Infection by *Ophiobolus graminis* of some perennial weed grasses.

by

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DECLARATION

I hereby declare that the following thesis is based on research done by me, that the thesis is my own composition and that it has not previously been presented for a higher degree.

The research was carried out in the Department of Agriculture of the University of Oxford under the direction of Dr. R.L. Lucas and Professor J.A. Macdonald, University of St. Andrews.

CERTIFICATE

We certify that Caroline M. Whiteman, B.Sc. has spent seven terms of research work under our supervision, and that she has fulfilled the conditions of Ordinance 51 (St. Andrews), and that she is qualified to submit the accompanying thesis in application for the Degree of Master of Science.

CONTENTS

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ABSTRACT

The appearance of serious outbreaks of take-all in wheat and barley crops can often be correlated with the presence of infected perennial grasses, such as Agropyron repens, Agrostis spp., and Holcus lanatus, growing amongst the crop. Holcus mollis is a weed prevalent amongst cereals growing on light, acid soils. Although this grass has been mentioned in the literature as a possible carrier of the disease, little experimental work appears to have been done to determine the susceptibility of the grass to the take-all fungus, Ophiobolus graminis Sacc.. An investigation was undertaken in this connection. Vegetative segments of H. mollis were found to be only slightly less susceptible than vegetative segments of Agropyron repens, which are heavily attacked by the fungus, but more susceptible than vegetative segments of Agrostis stolonifera.

A number of methods were used for estimating the amount of disease on these grasses. The symptoms of the disease, a blackening of the stele of the roots and ectotrophic runner hyphae, are less easy to discriminate on grass than on wheat roots, so an attempt was made to find a method particularly suitable for use with grasses. A method used extensively throughout this work was one of direct observation. Grass plants, seedlings or vegetative fragments, were viewed under water against a white background to determine the presence of stelar lesions. This was the most suitable method for differentiating between a discoloured, diseased root and one discoloured by some other cause. The presence of runner hyphae, on

what appeared to be healthy roots, could be seen when the roots were viewed under a binocular dissecting microscope under the same conditions as for stelar lesions. The percentage infection was calculated from the total number of roots and the total number infected with one or more stelar lesions. The runner hyphae were not used in this calculation but their number was noted. When present on Holcus spp. and A. stolonifera seedlings but without any stelar infection they were found to cause stelar lesions on subsequent wheat seedlings. These hyphae must, therefore, be considered a potential hazard to cereal crops.

There were seldom any above ground signs of attack by the fungus on the grasses; no withering of the leaves nor plate mycelium, which are generally associated with severe attacks of the fungus on wheat seedlings. However, A. repens did show some browning and withering of the leaves when the roots were heavily invaded.

Mature vegetative fragments of A. repens and A. stolonifera were found to be more susceptible to the disease than those nearer the terminal growing point of a rhizome or stolon. Seedlings of A. repens and Holcus spp. were found to vary in their susceptibility to the fungus, and this did not appear to be affected by the age of the seedlings, over the duration of time of the experiments.

Six mycelial isolates of Ophiobolus graminis were obtained from different parts of the country. Five of the isolates were

found to be highly pathogenic on wheat, but the sixth was only moderately so. This sixth isolate (3S) survived as chaffed inoculum in unsterile soil in a heated greenhouse for only four months, whereas the other five were still present, although at a low level, after eight months in the same soil under identical conditions. This isolate, was found on two occasions to cause more severe damage to A. repens vegetative segments than to wheat seedlings, a result which was contrary to those obtained with the other isolates.

Throughout this work an effort was made to use grass material from the same source in order to avoid variation wher-ever possible. Holcus mollis exists as a number of different cytotypes, tetraploid (4n = 28), pentaploid (5n = 35), hexaploid (6n = 42) and heptaploid (7n = 49). Triploid (3n = 21) and aneuploid plants are also frequently found. Rhizome material was collected from different habitats and localities throughout the British Isles. In order to determine the cytotype, chromosome counts of the rhizome material had to be made. Suitable lengths of rhizome were placed in shallow dishes of water, and when adventitious roots, which grew out from the nodes, were of sufficient length, the root tips were stained with aceto-orcein, squashed and the number of chromosomes counted. Seven clones of vegetative H. mollis were grown, three tetraploid, three pentaploid and one aneuploid clone. A comparison made of the vegetative characters of these clones showed that they

varied considerably. These differences could not have been entirely due to the different environmental conditions of the habitats from which they were collected, as they did not alter after a year in identical conditions in a heated greenhouse.

The clones were found to vary in their susceptibility to the six isolates of O. graminis. Vegetative fragments of the clones, all as similar as possible, were inoculated with each of the isolates. The results of the experiment were interesting in several respects. Three of the clones were found statistically to be significantly less susceptible to the isolates than the other three. (The aneuploid material was not included in the statistical analysis of the results owing to the limited amount available for inoculation). This is interesting in view of the fact that no one variety of wheat has been found to be more resistant to take-all than any other. The more resistant clones were not all of one cytotype, but two were pentaploid and one tetraploid. It appeared that the aneuploid was highly susceptible to the fungus, but few definite conclusions can be drawn from the results because of the small amount of material.

There was considerable variation of the results of some experiments, which could have been caused by differences in the O. graminis isolates as well as in the grass material. These isolates were mycelial and therefore not as pure as single ascospore isolates, the mycelium having possibly grown from a number of ascospores. This may have accounted for some of the variation in the cultural

characteristics of the isolates, and their varying pathogenicity in different experiments, as it has been found that mycelia grown from different ascospores from the same ascus, vary in their pathogenicity on wheat seedlings.

INTRODUCTION

Ophiobolus graminis Sacc., the fungus causing take-all disease of wheat and barley, has a wide host range among the Gramineae, (Brooks 1965, Garrett 1941, Walker 1945). Some perennial weed grasses, in particular Agropyron repens, Agrostis spp. and Holcus lanatus, have long been known to be carriers of O. graminis, and a high incidence of take-all in a wheat or barley crop after a break can often be correlated with infestation by one of these grass weeds or by Holcus mollis, (Garrett and Buddin 1947, Wehrle and Ogilvie 1955). The present investigation was undertaken in order to test, in detail, the effective perpetuation of O. graminis on these grasses, and also to examine further their susceptibility to the fungus. A number of methods for estimating the amount and severity of the disease were used in an endeavour to find one suitable for use with grasses.

A number of workers have given evidence of the susceptibility of these grasses. Buddin and Garrett (1944) suggested that effective perpetuation of the fungus on the underground parts of such grasses was due not so much to their specific susceptibility to infection as to their perennial habit and vigorous growth. However, Walker (1945) found that the infection of A. repens rhizomes was severe, while that of A. stolonifera stolons was less so. It was rare for lesions to appear on more than a few roots of A. stolonifera, but many of these roots were covered with runner hyphae. Robinson (1963) came to similar conclusions after experiments with

these grasses; Gottlieb et. al. (1958) and Sallans (1965) found Agropyron spp. very susceptible, and the former workers concluded that Agrostis spp. were resistant. Padwick and Henry (1933) found several Agropyron spp. to be susceptible to take-all but they found only A. repens infected in the field. Padwick (1935) found that Agropyron spp. encouraged the multiplication and survival of inoculum of the fungus in both sterilized and unsterilized soil.

Robinson (1963) found that seedlings of A. repens and A. stolonifera were more resistant to attack by O. graminis than regenerating vegetative segments of these grasses. This was further investigated using material of different ages.

(Although in spite of the fact that) there are several references in the literature to Holcus mollis being a possible carrier of O. graminis, (Buddin and Garrett, 1944, Garrett and Buddin, 1947, Wehrle and Ogilvie, 1955), there appears to be little experimental evidence to support this view. Holcus lanatus, the other species native to this country, has been shown by Brooks (1965) and Walker (1945) to be susceptible to attack by the fungus. H. mollis is often found as a weed among cereal crops in areas where the soil is acid and light, for example the lower Greensand around Woburn, (Mann and Barnes, 1947, Ovington and Scurfield, 1956). Fenton (1948) observed that this grass is tolerant of a wide range of soils, but suppressed on soils of high fertility by quicker growing species. Its capacity for

intensive rhizome and root production makes it a serious pest. Hubbard (1954) pointed out that a volume of infested sandy soil having area one square foot and depth six to eight inches may contain up to 110 feet of its rhizomes, while the weight of roots and rhizomes in such cases has been estimated at over seven and a half tons per acre. Grainger (1935) found that the length of the total roots within one square foot of ground was 770 feet.

