the series of 'survival' maps it can be seen that reduction in the percentage of crowns containing viable O. graminis was not associated with any particular part of the plot. There was little change in survival between January 28 and March 19. This was followed by a general disappearance of both high and intermediate survival areas during the following 5 months.

TABLE 28

The frequency distribution of percentage of crowns containing viable _O. graminis_ per site for 4 times of removal from Block E at Turretfield, 1970

Date	Time from 1969 harvest (wk)	Survival Category							
		0-16% 1	17-33% 2	34-50% 3	51-67% 4	68-83% 5	84-100% 6	0% -	100% -
Jan. 28	6	8	18	18	24	15	13	4	7
Mar. 19	13	12	10	21	21	26	6	5	2
May 28	23	31	29	17	10	7	0	16	0
Aug. 20	35	80	10	3	1	0	0	66	0

FIGURE 22

'SURVIVAL' MAP OF *O. GRAMINIS* AT TURRETFIELD,

BLOCK E, 1970.

(a) January 28

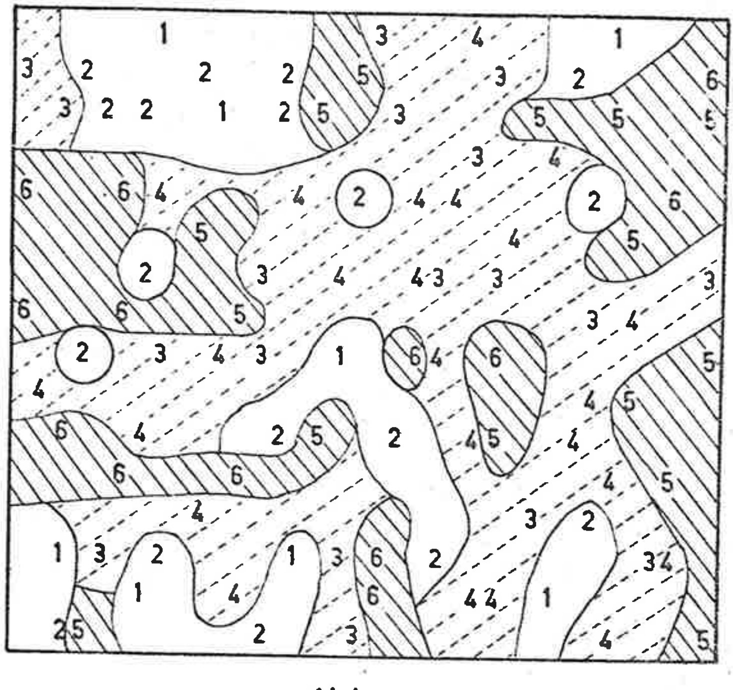
(b) March 19

'Survival' category (percentage of crowns infected per site)

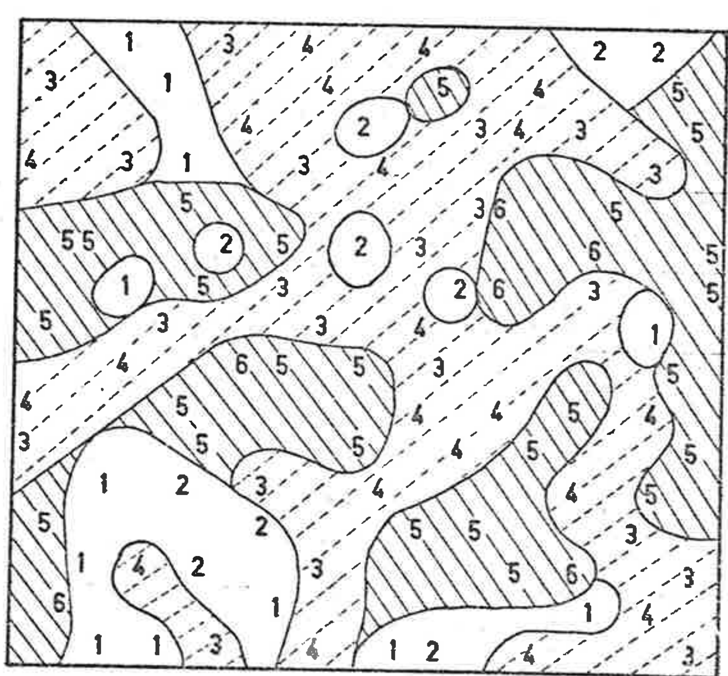
High survival	(5 = 68 to 83%, 6 = 84 to 100%)
Intermediate survival	(3 = 34 to 50%, 4 = 51 to 67%)
Low survival	(1 = 0 to 16%, 2 = 17 to 33%)

FIG. 22

(a)



(b)



'Survival' category

High Intermediate Low

Scale ⟝⟞ 30 cm

FIGURE 23

'SURVIVAL' MAP OF *O. GRAMINIS* AT TURRETFIELD,

BLOCK E, 1970

(a) May 28

(b) August 20

'Survival' category (percentage of crowns infected per site)

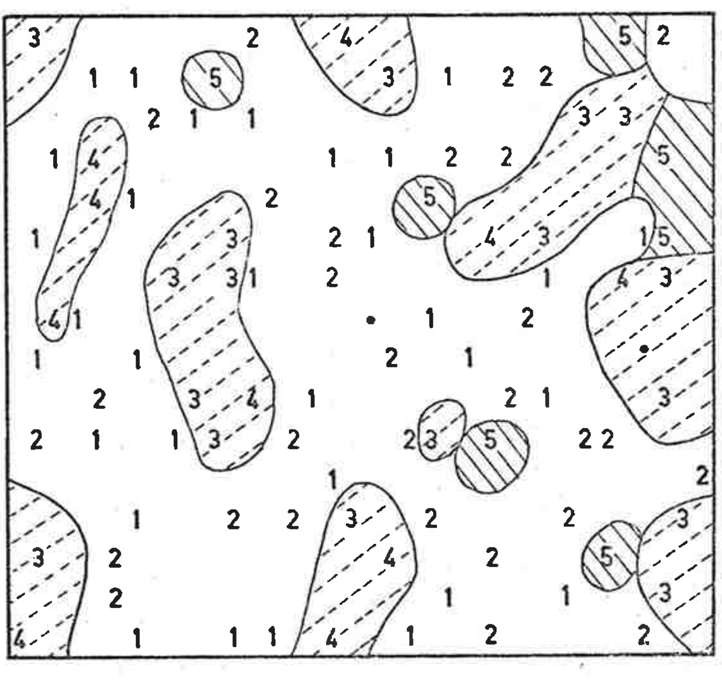
High survival (5 = 68 to 83%, 6 = 84 to 100%)

Intermediate survival (3 = 34 to 50%, 4 = 51 to 67%)

Low survival (1 = 0 to 16%, 2 = 17 to 33%)

FIG. 23

(a)



(b)

'Survival' category

High Intermediate Low

Scale ⊢ 30 cm

To prevent grazing stock trampling the stubble, the experimental block was fenced. As a consequence the block became overgrown during the winter of 1970 with a lush growth of African Capeweed (*Crystostemma calendula* Druce). There was also a considerable number of self-sown wheat plants and a few Wimmera Ryegrass (*Lolium rigidum* Gaud.). The adequate rainfall and the ground cover helped to maintain moist conditions on the soil surface, and this may have made conditions unfavourable for the survival of *O. graminis*. Although the decline in viable *O. graminis* was rapid, there would still have been a reservoir of fungus available to infect crops sown in late May or early June of 1970.

A planned core removal experiment at Turretfield was abandoned because the hardness of the soil during the summer months made it extremely difficult to remove cores.

(2) Survival of *O. graminis* in wheat stubble at Ceduna.

This experiment was conducted in a similar manner and with the same size block as the experiment at Turretfield. The experimental block (H in Fig. 3, page 28) was situated in a take-all patch where most plants were stunted, although the plants towards the eastern side of the block were more vigorous. The yield results for the 1969 crop are shown in Appendix F.

On 5 occasions (February 3, April 14, June 23, September 1 and November 10, 1970) all wheat stubble was removed from 1 site per macro-site and bioassayed for the presence of *O. graminis*. As the 1969 crop was harvested in the last week of November 1969, the above dates are 10, 20, 30, 40 and 50 weeks after harvest. The frequency distribution for

the percentage of crowns containing viable O. graminis, for the times of removal, is given in Table 29. (The contribution of each element to the chi-square is shown in Appendix F). Examination of the results shows that there was a reduction in the number of crowns containing viable O. graminis during the period of the experiment.

The 'survival' maps for February 3 and June 23 are shown in Figure 24 and for September 1 and November 10 in Figure 25. The map for April 14 was very similar to that for February 3 and has not been included.

From Figure 24a it can be seen that a large proportion of the macro-sites in the experimental block contained crowns carrying O. graminis. By April 14, the position had not changed markedly. The situation was much the same on June 23 (Fig. 24b) though the number of macro-sites in categories 1 and 2 had increased. In particular, the area of low survival along the northern edge of the block had extended further south. An area of low survival had also developed along the southern boundary of the block. There was a considerable reduction in the number of macro-sites in the 2 highest survival categories between June 23 and September 1, but no reduction between September 1 and November 10 (Table 29). I am unable to explain the marked difference in the central regions of Figures 25a and b. It seems unlikely there would be an increase in viable O. graminis within stubble in the region; a more likely explanation is that the discrepancy is due to natural variability. In the central region, where the difference is most marked, the low survival macro-sites in Figure 25a are in different drill rows from the high survival macro-sites in Figure 25b. As O. graminis tends to spread along the row (Adam and Colquhoun, 1936)

TABLE 29

The frequency distribution of percentage of crowns containing viable *O. graminis* per site for 5 times of removal from Block H at Ceduna, 1970

Date	Time from 1969 harvest (wk)	Survival category							
		0-16%	17-33%	34-50%	51-67%	68-83%	84-100%	0%	100%
		1	2	3	4	5	6	-	-
Feb. 3	10	10	4	6	4	5	60	9	55
April 14	20	10	5	7	8	15	48	8	46
June 23	30	15	8	3	7	11	51	13	43
Sept. 1	40	19	5	8	15	15	30	16	28
Nov. 10	50	20	6	5	12	14	36	17	33

FIGURE 24

'SURVIVAL' MAP OF O. GRAMINIS AT CEDUNA, BLOCK H, 1970

(a) February 3

(b) June 23

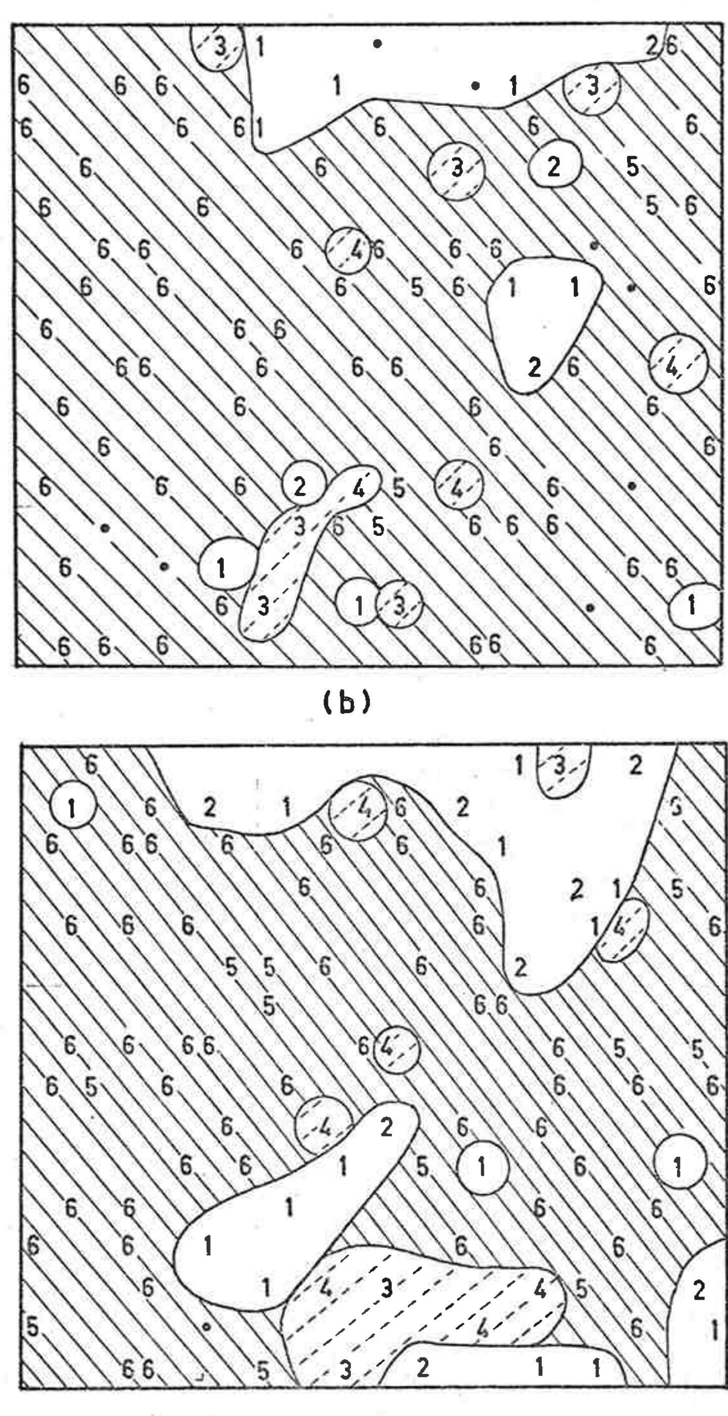
'Survival' category (percentage of crowns infected per site.)

High survival (5 = 68 to 83%, 6 = 84 to 100%)

Intermediate survival (3 = 34 to 50%, 4 = 51 to 67%)

Low survival (1 = 0 to 16%, 2 = 17 to 23%)

FIG. 24



'Survival' category

High Intermediate Low

Scale ⟼ 30 cm

FIGURE 25

'SURVIVAL' MAP OF O. GRAMINIS AT CEDUNA, BLOCK H, 1970

(a) September 1

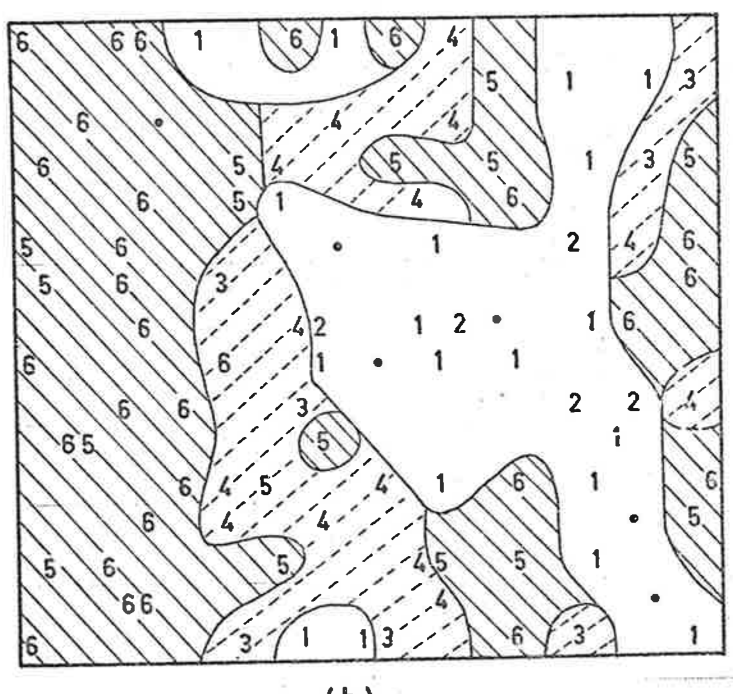
(b) November 10

'Survival' category (percentage of crowns infected per site)

High survival	(5 = 68 to 83%, 6 = 84 to 100%)
Intermediate survival	(3 = 34 to 50%, 4 = 51 to 67%)
Low survival	(1 = 0 to 16%, 2 = 17 to 23%)

FIG. 25

(a)



(b)

'Survival' category

High Intermediate Low

Scale ⟼ 30 cm

and infected plants tend to be clustered along the row (White, 1945), it is possible that the plants in some rows would all be infected, while plants in adjacent rows could be free of O. graminis. This possibility emphasises a technical problem associated with this type of mapping.

The results show that over all there was a gradual reduction in the number of crowns containing viable O. graminis during the period of the experiment. However, on November 1 stubble from 82 per cent of the macro-sites still contained viable O. graminis, and in 35 per cent of the macro-sites all crowns still contained viable fungus. This is in contrast to the results obtained at Turretfield, where by August 20 crowns from only 30 per cent of the macro-sites still contained viable O. graminis.

(3) Survival of O. graminis in soil at Ceduna.

The block used in this experiment (K in Fig. 3, page 28) was the same size and directly south of that used in the previous experiment. The block was in a take-all patch but contained several regions towards the eastern boundary which contained relatively vigorous plants. Yield results for 1969 are given in Appendix G.

As it was planned to remove samples at 5-weekly intervals throughout most of 1970, the 96 macro-sites were each divided into 12 core-sites (1 drill row x 15 cm). One core was taken on each occasion from the centre of 1 randomly selected core-site per macro-site. Samples were removed on February 3, March 9, April 13, May 19, June 24, July 29, September 1, October 5 and November 10, 1970, and bioassayed for the presence of O. graminis. The first sample was taken 10 weeks after the 1969

harvest.

The frequency distribution for the percentage of infected roots for seedlings grown in the cores is shown in Table 30. To increase the frequency per incidence category to greater than 5, to allow chi-square analysis, the number of categories was reduced from 8 to 4. The contribution of each element to the chi-square for the 4 incidence categories and the frequency distribution within the 8 incidence categories are shown in Appendix G. The average percentage of seedlings and roots infected per core is shown in Table 31.

'Incidence-survival' maps for February 3, May 19, September 1 and November 10 (10, 25, 40 and 50 weeks respectively) are shown in Figures 26 and 27, and those for the remaining dates are given in Appendix G. As the core bioassay determines the level of _O. graminis_ in the soil, on the basis of disease incidence on seedlings, the maps have been called 'incidence-survival' maps to differentiate them from the 'survival' maps obtained by the crown bioassay. Incidence levels grouped in these maps were: low, 0-25 per cent of roots infected per core; intermediate, 26-50 per cent; and high, 51-100 per cent. These delimiting points are the same as those used in the experiment at Ceduna comparing the incidence of _O. graminis_ and yield (Figs. 11b and 13b).