Several workers have studied the characteristics of a number of isolates of O. graminis. Padwick (1936) found cultural differences between a number of isolates of O. graminis, but White (1942), using eight single-spore isolates from one ascus, found that their cultural characteristics remained constant over a three year period during which many sub-cultures were made. Bussmann (1936) and Russell (1934) reported that isolates of O. graminis differed widely in their pathogenicity on wheat. Turner (1952) stated that the results of pathogenicity tests on a number of different grass species showed considerable variation in pathogenicity between different isolates of the same variety of O. graminis.

Isolations of this fungus were made in different years from cereals infected with take-all growing in the Oxford area, and these were supplemented with an isolate from Nottingham University. Tests were carried out on six such isolates in order to determine any

significant differences between them. In particular, the pathogenicity of the isolates on different species of perennial grasses was investigated.

A study was carried out to determine the possible variation between different cytotypes of H. mollis and their susceptibility to O. graminis was investigated. Ovington and Scurfield (1956) mention a number of different varieties of H. mollis, but the taxonomic status of these varieties is doubtful. This grass is variable as individual characteristics show wide differences, and Jones (1958) reports that it exists as a number of cytotypes, some of which he considers to be of hybrid origin. The cytotypes are tetraploid (4n = 28), pentaploid (5n = 35), hexaploid (6n = 42) and heptaploid (7n = 49). Triploid and aneuploid plants are also frequently found. Jones found the pentaploid to be the most vigorous and most dominant cytotype in the British Isles; these traits being, in his view, unique for an uneven polyploid of low fertility status. H. mollis is usually cross-pollinated and all the cytotypes, with the possible exception of the tetraploid, are only moderately fertile. Ovington and Scurfield (1956) found that flowering is considerably reduced in plants growing in moderate shade compared with those in open situations. Carroll and Jones (1962) found no very obvious character which could be used to distinguish between the cytotypes. Their work showed no evidence that size increased with chromosome

number, the pentaploid being larger than the hexaploid, which in turn was larger than the tetraploid.

An examination of the invasion of H. mollis by O. graminis necessitated the growing of clonal material of the different cytotypes, to eliminate as far as possible biological variations. It was postulated that because of the differences between the cytotypes, mentioned above, they might show considerable variation in their susceptibility to the take-all fungus. This theory was investigated by an examination of clonal material of the tetraploid and pentaploid cytotypes, and of one aneuploid clone, using material collected from various habitats and localities in the British Isles.

MATERIALS AND METHODS

PART 1. MATERIALS.

A. Plant material.

Whenever possible, all the vegetative grass material used in the experiments described in this work was originally collected from the same source. If not, this is stated in the text. This eliminated a certain amount of variation in the experimental material. After collection, vegetative material was planted in soil in seed boxes one foot square by six inches deep, in a heated greenhouse, and periodically transplanted. Agrostis stolonifera, for example, needed to be transplanted at frequent intervals owing to its rapidity of spread.

Vegetative grass material was collected from the following places:-

Agropyron repens	Department of Agriculture, Oxford.
	An allotment in Oxford.
Agrostis stolonifera	Harpers Field, Oxford University Field Station, at Wytham.
Holcus mollis	Shotover, near Oxford. This material was used in the work described in Section 1. Clonal material was used for the work described in Section 4.

Seed was obtained from the following places:-

Agropyron repens	An allotment in Oxford.
	Agricultural Research Council, Weed Research Organization, Begbroke Hill.
Agrostis stolonifera	Dunns Farm Seeds Ltd., Salisbury.
Holcus mollis	Shotover, near Oxford.
	Botany Department, Newcastle University.
Holcus lanatus	Shotover, near Oxford.
	Several places described in Section 4.

All the seed was stored in waxed paper or polythene bags in a heated greenhouse. The wheat seed used in all the experiments described in this work was the variety Cappelle.

B. *Ophiobolus graminis* isolates.

In the work described in Section 1. an isolate obtained from the Botany Department at Cambridge University was used as inoculum. Six isolates were used in Sections 2., 3. and 4.. The following table gives the details of these isolates.

Isolate	Host plant from which obtained	Locality	Date of isolation
1W	Wheat	Oxford University Field Station, Wytham.	August, 1965
2B	Barley	"	" "
3S	Wheat	Lockinge Estate near Oxford	September, 1964

Table Continued,

Isolate	Host plant from which obtained	Locality	Date of isolation
4N	?	Nottingham University	1956
5Bg	Barley	Agricultural Research Council, Weed Research Organization, Begbroke Hill.	September, 1965
6Sh	Wheat	Shrivenham, Wilts.	September, 1965

C. Soil mixture.

The soil used in all the experiments described was a mixture of one part of loam soil to three parts of fine sand by volume, unless otherwise stated in the text.

PART 2. METHODS.

A. Method used for isolating Ophiobolus graminis from infected wheat or barley roots. (Davies, 1935).

Suitable lengths of infected roots were washed overnight in tap water, surface sterilized in a one per cent silver nitrate solution for half a minute, and washed in a sterile one per cent sodium chloride solution to precipitate the surface sterilant. The roots were then placed on Petri dishes of potato dextrose agar (see Appendix A) acidified with one drop of lactic acid per Petri dish to inhibit bacterial growth. After two or three days mycelium of O. graminis grew out from the cut ends of the roots. Small amounts

of the mycelium were removed by means of a sterile glass needle under a binocular dissecting microscope, and placed on potato dextrose agar in Petri dishes. After further growth, test tubes of sterile potato dextrose agar were inoculated with the mycelium and plugged with cotton wool, incubated at 23°C until the surface of the medium had been colonized by the mycelium and then stored in the dark at 15°C.

B. Preparation of Ophiobolus graminis inoculum.

Two different inoculum media were used in this work, wheat chaff and agar disks.

a) Wheat chaff inoculum. This was prepared by sieving wheat chaff to eliminate any long straw and steaming it in a solution of two per cent glucose and one per cent sodium nitrate in distilled water for five hours. This soaking ensured that the O. graminis had sufficient nitrogen and carbohydrate requirements for complete colonization of the chaff. The following day the liquid was poured off and wide-necked milk bottles three quarters filled with the soaked chaff. These were plugged with cotton wool and autoclaved for two hours at 20lb. per square inch pressure on two consecutive days. Each bottle was then inoculated under aseptic conditions with an agar disk of O. graminis, fifteen millimetres in diameter, cut from the edge of a growing colony. The bottles were laid on their sides to give the fungus the maximum possible surface area to colonize and left at 23°C. After two weeks each bottle was shaken to promote even colonization of the chaff. Seed boxes, 9" x 14", were filled to a depth of three

field ; pasteurised or g.h. ?

centimetres with soil. A half centimetre layer of chaff was laid on this and covered with a half centimetre layer of fine sand. The plant material, seeds or vegetative material, to be inoculated was laid on this sand and the seed boxes filled with soil.

b) Agar disk inoculum. Sterile plastic Petri dishes were filled to a depth of half a centimetre with potato dextrose agar and when this had set, each dish was inoculated with an agar disk of O. graminis, four millimetres in diameter. The dishes were placed in a 23°C. incubator. One to two weeks later disks, eleven millimetres in diameter, were cut out from the edge of the still growing colony with a cork borer. These disks were placed in rows in a 9" x 14" seed box filled to within one centimetre of the top with soil. The plant material to be inoculated was placed on the disks, one seed or vegetative segment on each disk, and the boxes filled with soil.

C. Methods for estimating the disease on grasses.

Several methods were used for estimating the disease on grasses caused by the take-all fungus, O. graminis. Those which were used once only are explained in the text.

a) Method 1. Visual Method.

This method was used in combination with Method 2. Inoculated plants were dug up individually, taking care to extract as much of the root as possible. They were washed free of soil and examined under water against a white background. Each plant was scored for infection as follows:-

No infection (stelar or runner hyphae)	0 points
Infection slight (stelar lesions small or runner hyphae)	1 point
Infection slight to medium (lesions on a minority of roots)	2 points
Infection medium to severe (lesions on a majority of roots)	3 points
Infection severe (all roots severely blackened)	4 points

b) Method 2. Bioassay Method.

The roots of each plant were cut off at the base of the shoot or above the rhizome piece with a scalpel and rolled into a flat circle about one and a half to two centimetres in diameter. These were placed, in the same order in which they had been examined for scoring by the infection scale, in a seed box containing sand to a depth of two centimetres. Each root circle was covered with a layer of fine sand upon which a wheat seed was placed. The box was filled with sand. Four weeks later the test wheat seedlings were harvested, washed clean and marked as positive or negative depending on presence or absence of lesions on their seminal roots. The total number of test wheat seedlings per box showing stelar lesions was expressed as a percentage of the total number of germinated test wheat seedlings from the box.

c) Method 3. Observation Method.

Harvested plants were washed free of soil and floated in water against a white background. The total number of roots and the number of roots with one or more stelar lesions, were counted and

infection expressed as a percentage. Where no lesions were present the roots were further examined under a binocular dissecting microscope for runner hyphae. The presence of these was noted and the size, and infection symptoms of each sample of plants briefly described. This method was useful in that the actual extent of the lesions was noted.

d) Discussion.

The aim with these experiments was to find a suitable method for determining the amount of disease on grasses. Grasses are extremely difficult to examine for signs of stelar infection as they produce large numbers of roots which often obscure the areas of invasion. Infection is usually limited to roots initially in contact with the inoculum and is often small in amount. Adventitious roots of seedlings produced at nodes above the crown are seldom infected. The fungus probably only attacks these roots when they contact an already diseased seminal or crown root. There is generally a lack of stelar infection on grass seedlings, but runner hyphae may be present, sometimes completely ensheathing the roots.

The economic importance of grasses as host plants for O. graminis lies not only in the degree to which they are invaded by the fungus, but the amount of the fungus they carry over from one cereal crop to another. When devising methods for determining the disease on grasses it is important to take into account both the invasion

which has occurred, and that which could occur (from runner hyphae) either on the roots under examination or on cereal plants. It is shown (see Section 1, page 39) that runner hyphae present on the roots of grass seedlings can cause serious damage to subsequent wheat seedlings. It is essential therefore that these hyphae be considered when estimating the amount of disease on grasses.

As two of the methods had not been used before it was impossible to foresee (anticipate) some of the difficulties involved. The weight method (see Section 1, page 33) was not suitable, owing to the time involved in examining the large numbers of plants inoculated in each experiment. The Visual and Bioassay methods overcame the disadvantage of speed. These methods were excellent for use with large numbers of plants, providing a sufficient number of test wheat seedlings germinated. But even at a low level of infection of the host plants the test wheat seedlings often became positive for infection, so giving an overestimate of the amount of disease actually present on the host plants. Complete blackening of the host roots leading to a limited number of these roots resulted in the test wheat seedling seminal roots missing the infected host root circle with a resulting lack of infection of the wheat. This underestimated the disease present.

Robinson and Lucas' (1963) radioactive method was not used. This method involved incorporation of radioactive phosphorus into

the fungal inoculum. Autoradiography of infected plants showed the stelar lesions clearly and the ectotrophic extension of the fungus could be measured. Each plant had to be handled separately, and specially mounted for autoradiography. This was time consuming, and again not suitable for use with large numbers of plants. Also experiments could only be of limited duration because of the rapid decay of the radioactive phosphorus.

D. Method and procedure for staining and squashing Holcus mollis root tips in order to examine the chromosomes.

1. One centimetre lengths of root tips were immersed in a saturated solution of mono-bromonaphthalene (see Appendix E) in a specimen tube for four hours. Mono-bromonaphthalene inhibits spindle formation and stops mitosis at the metaphase stage. (see O'Mara, 1948).

2. The root tips were transferred to a mixture of nine parts of two per cent aceto-orcein in 45% acetic acid and one part of Normal hydrochloric acid (see Appendix E).in a watch-glass. (see Tjio and Levan, 1950).

3. The watch-glass was heated once over a low bunsen flame.

4. One or two drops of Normal hydrochloric acid were added to the watch-glass so making the material more easy to macerate.

5. The watch-glass was heated again two or three times until the solution steamed.

6. One to two millimetres of a root tip so treated was placed on a

clean, grease free glass slide in one drop of one per cent aceto-orcein. It was covered with a clean glass cover-slip and squashed gently but firmly with a blunt pencil.

7. The slide was heated gently and excess stain blotted up with filter paper.

8. Individual cells were gently tapped out with the point of a dissecting needle whilst the slide was constantly examined under a microscope.

9. The cover slip was held firmly in place with a thick layer of filter paper, and great pressure exerted on the paper in order to squash the root tip cells. The cover slip was then ringed with a layer of colourless nail varnish to exclude the air.

For a more permanent preparation. (see Rattenbury, 1956).

10. The nail varnish was omitted. Fresh squashes were thoroughly irrigated with 45% acetic acid to remove excess stain. This was done by wiping one edge of the cover slip with the acid while holding down one corner firmly.

11. A small drop of 10% glycerol in 45% acetic acid was allowed to flow under the cover slip.

12. The slide was blotted and examined.

SECTION 1

The susceptibility of Holcus mollis to the take-all fungus, Ophiobolus graminis, compared with Agropyron repens and Agrostis stolonifera which are known carriers of the disease.

Holcus mollis is a common rhizomatous weed, found generally on light acid soils. Its capacity for intensive rhizome and root production makes it a serious pest, particularly where it is present as a weed in cereal crops. There is little experimental evidence from the literature to show that H. mollis is a carrier of O. graminis. Thomas (1966) whilst inspecting a field of barley in Wales devastated with take-all, found the crop to be infested with graminaceous weeds, which included Agrostis spp. and H. mollis. Holcus mollis was found in the summer of 1966 growing amongst the crop in a field of barley near Leighton Buszard. The grass in this case was over a foot higher than the barley plants, and was producing numerous inflorescences.

Experiment 1.

In order to test the susceptibility of Holcus mollis to attack by Ophiobolus graminis, an experiment was designed to determine the extent to which rhizome fragments of this grass may be invaded by the fungus by comparison with similar vegetative material of Agropyron repens and Agrostis stolonifera. These latter two grasses are known to be susceptible to attack by the fungus, (Walker, 1945, and Robinson, 1963). Wheat seeds were inoculated to test the pathogenicity of the fungus during the experiment.

Vegetative material of the three grasses had been collected; H. mollis from Shotover, Nr. Oxford, A. repens from the grounds of the Department of Agriculture, Oxford, and A. stolonifera from the Oxford University Field Station at Wytham. The grasses had been planted in boxes of soil in a greenhouse for use in experimental work. H. mollis and A. repens rhizomes and A. stolonifera stolons were washed and cut into two centimetre lengths, each with a central node.

The inoculum was prepared by growing the fungus on potato dextrose agar plus 0.3% yeast extract (Appendix A) in Petri dishes at 25°C. for ten days. Disks, eleven millimetres in diameter, were cut from the edge of the colonies, and the inoculation of the plant material was carried out as described in the Materials and Methods, page 21. Twenty-eight rhizome pieces of H. mollis were inoculated in each of two boxes and 28 pieces laid on the soil in two boxes as uninoculated controls. Rhizome pieces of A. repens, stolon pieces of A. stolonifera, and wheat seeds were treated similarly. This gave a total of 56 inoculated and 56 control vegetative fragments or seeds for each species.

After three weeks one box of inoculated and one of uninoculated plants of each species were harvested, and the roots washed clean for inspection. They were examined in water against a white background, (Garrett, 1936). Plants with one or more stelar lesions visible to the naked eye were scored as positive for infection. Those with no lesions were further examined under a binocular dissecting microscope for

runner hyphae. The presence of these was noted. From this information the percentage of infected plants showing stelar lesions was calculated for each box. After five weeks the remaining plants were harvested and examined similarly. The results are shown in Table 1.

Table 1.
Infection by Ophiobolus graminis of vegetative fragments of Holcus mollis, Agropyron repens, Agrostis stolonifera, and wheat seedlings inoculated as a susceptible control.

Host plant	Total no. plants/ box		No. uninf. plants		No. plants with R.H. only		No. plants with stelar infection		% stelar inf.	
	I.	U.	I.	U.	I.	U.	I.	U.	I.	U.
H. mollis										
3 wk. harvest	28	28	0	28	3	0	25	0	89	0
5 wk. harvest	28	23	0	23	0	0	28	0	100	0
Mean % stelar inf.									95	0
A. repens										
3 wk. harvest	28	27	0	26	0	1	28	0	100	0
5 wk. harvest	25	27	0	27	0	0	25	0	100	0
Mean % stelar inf.									100	0
A. stolonifera										
3 wk. harvest	23	25	2	23	12	2	9	0	39	0
5 wk. harvest	24	15	1	15	9	0	14	0	58	0
Mean % stelar inf.									49	0
Wheat										
3 wk. harvest	28	26	0	26	1	0	27	0	96	0
5 wk. harvest	26	28	0	28	0	0	26	0	100	0
Mean % stelar inf.									98	0

I. - inoculated. U. - Uninoculated. R.H. - runner hyphae.

In all possible cases infection of inoculated plants had increased at the fifth week harvest. All the inoculated A. repens rhizome pieces were 100% infected, followed by wheat (98%), H. mollis (95%), and A. stolonifera (49%). These results show that H. mollis is highly susceptible to attack by O. graminis. Observation of these infected rhizome pieces showed all the typical signs of the disease. There were lesions, perithecia and runner hyphae present on both the roots and rhizome pieces. These plants, as well as those of A. repens, were almost completely destroyed by the fungus. The intense infection of the wheat seedlings points to the high pathogenicity of the fungus during the experiment.