An examination of the average percentage of roots infected per core (Table 31) for the period of the experiment shows that there was an overall decline in the level of _O. graminis_ detected in the soil. From February 3 to July 29, there was no consistent change in incidence, but between July 29 and November 10 there was a reduction in incidence. It

TABLE 30

The frequency distribution of the percentage of infected roots on wheat seedlings grown in cores removed at intervals from Block K at Ceduna, 1970

Date	Time from 1969 harvest (wk)	Incidence category			
		0-25%	26-50%	51-75%	76-100%
Feb. 3	10	31	11	26	28
March 9	15	28	9	21	38
April 13	20	41	8	19	28
May 19	25	39	12	16	29
June 24	30	34	18	10	34
July 29	35	40	12	10	34
Sept. 1	40	50	12	14	20
Oct. 5	45	52	13	16	15
Nov. 10	50	58	13	13	12

TABLE 31

The incidence of *O. graminis* on wheat seedlings grown in cores removed at intervals from Block K at Ceduna, 1970 (average of 96 cores)

Date	Time from 1969 harvest (wk)	Percentage of:	
		seedlings infected	roots infected
Feb. 3	10	68	48
March 9	15	71	56
April 13	20	59	44
May 19	25	59	44
June 24	30	63	46
July 29	35	60	45
Sept. 1	40	51	35
Oct. 5	45	45	31
Nov. 10	50	40	27

FIGURE 26

'INCIDENCE-SURVIVAL' MAP OF O. GRAMINIS AT CEDUNA,

BLOCK K, 1970

(a) February 3

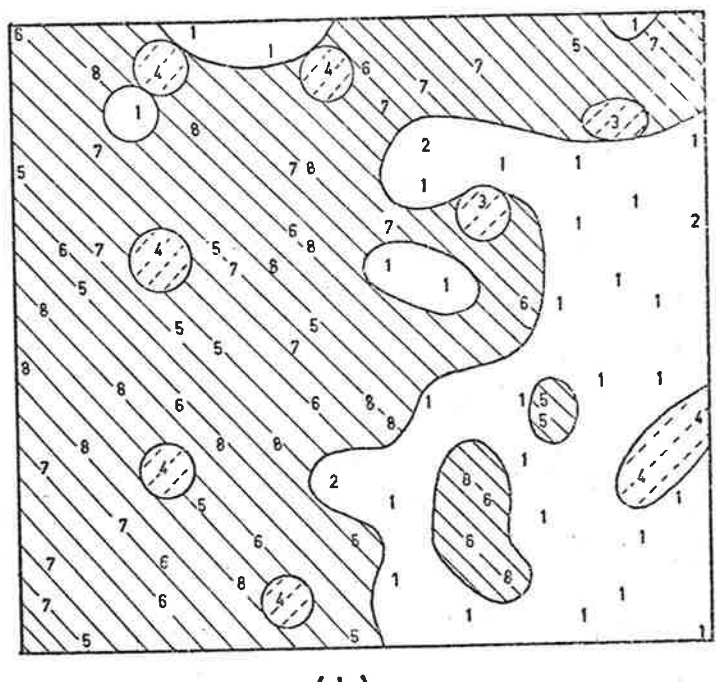
(b) May 19

'Incidence-survival' category (percentage of roots infected per core)

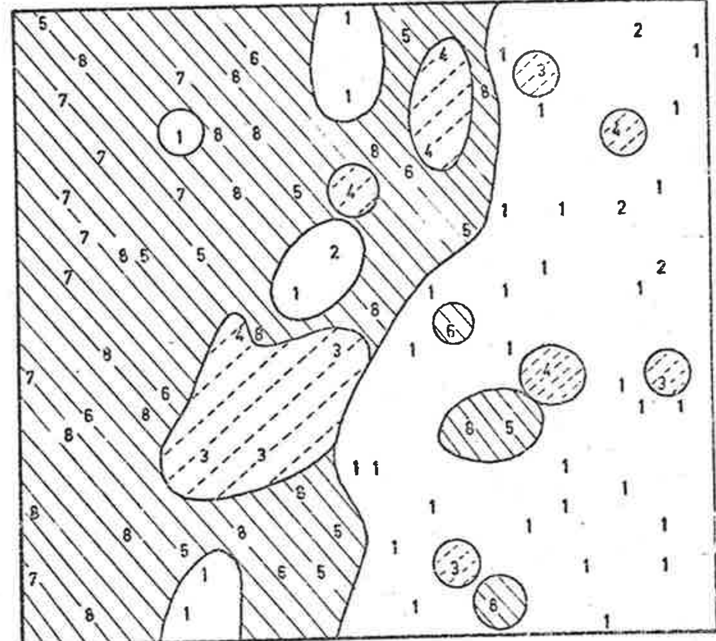
High incidence	(7 = 76 to 87%, 8 = 88 to 100%)
	(5 = 51 to 62%, 6 = 63 to 75%)
Intermediate incidence	(3 = 26 to 37%, 4 = 38 to 50%)
Low incidence	(1 = 0 to 12%, 2 = 13 to 25%)

FIG. 26

(a)



(b)



'Incidence - survival' category

High Intermediate Low

Scale ⊢⊣ 30 cm

FIGURE 27

'INCIDENCE-SURVIVAL' MAP OF *O. GRAMINIS* AT CEDUNA,

BLOCK K, 1970

(a) September 1

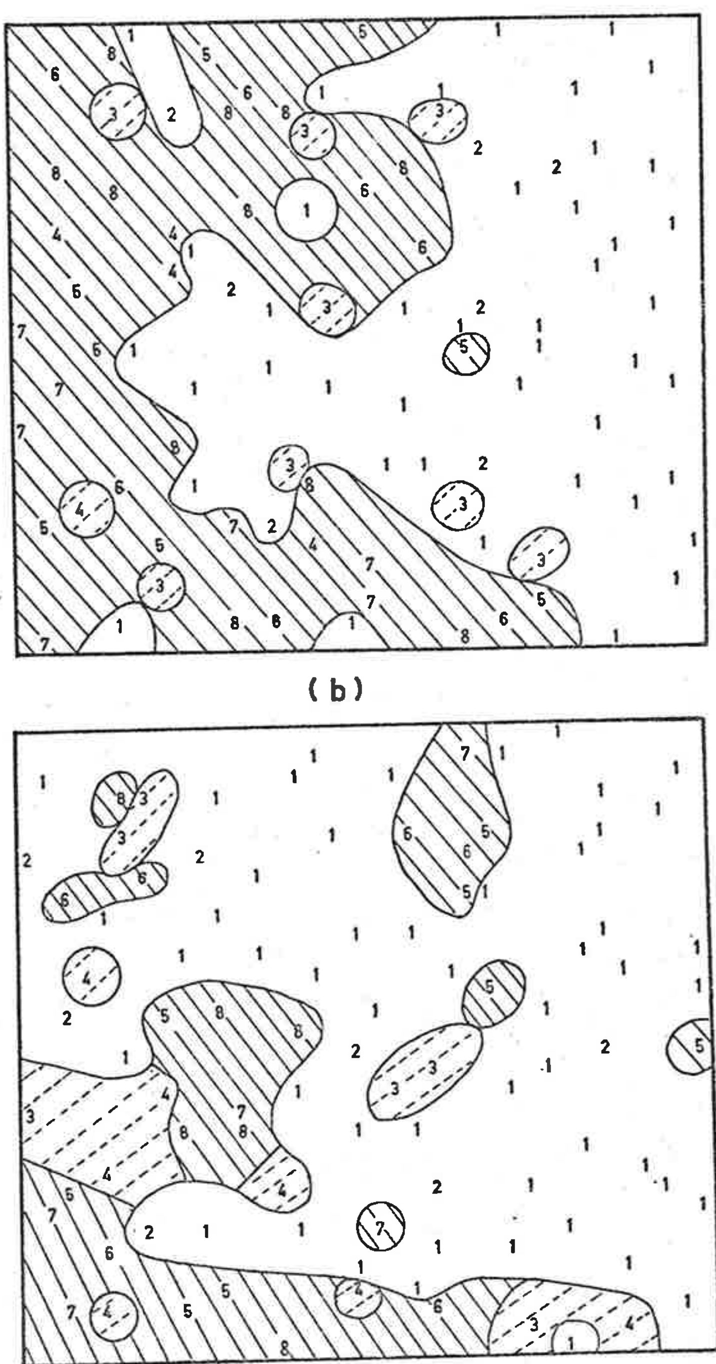
(b) November 10

'Incidence-survival' category (percentage of roots infected per core)

High incidence	(7 = 76 to 87%, 8 = 88 to 100%)
	(5 = 51 to 62%, 6 = 63 to 75%)
Intermediate incidence	(3 = 26 to 37%, 4 = 38 to 50%)
Low incidence	(1 = 0 to 12%, 2 = 13 to 25%)

FIG. 27

(a)



'Incidence- survival' category

High Intermediate Low

Scale ⊢⊣ 30 cm

appears that environmental conditions were most unfavourable for the survival of *O. graminis* during the month of August.

Examination of the 'incidence-survival' maps (Figs. 26 and 27) reveals that regions of low incidence appeared on the eastern edge and gradually expanded across the block during the period of the experiment. By November 10 the high incidence area was confined to the south-western corner of the block.

In this and the previous experiment at Ceduna, it was noted that the areas in the plot with consistently high levels of viable *O. graminis* were the same areas in which the least vigorous plants were observed the previous season. This observation adds support to Gerlagh's (1968) contention that take-all 'decline' is not due to a reduction in the inoculum level in the soil caused by a rapid rate of decomposition of severely infected and less vigorous plants.

My hypothesis that there would be a gradual reduction in the level of *O. graminis* during summer and early winter, followed by an increase during late winter was not fulfilled. The dry conditions at Ceduna during the first half of the year may account for the survival during that period, while the scarcity of grasses and self-sown wheat in the experimental block would not encourage a build-up of *O. graminis* during the latter part of winter. (Although the block was fenced, the dry season prevented a lush growth of vegetation. The dominant species was Harbinger Medic (*Medicago littoralis* Rhode c.v. Harbinger) with a few Wimmera Ryegrass (*Lolium rigidum* Gaud.) and self-sown wheat plants).

The above experiments have investigated the survival of

O. graminis in the field. Studies on the survival of *O. graminis* in undisturbed cores stored under controlled conditions are reported in the next section.

B. Survival under controlled conditions

The effect of moisture and temperature on the survival of *O. graminis* in naturally infested soil was investigated. Incidence of *O. graminis* after different periods of time was assessed using the core bioassay. Cores were collected along the drill row from a take-all patch at Ceduna in February, 1970. It was planned to remove cores from the experimental area at Turretfield, but the hardness of the soil in mid-summer made their removal so difficult that this part of the investigation was abandoned.

The storage conditions chosen were moisture tensions of pF2 and pF6 and temperatures of 15°C and 35°C. These were used in all 4 combinations. The tension of pF2 was chosen to represent the moisture tension in field soil in mid-winter. However, recordings of soil moisture at Ceduna revealed that this was too high (Table 2). For this reason a fifth experiment (pF3.6 and 15°C) was later included using cores that had been collected at Ceduna in April 1970. The soil moisture tension of pF6 (1.5%) was the same as that in the field at Ceduna in mid-summer (Table 2).

Fifteen and 35°C were used to represent winter and summer temperatures respectively. Surface soil temperatures in South Australian wheat growing areas are below 15°C for most of the time in mid-winter (Table 32). Although it may have been preferable to have storage conditions

TABLE 32

Soil temperatures at Waite Institute in bare soil at a depth of 2.5 cm.

	Date	Day							Total
		M	T	W	T	F	S	S	
Hours above 35°C	Dec. 30	6	0	6	8	8	9	10	47
	1967	10	9	7	7	8	9	8	58
		10	10	10	11	0	0	1	42
		7	9	8	8	9	10	10	61
	to	10	11	12	9	8	8	10	68
		9	8	8	6	8	8	8	55
		8	10	9	9	10	10	10	66
	March 2	10	0	3	7	8	9	8	45
	1968	2	0	2	6	2	6	6	24
Hours above 15°C	June 3	4	0	4	6	7	5	4	30
	1968	0	7	7	7	0	7	6	34
		7	0	0	0	0	3	4	14
		0	0	3	0	4	0	0	7
	to	0	0	0	0	4	6	5	15
		4	0	0	4	5	4	2	20
	July 29	3	0	0	5	6	1	0	15
	1968	3	5	2	0	0	0	4	14

with daily temperature fluctuations similar to those in the field, these conditions were not available. Constant 15°C was employed because storage space was available at that temperature. In mid-summer, surface soil temperatures in the South Australian wheat belt are above 35°C for a considerable part of the time (Table 32). As there was storage space available at constant 35°C, this temperature was used. Temperatures in the mid-thirties are near the upper limit for the growth of many mesophilic micro-organisms (Alexander, 1961). I wanted to know whether this would affect the survival of _O. graminis_ in natural soil.

(1) Survival in undisturbed cores stored at pF2 and 15°C.

In this experiment the cores were watered fortnightly to a constant weight equivalent to pF2 (24.4%). Average loss in weight per core during storage was 43g per fortnight. This is equivalent to a rise in soil moisture tension from pF2 to pF2.3. Seedlings that appeared in the cores during storage were removed.

At intervals of 9 weeks, 1 set of cores (7 replications) was removed and bioassayed. The results are shown in Table 33. Although there was a significant reduction in the incidence of _O. graminis_, there was still viable fungus remaining after 45 weeks of storage. In this experiment there was ample moisture for microbiological activity. Without further investigation it is impossible to determine whether the reduction in incidence is due to exhaustion of nutrient supply, the production of substances antagonistic to _O. graminis_ or some other factor.

(2) Survival in undisturbed cores stored at pF6 and 15°C.

An examination of Table 2 indicates that the soil moisture con-

TABLE 33

The effect of storage at pF2 and 15°C on the incidence of O. graminis on wheat seedlings grown in cores removed from a take-all patch at Ceduna (mean of 7 replications)

Period of storage (wk)	Percentage of:			
	seedlings infected	Arcsine (radians)	roots infected	Arcsine (radians)
0	87	1.33	76	1.12
9	90	1.33	75	1.09
18	74	1.08	51	0.78
27	29	0.45	16	0.32
36	26	0.41	13	0.27
45	31	0.52	12	0.28
Standard error		0.137		0.107
L.S.D. p=0.05		0.28		0.22
=0.01		0.38		0.29

tent remained about 1.5 per cent (or pF6) for 6 months during 1969-1970 at Ceduna. This combination of moisture tension and temperature may be encountered for short periods in most years and in drought years very dry conditions could extend beyond the end of April in to the cool winter months.

During storage, the cores received no treatment. The relative humidity in the place of storage was greater than 70 per cent, consequently, the soils absorbed a small amount of moisture. They gained an average of 5 g during the 45 weeks of the experiment; this is equivalent to a drop in soil moisture tension from pF6 to pF5.4. The results of the bioassay are shown in Table 34. There was no significant change in the incidence of O. graminis during the period of the experiment. Chen and Griffin (1966) found that microbiological activity in soil ceases below pF5.6 (relative humidity 75%). In this experiment, where the activity of other micro-organisms would be minimal, the survival of O. graminis was prolonged, possibly due to the cool conditions.

(3) Survival in undisturbed cores stored at pF6 and 35°C.

At the time of collection, the cores used had a soil moisture content of about 1.5 per cent or pF6. The average loss of moisture per core during the 45 week period of storage was 9 g, thus soil moisture dropped from 1.5 to 0.7 per cent. During storage the cores received no treatment.

The results of this experiment are shown in Table 35. There was a significant reduction in O. graminis during the period of the experiment. The reduction in incidence was greater than that obtained in cores

TABLE 34

The effect of storage at pF6 and 15°C on the incidence of *O. graminis* on wheat seedlings grown in cores removed from a take-all patch at Ceduna (mean of 7 replications)

Period of storage (wk)	Percentage of: seedlings infected	Arcsine (radians)	roots infected	Arcsine (radians)
0	87	1.33	76	1.12
9	69	1.03	47	0.72
18	98	1.51	67	1.00
27	71	1.16	49	0.77
36	69	1.02	40	0.64
45	91	1.40	55	0.85
Standard error		0.165		0.125
L.S.D.		ns		ns

TABLE 35

The effect of storage at pF6 and 35°C on the incidence of O. graminis on wheat seedlings grown in cores removed from a take-all patch at Ceduna (mean of 7 replications)

Period of storage (wk)	Percentage of: seedlings	infected Arcsine (radians)	roots	infected Arcsine (radians)
0	87	1.33	76	1.12
9	64	0.93	34	0.58
18	60	0.96	45	0.72
27	39	0.59	30	0.48
36	35	0.52	20	0.35
45	41	0.69	24	0.44
Standard error		0.161		0.128
L.S.D. p=0.05		0.33		0.26
=0.01		0.44		0.35

removed from the field at Ceduna during the summer months (Table 31). In the field experiment there was only a small change in the level of viable O. graminis present in the soil during the summer months. Although the soil moisture conditions were similar in both experiments, temperatures at Ceduna would not be as consistently high (Table 2). This suggests that temperature was the main factor affecting survival in this experiment. It is unlikely that reduction in the level of viable O. graminis can be attributed to the activity of other micro-organisms (Chen and Griffin, 1966).

(4) Survival in undisturbed cores stored at pF2 and 35°C.

This combination of moisture tension and temperature is unlikely to exist for more than a very short time in any part of the wheat growing areas of South Australia. However attempts have been made to grow wheat in tropical parts of Western Australia (Beech and Norman, 1966). High soil moisture and soil temperatures are also experienced during summer in the wheat growing areas of the Darling Downs in Queensland (Purss, 1971).

The cores were watered to a constant weight equivalent to pF2 (24.4%) every third day. Average loss in weight per core during this period was 44 g. This is equivalent to a pF rise from 2 to 2.3. All seedlings that appeared during storage were removed.

A bioassay was performed on 1 set of cores after 4 weeks storage and another after 9 weeks. As in both instances, all seedlings in the bioassay were free of infection, the experiment was terminated. The cause of the rapid elimination of O. graminis is unknown. There is the possi-

bility that 35°C is lethal for O. graminis maintained in wet soil. Further investigation would be required to determine the factors involved. It would be of interest to determine whether the poor survival of the fungus in hot wet soil was a contributing factor to O. graminis being of minor importance in Queensland (McKnight, 1960).