Robinson (1963) found that A. repens rhizome fragments had intense stelar infection of their roots, but infection of A. stolonifera was mainly by runner hyphae. The maximum runner hyphal extension was 52 millimetres five weeks after inoculation on A. stolonifera, compared with 33 millimetres on A. repens. Table 1 shows that fewer A. stolonifera plants had stelar lesions than the other host plants. As only three uninoculated plants showed any infection at all, and this consisted of runner hyphae only, it was concluded that there was no significant amount of viable O. graminis already present in the soil.

Experiment 2.

The first experiment demonstrated the susceptibility of H. mollis

rhizome pieces to attack by O. graminis, but Robinson (1963), using seedlings of A. repens and A. stolonifera, found them to be more resistant than vegetative fragments of these grasses. In view of Robinson's results the following experiment was set up to compare the susceptibility of H. mollis seedlings and regenerating rhizome pieces. Seedlings of different maturity were compared to see whether age affected their susceptibility to attack by O. graminis.

The method used for estimating the amount of disease in Experiment 1 was discarded in favour of a more precise one. The first method gave no indication of the severity of infection, and measured only the presence or absence of the disease. A new criterion was devised for Experiment 2; namely the relative intensity of infection of the plant roots. Those parts of the root system bearing stelar lesions were cut off and weighed, and infection was calculated by comparing this weight with the total root weight for each plant. This gave a more exact measurement of the degree of stelar infection of each plant.

Seedlings were inoculated after growing for two, three or six weeks, and rhizome pieces were similarly treated after being left to shoot in soil for four weeks. Inoculation was carried out in a similar manner to that in Experiment 1. Disks, eleven millimetres in diameter, were used as inoculum. A total of ten seed boxes were used. Two boxes of rhizome pieces, each two centimetres in length with a central node, and two boxes of seedlings for each age group were inoculated. In the final two boxes wheat seeds were inoculated as

susceptible controls. The plants were harvested four and seven weeks later.

The plants were carefully dug up to preserve the maximum possible amounts of root per plant. They were rinsed and stored in polythene bags in a deep freeze until examination. The root system of each plant was then washed free of soil, superficially dried and cut off at the base of the shoot, or at the point of emergence from the rhizome. The roots were weighed, and then examined in water under a binocular dissecting microscope. All the blackened root material was removed with a sharp scalpel and the fresh weight determined. The weight of infected roots was expressed as a percentage of the total root weight. The results are shown in Table 2, page 34 .

Infection in all cases was irregular, and varied enormously even within each sample. Subsequent experience suggested that the seed sample may have been contaminated with seed of Holcus lanatus. These irregularities reinforce the contention that a seed mixture was used. Holcus lanatus seedlings produce more adventitious roots than H. mollis in a given length of time, and probably at a rate faster than they can be infected by O. graminis. This might account for the low or non-existent infection of some of the seedlings which produced large numbers of roots.

It is difficult to differentiate between the seedlings of these two species at the stage at which they have been used for inoculation, and it is essential that seed be collected from positively identified

Table 2.
Infection by Ophiobolus graminis of Holcus mollis rhizome pieces and seedlings of different ages, and wheat seedlings inoculated as a susceptible control. Infection is calculated as the weight of roots with one or more stelar lesions expressed as a percentage of the total root weight.

Host plant (age at inoc.)	Harvest time	Mean total lengths of plants (cms.)	Mean weight total roots (g.)	Mean weight infected roots (g.)	% infection
Holcus spp. seedlings					
(2 weeks)	4 weeks	20.9	0.008	0.001	12.5
	7 weeks	29.4	0.013	0.001	7.7
(3 weeks)	4 weeks	24.6	0.014	0.001	7.1
	7 weeks	23.2	0.012	0.001	8.3
(6 weeks)	4 weeks	29.2	0.021	0.003	14.3
	7 weeks	37.5	0.031	0.002	6.5
H. mollis rhizome pieces	4 weeks	6.0	0.005	0.002	40.0
	7 weeks	3.7	0.002	0.001	50.0
Wheat seedlings	4 weeks	30.4	0.022	0.009	40.9
	7 weeks	30.5	0.020	0.007	35.0

parents. *Holcus lanatus* and *Holcus mollis* are sometimes difficult to distinguish, but there are two main differences between the species:-

a) The awn on the upper lemma of *H. mollis* is longer (3.5 - 5 mm.) than that of *H. lanatus* (up to 2 mm.). *Holcus lanatus* awn becomes recurved like a fish-hook when dry, while that of *H. mollis* remains

straight when dry.

b) Holcus lanatus ligules are hairy on the abaxial surface. Holcus mollis ligules are never hairy.

These two species hybridize readily, and seed collected from mixed populations of H. mollis and H. lanatus is likely to be either triploid or aneuploid. The presence of hybrids and the differences between the species are expanded in Section 4.

The results of Experiment 2 showed that H. mollis rhizome pieces were much more heavily infected than the seedlings, which agrees with Robinson's results for A. repens and A. stolonifera. Table 2 shows that, at least for the duration of the experiment, age did not affect seedling susceptibility. The more mature seedlings were no more heavily infected than the smaller, less developed ones. It is possible that susceptibility or resistance to take-all is a condition inherent in individual plants, or groups of plants, irrespective of age. It must be stressed that comparison between the seedlings and rhizome pieces was made difficult by the fact that not all seedlings were of the same species. The rhizome pieces were as heavily infected as the wheat seedlings. Infection had not increased by the second harvest. This may have been due, at least with the seedlings, to the rate at which they produced new roots. The weight of the total roots generally increased at the second harvest. This increase in root weight, which was correlated with an increase in root numbers, shows that the plants were producing new roots at a rate faster than they

were infected as there was no increase in infected root weight at this harvest.

The method of assessment used in this experiment was satisfactory in that it gave a useful basis for comparison. However, it did not take into account runner hyphae. Some roots were densely covered with these hyphae, whilst others had only minutely blackened areas which were too small to weigh. This blackening may have been small in amount, but it could spread. Other disadvantages of the method are that it is time consuming and laborious, and to give reliable results all the roots have to be very carefully excavated from the soil. This is difficult with brittle, heavily infected roots.

Other disease assessment methods were used in later experiments as there were usually such large numbers of plants to be examined. In an attempt to overcome the disadvantages of the Weight method, the Visual method was used, combined with a Bioassay using test wheat seedlings, (see Materials and Methods, page 21). These methods had the advantage of speed, were easy to execute, and took into account the presence of runner hyphae.

Experiment 3.

It has been shown that A. repens and H. mollis rhizome pieces and A. stolonifera stolon pieces are susceptible to attack by O. graminis. Robinson (1963) using lengths of A. repens and A. stolonifera rhizome or stolon still attached to the parent plant, found them to be less

susceptible to attack than the vegetative fragments. In order to investigate this with regard to H. mollis a comparison was made of the susceptibility of multinodal and uninodal rhizome pieces and seedlings of this grass. This susceptibility was compared with that of similar material of A. repens and A. stolonifera.

The O. graminis isolate used previously appeared to have lost most of its pathogenicity. It had been kept in culture, on agar slopes, for over twelve months. A new culture was obtained from Nottingham University (isolate 4N, see Materials and Methods, page 19) which was unusual in several respects:-

a) It produced perithecia containing viable ascospores on potato dextrose agar.

b) It produced numerous runner hyphae, as well as stelar lesions, on wheat roots.

c) It was a virulent pathogen on wheat, although it had been in culture for over nine years.

In January 1965, two months before the start of the experiment, two centimetre lengths of young rhizome of A. repens and H. mollis, and stolon pieces of A. stolonifera were planted in seed boxes in soil. These were kept in continuous artificial light, since shooting occurs more rapidly under these conditions. Whole lengths of rhizome (or stolon) each with about seven nodes, were treated similarly. Holcus spp. and A. stolonifera seed was sown in pots six and ten weeks, respectively, before the start of the experiment. The pots were

kept initially in a warm dark place to hasten germination, but when the seedlings reached a height of one centimetre were transferred to continuous light. Unfortunately A. repens seed, collected in 1964, did not germinate.

Inoculation of the host species was by two methods:-

a) Chaff.

The chaff was completely colonised after five weeks growth, (see Materials and Methods, page 20). The bottles were emptied and the contents thoroughly mixed together. Seed boxes were filled with a three centimetre layer of soil and a half centimetre layer of chaff laid on the soil. This was covered with a layer of sand so that the plant roots were not in initial direct contact with the chaff. The material for inoculation was dug up and washed. Twenty-four seedlings of H. mollis were placed in rows in a seed box and covered with soil. Twenty-four rhizome pieces of the grass were treated similarly, and four long pieces of rhizome of H. mollis were placed in a third box and covered with soil. Plant material of A. repens and A. stolonifera was treated similarly. One box of 24 wheat seeds was inoculated as a susceptible control. This gave a total of nine boxes inoculated with chaff.

b) Agar Disks.

Inoculation with agar disks was undertaken as described previously, with two minor differences. A thin layer of sand was placed on each agar disk before the plant material was held in place over each disk.

This meant that neither the plants inoculated with chaff nor those inoculated with agar disks were in direct contact with the inoculum. Each node of the lengths of vegetative material was individually held in place over an agar disk with fine sand. There was a total of nine boxes, containing the same plant material as those inoculated with chaff. All eighteen boxes were randomised and kept moist in a greenhouse. Six weeks later the plants from each box were harvested, and examined using the Visual and Bioassay methods, (see Materials and Methods, page 21). The rhizome or stolon lengths were cut halfway along each internode into single nodal pieces for infection scoring.

It has been stated that seedlings of A. repens, H. mollis and A. stolonifera are less susceptible to attack by O. graminis than are the rhizome or stolon pieces of these grasses. Results presented in Table 3 appear to contradict this. The wheat seedlings sown over the Holcus spp. seedling roots were more heavily attacked than those planted over the rhizome pieces or lengths of this grass. This may be explained by the fact that grass seedlings produce larger numbers of fibrous roots than rhizomes or stolons, and A. stolonifera has been shown by Robinson (1963) to support enormous numbers of runner hyphae on its roots with few stelar lesions. The O. graminis isolate used in this experiment has been observed to produce considerable numbers of these hyphae without necessarily producing stelar lesions. The fungus may have spread densely over the Holcus spp. seedling roots.

Table 3.
Infection by Ophiobolus graminis of Holcus mollis, Agropyron repens and Agrostis stolonifera compared with wheat seedlings. Assessment of infection using the Visual and Bioassay methods.

Host plant	Av. rating 0-4 scale	No. wheat plants germinated	No. wheat plants infected	% infection wheat seedlings
A. repens rhizome pieces				
CHAFF	3	15	8	53
DISKS	3	15	13	87
A. repens rhizome lengths				
CHAFF	3	23	8	35
DISKS	3	24	19	79
A. stolonifera seedlings				
CHAFF	2	23	14	61
DISKS	1	18	15	83
A. stolonifera stolon pieces				
CHAFF	1	9	5	56
DISKS	1	9	8	89
A. stolonifera stolon lengths				
CHAFF	1	7	6	86
DISKS	1	17	8	47
Holcus spp. seedlings				
CHAFF	2	23	20	87
DISKS	2	24	23	96
H. mollis rhizome pieces				
CHAFF	3	12	6	50
DISKS	2	10	6	60

Table 3 continued.

Host plant	Av. rating 0-4 scale	No. wheat plants germinated	No. wheat plants infected	% infection wheat seedlings
H. mollis rhizome lengths				
CHAFF	3	17	8	47
DISKS	3	15	10	67
Wheat seedlings				
CHAFF	2	23	22	96
DISKS	2	23	22	96

The results in Table 3 show that the initial rating for these seedlings was low, but that the subsequent Bioassay seedlings were heavily infected. As there were a large number of roots which were covered by runner hyphae, this may account for the high infection of the Bioassay wheat seedlings. Similarly this explanation may account for the high infection of the Bioassay seedlings sown over the A. stolonifera seedling roots. Smaller numbers of roots were produced by the rhizomes and stolons, thus the root circles were smaller and some of the wheat seedling seminal roots may have grown round, rather than through, the root circle so escaping infection. This may be the cause of the low infection of the wheat seedlings sown over the A. repens rhizome pieces and lengths, as the original disease rating for this material by the Visual method was high.

The results of the experiment show that, contrary to Robinson's (1963) results for A. stolonifera, the multinodal pieces of this grass and of H. mollis were as susceptible to O. graminis as the uninodal

pieces. But the results with A. repens agree with those of Robinson for this grass; namely the multinodal pieces were less susceptible to O. graminis than were the uninodal.

The Bioassay method does not discriminate between the amounts of infection present on the test wheat seedlings, which varied from pin-point lesions to whole root systems blackened. The percentage infection shown in Table 3 can be taken therefore only as an approximate indication of the amount of infection present.

The plants were generally more infected when inoculated with agar disks than with chaff, the exception being A. stolonifera stolon lengths. There was no difference in the infection of the wheat by either inoculum medium.

The results of Experiment 3 show:-

a) Under certain controlled conditions grass seedlings, rhizomes or stolons may be equally capable of supporting sufficient O. graminis to produce severe infection of a subsequent cereal host.

b) Agropyron repens rhizome lengths are possibly not so susceptible to infection by this fungus as are the fragments of this grass, but infection of whole or fragmented rhizomes or stolons is similar in both H. mollis and A. stolonifera.

c) Holcus spp. and A. stolonifera seedlings may carry large numbers of runner hyphae on their roots, and this mycelium may be a potential menace to subsequent cereal crops. However, these grasses appear to

spread almost exclusively by vegetative means. Much of the seed they produce is non-viable so that the potential danger to succeeding cereal crops is not so real as it may appear from this experiment.

Experiment 4.

The Visual method for estimating the amount of disease which may be transmitted from grass hosts to succeeding cereal plants is obviously open to criticism. Incomplete washing of the grass host roots, leaving minute fragments of agar or chaff inoculum adhering to them, could exaggerate the assessment of infection on these roots when later tested by Wheat seedlings. The importance of this was investigated by comparing two resistant species, Avena sativa and Phleum pratense with wheat as a susceptible control.

Garrett (1941) tested several ley grass seedlings and classified them as resistant or susceptible to attack by the take-all fungus. He found that P. pratense was resistant and for this reason it was selected for this experiment. Oats are resistant to infection by O. graminis in so far as only O. graminis var. avenae causes severe stelar lesions on A. sativa, (Turner, 1940), although O. graminis may produce runner hyphae and superficial lesions on the seminal roots of oat seedlings.

Three boxes each of P. pratense seedlings, oats and wheat seeds were inoculated with agar disks of the fungus. One third of the seedlings were harvested four, five and six weeks, respectively, after inoculation. Results are given in Table 4.

Table 4.
Infection by Ophiobolus graminis (isolate 3S) of Phleum pratense, oat and wheat seedlings.

Host plant	Av. rating 0-4 scale	No. wheat plants germinated	No. wheat plants infected	% infection wheat seedlings
P. pratense seedlings				
4 wk. harvest	0	15	0	0
5 wk. harvest	0	20	2	10
6 wk. harvest	0	18	4	22
Mean % inf.				11
Oats seedlings				
4 wk. harvest	0	11	2	18
5 wk. harvest	0	4	1	25
6 wk. harvest	0	8	2	25
Mean % inf.				23
Wheat seedlings				
4 wk. harvest	0	21	8	38
5 wk. harvest	0	17	6	35
6 wk. harvest	0	9	3	33
Mean % inf.				35

Infection of the oats and P. pratense Bioassay seedlings was unexpectedly high. Isolate 3S of O. graminis, (see Materials and Methods, page 18), was used as inoculum. When first tested it was moderately pathogenic, but its pathogenicity fell after a few months in culture, and it can be seen from Table 4 that the mean infection of the wheat Bioassay seedlings was only 35%. Several lesions were found on the oats and P. pratense Bioassay seedlings, so in respect of these and of the low infection of the wheat, and the poor germination of some of the Bioassay seedlings, the experiment was repeated using isolate 1W of O. graminis. The pathogenicity of this isolate was

tested a few days after it was isolated and all the wheat roots examined one month after inoculation were severely infected.

Experiment 5.

This was carried out in a similar manner to Experiment 4, and the results are shown in Table 5.

Table 5.
Infection by Ophiobolus graminis (isolate 1W) of Phleum pratense, oats and wheat seedlings.

Host plant	Av. rating 0-4 scale	No. wheat plants germinated	No. wheat plants infected	% infection wheat plants
P. pratense seedlings				
4 wk. harvest	0	22	3	14
5 wk. harvest	1	23	7	30
6 wk. harvest	0	20	3	15
Mean % inf.				20
Oats seedlings				
4 wk. harvest	0	19	0	0
5 wk. harvest	0	18	0	0
6 wk. harvest	0	18	0	0
Mean % inf.				0
Wheat seedlings				
4 wk. harvest	3	11	4	36
5 wk. harvest	3	11	3	27
6 wk. harvest	2	15	6	40
Mean % inf.				34

On harvesting, some of the oats and P. pratense seedlings had hyphae on their roots which may have been those of O. graminis, and some of the P. pratense Bioassay seedling roots were infected with stelar lesions. The oats Bioassay wheat seedlings were completely uninfected.