(5) Survival in undisturbed cores stored at pF3.6 and 15°C.

The cores used in this experiment were watered to constant weight (12% soil moisture) once a fortnight. The average loss in weight per core was 16 g per fortnight. This is equivalent to an increase in pF from 3.6 to 3.85. The cores received no other treatment apart from the removal of any seedlings that appeared.

Cores were removed and bioassayed for the presence of O. graminis at 5-weekly intervals. Results are recorded in Table 36. There was no significant change in incidence of the fungus throughout the period of the experiment. This is in contrast to the results obtained when naturally infested soil was maintained at pF2 and 15°C (Table 33). The relative dryness of the soil at pF3.6 apparently had a restricting effect on the factor (s) reducing the survival of O. graminis in the wet soil (pF2).

* * * * * * * *

Most studies on the survival of O. graminis in naturally infested soils have shown that this fungus remains viable for periods of 2 or more years (Russell, 1934; Fellows, 1941), although Clark (1942) reported that O. graminis disappeared after 3 months storage under "moisture and temperature conditions favourable for microbial activity". Clark did not specify

TABLE 36

The effect of storage at pF3.6 and 15°C on the incidence of O. graminis on wheat seedlings grown in cores removed from a take-all patch at Ceduna (mean of 7 replications)

Period of storage (wk)	Percentage of: seedlings infected	Arcsine (radians)	roots infected	Arcsine (radians)
0	98	1.51	76	1.10
5	85	1.31	65	0.94
10	100	1.57	91	1.33
15	92	1.45	83	1.27
20	98	1.51	82	1.18
25	76	1.21	59	0.87
31	86	1.35	73	1.11
Standard error		0.118		0.119
L.S.D.		ns		ns

what these conditions were.

Studies on the survival of *O. graminis* in naturally infested soil are reported in Part II. At Turretfield there was a small reduction in the level of *O. graminis* during summer. The percentage of sites with crowns containing viable fungus dropped from 96 in late January to 83 at the end of May. However, during the winter there was a rapid reduction in inoculum, so that by the end of August only 30 per cent of the sites contained crowns with viable *O. graminis*. This is in contrast to the results from Ceduna where the percentage of sites with crowns containing viable fungus dropped from 90 in early February to 82 in mid November.

There was a small drop in the incidence of *O. graminis* in cores removed at regular intervals from the experimental area at Ceduna. The average percentage of roots infected per core remained about 45 from early February to late July. From the end of July it gradually fell to 27 in mid November. When cores removed from Ceduna in summer were stored, there was no significant change in the incidence of *O. graminis* if the soil was maintained in a cool dry or cool moist condition. Even when maintained in a hot dry or cool wet condition there was considerable viable fungus remaining after 45 weeks of storage. Only when the soil remained hot and wet did *O. graminis* disappear rapidly.

It is unfortunate that the hardness of the soil at Turretfield prevented core experiments at that location. The differences in the survival of *O. graminis* at Turretfield and Ceduna revealed by the crown removal experiments may have given some interesting results in core experiments.

The levels of survival reported in Part II are in contrast to

those reported in most studies using straws artificially colonized by *O. graminis*. The longest period of survival in artificially colonized straw appears to be that recorded by Butler (1959), when only 3 per cent of the straws contained viable *O. graminis* after 52 weeks of burial in soil. To clarify this situation I undertook an investigation using artificially colonized straws buried in naturally infested field soil. These studies, reported in Part III, allow a comparison of the survival of *O. graminis* in both artificially colonized plant material and naturally infested debris in soil.

PART III

SURVIVAL OF *OPHIOBOLUS GRAMINIS* IN ARTIFICIALLY COLONIZED STRAWS BURIED IN FIELD SOIL

Garrett (1938) introduced the use of standardized artificially colonized straw for the study of the survival of *O. graminis*. Since then, this convenient method has been used in most studies. However, Chambers (1971b) has drawn attention to some of the problems of using artificially colonized straw in survival studies. For instance, he found that the method of sterilization and the type of straw used both influenced survival.

The results in Part II indicate that there is a case for conducting more survival investigations using naturally infested soil. The investigations reported here combine both artificially colonized straws and naturally infested soil. Although the primary aim was to study the survival of *O. graminis*, the opportunity was taken to broaden the scope of the investigation to include a study of the effect of cropping history on survival. Inoculum potential and virulence of the surviving fungus was also investigated.

The most commonly used survival test (Garrett, 1938) is based on the assumption that no infection indicates no surviving *O. graminis* present in the straw. In Garrett's test the seed is placed in the lumen of the straw to give maximum opportunity for the roots of the test seedling to come in contact with the fungus. In the field the fungus rarely

has such favourable conditions for contact with roots of a host. In the survival test used in my investigations, a seedling is grown in contact with, but not in the lumen of, a colonized straw. The fungus and the straw is treated as a unit with the potential to cause disease to a wheat seedling. Reduction in the effect of the unit on the growth of the seedling with increasing period of burial of the unit would indicate a reduction in inoculum potential, a loss of virulence, or both. Lack of infection would indicate that the fungus has failed to survive in the straw, that inoculum potential has fallen below a minimal level or that the fungus has become avirulent.

A. Tests to determine survival and virulence of O. graminis

The straws used for colonization were clean bright nodal sections (approximately 15 mm long by 3 to 5 mm diameter at the node) of the Gabo variety. Supplies of straws were prepared by autoclaving (1½ hours at 1 atmosphere) approximately 150 straws and 30 ml of distilled water in 100 ml Erlenmeyer flasks. The fungus was grown for 6 to 8 days on about 10 ml of PMDA in a 500 ml Erlenmeyer flask. The straws were then added to this flask and colonized at 20°C for 7 to 8 weeks. Four weeks after the addition of the straws, the flasks were shaken thoroughly to mix the straws. After colonization the straws were washed in sterilized water to remove any adhering mycelium and blotted dry prior to use.

Soil cores were used as the storage media. Fifty straws were pressed vertically into the soil leaving about 1 mm of straw showing

above the surface. They were inserted in this manner to simulate the lower stem region of infected stubble standing in soil. Although not completely covered the term 'buried' is used to describe the position of the straws in the soil. Straws were inserted at approximately 1 cm centres on a square grid pattern. For all treatments 4 cores (i.e. 4 replications) were used. Limits on controlled environment space for both storage and the survival test prevented the use of more than 4 replications. For each survival test, 5 straws were removed at random from each core.

The tests used to determine survival or virulence are similar. For the survival test, colonized straws were removed from the core, washed with a jet of water to remove attached soil and then used. For the virulence test, straws were used immediately after colonization. Five straws were evenly distributed on the surface of 'soil mixture' (275g in a plastic drinking cup) and pressed horizontally into moist soil to a depth of 2.5 cm. A seed was placed in contact with the straw and the soil was pressed around the seed and straw. The moisture level of the soil was then brought to 10 per cent of oven-dry weight (pF 1.8). Cups were placed in a controlled environment (described previously) and covered with plastic sheets for the first 5 days to minimise evaporation; thereafter, they were watered daily to constant weight.

Cups were randomized within 4 replicated blocks. Within each block, 2 extra cups containing only soil and seeds were incorporated to act as uninoculated 'standards'. These were included in the tests so that comparisons between different tests could be made; the double number of

cups was used to minimize the possibility of the comparison being jeopardized by a chance failure of, or accident to, some of the cups. In both survival and virulence tests, the 'standards' were not included in the analysis of variance. The 'standard' quoted in the text for both tests is a mean of 8 cups.

After 3 weeks, the seedlings were washed free of soil and the following parameters recorded per cup: (1) number of seedlings, (2) percentage of seedlings infected in the scutellar-node region, and (3) total root dry weight. As there is a strong possibility of cross infection of seedlings within the cup, estimates of survival are based on a survival rating of positive or negative infection of seedlings per cup rather than per straw. The percentage of seedlings infected in the scutellar-node region per cup was recorded and has been included in the text with the proviso that it be treated only as a guide to the level of survival. Root dry weight was chosen as the parameter to measure effect of the fungus on the host because it was more easily obtained than length of discoloured root. A preliminary experiment demonstrated a highly significant correlation ('r' = 0.9136) between root dry weight and length of discoloured root. Seedling emergence was recorded to ensure that differences in total weight were not due to differences in emergence. Analysis of variance of emergence data from all tests, although not strictly legitimate, was performed as routine throughout the investigation without any significant differences being obtained.

B. Survival experiments

The soils used as storage media in the experiments were obtained

from 2 adjacent farms 16 km west of Ceduna. Three areas are on the property of Mr. E. Hoffrichter and the remaining area is on Mr. W. Hoffrichter's property. Although the areas are scattered over the 2 farms (2 are in adjacent fields, while 1 is 4 km east and the other 2.5 km west of the above 2) their soils are very alike. The similarities of the physical and chemical properties, and moisture characteristic (drying boundary) figures for the 4 soils are shown in Table 37. (Their similarity to the soil from Ceduna is also shown). All these soils are classified as brown calcareous earths (Northcote, 1960).

The sites chosen for the collection of cores had the following cropping histories: Hoff. 1 had pasture 1964, wheat 1965 and 66, pasture 1967 and 68, and wheat 1969; Hoff. 2 had wheat 1964, pasture 1965 to 67, wheat 1968 and 69; Hoff. 4 had wheat 1962, pasture 1963, wheat 1964, pasture 1965, wheat 1966 to 69; Hoff. 8 wheat 1962 to 69. Cores were collected along the drill rows in February 1970 and had a soil moisture tension of pF6 at the time of collection.

A random selection of cores from each site was bioassayed prior to the commencement of the survival experiment. The results shown in Table 38 indicate that there was viable _O. graminis_ in all soils. This despite the fact that the farmers reported severe take-all in Hoff. 1, moderate take-all in Hoff. 2 and no take-all in Hoff. 4 or Hoff. 8.

Experiment I: Soils collected after the 1969 harvest and maintained in the open.

Straws colonized by isolates 2C or 044 were inserted in the soils. The cores were placed on benches outside at the Waite Institute. Soils

TABLE 37

Comparison of the physical and chemical properties, and moisture tensions of 4 soils used to bury colonized straws. Soils also compared with soil from Ceduna experimental area

Details	Hoff. 1	Hoff. 2	Hoff. 4	Hoff. 8	Ceduna
Hue	7.5YR(5/3)	7.5YR(5/4)	7.5YR(5/4)	7.5YR(4/3)	7.5YR(5/4)
Particle size <2µ(clay) %	15	17	14	14	17
2-20µ(silt) %	6	6	8	9	8
>20µ(sand) %	79	77	78	77	75
pH	8.7	8.5	8.7	8.7	8.5
Specific conductivity (reciprocal ohms)	0.00109	0.00157	0.00147	0.00135	0.00160
NaCl equivalents %	0.41	0.59	0.55	0.51	0.60

TABLE 37 (contd.)

Details		Hoff. 1	Hoff. 2	Hoff. 4	Hoff. 8	Ceduna
Total Nitrogen	%	0.103	0.128	0.100	0.123	0.100
Total Phosphorus	ppm	140	228	203	245	248
Total carbon	%	1.38	1.24	1.06	1.37	0.99
C : N ratio		13.4	9.7	10.6	11.1	9.9
Moisture characteristic Sieved soil						
pF 1.73 Soil moisture %		25.7	26.5	29.7	30.8	31.3
1.99 "		16.0	16.9	18.2	18.4	22.8
2.47 "		12.8	13.2	15.1	13.2	18.7
2.76 "		11.4	11.3	12.2	10.6	16.5
5.36 "		2.0	2.2	2.2	2.0	2.0
6.00 "		1.3	1.4	1.6	1.5	1.3
Undisturbed core pF 2.00 Soil moisture %		20.6	21.4	23.0	21.3	24.4

TABLE 38

Incidence of *O. graminis* in cores removed from the 4 Hoffrichter sites (mean of 7 replications)

Soil	Percentage of:			
	seedlings infected	Arcsine (radians)	roots infected	Arcsine (radians)
Hoff. 1	81	1.24	59	0.89
Hoff. 2	74	1.17	52	0.82
Hoff. 4	48	0.70	30	0.48
Hoff. 8	58	0.88	41	0.62
Standard error		0.214		0.183
L.S.D.		ns		ns

from different sites were separated by a distance of 4.5m to minimize cross contamination by rain splash. Cores were in cans with drainage holes and the cans in turn were placed in boxes and surrounded by fine gravel to minimize temperature fluctuations. For each soil type, 2 extra cores were included. These were weighed at regular intervals to determine changes in soil moisture. The screen air temperatures and rainfall details at the Waite Institute are shown in Figure 28 along with the pF values for Hoff. 1. The other soils had similar pF values. The cores were placed in the open on May 1, 1970 and received no treatment other than the removal of any seedlings that appeared.

Straws were removed after 5, 10, 15, 20, 27, 32 and 37 weeks of burial and used in survival tests. The number of replications containing infected seedlings and the mean percentage of infected seedlings per replication are shown in Tables 39 and 40 . The results for weeks 10, 15 and 20 were checked by isolation of O. graminis from straws. At these times 1 extra straw was removed from each core, washed, surface sterilized with silver nitrate, cut longitudinally and plated surface downwards on PMDA plus 30 ppm Aureomycin hydro-chloride. The number of replications from which O. graminis was isolated after 12 days incubation at 20°C is shown in Table 41. The results show that survival estimates by both infection and isolation are similar.

After the last removal of straws at week 37, the cores were bioassayed for the presence of O. graminis. Prior to the bioassay, the top 2 cm of soil was removed and sieved (2.0 mm mesh) to remove any of the original straws that may have remained in the soil. The soil was

FIG. 28

WEEKLY AIR TEMPERATURE, RAINFALL AND SOIL MOISTURE TENSION OF HOFF. 1 CORES, AT THE WAITE INSTITUTE, 1970

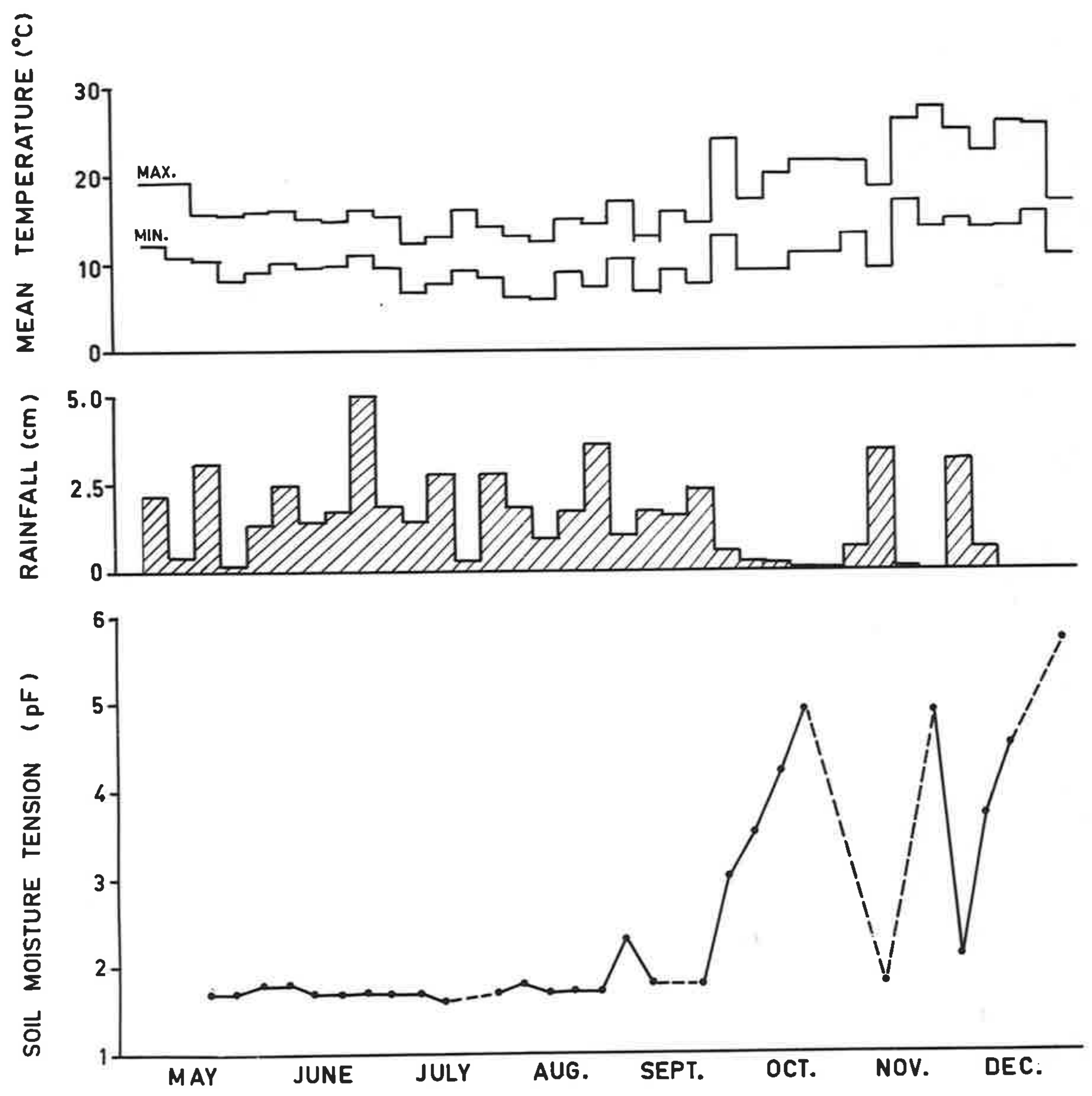


TABLE 39

Number of replications containing infected wheat seedlings when grown in contact with straws artificially colonized with <u>O. graminis</u> and buried for different periods in 1 of 4 soils maintained in the open (maximum of 4 replications)

Isolate	Soil	Period of burial (wk)						
		5	10	15	20	27	32	37
2C	Hoff. 1	4	4	4	4	4	4	4
	Hoff. 2	4	4	4	4	4	4	4
	Hoff. 4	4	4	4	2	0	0	0
	Hoff. 8	4	4	4	4	4	4	4
044	Hoff. 1	4	4	4	4	4	4	4
	Hoff. 2	4	4	4	4	2	2	0
	Hoff. 4	4	2	1	0	0	0	0
	Hoff. 8	4	4	1	0	0	0	0

TABLE 40

Percentage of infected seedlings per replication for wheat seedlings grown in contact with straws artificially colonized with *O. graminis* and buried for different periods in 1 of 4 soils maintained in the open (mean of 4 replications)

Isolate	Soil	Period of burial (wk)						
		5	10	15	20	27	32	37
2C	Hoff. 1	92	95	100	84	100	94	100
	Hoff. 2	90	94	100	100	85	95	74
	Hoff. 4	95	75	39	15	0	0	0
	Hoff. 8	100	100	95	84	80	79	82
044	Hoff. 1	90	100	95	80	76	88	95
	Hoff. 2	100	84	85	45	38	18	0
	Hoff. 4	85	10	13	0	0	0	0
	Hoff. 8	100	64	25	0	0	0	0

TABLE 41

Isolation of *O. graminis* from artificially colonized straws after different periods of burial in 1 of 4 soils maintained in the open.