This lack of infection of the oats Bioassay seedling roots shows that infection of test wheat seedlings arises from the original host roots, and not from pieces of inoculum adhering to the host roots; assuming that there is no wild inoculum present in the soil. ∴ unsterilised?

SECTION 2

The effects of age and different isolates of Ophiobolus graminis on the susceptibility of Agropyron repens and Agrostis stolonifera to take-all infection.

Agropyron repens and Agrostis stolonifera spread almost exclusively by means of vegetative structures, rhizomes or stolons. In one growing season Palmer (1958) found that rhizomes of A. repens may attain a length of two metres or more. This is rare, and lengths of five to fifteen centimetres are more common for plants in closed communities. The following experiments were carried out to determine whether the position of a node on a rhizome or stolon affects its susceptibility to take-all infection by Ophiobolus graminis. Age of seedlings of Holcus spp. was found by an experiment described in Section 1. not to affect their attack by O. graminis, so similar tests were applied to seedlings of A. repens. Four different isolates of O. graminis were used in these experiments, and comparison made of their invasion of the host plants.

The intention with the following experiment was to investigate the susceptibility of different ages of A. stolonifera vegetative material. "Old" and "young" stolon pieces were required, and in order to reduce variability, stolons were used from one source only. Comparable material had to be used, and all the stolon material had adventitious roots and shoots about four centimetres high.

Because of the basis for comparison in these experiments only small numbers of plants could be used, as only a limited amount of vegetative material was available.

Stolons of A. stolonifera were taken from boxes in the greenhouse. The distal four nodes were designated "young" and nodes proximal to these were termed "old", and each such "old" node had adventitious roots and a shoot about four centimetres high. Stolon pieces were cut so as to leave one centimetre on either side of a node, and were planted in boxes of soil. After one month all the stolon pieces were dug up and washed free of soil. Some of the "young" nodes had roots and shoots and these were selected, together with the "old" nodes for inoculation with O. graminis.

Isolate 1W (see Materials and Methods, page 18) of the fungus was grown on potato dextrose agar plus one per cent malt extract and one per cent yeast extract (see Appendix A). Inoculation was carried out using disks eleven millimetres in diameter. There were two boxes each of "young" stolon pieces, "old" stolon pieces and wheat as a control. One month later the plants were dug up and examined using the Visual and Bioassay methods (see Materials and Methods, page 21). The wheat seedlings used for the Bioassay were dug up four (Box 1.) and five (Box2.) weeks later and examined for infection. The results are shown in Table 6.

Table 6.
Infection by Ophiobolus graminis (isolate 1W) of Agrostis stolonifera "old" and "young" stolon pieces compared with wheat seedlings.

Host plant	Average rating 0-4 scale	No. wheat plants germinated	No. wheat plants infected	% infection wheat plants
A. stolonifera stolon pieces "Old"				
Box 1.	3	16	14	88
Box 2.	2	16	15	94
Mean % inf.				91
A. stolonifera stolon pieces "Young"				
Box 1.	2	18	14	78
Box 2.	2	17	14	82
Mean % inf.				80
Wheat seedlings				
Box 1.	4	18	13	72
Box 2.	4	22	19	86
Mean % inf.				79

Although it is not evident from the table, there was a great variation of infection of the "young" stolon pieces, and the figures given represent the average infection rating. The rating for individual plants varied between one and three, while for the "old" pieces it was more consistently three. (see Infection scale, Method 1, Materials and Methods, page 22). The older pieces were more heavily/extensively infected than the younger ones, and showed considerably more lesions and blackening of the stolon pieces. The shoots of the wheat, inoculated as a control, were shrivelled and yellow and the roots blackened and brittle, the fungus having almost completely destroyed the plants.

The test wheat seedlings were heavily infected in all cases. This infection was particularly severe for the "old" stolon pieces, and was 10% higher than for the "young" pieces. However, the figures for the test wheat seedlings sown over the wheat roots are inconsistently low when compared with the high original rating. This is probably due to disease escape by the test wheat seedlings' seminal roots missing the small root circle, so leading to loss of direct contact with the severely infected roots. The percentage figures for the "young" stolon pieces are similar to those for the wheat.

It appears from this experiment that position of nodes on a stolon is important with regard to their susceptibility. Although at such severe levels of infection differences in amounts of discolouration of the stolon roots were less easy to discriminate, nevertheless it was apparent from observation that the "old" stolon pieces were more severely attacked by O. graminis than the younger ones. This was substantiated by the heavy infection of the test wheat seedlings sown over the "old" stolon pieces. These results suggest that nodes nearest the tip of an advancing stolon are more resistant, and those more established shooting nodes further behind more susceptible, to attack by this fungus.

This heavy invasion of all the A. stolonifera plants is unusual. Walker (1945) considers A. stolonifera to be an important carrier of take-all not because of the degree to which it is

attacked, but because of its wide-spread distribution, perennial habit and vigorous growth. The fungus spreads along the outside of roots of this grass, but these are not usually severely invaded. There are three possible explanations of this high infection:-

a) Extreme virulence of this isolate of O. graminis.

b) Favourable conditions for spread of the fungus along the host roots.

c) Unusually high susceptibility of the grass.

a) Isolate 1W was very pathogenic on wheat, as is shown by the destruction of the control wheat seedlings.

b) Conditions in the greenhouse where this experiment was carried out were identical as far as possible for all the boxes, so there is no reason why this hypothesis should stand. Nevertheless it is a possibility and therefore cannot be discounted.

c) The grass material used in this experiment came from the same source as that used in former experiments, and in all previous work with this grass, stelar infection of the grass roots had been slight. This is substantiated by work done by Robinson (1963) and Walker (1945). Garrett (1950) considers that O. graminis can survive almost indefinitely on the roots of various perennial grass hosts, for example Agrostis spp.. It rarely kills these grasses, and although infected they are often found as dominants of their grassland associations.

This evidence suggests that the virulent nature of the fungus isolate, together with favourable conditions for infection may have been the cause of the heavy infection of the A. stolonifera plants, and not the unusual susceptibility of the grass.

In view of the interesting results obtained with A. stolonifera the experiment was repeated using Agropyron repens. A comparison was made not only of rhizome pieces but of seedlings of different ages, using a chaff inoculum of isolates 3S and 5Bg. (see Materials and Methods, page 18).

Rhizomes were cut into uninodal pieces. The distal four nodes of a rhizome were termed "young". The "old" pieces were from the tougher, stouter, central part of a rhizome. All these pieces were planted in soil four weeks before inoculation, in order that all the rhizome segments used in the experiment might have roots and a shoot. Seed was sown in pots of sand at three different times, nine, ten and eleven weeks before inoculation. The chaff inoculum was prepared. One seed box for each different age of seedling or rhizome pieces, and one box for wheat as a susceptible control were inoculated with each of the two fungal isolates. This gave a total of twelve boxes. The plants were harvested four weeks after inoculation and examined as in Method 3. (see Materials and Methods, page 22). The results are shown in Table 7.

Table 7.
Infection by Ophiobolus graminis (isolates 3S and 5Bg) of Agropyron repens "old" and "young" rhizome pieces and seedlings of different ages compared with wheat seedlings.

Host plant	Total number plants per sample	% infection
Isolate 5Bg.		
A. repens seedlings		
Age at 9 weeks old	24	58
time of 10 weeks old	23	59
inoc. 11 weeks old	24	44
A. repens rhizome pieces		
"Old"	15	78
"Young"	13	68
Wheat seedlings	16	73
Isolate 3S		
A. repens seedlings		
Age at 9 weeks old	24	32
time of 10 weeks old	24	43
inoc. 11 weeks old	25	29
A. repens rhizome pieces		
"Old"	15	56
"Young"	14	44
Wheat seedlings	18	36

Extension to Table 7 in Appendix B.

The older rhizome pieces were slightly more susceptible to attack by O. graminis than the younger ones. This is in agreement with the results obtained for A. stolonifera. There was no difference in the infection of the seedlings, although with both

take-all isolates the eleven week seedlings were slightly less infected. This may have resulted from the smaller root systems of the oldest seedlings at the time of inoculation owing to the unexplained higher germination rate of the first sample of seed and the resultant root competition between seedlings.

Isolate 5Bg caused considerably more damage to the wheat than to the grass seedlings. Most of the wheat seminal roots were attacked, and although the shoots were healthy there was plate mycelium on some plants, and few fibrous roots. However, the "old" rhizome pieces were heavily infected. The roots had extensive lesions and the rhizome pieces were extensively blackened. In spite of this, the shoots were healthy, new uninfected tillers and rhizomes being produced, and the plants did not appear to be unduly affected by the presence of the parasite. Infection of the younger rhizome pieces was more variable. The shoots were healthy and new uninfected roots were being produced. But the lesions, where present, were extensive. It has been mentioned above that the eleven week old seedlings were small. Lesions on the roots of these plants were not extensive and the plants appeared very healthy. By comparison, the ten and nine week old seedlings were more severely infected. The nine week seedlings were by far the largest of the three ages, and were producing numerous new tillers and rhizomes. Age of seedlings in this experiment was no guide to

developmental stage and it seems unreasonable to expect any positive correlation between age and susceptibility, since the youngest were most advanced, and the oldest were least advanced.

Infection of the wheat caused by isolate 3S was less severe. The parasite had not reached the crown of many of the plants, and they had produced secondary roots. Infection of the "old" vegetative fragments was variable; lesions, when they occurred, were extensive, and new uninfected tillers and rhizomes were being produced by both the "old" and "young" rhizome pieces. Variable infection was also shown by the "young" fragments, and the lesions were very small. 56% of the roots of the "old" pieces were infected compared with 44% of the "young" pieces. The ten week old seedlings were the most susceptible of the seedlings showing extensive lesions and 43% of the roots were infected. Figure 1. shows infection of vegetative segments of A. repens and A. stolonifera.

This investigation was extended by comparing the susceptibility of different ages of seedlings and of rhizome pieces of A. repens which had been left to shoot for different times. A chaff inoculum of isolate 5Bg was used. The A. repens seedlings were five, six and seven weeks old, and the rhizome pieces had been left in boxes to shoot for four and five weeks before inoculation. The plants were harvested after four weeks and estimation of disease made by Method 3. The results are shown in Table 8.

Figure 1.

Infection by *Ophiobolus graminis* of *Agropyron repens* rhizome pieces and *Agrostis stolonifera* stolon pieces.

Agropyron
repens

Agrostis
stolonifera

Table 8.
Infection by Ophiobolus graminis (isolate 5Bg) of Agropyron repens seedlings of different ages and rhizome pieces which had been left to shoot for different lengths of time, compared with wheat seedlings.

Host plant		Total number plants per sample	% infection
A. repens seedlings			
Age at time of inoc.	5 weeks old	24	72
	6 weeks old	24	80
	7 weeks old	22	81
A. repens rhizome pieces			
	4 weeks shooting	22	92
	5 weeks shooting	15	96
Wheat seedlings		17	79

Extension to Table 8 in Appendix B.

The rhizome pieces were much more heavily infected than the seedlings of A. repens or the wheat seedlings. In spite of the almost total destruction by the fungus of the adventitious roots formed by the rhizome pieces, the plants were producing new uninfected rhizomes and did not appear to be suffering unduly from the presence of the parasite. But there was evidence from the shoots of severe infection as some of the leaves were slightly brown and shrivelled. The A. repens seedling shoots did not show any sign of the disease, but the roots were blackened by the extensive lesions. These results point to the virulent nature of this isolate of O. graminis. There was no difference in the susceptibility of the rhizome pieces which had been shooting for different times,

or between the seedlings of different ages.

The A. repens seedlings used in the last two experiments were of different ages at the time of inoculation. The results show that this age difference did not affect their susceptibility. The following experiment was designed to determine whether the amount of seedling root infection increased by leaving them in contact with the inoculum for an increasing length of time.

Eleven week old A. repens seedlings were inoculated with agar disks of O. graminis. Wheat seeds were inoculated as a control. Two isolates of the fungus were used, 4N and 5Bg. Both these isolates had been used before and had been shown to be virulent pathogens. Three boxes of A. repens seedlings and three boxes of wheat seeds were inoculated with each of the two isolates. This gave a total of twelve boxes. One box of A. repens seedlings and one of wheat seedlings inoculated with each isolate were harvested four, five and six weeks later. The amount of disease was estimated by Method 3.

The results are shown in Table 9, page 59. Both isolates were moderately pathogenic on the A. repens seedlings, and 5Bg on the wheat seedlings. The pattern of infection was similar with both the wheat and A. repens seedlings. There is an indication of progressive infection of the wheat with isolate 4N, but this may be the consequence of an anomalous low infection (34%) after four

Table 9.
Infection by Ophiobolus graminis (isolates 4N and 5Bg) of Agropyron repens seedlings compared with wheat seedlings.

Host plant	Total number plants per sample	% infection
Isolate 4N.		
A. repens seedlings		
4 week harvest	26	67
5 week harvest	24	87
6 week harvest	26	61
Wheat seedlings		
4 week harvest	18	34
5 week harvest	17	58
6 week harvest	16	68
Isolate 5Bg		
A. repens seedlings		
4 week harvest	25	79
5 week harvest	26	68
6 week harvest	24	82
Wheat seedlings		
4 week harvest	18	67
5 week harvest	22	90
6 week harvest	19	69

Extension to Table 9 in Appendix B.

weeks rather than higher fifth and sixth week figures. It seems from this experiment that increasing the length of contact of seedling roots of A. repens with O. graminis inoculum does not increase the amount of infection produced by the fungus.

SECTION 3

A comparison of some of the morphological and physiological characters of six isolates of Ophiobolus graminis.

The preceding sections have dealt with the infection of different grasses by Ophiobolus graminis. A number of isolates of the fungus were used as inoculum for these experiments. The severity of infection varied according to the isolate used as inoculum. In order to achieve a better understanding of the results, an extensive study was made of the growth rate, pathogenicity and survival in soil of six isolates of O. graminis, four of which had been used in previous experiments. Details of the isolates are given in Materials and Methods, page 18.

Confirmation of the identity of five of these isolates was determined by measurements of ascospore length. Perithecia were obtained using the method described by Brooks (1965a). When mature, the perithecia were gently crushed in water to expel the asci. Fifteen minutes later the ascospores had been liberated from the asci, and these were mounted in water or cotton blue on a slide. The length of ten ascospores from each of ten randomly selected perithecia was measured under a microscope, using an eye-piece micrometer, and a x40 objective.

Turner (1940) gives the range of length of ascospores of O. graminis as 79 - 86 µ and of O. graminis var. avenae as 101 - 117 µ. The mean ascospore length for five of the isolates falls

within this range for O. graminis. (see Table 10). It was therefore concluded that these five isolates were all O. graminis. The range in length of the ascospores of the different isolates varies considerably, that for isolate 4N (57 - 110 μ) being much larger than the other isolates. Isolate 1W did not produce perithecia by this or any other method, but its cultural characteristics were similar to those of other isolates of O. graminis. It infected wheat seedlings producing lesions typical of those caused by O. graminis, and it did not infect oats. For these reasons it was considered to be Ophiobolus graminis.

Table 10.
Details of ascospores of the Ophiobolus graminis isolates.

Isolate	Mean spore length μ	Modal length μ	Range in length μ
1W	-	-	-
2B	80	80	60 - 100
3S	71	70	60 - 90
4N	81	84	57 - 110
5Bg	79	83	56 - 99
6Sh	78	80	57 - 93

Isolate 4N was unusual in that it readily produced perithecia in culture on Potato dextrose agar. None of the other isolates formed perithecia on this medium, but isolate 6Sh produced aggregations of hyphae around the edge of the Petri dish on

oatmeal agar. (see Appendix A). A week or so later, on the same Petri dishes, dark hyphal masses were produced on the surface of the agar from the point of inoculation outwards. These may have been perithecial initials, but although these hyphal masses were produced by this isolate on oatmeal agar on several occasions no mature perithecia were found. Weste and Thrower (1963) produced fertile perithecia on a medium containing one per cent glucose and just under one per cent asparagine. But there was no perithecial formation by any of the isolates when grown on Lilley and Barnetts' medium (see Appendix A) which also contains glucose and asparagine. The mycelium produced on this medium was in all cases light coloured and thin.