Soil	Number of straws yielding *O. graminis* (max. 4)					
	Isolate 2C			Isolate 044		
	Period of burial (wk)			Period of burial (wk)		
	10	15	20	10	15	20
Hoff. 1	4	4	4	4	4	4
Hoff. 2	4	4	3	4	4	3
Hoff. 4	4	3	3	4	0	0
Hoff. 8	3	4	4	4	3	1

then replaced. The results of the bioassay are shown in Tables 42 and 43. Both the soil and the isolate used to colonize the straws affected the level of viable _O. graminis_ surviving in the soil. The results of these bioassays show that in only 1 treatment had the native _O. graminis_ completely disappeared after 37 weeks of storage. This is in contrast to the survival of the fungus in artificially colonized straws where _O. graminis_ failed to survive to 37 weeks in 4 treatments.

Details of the results for root dry weights are shown in Tables 44 and 45. There was a highly significant ($p = 0.001$) effect on root growth due to period of burial, type of soil and isolate used to colonize the straws. These effects can be seen in Figure 29 where the root weights of the various treatments are shown as percentages of the 'standard'.

The 'soil effect' for isolate 2C is evident in Figure 29a where the root growth of seedlings in contact with straws from Hoff. 4 is similar to the 'standard' from week 20 to the end of the experiment; root growth from the remaining 3 soils reached a peak at about 10 weeks and continued thereafter at about the same percentage of the 'standard'. With isolate 044 the 'soil effect' (Fig. 29b) was not as clear cut as with isolate 2c. From week 15 onwards only those seedlings in contact with straws from Hoff. 1 were consistently different from the 'standard'.

The virulence of the surviving _O. graminis_ was determined. One reisolate per treatment was obtained from the 20-week straws, an exception being 044 in Hoff. 4, where the reisolate used was that obtained on the last occasion _O. graminis_ was isolated. The straws used in the virulence test were prepared by placing 20 sterilized straws on a colony of

TABLE 42

Incidence of native O. graminis in cores used for burial of straws artificially colonized with O. graminis (isolate 2C or 044)

Soil	Percentage of: seedlings infected		seedlings infected Arcsine (radians)		roots infected		roots infected Arcsine (radians)	
	Isolates		Isolates		Isolates		Isolates	
	2C	044	2C	044	2C	044	2C	044
Hoff. 1	57	11	0.86	0.24	25	5	0.49	0.16
Hoff. 2	55	34	0.84	0.55	25	14	0.52	0.30
Hoff. 4	8	0	0.20	0	2	0	0.10	0
Hoff. 8	55	12	0.84	0.25	15	3	0.39	0.12
Standard error			0.145				0.086	

TABLE 43

Results of analysis of variance (factorial) of percentage of seedlings and roots infected shown in Table 42.

Soil means (radians)

Parameter	Hoff. 1	Hoff. 2	Hoff. 4	Hoff. 8	F	SE	DF
Seedlings infected	0.55	0.70	0.10	0.55	6.3**	0.102	3 and 21
Roots infected	0.32	0.41	0.05	0.26	6.3**	0.061	3 and 21

Isolate means (radians)

Parameter	Isolate 2C	Isolate 044	F	SE	DF
Seedlings infected	0.69	0.26	17.2***	0.072	1 and 21
Roots infected	0.37	0.14	14.3**	0.043	1 and 21

TABLE 44

Root dry weight (mg) of wheat seedlings grown in contact with straws artificially colonized with O. graminis and buried for different periods in 1 of 4 soils maintained in the open (mean of 4 replications)

Isolate	Soil	Period of burial (wk)							
		0*	5	10	15	20	27	32	37
2C	Hoff. 1	38	67	85	89	83	83	84	75
	Hoff. 2	38	91	77	88	85	70	73	86
	Hoff. 4	38	83	107	113	133	117	116	112
	Hoff. 8	38	59	75	85	80	87	86	69
044	Hoff. 1	34	77	94	100	107	89	95	99
	Hoff. 2	34	89	82	117	120	100	108	110
	Hoff. 4	34	96	123	134	118	126	122	119
	Hoff. 8	34	77	104	125	130	118	136	114
Standard error			9.4						
'Standard'		126	127	124	133	125	115	129	121

* Not included in analysis of variance.

TABLE 45

Results of analysis of variance (factorial) of root dry weight (mg) shcwn in Table 44.

Period of burial means

5	10	15	20	27 (wk)	32	37	F	SE	DF
80	93	106	110	99	103	98	8.8***	3.3	6 and 165

Soil means

Hoff. 1	Hoff. 2	Hoff. 4	Hoff. 8	F	SE	DF
88	93	116	97	23.6***	2.5	3 and 165

Isolate means

2C	044	F	SE	DF
88	109	73.4***	1.8	1 and 165

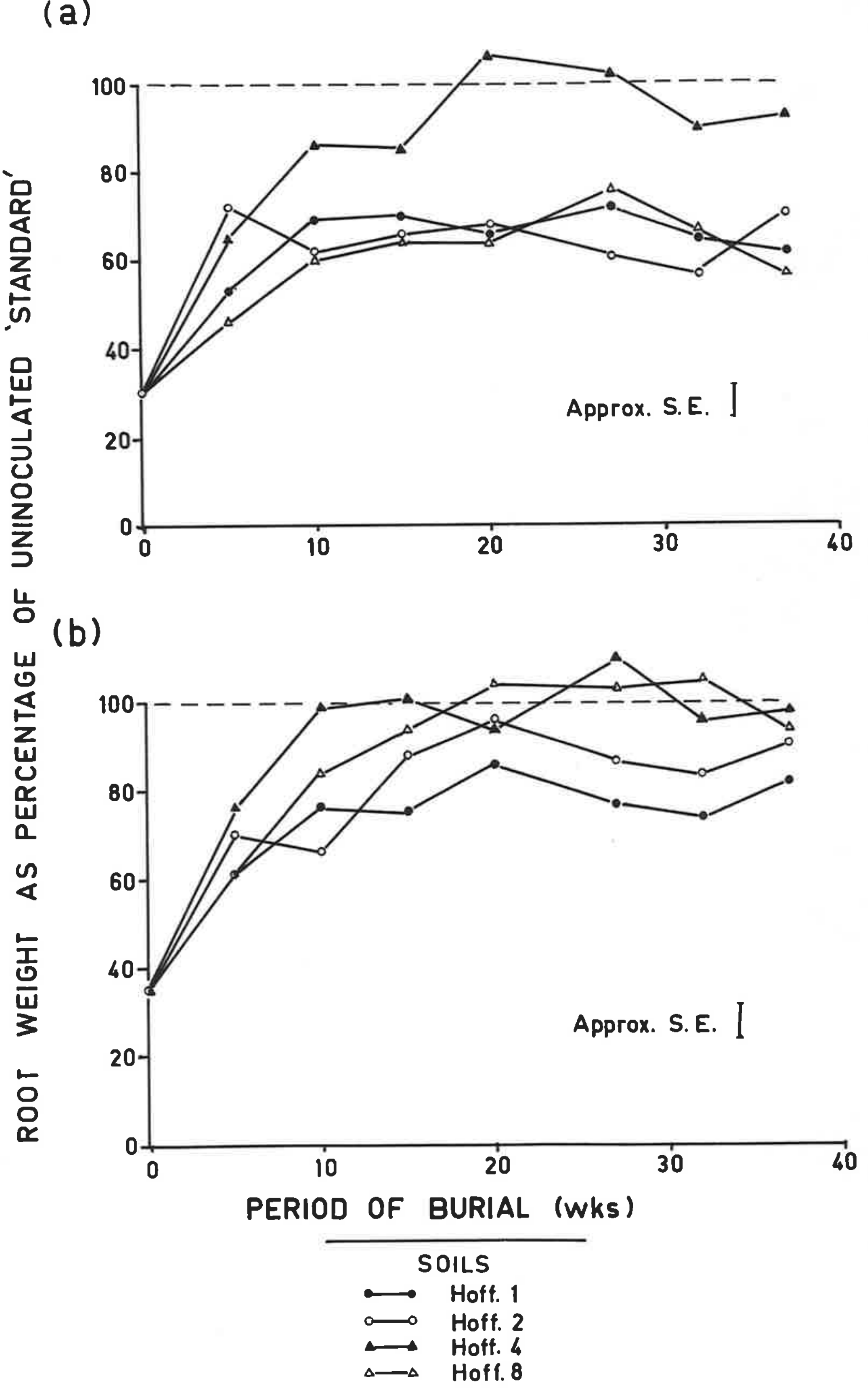
FIGURE 29

ROOT DRY WEIGHT OF SEEDLINGS GROWN IN CONTACT WITH STRAWS BURIED FOR VARIOUS PERIODS IN SOILS MAINTAINED IN THE OPEN

(a) Straws colonized by isolate 2C

(b) Straws colonized by isolate 044

FIG. 29



the reisolate on PMDA and incubating for 2 weeks at 20°C. The results of the test are shown in Table 46. Both 2C and 044 maintained their virulence during burial.

Experiment II: Soils collected after the 1969 harvest and maintained at pF2 and 15°C.

Straws colonized by isolates 2C or 044 were inserted in the soils as described previously. An added storage medium, autoclaved coarse sand, was included to provide a control unaffected by micro-organisms. The sand was sealed in McCartney bottles. The precautions to maintain sterility made the conditions of storage different from those in the cores, but this was unavoidable.

Cores were watered fortnightly to constant weight. Water losses between waterings were similar to those reported in a previous experiment (page 152). As the soil dried between waterings, small deposits of salts formed on the high parts of the soil surface. The amount of deposition was similar for all 4 field soils. The cores received no treatment other than the removal of all seedlings that emerged.

Straws from all treatments were removed after 5, 10, 15 and 20 weeks of burial and used in survival tests. The number of replications containing infected seedlings and the mean percentage of infected seedlings per replication are given in Tables 47 and 48. These results were checked by direct isolation from straws. The number of straws from which O. graminis grew in 12 days incubation is shown in Table 49. Again there was a good correlation between host infection and direct isolation.

Root dry weight results are shown in Tables 50 and 51. As

TABLE 46

Virulence of isolates of _O. graminis_ before and after reisolation from straws buried for 20 weeks in 1 of 4 soils maintained in the open.

Soil	Root dry weight (mg)	
	Isolate	
	2C	044
Original isolate*	27	24
Reisolate from:		
Hoff. 1	31	24
Hoff. 2	27	31
Hoff. 4	30	31
Hoff. 8	34	28
Standard error	4.1	
L.S.D.	ns	
'Standard'	128	

* not included in analysis of variance; 'standard' = 124 mg.

TABLE 47

Number of replications containing infected wheat seedlings when grown in contact with straws artificially colonized with *O. graminis* and buried for different periods in 1 of 5 soils maintained at pF2 and 15°C (maximum of 4 replications)

Isolate	Soil	Period of burial (wk)			
		5	10	15	20
2C	Hoff. 1	4	4	4	4
	Hoff. 2	4	4	4	4
	Hoff. 4	4	4	2	1
	Hoff. 8	4	4	4	4
	Sand	4	4	4	4
044	Hoff. 1	4	4	4	4
	Hoff. 2	4	4	4	4
	Hoff. 4	3	0	0	0
	Hoff. 8	4	3	2	1
	Sand	4	4	4	4

TABLE 48

Percentage of infected seedlings per replication for wheat seedlings grown in contact with straws artificially colonized with O. graminis and buried for different periods in 1 of 5 soils maintained at pF2 and 15°C (mean of 4 replications)

Isolate	Soil	Period of burial (wk)			
		5	10	15	20
2C	Hoff. 1	95	100	100	100
	Hoff. 2	100	100	90	95
	Hoff. 4	100	55	38	10
	Hoff. 8	100	95	95	100
	Sand	100	94	94	88
044	Hoff. 1	100	95	100	83
	Hoff. 2	100	95	85	74
	Hoff. 4	40	0	0	0
	Hoff. 8	100	44	31	5
	Sand	90	95	68	61

TABLE 49

Isolation of *O. graminis* from artificially colonized straws after different periods of burial in 1 of 5 soils maintained at pF2 and 15°C.

Soil	Number of straws yielding *O. graminis* (max. 4)							
	Isolate 2C				Isolate 044			
	Period of burial (wk)				Period of burial (wk)			
	5	10	15	20	5	10	15	20
Hoff. 1	4	4	4	4	4	4	4	4
Hoff. 2	4	4	4	4	4	4	4	4
Hoff. 4	4	4	3	0	3	4	0	0
Hoff. 8	4	4	4	4	4	4	2	2
Sand	4	4	4	4	4	4	4	4

TABLE 50

Root dry weight (mg) of wheat seedlings grown in contact with straws artificially colonized with O. graminis and buried for different periods in 1 of 5 soils maintained at pF2 and 15°C (mean of 4 replications)

Isolate	Soil	Period of burial (wk)				
		0*	5	10	15	20
2C	Hoff. 1	38	48	59	102	73
	Hoff. 2	38	60	82	108	94
	Hoff. 4	38	77	124	131	127
	Hoff. 8	38	48	66	85	87
	Sand	38	44	85	106	99
044	Hoff. 1	34	64	84	104	88
	Hoff. 2	34	51	92	110	116
	Hoff. 4	34	100	118	125	114
	Hoff. 8	34	62	104	108	127
	Sand	34	82	99	92	89
Standard error			9.5			
'Standard'		126	127	124	133	125

* Not included in analysis of variance.

TABLE 51

Results of analysis of variance (factorial) of root dry weight (mg) shown in Table 50

Period of burial means

5	10	15	20	F	SE	DF
(wk)						
63	91	107	101	40.9***	3.0	3 and 117

Soil means

Hoff. 1	Hoff. 2	Hoff. 4	Hoff. 8	Sand	F	SE	DF
78	89	112	87	87	14.7***	3.4	4 and 117

Isolate means

2C	044	F	SE	DF
85	96	13.3***	2.1	1 and 117

previously, there was a highly significant effect on root growth due to period of burial, type of soil and isolate used to colonize the straws. These effects can be seen in Figure 30. The virulence of reisolates at week 20 (Hoff. 4 last occasion isolated) was determined (Table 52). The virulence of both isolates was maintained during survival within straws buried in all test soils.

As both the survival and root growth results in this experiment were similar to those in Experiment I, the experiment was terminated at 20 weeks. The similarity of moisture and temperature (Fig. 28) in both experiments during the first 20 weeks could account for the parallel results.

Experiment III: Soils collected after the 1969 harvest and maintained at pF6 and 35°C.

Isolates 2C or 044 were again used to colonize the straws inserted in the soils. Coarse autoclaved sand in cotton-plugged McCartney bottles was used as a control. The cores and the sand were placed in a 35°C storage cabinet and received no further treatment.

When the cores were placed in storage, the relative humidity within the cabinet rose from 30 to 45 per cent during the first 3 hours. Over the next 13 hours, the relative humidity gradually returned to 30 per cent. As the soil moisture tension was already at pF6, this increase in humidity was due to evaporation from the straws. This indicates that a rapid initial drying out of the straws took place. The straws in the coarse sand in the McCartney bottles dried out more slowly than those in the cores. Half the moisture lost occurred during the first 35 hours,

FIGURE 30

ROOT DRY WEIGHT OF SEEDLINGS GROWN IN CONTACT WITH STRAWS BURIED FOR VARIOUS PERIODS IN SOIL MAINTAINED AT pF2 AND 15°C

(a) Straws colonized by isolate 2C

(b) Straws colonized by isolate 044

FIG. 30

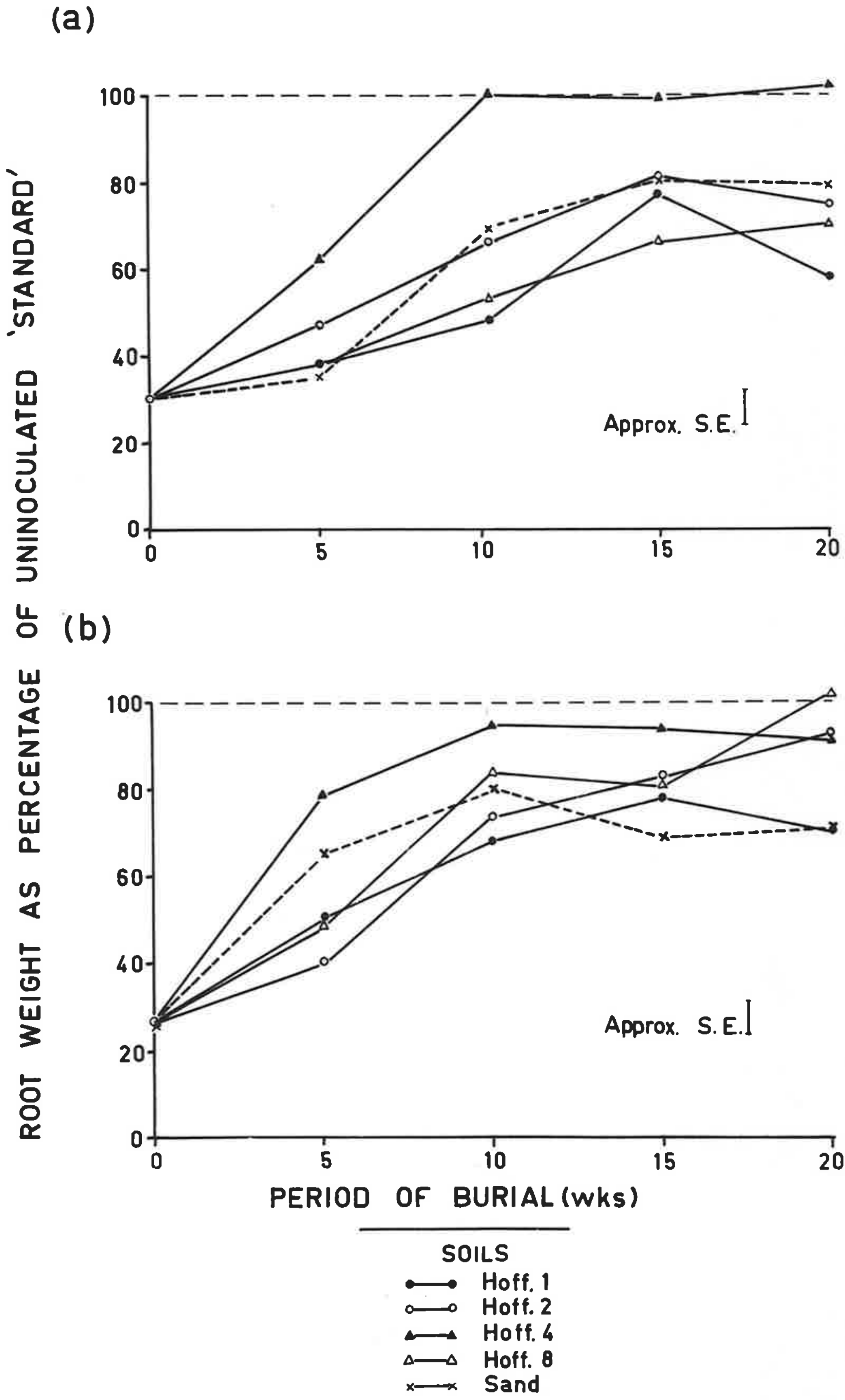


TABLE 52

Virulence of isolates of *O. graminis* before and after reisolation from straws buried for 20 weeks in 1 of 5 soils maintained at pF2 and 15°C.

Soil	Root dry weight (mg)	
	Isolate	
	2C	044
Original isolate*	27	24
Reisolate from:		
Hoff. 1	28	27
Hoff. 2	29	29
Hoff. 4	36	36
Hoff. 8	36	43
Sand	18	31
Standard error	6.2	
L.S.D.	ns	
'Standard'	128	

* not included in analysis of variance; 'standard' = 124 mg.

while the remainder was lost during the next 24 days of storage.

Straws were removed after 5, 10, 15, 20 and 27 weeks of burial and used in survival tests. The number of replications containing infected seedlings and the mean percentage of infected seedlings per replication are given in Tables 53 and 54. The survival results to week 20 were checked by direct isolations from straws. The results (Table 55) confirm those obtained by host infection.

By week 27 only straws from the sand contained viable O. graminis. The differences in the survival of O. graminis in dry sand and dry wheat-field soil might be attributed to microbiological activity on the straws in natural soil during the initial drying period or to the rapid drying of straws. The short period for microbiol activity and the uniformity of the rate of reduction of survival in 4 different wheat-field soils makes the microbial interpretation seem unlikely. The explanation appears to involve the method of storage. Although both types of container were in the same controlled environment, the rate of reduction in moisture content of straws stored in the sand was slower than that of the straws in the cores. The slower rate of drying may have allowed time for hyaline hyphae occupying the freshly colonized straw to be replaced by the more resistant (Padwick, 1936) dark hyphae. Examination of straws after 20 weeks of burial showed that dark hyphae were found only rarely in any of the straws, although some thick hyaline hyphae were observed in straws from the autoclaved sand. The latter may be more resistant to desiccation than the thin hyaline hyphae. The slower rate of drying may have also caused less damage to the hyphae in the straw.

TABLE 53

Number of replications containing infected wheat seedlings when grown in contact with straws artificially colonized with _O. graminis_ and buried for different periods in 1 of 5 soils maintained at pF6 and 35°C (maximum of 4 replications)

Isolate	Soil	Period of burial (wk)				
		5	10	15	20	27
2C	Hoff. 1	4	4	2	2	0
	Hoff. 2	4	4	4	0	0
	Hoff. 4	4	4	3	1	0
	Hoff. 8	4	4	3	1	0
	Sand	4	4	4	4	4
044	Hoff. 1	4	4	3	1	0
	Hoff. 2	4	4	2	2	0
	Hoff. 4	4	4	2	0	0
	Hoff. 8	4	4	3	1	0
	Sand	4	4	4	4	4

TABLE 54

Percentage of infected seedlings per replication for wheat seedlings grown in contact with straws artificially colonized with O. graminis and buried for different periods in 1 of 5 soils maintained at pF6 and 35°C (mean of 4 replications)

Isolate	Soil	Period of burial (wk)				
		5	10	15	20	27
2C	Hoff. 1	100	61	15	14	0
	Hoff. 2	100	90	35	0	0
	Hoff. 4	100	80	34	5	0
	Hoff. 8	100	75	28	8	0
	Sand	100	95	100	80	89
044	Hoff. 1	100	92	15	5	0
	Hoff. 2	100	68	30	11	0
	Hoff. 4	100	73	35	0	0
	Hoff. 8	100	95	53	5	0
	Sand	100	100	85	83	75

TABLE 55

Isolation of O. graminis from artificially colonized straws after different periods of burial in 1 of 5 soils maintained at pF6 and 35°C.

Soil	Number of straws yielding O. graminis (max 4)							
	Isolate 2C				Isolate 044			
	Period of burial (wk)				Period of burial (wk)			
	5	10	15	20	5	10	15	20
Hoff. 1	4	2	3	1	4	3	0	0
Hoff. 2	4	4	2	0	4	4	1	2
Hoff. 4	4	4	1	1	4	4	1	2
Hoff. 8	4	4	0	2	4	4	3	1
Sand	4	4	4	3	4	4	4	4

Root dry weight results are shown in Tables 56 and 57. The increase in root weight with period of burial was highly significant ($p = 0.001$) and can be clearly seen from the results (Table 56). After 5 weeks of burial the root weight for each treatment was still similar to the weight recorded at zero time. By week 20, all but the sand treatments were similar to the root weight of the standard. Examination of the analysis of the soil means, reveals significant differences. However, an analysis on the results for the field soils alone shows that root weights are not significantly different, thus the significant difference shown in Table 57 can be attributed to the sand treatment. There is no effect on root weight that can be attributed to the isolates.

The virulence of reisolates at week 20 (or last occasion isolated) was determined (Table 58). In contrast to the previous virulence tests, there were highly significant differences between reisolates in this test. Reisolate 2C from Hoff..2 was different from all others at $p = 0.05$ and all but 1 other at $p = 0.01$. When this reisolate was retested the root dry weight was 46 mg, indicating that this reisolate had lost some virulence either during burial or subsequent storage on glucose-asparagine medium (Table 55). All other reisolates appear to have maintained their virulence although some from field soils were significantly different from those from the sand.

Experiment IV: Soils collected after the 1970 harvest and maintained at pF2 and 15°C.

This experiment was conducted to determine whether the difference in the survival of isolates 2C and 044 observed in Experiments I and II

TABLE 56

Root dry weight (mg) of wheat seedlings grown in contact with straws artificially colonized with O. graminis and buried for different periods in 1 of 5 soils maintained at pF6 and 35°C (mean of 4 replications)

Isolate	Soil	Period of burial (wk) 0*	5	10	15	20
2C	Hoff. 1	38	51	97	122	116
	Hoff. 2	38	58	95	132	113
	Hoff. 4	38	49	91	101	123
	Hoff. 8	38	41	84	104	123
	Sand	38	39	56	58	84
044	Hoff. 1	34	46	78	137	137
	Hoff. 2	34	49	90	127	131
	Hoff. 4	34	51	89	118	110
	Hoff. 8	34	56	85	120	116
	Sand	34	44	53	70	89
Standard error			9.5			
Standard		126	127	124	133	125

* Not included in analysis of variance.

TABLE 57

Results of analysis of variance (factorial) of root dry weight (mg) shown in Table 56.

Period of burial means

5	10	15 (wk)	20
48	81	109	114

F	SE	DF
99.4***	3.0	3 and 117

Soil means

Hoff. 1	Hoff. 2	Hoff. 4	Hoff. 8	Sand
98	99	91	91	62

F	SE	DF
19.7***	3.4	4 and 117

Isolate means

2C	044
87	90

F	SE	DF
0.9	2.1	1 and 117

TABLE 58

Virulence of isolates of O. graminis before and after reisolation from straws buried for 20 weeks in 1 of 5 soils maintained at pF6 and 35°C.

Soil	Root dry weight (mg)	
	Isolate	
	2C	044
Original isolate*	27	24
Reisolate from:		
Hoff. 1	29	35
Hoff. 2	52	28
Hoff. 4	33	31
Hoff. 8	26	38
Sand	20	18
Standard error	3.8	
LSD p = .05	11.2	
= .01	15.2	
= .001	20.3	
'Standard'	128	

* Not included in analysis of variance; 'standard' = 124 mg.

could be confirmed. Another aim was to continue the study of the effect of cropping history on survival using soils collected after another season.

Cores were collected in November, 1970. The cropping histories of the sites chosen were as follows: Hoff. 1 + S was Hoff. 1 with the intervening season as stubble-pasture; Hoff. 4 + W was Hoff. 4 with the intervening season wheat; Hoff. 4 + S was near to Hoff. 4 but left as stubble-pasture in the intervening season; Hoff. 8 + W was Hoff. 8 with the intervening season as wheat. Although the crops on Hoff. 4 + W and Hoff. 8 + W were vigorous, crown bioassays revealed that 78 and 82 per cent respectively of the stubble carried viable _O. graminis_. Autoclaved coarse sand was again included as a control.

Straws colonized by isolates 2C or 044 were inserted in the soils. Cores were watered to constant weight every 4 weeks. The average loss in weight during this period was 108 g. This is equivalent to a rise in soil moisture tension from pF2 to pF2.7. Cores received no treatment other than the removal of any seedlings that appeared.

Straws were removed after 4, 8 and 12 weeks of burial and used in survival tests. The number of replications containing infected seedlings and the mean percentage of infected seedlings per replication are given in Tables 59 and 60. Survival was again affected by the isolate used to colonize the straws and the soil used to bury the straws. As previously, isolate 2C survived better than isolate 044. The survival of both isolates in Hoff. 1 + S was similar to that previously recorded in Hoff. 1 (Table 47). Survival of isolate 044 in Hoff. 4 + W and Hoff. 4+S

TABLE 59

Number of replications containing infected wheat seedlings when grown in contact with straws artificially colonized with O. graminis and buried for different periods in 1 of 5 soils maintained at pF2 and 15°C (maximum of 4 replications)

Isolate	Soil	Period of burial		
		4	8	12
2C	Hoff. 1 + S	4	4	4
	Hoff. 4 + W	4	4	4
	Hoff. 4 + S	4	4	4
	Hoff. 8 + W	4	4	1
	Sand	4	4	4
044	Hoff. 1 + S	4	4	4
	Hoff. 4 + W	4	3	3
	Hoff. 4 + S	4	4	2
	Hoff. 8 + W	2	0	0
	Sand	4	4	4

TABLE 60

Percentage of infected seedlings per replication for wheat seedlings grown in contact with straws artificially colonized with O. graminis and buried for different periods in 1 of 5 soils maintained at pF2 and 15°C (mean of 4 replications)

Isolate	Soil	Period of burial (wk)		
		4	8	12
2C	Hoff. 1 + S	100	100	100
	Hoff. 4 + W	100	100	90
	Hoff. 4 + S	100	100	100
	Hoff. 8 + W	100	85	5
	Sand	100	100	94
044	Hoff. 1 + S	100	100	95
	Hoff. 4 + W	90	59	20
	Hoff. 4 + S	95	78	25
	Hoff. 8 + W	15	0	0
	Sand	100	100	100

was similar and was better than this isolate in Hoff. 4. Both isolates did not survive as well in Hoff. 8 + W as they had in Hoff. 8. Although there were some differences in survival between soils collected after the 1969 and 1970 crops, the general pattern of survival being reduced with increased numbers of consecutive crops was maintained.

Details of the results for dry root weight are given in Tables 61 and 62. Once again there was a highly significant effect on root growth due to period of burial, type of soil and isolate used to colonize the straws. These effects can be seen in Figure 31.

C. Morphological differences between isolates used in survival studies

Chambers and Flentje (1967b) have shown that isolates of *O. graminis* which differ in virulence may also differ in their ability to survive. Isolates 2C and 044 have similar virulence but show differences in survival. An investigation was undertaken to determine whether any morphological differences between these isolates could account for differences in survival.

During colonization of straws for the survival studies, it was observed that isolate 2C produced abundant microconidia, while 044 produced none. This suggests that these isolates may be different strains. The distance between the original sources of these isolates makes this a possibility. No attempt was made to induce 044 to produce microconidia.

Young cultures of both isolates were morphologically similar when grown on PMDA. Old cultures of isolate 044 were slightly flatter and darker than those of isolate 2C. Both isolates were examined for strands containing 2 or more dark hyphae. Two subcultures of each isolate

TABLE 61

Root dry weight (mg) of wheat seedlings grown in contact with straws artificially colonized with O. graminis and buried for different periods in 1 of 5 soils maintained at pF2 and 15°C (mean of 4 replications).

Isolate	Soil	Period of burial (wk)			
		0*	4	8	12
2C	Hoff. 1 + S	30	30	43	55
	Hoff. 4 + W	30	43	39	73
	Hoff. 4 + S	30	43	36	64
	Hoff. 8 + W	30	36	90	144
	Sand	30	28	46	46
044	Hoff. 1 + S	34	52	64	63
	Hoff. 4 + W	34	87	98	135
	Hoff. 4 + S	34	77	101	135
	Hoff. 8 + W	34	125	133	138
	Sand	34	34	33	54
Standard error			9.7		
'Standard'		129	125	129	132

* Not included in analysis of variance.

TABLE 62

Results of analysis of variance (factorial) of root dry weight (mg) shown in Table 61.

Period of burial means

4	8 (wk)	12	F	SE	DF
55	66	92	38.3***	3.1	2 and 87

Soil means

Hoff. 1 + S	Hoff. 4 + W	Hoff. 4 + S	Hoff. 8 + W	Sand	F	SE	DF
51	79	76	110	40	47.6***	4.0	4 and 87

Isolate means

2C	044	F	SE	DF
54	88	91.9***	2.5	1 and 87

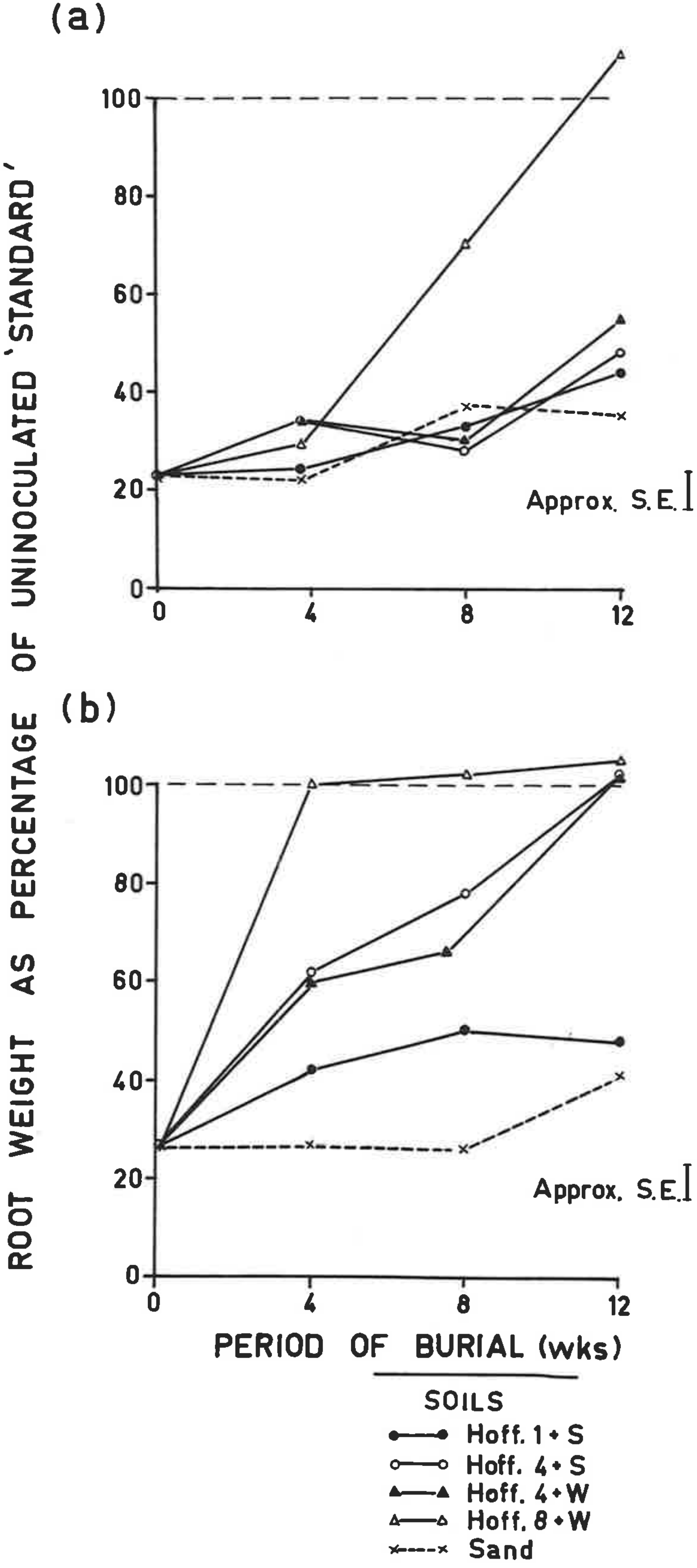
FIGURE 31

ROOT DRY WEIGHT OF SEEDLINGS GROWN IN CONTACT WITH STRAWS BURIED FOR VARIOUS PERIODS IN SOIL MAINTAINED AT pF2 AND 15°C.

(a) Straws colonized by isolate 2C.

(b) Straws colonized by isolate 044.

FIG. 31



were grown on PMDA for 15 days at 23°C. Six sites at 30 degree angles and at 1 and 2 cm from the centre of each subculture were chosen. At each site the number of strands crossing a 0.375 mm line was recorded. The results are shown in Table 63. Isolate 044 produced more common strands of dark hyphae than isolate 2C. This is in contrast to what happens on straws stored in moist autoclaved sand. In sand, isolate 2C produced a mat of dark hyphae over the surface of the straw, while the surface of straws containing 044 remained free of dark hyphae. The dark hyphae from isolate 2C advanced into the surrounding sand and grew over some parts of the inside of the bottle below the level of the soil surface.

Chambers and Flentje (1967b) reported differences in the development of hyphae in straws colonized by highly virulent and weakly virulent isolates of O. graminis. The former produced dark hyphae in the internodal segment during storage, while the latter did not. Other work of Chambers and Flentje (1968, 1969) also suggests that the production of dark hyphae and survival are related.

An investigation was made of the production of hyphae in straws colonized by isolates 2C or 044. Straws were homogenized in 75 per cent glycerol and observed microscopically. An examination of freshly colonized straws revealed that both isolates produced thin hyaline hyphae, a small amount of thick hyaline hyphae, but no dark hyphae.

Straws from Experiment IV were examined after 12 weeks burial. There were no consistent differences in the number of thin hyaline hyphae, but there were differences in the numbers of thick hyaline hyphae and dark

TABLE 63

Number of strands with 2 or more dark hyphae crossing an 0.375 mm line (mean of 12 observations)

Distance from centre of culture	Isolate 2C		Isolate 044	
	Mean	Range	Mean	Range
1 cm	1.2	0-3	2.5	1-5
2 cm	1.3	0-2	2.3	1-4

hyphae. The straws stored in autoclaved sand contained low numbers of dark hyphae but high numbers of thick hyaline hyphae. This may indicate that thick hyphae had formed but pigmentation had failed to develop to the same extent as in hyphae in the natural soils. There were no observed differences in the number of dark hyphae in straws in 3 of the natural soils that would account for the differences in the survival of isolates 2C and 044. The exception was in Hoff. 8 + W, where isolate 2C produced more dark hyphae than isolate 044. As the survival of these isolates within this soil was markedly different (Table 59), further detailed examinations of straws from this soil were made.

One straw per replication per time of burial was selected at random from preserved straws from Hoff. 8 + W and examined. The number of quadrats (0.375 x 0.375 mm) out of 40 containing dark hyphae was recorded for each straw. The results are shown in Table 64. Straws colonized by isolate 2C contained significantly more dark hyphae than those colonized by isolate 044. The number of dark hyphae in straws colonized by isolate 2C increased with time while the number of dark hyphae in straws containing isolate 044 remained unchanged. There was no way of determining whether the dark hyphae counted were viable, however if isolate 2C is able to produce more dark hyphae and produce them over a longer period than isolate 044, this may account for the differences in survival in Hoff. 8 + W.

Preserved straws removed from Experiment I after 27 and 37 weeks of burial were also examined. Straws from Hoff. 8 containing isolate 2C had significantly more dark hyphae than those containing isolate 044 (Table 65). In this examination it was evident that many of the dark

TABLE 64

Number of quadrats out of 40 containing dark hyphae. Artificially colonized straws buried for various periods in Hoff. 8 + W maintained at pF2 and 15°C (mean of 4 replications)

Isolate	Period of burial (wk)		
	4	8	12
2C	13.5	19.0	24.0
044	10.5	9.5	8.8

Results of analysis of variance (factorial)

Period of burial means

4	8	12
12.0	14.2	16.2

F	SE	DF
2.2	1.4	2 and 15

Isolate means

2C	044
18.8	9.6

F	SE	DF
30.7***	1.2	1 and 15

TABLE 65

Number of quadrats out of 40 containing dark hyphae. Artificially colonized straws buried for 27 or 37 weeks in Hoff. 8 maintained in the open (mean of 4 replications)

Isolate	Period of burial (wk) 27	37
2C	8.5	8.0
044	5.2	5.0

Results of analysis of variance (factorial)

Period of burial means

27	37
6.9	6.5

F	SE	DF
0.1	0.8	1 and 9

Isolate means

2C	044
8.3	5.1

F	SE	DF
8.4*	0.8	1 and 9

hyphae, especially in straws colonized by isolate 044, were disintegrating or had collapsed. But even after 37 weeks some dark hyphae in straws containing 044 were still intact. These differences in numbers of dark hyphae may have contributed to the marked differences in survival of the 2 isolates in Hoff. 8 (Table 39). The reason for the differences in numbers of dark hyphae is unknown.

* * * * * * *

In Part III a comparison has been made of the survival of _O. graminis_ in artificially colonized straws and in naturally infested soil. There were some marked differences; the fungus in the naturally infested soil generally survived better than the fungus in the straw. This suggests that there is a case for using natural media for the study of the survival of _O. graminis_.

The effect of cropping history on the survival of _O. graminis_ in artificially colonized straws was also studied. Marked differences in the survival of the fungus in similar soils with different cropping histories were observed. Generally survival was negatively correlated with increased numbers of continuous crops. It was also found that 1 of the 2 isolates of _O. graminis_ used in these investigations always survived better than the other.

During these investigations it was found that _O. graminis_ surviving in buried straws maintained its original virulence. However when straws containing viable fungus were maintained in cool wet soil, the inoculum potential of the infection unit (i.e. straw containing _O. graminis_)

dropped during the first 10 to 15 weeks of burial. Thereafter the inoculum potential could remain constant, in some soils, for as long as a further 27 weeks.

DISCUSSION

Throughout the investigations reported in this dissertation, I have placed emphasis on the use of natural soil and inoculum. In the literature there are reports of studies of the incidence of O. graminis in the field (White, 1945, 1947), and of its survival in naturally infested soil (Fellows, 1941). However, many of the investigations of O. graminis have been made using artificial inoculum either in the field (Kirby, 1925; Cunningham, 1967) or in the glasshouse and laboratory (Davis, 1925; Garrett, 1934a). Many of the studies on the survival of O. graminis have been conducted using artificially colonized straws (Hynes, 1937; Garrett, 1938; Gerlagh, 1968). Although there is a place for laboratory experiments, I have endeavoured to work from the general towards the specific; from the field to the use of natural soil in controlled environments. To undertake these studies several techniques were developed.

One of the techniques, the core bioassay, was based on the assumption that the roots of wheat seedlings will explore a large part of the soil mass within the core. However, it seems unlikely that there would have been enough root exploration in 4 weeks to ensure contact with every piece of debris containing viable O. graminis. This would be especially so if O. graminis was unable to grow from debris as suggested by Lucas (1955). Even if O. graminis can grow from its habitat to meet approaching roots (Brown and Hornby, 1971), the bioassay should only be

treated as a relative estimate of O. graminis incidence in the soil.

Hornby (1969a, b, c) separated infested soil into its various components, and estimated the number of infective units present. Although this approach will give a comparison of the numbers of infective units in various soils, the usefulness of the comparison is limited by the large variation in size of the units colonized by the fungus; these range from whole crowns to small pieces of debris. In the field, a large infective unit may provide the infection point for several roots or even several plants.

In the approach used by Slope, Henden and Etheridge (1969) and independently by me, no attempt was made to calculate the number of infective units present, but rather to treat each core as a unit with a potential for causing a certain level of disease. The level of disease was called "infective index" by Slope, Henden and Etheridge (1969) and has been designated incidence of O. graminis by me. The results of the bioassay should be similar to those obtained by growing seedlings in the same unit of soil in situ, although in the field roots from seedlings outside the unit may also explore the soil. The environment will also influence the results, but the differences between the level of infection in the field and in the bioassay may not be very large in a short period of growth (4 weeks). However, the effect of the environment on symptom expressions, between the time of the bioassay and maturity, could be considerable.

Workers at Rothamsted found that the "infection index" was unreliable for advisory purposes (Hornby, 1969b); there was a lack of cor-

relation between the index and the amount of disease in the crop. I was unable to correlate differences in *O. graminis* incidence at the time of the bioassay with yield from plants in cores transferred to soil in the glasshouse and grown to maturity. It was apparent that any level of infection at the time of the bioassay led to a marked reduction in yield at maturity. The buckets used in the glasshouse may have placed a limit on nutrients that would not be encountered in the field where roots would not be so confined. Lack of nutrients may allow a similar level of infection in seedlings to cause marked differences in yield between the glasshouse and field. However my mapping experiments suggest that at least some of the variation in yield within take-all patches was due to differences in time of infection of the plants by *O. graminis* rather than the level of infection on the seedling.

Variability in levels of infection in cores from the same site was possibly a reflection of the natural variability in the field. This suggests it would be difficult to use the bioassay to sample broad-acres for the prediction of disease incidence in the following crop. However with intensive sampling in a small block, as undertaken in the survival study at Ceduna, the bioassay proved to be a useful tool.

The use of cores from take-all patches, to study the effect of different treatments on *O. graminis*, has many possibilities. However, when using naturally infested soils, rather than artificially inoculated soils, there was the problem of lack of uniformity in levels of inoculum. The use of cores taken over plant remains helped to minimize variability of inoculum level. This allowed high, and reasonably uniform, levels of

inoculum to be obtained. However, cores with a reliable and predictable range of inoculum level, were not obtained.

Another problem with the use of cores is to express the soil moisture level in meaningful terms. The moisture-characteristic curve is the best method of expressing soil moisture (Griffin, 1963), but the establishment of curves using large cores is difficult. Theoretically, at tensions greater than pF3, soil structure should have no effect on moisture characteristic (Salter and Williams, 1965). Some time was spent in preliminary experiments trying to establish the drying boundary curve (below pF3) for soils in cores, but moisture equilibrium was not obtained. The possibility of using tensiometers to measure suctions below pF 2.9 (Marshall, 1959) was not investigated.

Another technique, the bioassay of wheat stubble for the presence of O. graminis, was shown to be suitable for the study of both the incidence and survival of the fungus in the field. However results obtained by this bioassay revealed nothing about the type and location of propagules present or the amount of inoculum present. The intensity of infection in terms of effect on seedling growth was recorded in a preliminary experiment, but the assessment was too time-consuming to be employed in large scale experiments.

The other major technique developed was the 'incidence-yield' regression. This was based on the assumption that yield is a reflection of incidence. Correlating yield with incidence could be confounded by other pathogens, and by variability of the soil and differences in moisture stress. The same level of incidence may give different yields in

different years. There may also be differences in an individual crop due to soil and moisture variability. The first of these problems will be overcome by adhering to the 2 principles mentioned earlier, *viz.* close scrutiny of the experimental area to establish that no other major pathogens are affecting yield, and establishment of the 'incidence-yield' regression from sites within or around the experimental area on each occasion the experiment is conducted. The problem of variability of soil type within the experimental area is more important. The area selected for experiments should have a uniform soil type. If different soil types are present, it would be preferable to conduct separate experiments on each soil type. If there is considerable variability and the different soil types are too diverse to conduct individual experiments, the 'incidence-yield' approach should not be employed.

It was unfortunate that the consecutive cropping experiments at Turretfield and Ceduna could not be maintained for more than 2 seasons. To determine whether the 'incidence-yield' technique is a satisfactory method of studying changes in the incidence of take-all and *O. graminis* a trial period of 4 or 5 seasons may be necessary. This would allow testing under a range of climatic conditions and possibly a range of take-all and *O. graminis* relationships.

The survival experiments in the field at Turretfield and at Ceduna revealed an interesting contrast between the survival of *O. graminis* at both places. The reason for the difference was not established, but may have been due to factors like differences in climate or soil type. A similar study over a period of several years could give valuable infor-

mation about the possibility of take-all 'decline' being associated with factors affecting the survival of O. graminis. These studies could be profitably linked with a study of the type and condition of the fungus occupying the crown and root tissue of stubble remaining in the field.

For short term survival experiments, the removal of wheat stubble is more convenient than the removal of cores. In long term experiments, extending over several seasons, it would become increasingly difficult to locate the remains of the plant. Although cores would be superior in long term experiments, there are disadvantages in using them in survival studies. Firstly, cores are very difficult to remove from heavy soils, thus limiting the scope of the study; secondly the survival studies may be confounded by the presence of grasses and self-sown cereals which grow during the winter. The latter could be overcome with herbicides, but these might affect survival (Wilkinson, 1969). Despite the difficulties, cores appear to be a useful method for studying the survival of O. graminis in the field.

Studies on the survival of O. graminis in naturally infested soils and in artificially colonized straws buried in naturally infested soil raised the question of why the fungus generally survived best in the natural medium. Although this was not answered in these investigations, survival appeared to be linked to the type of hyphae within the 2 media. An examination of naturally infected plant remains from the field showed that they contained mainly dark hyphae, while freshly prepared, artificially colonized straws contained no dark hyphae.

The fungus established as resistant dark hyphae (Padwick, 1936)

prior to treatment, would have an advantage over the fungus that had to form dark hyphae after the treatment commenced. In cool wet soil, dark hyphae may be more resistant to attack by micro-organisms or less affected by antagonistic substances than hyaline hyphae. In hot dry soil where micro-organisms are unlikely to be involved, the resistance of dark hyphae to desiccation would give the fungus in natural medium an advantage. Differences in hyphae and in the way the habitats are colonized, could be expected to cause differences between the survival of O. graminis in artificially colonized straws and in natural debris.

The results of my experiments suggest that the results from other experiments (where only artificially colonized straws were used) may need to be re-examined. Use of artificially colonized straws may reveal survival patterns that have no application in the field. The use of cores allowed the study of O. graminis in a natural form.

The results of my survival experiments using artificially colonized straws, necessitate explanations for the following: (1) why there were marked differences in survival in the different soils; (2) why isolate 2C always survived better than isolate 044 and why this difference was so marked in Hoff. 8 and Hoff. 8 + W; (3) why in some instances the inoculum potential of O. graminis dropped during the first 10 to 15 weeks after burial, but thereafter remained constant for the rest of the experiment.

Differences in survival did not appear to be associated with differences in breakdown of the straws. Visually, the rate of disintegration of the straws containing either isolate 2C or 044 was similar in

all 4 field soils. Comparison of the physical and chemical properties of the soils used in these experiments suggested that differences in survival of *O. graminis* could not be attributed to differences in these properties (Table 37). Although there were small differences between the soils there was no correlation between survival and any other property. Unless there were subtle interactions of physical or chemical properties, differences in survival associated with the soils were most likely due to the cropping history of the soils.

In the experiments reported by Butler (1959) where straws colonized by *O. graminis* were stored in natural soil, differences in survival were attributed to differences in soil fertility. Although the fertile soil (6 years of legume ley) had a total nitrogen content of 0.13 per cent compared to 0.07 per cent in the adjacent infertile area (50 years of cereal pasture rotation), the C:N ratio for both areas was similar. There is a possibility that the reduction in survival in the infertile soil was associated with cropping history rather than fertility.

Gerlagh (1968) found that continuous cropping led to a build up within the soil of an 'antagonism' to *O. graminis*. This 'antagonism' was induced in soil in the glasshouse by adding *O. graminis* inoculum (colonized wheat-seed and wheat-straw mixture) to the soil on each occasion that a consecutive crop was sown (3 monthly). He also found that the survival of *O. graminis* in artificially colonized straws stored in soil with induced 'antagonism' was reduced with increasing numbers of consecutive crops. The soils used in my experiments were natural soils. Any differences between the soils in levels of antagonism or other factor(s)

contributing to a reduction in survival of *O. graminis*, appear to be associated with cropping history and not with any addition to the soil.

The hypothesis is advanced here that continuous cropping causes a 'factor' to develop in the soil which affects the saprophytic survival of *O. graminis* in artificially colonized straws. This 'factor' may be antibiotic antagonism as suggested by Gerlagh (1968) but could be something else. For instance, Lapierre *et al*. (1970) have found a virus that affects the parasitic phase of *O. graminis*. The same or other viruses may affect saprophytic survival. The 'factor' causing a reduction in survival in my experiments is not necessarily removed by a 1 year break in the crop (Table 59). Results from Experiments I, II and IV indicate that the 'factor' may not continue to increase in intensity. It reached a maximum after 3 or 4 consecutive crops and thereafter varied slightly in effect between seasons.

The above hypothesis may explain the differences in survival between the soils, but there remains the question of why isolate 2C survived better than isolate 044. Part of the explanation appeared to be related to production of dark hyphae. Some evidence was found that isolate 2C produced more dark hyphae in straws buried in Hoff. 8 or Hoff. 8 + W than isolate 044. It is also possible that the isolates are different strains. A completely satisfactory explanation for the difference in the survival of the 2 isolates has not been found. A more detailed investigation of the genetic make up of the 2 isolates and a more exhaustive study of their production of dark hyphae may provide an answer.

I have been unable to find an explanation for the drop in inoculum potential of the infection unit during the first 10 to 15 weeks after burial in some soils. The drop in inoculum potential did not appear to be due to a reduction in virulence of the isolates surviving in the straws. One possibility that could be investigated would be to study the effect of the ratio of thin hyaline hyphae to dark hyphae on inoculum potential. It could be proposed that the initial high inoculum potential obtained with freshly colonized straws is due to the hyaline hyphae. During the first 10 to 15 weeks after burial in wheat-field soil there is a natural reduction in the number of viable hyaline hyphae in the straws. This is accompanied by a reduction in inoculum potential. During this period the production of resistant dark hyphae commences, and the drop in inoculum potential is halted as the dark hyphae take over from the hyaline hyphae. In the autoclaved sand the same sequence of events may take place, with the thick hyaline hyphae acting in the same manner as the dark hyphae.

The effect of the presence of isolate 044 on the survival of native _O. graminis_ (Table 42) remains unexplained. Possible explanations might include: (1) the introduction of isolate 044 into natural soil stimulated the production by soil micro-organisms of a substance(s) which reduced the saprophytic survival of _O. graminis_; (2) isolate 044 produced an auto-toxin(s) which also affected native _O. graminis_; (3) in natural soil isolate 044 produced or stimulated the production of a substance(s) which inhibited the parasitic phase (or infection processes) and the results were thus an artifact unrelated to survival; (4) isolate 044 contained an entity which affected survival and this factor was passed

to the native *O. graminis*.

In conclusion, it has been established that continuous cropping at Ceduna caused a build up of a 'factor' antagonistic to the saprophytic survival of *O. graminis* in artificially colonized straws. The importance of this observation is unknown, but possibly it has no significance in the field. The antagonistic 'factor' may only affect the survival of hyaline hyphae as found in artificially colonized straws. It may have no effect on 'parasitic' hyaline hyphae. By the time the survival phase is reached in the field, hyaline hyphae will have been replaced with dark hyphae. The level of native *O. graminis* in all the soils from Ceduna (Table 38 and page 201) suggests that continuous cropping did not eliminate the fungus. However, there may have been another 'factor' present, which prevented the fungus causing severe take-all in those fields with 4 or more consecutive crops. The take-all 'decline' observed at Ceduna may have been caused by inhibition of infection or prevention of lesion extension (Cox, 1963; Glynne, 1965), rather than reduction in growth during the parasitic phase or elimination during the saprophytic phase (Gerlagh, 1968). This matter needs further investigation.

Even if it can subsequently be shown that continuous cropping affects the survival of native *O. graminis*, this may be of only limited significance in South Australia. The dryness of the soil during the summer in South Australia and in most Australian wheat growing areas would prevent the antagonistic 'factor' affecting survival (Table 53). In those parts of the world where the soil remains moist for most of the year, antagonism to the saprophytic phase could be of considerable importance.

This may be part of the reason why take-all 'decline' is reported so frequently from European countries and so seldom in Australian literature.

A P P E N D I C E S

APPENDIX A

Preparation of culture media

The following culture media were used in this investigation:

Glucose-asparagine medium (Lilly and Barnett, 1951)

Ingredients:			
	glucose	10	g
	asparagine	2	g
	KH_2PO_4	1	g
	$MgSO_4.7H_2O$	0.5	g
	Fe^{+++} (as citrate)	0.2	mg
	Mn^{++} (as sulphate)	0.1	mg
	Zn^{++} (as sulphate)	0.2	mg
	biotin	5	μg
	thiamine	100	μg
	agar	20	g
	distilled water	1000	ml

Method: Melt agar in water, add other constituents, make volume up to 1 litre, dispense and autoclave.

Potato-"Marmite"-dextrose agar (PMDA)

Ingredients:			
	Non-milk type instant mashed potatoes ("Deb" Brand, Rosella Foods Pty. Ltd., Melbourne)	22	g

"Marmite" (concentrated yeast extract, Sanitarium Health Food Co., Aust.)	1	g
agar (Davis)	15	g
distilled water	1000	ml

Method: Autoclave potato in 1 litre of water at 1 atmosphere for 10 minutes, filter through macerated filter paper, melt agar in filtrate, disperse "Marmite" and dextrose in small quantity of water, add dispersion to filtrate, make up volume to 1 litre, dispense and autoclave.

APPENDIX B

Data for Part I,

Section C. (1).

TABLE B1

GRAIN YIELD (g) FROM EACH OF 144 SITES

FOR BLOCK A AT TURRETFIELD

Sites \ Drill rows	1	2	3	4	5	6	7	8	9	10	11	12
1	11	16	21	8	3	8	20	7	15	16	12	9
2	14	17	17	5	10	10	16	11	7	22	15	10
3	14	15	20	8	22	15	14	12	9	14	14	12
4	12	12	15	17	15	9	12	14	5	7	13	13
5	13	18	14	6	11	11	16	14	11	22	12	11
6	19	11	17	11	12	11	9	14	14	10	14	15
7	10	14	6	15	15	14	14	9	11	13	11	12
8	12	20	8	8	10	11	11	9	9	9	9	10
9	13	10	17	10	5	11	13	5	10	13	12	10
10	15	12	4	9	17	16	15	14	6	13	11	10
11	20	15	22	12	12	18	14	19	10	9	12	13
12	9	14	8	8	10	11	15	3	9	10	10	14

TABLE B2

GRAIN YIELD (g) FROM EACH OF 144 SITES

FOR BLOCK B AT TURRETFIELD

Sites	Drill rows 1	2	3	4	5	6	7	8	9	10	11	12
1	4	0	3	1	1	3	3	0	2	0	2	2
2	2	0	2	2	4	3	8	0	1	0	2	4
3	4	5	4	0	2	1	2	0	2	0	1	1
4	3	4	3	1	1	8	0	3	1	0	0	4
5	2	5	4	1	0	4	1	0	0	1	1	3
6	1	3	6	1	2	1	0	4	1	12	1	14
7	2	3	1	3	2	1	1	2	1	4	1	4
8	2	7	1	1	1	1	2	6	2	4	1	2
9	1	4	1	9	1	0	3	2	1	1	1	1
10	2	0	3	1	0	1	2	2	4	2	0	1
11	4	2	1	0	2	1	3	2	1	6	4	1
12	6	1	0	1	4	1	1	1	0	4	0	1

TABLE B3

GRAIN YIELD (g) FROM EACH OF 144 SITES

FOR BLOCK C AT TURRETFIELD

Sites	Drill rows 1	2	3	4	5	6	7	8	9	10	11	12
1	1	6	2	8	0	12	14	3	2	7	0	17
2	4	5	13	6	7	2	23	9	6	3	7	9
3	13	11	6	14	3	22	12	14	9	9	9	17
4	9	11	2	10	11	7	9	19	13	13	16	15
5	1	9	5	7	16	4	6	7	10	14	8	16
6	7	2	2	16	10	6	8	11	7	8	11	6
7	4	0	6	2	7	3	10	11	9	8	9	10
8	1	4	5	3	5	13	9	11	15	7	14	12
9	5	2	7	2	2	5	4	9	15	12	9	13
10	3	3	4	3	2	2	4	17	11	6	15	11
11	3	5	5	2	4	8	5	8	13	13	13	11
12	7	11	4	7	1	3	6	9	11	16	15	11

APPENDIX C

Data for Part I,

Section C.(2).

TABLE C1

GRAIN YIELD (g) FROM EACH OF 256 SITES

FOR BLOCK F AT CEDUNA

Drill rows

Sites	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	0	0	0	0	0	0	0	0	2	2	3	1	1	3	0	2
2	0	0	0	0	0	0	0	1	0	4	8	0	1	4	1	9
3	0	1	0	1	0	0	0	0	0	1	1	1	2	1	2	4
4	0	3	1	0	0	0	0	0	2	0	7	3	4	1	6	4
5	0	0	0	0	0	0	0	0	3	0	3	4	5	3	2	4
6	0	0	0	0	0	0	0	1	1	0	2	7	7	7	2	7
7	0	1	0	0	1	0	0	0	0	0	0	8	3	6	0	4
8	0	0	0	1	1	1	0	0	1	0	2	6	4	5	3	5
9	0	3	1	3	1	6	1	1	6	0	1	7	7	7	6	8
10	0	1	0	3	5	2	2	4	5	0	1	6	9	2	6	3
11	2	2	0	0	3	5	2	4	4	0	4	6	5	7	6	3
12	1	2	2	2	5	7	5	4	4	2	4	3	5	4	6	3
13	0	4	0	7	7	3	6	5	4	4	3	8	7	4	7	7
14	3	2	3	3	2	4	7	7	5	1	6	5	10	2	5	2
15	1	2	1	4	3	4	4	2	2	4	5	4	5	3	3	6
16	4	2	2	5	3	3	7	4	6	1	5	2	8	11	5	5

TABLE C2

PERCENTAGE OF CROWNS INFECTED WITH O. GRAMINIS FROM EACH OF 128 SITES FOR BLOCK F AT CEDUNA

Sites	Drill rows															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	100		100		100		100		100		100		100		100	
2		100		82		100		100		100		100		50		20
3	100		100		75		100		100		50		100		50	
4		89		100		100		100		100		40		100		0
5	100		100		100		100		100		50		25		100	
6		100		100		100		100		100		20		50		67
7	100		100		100		100		100		100		75		100	
8		100		100		100		100		100		0		20		0
9	100		100		100		100		100		100		0		57	
10		100		80		100		86		100		0		83		0
11	75		100		86		100		75		60		50		40	
12		75		100		55		67		100		50		37		0
13	100		100		40		33		25		92		0		33	
14		100		80		100		50		100		25		0		0
15	100		100		80		50		75		0		0		100	
16		100		36		67		67		100		60		21		0

TABLE C3

BIOASSAY OF SOIL CORES (PERCENTAGE OF SEEDLINGS INFECTED PER CORE) TAKEN FROM THE CENTRE OF EACH OF 128 SITES FOR BLOCK F AT CEDUNA

Drill Rows

Sites	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1		100		100		100		100		100		100		100		100
2	100		100		100		100		100		100		100		100	
3		100		43		86		100		100		100		29		14
4	100		100		100		100		100		0		0		71	
5		100		100		100		60		100		0		100		0
6	100		100		100		100		100		100		43		67	
7		100		100		100		100		100		100		29		100
8	100		100		100		100		100		100		83		43	
9		100		100		17		100		100		100		29		86
10	100		100		43		100		57		0		100		43	
11		100		100		100		86		100		100		100		0
12	100		100		100		43		100		100		33		57	
13		100		86		100		0		100		67		22		29
14	86		100		100		100		50		0		0		0	
15		71		14		100		0		71		14		43		33
16	100		100		72		29		100		0		43		0	

TABLE C4

BIOASSAY OF SOIL CORES (PERCENTAGE OF INFECTED ROOTS PER CORE) TAKEN FROM THE CENTRE OF EACH OF 128 SITES FOR BLOCK F AT CEDUNA

Drill rows

Sites	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1		97		95		58		88		91		100		77		100
2	91		79		83		69		72		88		92		97	
3		79		10		27		68		100		100		8		3
4	100		81		87		76		96		0		0		33	
5		100		66		86		53		93		0		61		0
6	93		52		70		100		46		77		11		33	
7		100		73		90		83		71		54		7		91
8	90		84		90		70		97		100		38		13	
9		94		87		8		100		97		38		7		36
10	86		77		10		88		30		0		82		11	
11		68		79		67		42		100		38		38		0
12	71		77		93		13		71		65		17		37	
13		78		41		91		0		70		32		17		7
14	79		59		95		100		25		0		0		0	
15		30		11		67		0		55		3		27		12
16	97		74		44		7		93		0		10		0	

APPENDIX D

Data for Part I,

Section D. (2).

TABLE D1

PERCENTAGE OF INFECTED PLANTS, TOTAL TOP DRY WEIGHT, AND AVERAGE TOP DRY WEIGHT PER MACRO-SITE FROM BLOCK G AT CEDUNA, 1970. TOTAL GRAIN YIELD FOR THE 1969 CROP FROM THE SAME BLOCK ALSO SHOWN.

Macro-row	Macro-site	Percentage of plants infected	Total top dry weight (g)	Average top dry weight (g)	Grain yield previous year (g)
1	1	85	3.5	.18	5
	2	100	3.8	.24	1
	3	98	8.0	.19	3
	4	100	4.9	.14	1
	5	100	2.9	.18	1
	6	82	5.5	.24	0
	7	94	4.0	.25	1
	8	100	5.4	.23	0
	9	100	2.1	.21	7
	10	96	5.7	.21	0
	11	100	8.1	.27	1
	12	97	6.9	.18	3
	13	88	5.8	.24	8
	14	46	6.3	.29	6
	15	29	7.8	.25	12
	16	75	8.2	.26	24
	17	40	6.8	.27	18
	18	57	0.8	.11	19
	19	59	9.3	.32	24
	20	36	7.2	.22	16

TABLE D1 (cont.)

Macro-row	Macro-site	Percentage of plants infected	Total top dry weight (g)	Average top dry weight (g)	Grain yield previous year (g)
2	1	96	7.2	.27	6
	2	100	3.9	.30	1
	3	100	7.2	.14	4
	4	97	6.0	.15	8
	5	100	6.1	.24	3
	6	100	7.1	.29	5
	7	100	3.4	.16	0
	8	100	6.6	.22	0
	9	100	4.4	.22	2
	10	100	7.0	.33	1
	11	100	7.6	.29	3
	12	100	6.0	.14	2
	13	100	7.1	.25	7
	14	84	4.6	.24	6
	15	82	7.5	.27	12
	16	56	6.8	.27	15
	17	58	8.7	.28	17
	18	29	3.6	.51	16
	19	58	6.4	.25	16
	20	50	11.4	.30	28
3	1	97	6.3	.18	13
	2	100	2.7	.23	1
	3	100	5.6	.10	6
	4	100	4.9	.15	0
	5	100	4.8	.25	1
	6	100	6.1	.23	3
	7	100	8.2	.32	2
	8	100	6.8	.23	1

TABLE D1 (cont.)

Macro-row	Macro-site	Percentage of plants infected	Total top dry weight (g)	Average top dry weight (g)	Grain yield previous year (g)
3	9	92	2.7	.23	1
	10	95	4.9	.26	1
	11	94	8.0	.24	3
	12	100	3.2	.12	5
	13	97	5.6	.19	10
	14	74	7.0	.26	5
	15	58	7.7	.23	10
	16	63	6.2	.26	13
	17	76	6.1	.25	16
	18	29	1.8	.26	13
	19	93	7.6	.28	14
	20	72	7.0	.39	16
4	1	100	5.7	.25	1
	2	100	4.4	.31	2
	3	100	2.6	.11	0
	4	100	2.6	.11	0
	5	95	5.5	.14	1
	6	100	4.2	.35	2
	7	94	4.8	.28	1
	8	100	8.2	.22	0
	9	93	7.3	.25	1
	10	96	6.2	.28	0
	11	100	5.1	.22	5
	12	100	6.9	.18	1
	13	80	4.9	.20	13
	14	71	6.7	.32	12
	15	78	2.4	.27	7
	16	63	10.0	.29	10

TABLE D1 (cont.)

Macro-row	Macro-site	Percentage of plants infected	Total top dry weight (g)	Average top dry weight (g)	Grain yield previous year (g)
4	17	77	5.7	.26	11
	18	56	2.4	.27	14
	19	96	4.7	.19	17
	20	60	6.2	.25	13
5	1	100	6.0	.24	0
	2	100	2.9	.22	0
	3	100	4.0	.15	0
	4	100	4.8	.11	4
	5	100	1.9	.11	0
	6	100	3.2	.16	1
	7	100	6.0	.27	3
	8	100	6.0	.25	1
	9	80	2.5	.50	6
	10	94	7.2	.23	2
	11	91	5.8	.28	3
	12	100	5.6	.22	4
	13	88	6.9	.27	11
	14	65	5.1	.22	21
	15	69	8.5	.29	17
	16	73	8.2	.32	9
	17	80	5.9	.24	22
	18	81	6.2	.24	17
	19	95	4.2	.19	22
	20	72	5.0	.20	19
6	1	100	3.6	.21	0
	2	100	2.5	.36	0
	3	100	6.6	.18	0

TABLE D1 (cont.)

Macro-row	Macro-site	Percentage of plants infected	Total top dry weight (g)	Average top dry weight (g)	Grain yield previous year (g)
6	4	100	6.6	.13	0
	5	100	3.7	.14	0
	6	100	5.1	.17	0
	7	100	7.1	.24	3
	8	100	4.5	.24	1
	9	100	3.2	.40	9
	10	95	7.0	.33	5
	11	100	9.1	.28	9
	12	98	6.4	.16	5
	13	93	6.5	.24	17
	14	100	4.4	.21	10
	15	78	4.9	.21	8
	16	84	8.3	.26	19
	17	88	6.0	.23	11
	18	88	2.7	.17	14
	19	100	5.9	.23	13
	20	95	5.4	.24	19
7	1	100	4.2	.17	0
	2	100	2.9	.18	0
	3	100	7.3	.14	0
	4	100	8.2	.13	0
	5	100	1.8	.13	0
	6	100	5.8	.22	3
	7	96	5.9	.23	1
	8	100	5.7	.25	0
	9	80	1.5	.15	2
	10	95	9.1	.41	2
	11	100	5.9	.33	8

TABLE D1 (cont.)

Macro-row	Macro-site	Percentage of plants infected	Total top dry weight (g)	Average top dry weight (g)	Grain yield previous year (g)
7	12	100	8.5	.19	5
	13	100	2.7	.21	18
	14	86	9.2	.33	11
	15	100	6.8	.23	5
	16	95	7.7	.35	7
	17	97	9.1	.28	6
	18	100	4.4	.15	10
	19	100	4.9	.20	10
	20	97	6.4	.19	14
8	1	100	3.5	.12	2
	2	100	2.3	.15	1
	3	100	3.3	.11	2
	4	98	7.1	.15	0
	5	100	1.9	.10	0
	6	96	4.4	.18	0
	7	100	5.0	.18	0
	8	96	5.5	.20	0
	9	100	2.3	.19	3
	10	100	8.6	.28	1
	11	100	4.5	.15	6
	12	100	4.2	.14	6
	13	96	5.5	.20	14
	14	90	4.7	.16	6
	15	92	6.6	.28	7
	16	100	6.2	.26	12
	17	91	7.8	.34	10
	18	100	0.5	.25	16

TABLE D1 (cont.)

Macro-row	Macro-site	Percentage of plants infected	Total top dry weight (g)	Average top dry weight (g)	Grain yield previous year (g)
8	19	76	8.1	.32	17
	20	64	6.2	.28	24
9	1	100	2.8	.14	1
	2	100	4.3	.31	3
	3	100	5.0	.16	2
	4	100	8.0	.13	2
	5	100	4.1	.18	0
	6	93	7.1	.25	2
	7	100	5.1	.20	0
	8	100	5.1	.16	0
	9	100	3.2	.25	0
	10	96	6.3	.29	1
	11	100	5.7	.16	2
	12	100	5.8	.16	4
	13	92	5.9	.25	8
	14	75	3.6	.18	3
	15	100	5.0	.25	2
	16	100	6.8	.30	0
	17	96	7.4	.34	13
	18	100	2.5	.36	13
	19	95	5.9	.31	9
	20	82	6.3	.23	15
10	1	100	4.2	.17	1
	2	100	2.0	.15	1
	3	100	4.7	.13	1
	4	94	7.8	.11	1
	5	96	2.1	.10	1
	6	100	7.4	.23	5

TABLE D1 (cont.)

Macro-row	Macro-site	Percentage of plants infected	Total top dry weight (g)	Average top dry weight (g)	Grain yield previous year (g)
10	7	100	5.9	.25	4
	8	100	3.7	.22	1
	9	100	1.7	.11	2
	10	88	7.5	.31	2
	11	100	8.6	.28	10
	12	100	5.4	.16	10
	13	100	4.8	.17	11
	14	77	3.7	.17	1
	15	77	5.5	.21	8
	16	96	4.9	.22	7
	17	87	4.7	.16	4
	18	91	2.9	.13	7
	19	96	6.6	.29	8
	20	60	8.2	.41	7
11	1	100	4.8	.19	7
	2	100	2.7	.27	2
	3	100	4.7	.11	2
	4	96	7.6	.14	3
	5	100	2.4	.10	0
	6	96	6.9	.28	4
	7	100	6.5	.24	5
	8	100	4.0	.09	1
	9	100	1.6	.23	1
	10	90	9.4	.32	0
	11	100	5.3	.19	14
	12	97	4.8	.15	14
	13	100	5.2	.17	11
	14	95	4.4	.23	6

TABLE D1 (cont.)

Macro-row	Macro-site	Percentage of plants infected	Total top dry weight (g)	Average top dry weight (g)	Grain yield previous year (g)
11	15	100	7.0	.39	3
	16	100	7.1	.22	4
	17	100	3.1	.17	5
	18	77	1.6	.18	14
	19	96	3.9	.16	13
	20	76	4.2	.17	13
12	1	100	5.6	.23	2
	2	100	3.2	.23	0
	3	100	3.0	.08	1
	4	100	7.4	.14	0
	5	100	1.8	.13	0
	6	100	6.6	.19	3
	7	100	7.5	.31	6
	8	100	7.9	.28	0
	9	100	2.1	.19	1
	10	96	8.4	.31	0
	11	100	8.9	.29	9
	12	100	3.9	.13	7
	13	91	5.3	.23	11
	14	95	3.4	.17	6
	15	100	9.3	.25	0
	16	100	6.7	.26	0
	17	97	9.9	.28	3
	18	100	2.6	.29	14
	19	95	3.5	.18	11
	20	100	2.4	.14	7

APPENDIX E

Data for Part II,

Section A. (1).

TABLE E1

GRAIN YIELD (g) FROM EACH OF 96 MACRO-SITES FOR BLOCK E AT TURRETFIELD, 1969

	1	2	3	4	5	6	7	8	9	10	11	12
1	23	41	28	28	31	39	28	63	48	39	55	36
2	10	25	42	43	45	15	15	20	35	22	21	22
3	9	13	42	33	21	32	22	39	29	39	30	25
4	18	16	24	5	17	24	17	17	32	39	40	16
5	19	16	32	20	27	14	15	19	21	41	64	26
6	12	11	29	21	40	23	27	15	23	53	45	41
7	15	17	44	57	37	44	10	23	129	42	37	56
8	20	38	57	48	33	25	25	23	54	65	55	45

TABLE E2

THE CONTRIBUTION OF EACH ELEMENT TO THE CHI-SQUARE FOR THE FREQUENCY DISTRIBUTION OF CROWNS CONTAINING VIABLE O. GRAMINIS PER SITE FOR 4 TIMES OF REMOVAL FROM BLOCK E AT TURRETFIELD, 1970

Date	Time from 1969 harvest (wk)	Survival Category					
		0-16% 1	17-33% 2	34-50% 3	51-67% 4	68-83% 5	84-100% 6
Jan. 28	6	19.257	0.087	0.697	7.072	0.733	12.725
Mar. 19	13	13.656	2.748	2.609	3.455	16.218	0.194
May 28	23	0.120	9.224	0.382	1.090	2.022	5.013
Aug. 20	35	66.939	2.748	9.397	12.108	12.031	5.013
Chi-square expected frequency*		33	17	15	14	12	5

The value of chi-square is 205.53*** with 15 degrees of freedom.
* The actual expected frequency for individual times varies slightly due to missing sites.

APPENDIX F

Data for Part II,

Section A. (2).

TABLE F1

GRAIN YIELD (g) FROM EACH OF 96 MACRO-SITES FOR BLOCK H AT CEDUNA, 1969.

	1	2	3	4	5	6	7	8	9	10	11	12
1	2	3	3	12	9	9	13	7	11	18	29	6
2	0	3	3	13	6	8	6	4	12	13	19	9
3	1	6	9	10	3	4	3	9	14	11	20	4
4	0	3	1	14	17	4	17	13	14	13	10	13
5	0	1	4	6	12	17	22	8	9	16	12	20
6	0	2	3	8	16	17	28	13	9	4	20	11
7	0	1	3	11	20	15	24	12	26	9	11	16
8	0	0	3	8	17	23	21	19	24	18	3	19

TABLE F2

THE CONTRIBUTION OF EACH ELEMENT TO THE CHI-SQUARE FOR THE FREQUENCY DISTRIBUTION OF CROWNS CONTAINING VIABLE _O. GRAMINIS_ PER SITE FOR 5 TIMES OF REMOVAL FROM BLOCK H AT CEDUNA, 1970

Date	Time from 1969 harvest (wk)	Survival category					
		0-16% 1	17-33% 2	34-50% 3	51-67% 4	68-83% 5	84-100% 6
Feb. 3	10	1.270	0.360	0.031	2.667	3.721	6.400
Apr. 14	20	1.607	0.072	0.231	0.171	0.707	0.162
June 23	30	0.003	0.873	1.472	0.639	0.145	0.484
Sep. 1	40	1.234	0.059	0.857	3.723	0.780	4.892
Nov. 10	50	1.749	0.023	0.120	0.811	0.306	1.906
Chi-square expected frequency*		15	6	6	9	12	45

The value of chi-square is 37.48* with 20 degrees of freedom.

* The actual expected frequency for individual times varies slightly due to missing sites.

APPENDIX G

Data for Part II,

Section A. (3).

TABLE G1

GRAIN YIELD (g) FROM EACH OF 96 MACRO-SITES FOR BLOCK K AT CEDUNA, 1969

	1	2	3	4	5	6	7	8	9	10	11	12
1	0	2	4	18	11	16	16	16	27	21	7	22
2	0	3	2	5	19	17	16	21	19	28	19	19
3	1	4	1	4	7	17	29	18	34	12	20	22
4	1	5	2	2	10	12	15	19	32	9	30	14
5	2	3	2	2	15	15	15	16	23	20	24	24
6	2	4	1	3	12	12	15	9	23	11	30	12
7	5	3	1	12	16	10	20	9	17	12	24	19
8	1	4	0	3	10	7	20	20	23	16	17	17

TABLE G2

THE CONTRIBUTION OF EACH ELEMENT TO THE CHI-SQUARE FOR THE FREQUENCY DISTRIBUTION OF THE PERCENTAGE OF INFECTED ROOTS ON WHEAT SEEDLINGS GROWN IN CORES REMOVED AT INTERVALS FROM BLOCK K AT CEDUNA, 1970

Date	Time from 1969 harvest (wk)	Incidence category			
		0-25%	26-50%	51-75%	76-100%
Feb. 3	10	2.632	0.083	6.070	0.092
Mar. 9	15	4.361	0.750	1.484	5.049
Apr. 13	20	0.005	1.333	0.518	0.092
May 19	25	0.144	0.000	0.001	0.247
June 24	30	1.337	3.000	2.318	2.159
July 29	35	0.050	0.000	2.318	2.159
Sep. 1	40	1.766	0.000	0.277	1.570
Oct. 5	45	2.688	0.038	0.001	4.953
Nov. 10	50	6.613	0.038	0.601	7.890
Chi-square expected frequency		41	12	16	26

The value of chi-square is 62.73*** with 24 degrees of freedom.

TABLE G3

THE FREQUENCY DISTRIBUTION OF THE PERCENTAGE OF INFECTED ROOTS ON WHEAT SEEDLINGS GROWN IN CORES REMOVED AT INTERVALS FROM BLOCK K AT CEDUNA, 1970

Date	Time from 1969 harvest (wk)	Incidence Category							
		0-12% 1	13-25% 2	26-37% 3	38-50% 4	51-62% 5	63-75% 6	76-87% 7	88-100% 8
Feb. 3	10	29	3	2	7	13	13	13	15
Mar. 9	15	23	5	3	5	8	13	9	29
Apr. 13	20	32	9	4	4	12	7	12	16
May 19	25	35	4	6	6	10	6	9	20
June 24	30	33	1	3	14	3	7	18	16
July 29	35	34	6	5	7	6	4	13	21
Sep. 1	40	43	7	8	4	8	6	7	13
Oct. 5	45	48	4	2	11	11	9	7	8
Nov. 10	50	51	7	6	7	8	6	5	6

FIGURE G4

'INCIDENCE-SURVIVAL' MAP OF O. GRAMINIS AT CEDUNA,

BLOCK K, 1970

(a) March 9

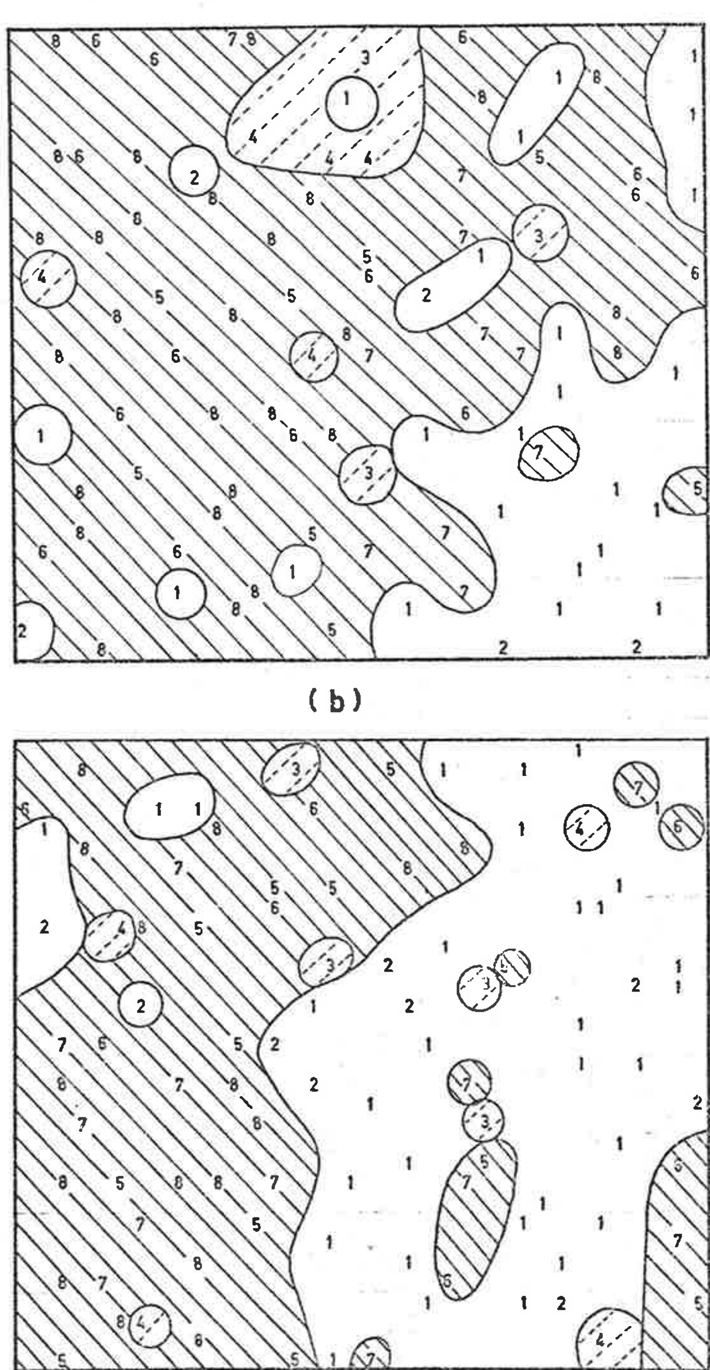
(b) April 13

'Incidence-survival' category (percentage of roots infected per core)

High incidence	(7 = 76 to 87%, 8 = 88 to 100%)
	(5 = 51 to 62%, 6 = 63 to 75%)
Intermediate incidence	(3 = 26 to 37%, 4 = 38 to 50%)
Low incidence	(1 = 0 to 12%, 2 = 13 to 25%)

FIG. G4

(a)



'Incidence- survival' category

High   Intermediate   Low

Scale ⟼ 30 cm

FIGURE G5

'INCIDENCE-SURVIVAL' MAP OF *O. GRAMINIS* AT CEDUNA,

BLOCK K, 1970

(a) June 24

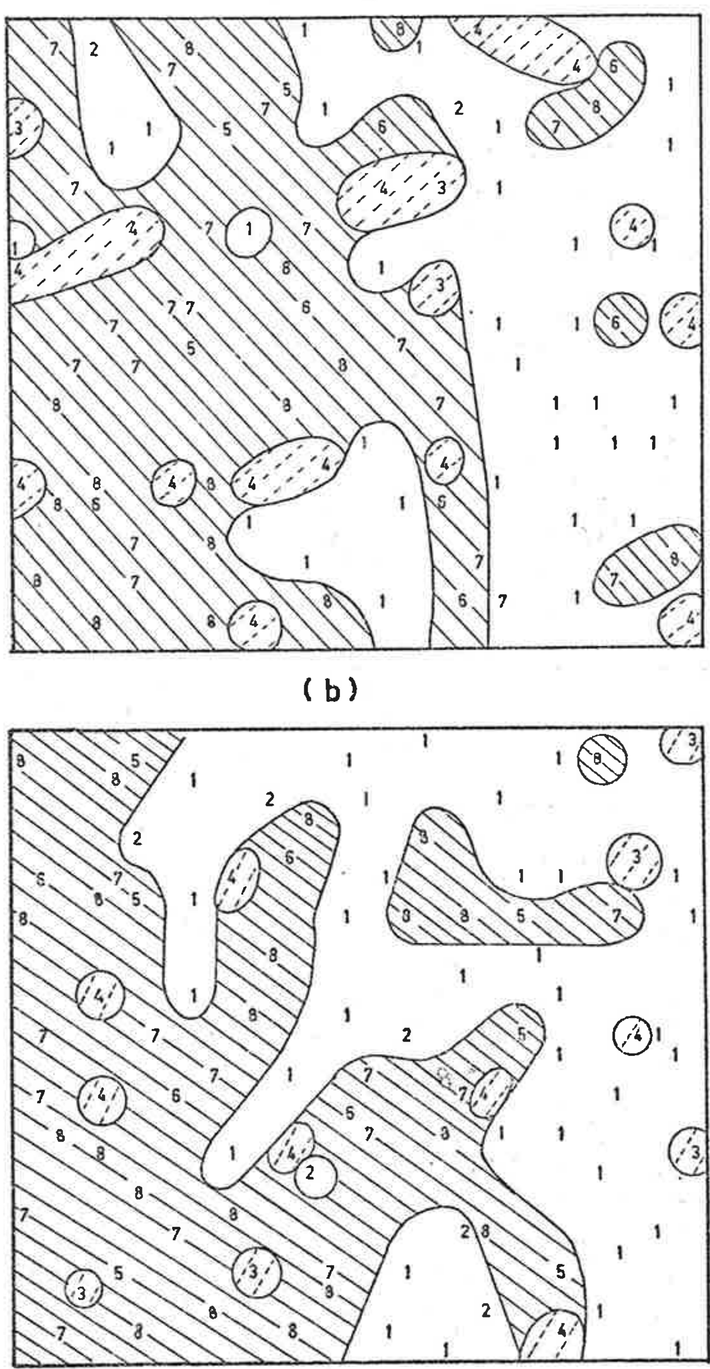
(b) July 29

' Incidence-survival' category (percentage of roots infected per core)

High incidence	(7 = 76 to 87%, 8 = 88 to 100%)
	(5 = 51 to 62%, 6 = 63 to 75%)
Intermediate incidence	(3 = 26 to 37%, 4 = 38 to 50%)
Low incidence	(1 = 0 to 12%, 2 = 13 to 25%)

FIG. G5

(a)



'Incidence- survival' category

High Intermediate Low

Scale ⊢ 30 cm

FIGURE G6

'INCIDENCE-SURVIVAL' MAP OF *O. GRAMINIS* AT CEDUNA,

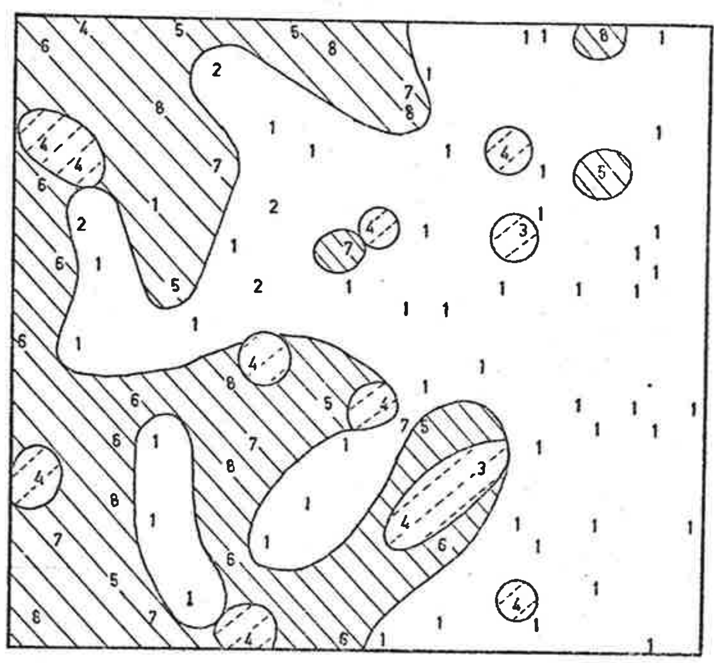
BLOCK K, 1970

October 5

'Incidence-survival' category (percentage of roots infected per core)

High incidence	(7 = 76 to 87%, 8 = 88 to 100%)
	(5 = 51 to 62%, 6 = 63 to 75%)
Intermediate incidence	(3 = 26 to 37%, 4 = 38 to 50%)
Low incidence	(1 = 0 to 12%, 2 = 13 to 25%)

FIG. G6



'Incidence- survival' category

High   Intermediate   Low

Scale ⊢⊣ 30 cm

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