Measurements were made of the daily increase of colony diameter of the isolates. Plastic Petri dishes, 85 millimetres in diameter, each containing potato dextrose agar, were inoculated centrally with a four millimetre disk. Each disk was inverted so that the mycelium was in direct contact with the agar surface. Four such dishes were inoculated for each isolate. Every 24 hours from the time of inoculation the diameter of the fungal colonies was measured. In the case of an irregularly shaped colony the mean of the greatest and the least diameter was taken. The daily mean increase in colony diameter for each isolate was calculated. This growth experiment was repeated on two later occasions, and during

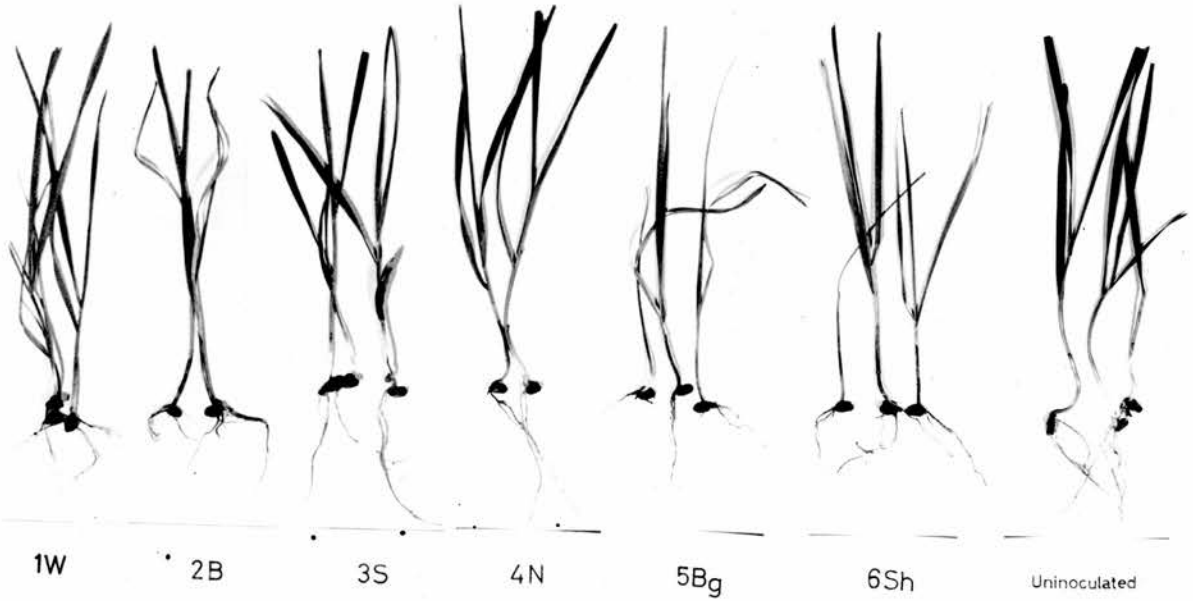
the three months interval between each experiment the isolates were kept in test tubes of potato dextrose agar at room temperature.

The results obtained from these experiments showed that there was little difference between the isolates with respect to the daily increase of colony diameter. The increase was about ten millimetres every 24 hours. But there was usually a 24 hour lag phase in the growth of isolates 1W, 3S and 6Sh which could usually be attributed to slow initial growth from the inoculum disk. Isolates 2B, 4N and 5Bg usually reached the edge of the Petri dish 24 hours before the other isolates.

At the end of each growth experiment the fungal colonies were used as inoculum for testing the pathogenicity of the isolates. Each experiment was set up as follows. A wheat seed was placed on each of 24 agar disks in a seed box of soil. One box of wheat seeds was inoculated with each isolate. The wheat seedlings were harvested four weeks later. The seedling seminal roots were examined under water against a white background, and the amount of disease estimated as in Method 3. The pathogenicity of each isolate was expressed as the percentage of wheat seedling seminal roots showing stelar lesions. (see Figure 2). After the first experiment each isolate was reisolated from infected wheat seedling roots and in the second and third experiments wheat seeds were inoculated with these reisolates as well as with the

Figure 2.

Infection by six isolates of Ophiobolus graminis of wheat seedlings. The seminal roots of the seedlings inoculated with isolate 3S appear to be less blackened by the fungus than those inoculated with the other isolates.


1W
2B
3S
4N
5Bg
6Sh
Uninoculated

original isolates. It was thought that the presence of the host plant might stimulate the isolates, so that when reisolated their pathogenicity would be markedly increased. The results of the experiments are shown in Table 11.

Table 11.
The pathogenicity of the six isolates of O. graminis, expressed as the percentage of wheat seedling seminal roots showing one or more stelar lesions.

Isolate	Experiment One	Two	Three
1W	97	45	80
Reisolate 1W	-	22	63
2B	92	2	90
Reisolate 2B	-	36	84
3S	50	8	61
Reisolate 3S	-	38	67
4N	100	47	90
Reisolate 4N	-	44	80
5Bg	100	18	86
Reisolate 5Bg	-	30	62
6Sh	94	26	80
Reisolate 6Sh	-	23	82

Extension to Table 11 in Appendix C.

These results show that five of the isolates were highly pathogenic on wheat. The sixth, 3S, was only moderately so. Infection was low in the second experiment. This was particularly pronounced with isolates 2B and 3S, and may have been caused by

different greenhouse conditions than those in the first and third experiments. The seedlings in the third experiment were again heavily infected, and there was only a slight drop in the pathogenicity in the six months interval between the first and third experiments. The pathogenicity of isolates 1W, 2B, 5Bg and 6Sh did not fall appreciably during their first six months in culture on agar slopes, and after nine years in culture for 4N. The pathogenicity of 3S was low at the time of isolation and had decreased steadily over the eighteen months it had been in culture.

Infection caused by the isolates and reisolates was similar with the exception of 2B and 3S in the second experiment. This could not have been due to the increased pathogenicity of the reisolates because in the third experiment the pathogenicity of the isolates and reisolates of 2B and 3S was almost the same. Reisolation of the isolates did not therefore affect their pathogenicity.

The next experiment was concerned with the persistence of the isolates in soil. Chaffed inoculum was buried in soil, in three proportions of inoculum to soil, and the survival of the fungus tested after one, two, four and eight months, and immediately the experiment was set up. At each sampling time wheat seedlings were sown in boxes of the inoculum soil mixture, and the survival of the isolates expressed as the percentage of seminal roots of

wheat seedlings showing stelar infection.

Chaff inoculum was prepared. The experimental procedure was carried out similarly for each isolate. After colonization, the inoculum was mixed with a loam soil in the following proportions on a volume basis:-

One part of inoculum to fifteen parts of soil

One part of inoculum to ten parts of soil

One part of inoculum to five parts of soil

Each of three deep seed boxes, one foot square, was filled with one of the inoculum-soil mixtures and a fourth with soil only as a control. This made a total of twenty four boxes, four for each of the six isolates. The boxes were laid out in rows on a table in the centre of a large greenhouse which was maintained at a temperature of about 21°C., and watered regularly. The boxes were sampled immediately and after one, two, four and eight months. At each sampling time approximately a sixth of the inoculum-soil mixture was removed from each box and placed in the bottom of a shallow seed box, which it half filled. Thirty wheat seeds were sown on the inoculum mixture in each box, and covered with a two centimetre layer of soil. They were left in an unheated greenhouse. Four weeks later the wheat seedlings were dug up and stored in polythene bags until examination. They were then washed and examined under water against a white background for stelar lesions

by Method 3. The results of the experiment are shown in Table 12.

Table 12.
Persistence of six isolates of Ophiobolus graminis in three proportions of chaffed inoculum to soil, expressed as a percentage of wheat seedling seminal roots with one or more stelar lesions.

Isolate	Proportion inoculum to soil	Months from inoculation 0	1	2	4	8
1W	1 : 5	94	75	59	33	2
	1 : 10	73	71	54	19	0
	1 : 15	68	64	47	16	0
2B	1 : 5	76	71	77	42	8
	1 : 10	72	64	48	10	7
	1 : 15	38	67	56	5	0
3S	1 : 5	41	32	16	0	0
	1 : 10	70	13	14	0	0
	1 : 15	50	9	7	0	0
4N	1 : 5	65	53	55	11	0
	1 : 10	67	58	53	16	6
	1 : 15	63	61	31	6	2
5Bg	1 : 5	73	89	68	37	5
	1 : 10	72	66	64	38	0
	1 : 15	62	63	71	32	2
6Sh	1 : 5	56	74	64	39	2
	1 : 10	53	77	59	35	2
	1 : 15	56	67	65	33	2
Mean infection of controls		0	9	17	0	0

Extension to Table 12 in Appendix C.

All the isolates were present, although at a low level, after eight months in the soil, with the exception of 3S. Infection of

the wheat seedlings by this isolate was low at the beginning of the experiment and fell rapidly, so that after four months it had almost completely disappeared. Infection was maintained at a higher level for the other isolates until the fourth month when there was a sudden drop in infection of about 30%.

At the first harvest the control seedlings could be picked out from the inoculated ones from the bright green colour of their shoots. The inoculated seedling shoots were shrivelled and yellow, and their roots blackened and brittle. The roots of the control seedlings were completely uninfected. The temperature dropped during the second week of seedling growth, and the length of daylight had shortened as it was then the end of November, so the temperature in the greenhouse was raised to 18°C. and artificial lights switched on. In spite of this, at harvest the seedlings were small and had only reached the two leaf stage.

In contrast the test seedlings for the one month sample were much larger, as a result of the higher temperature. This time there was little above ground evidence of the disease although the roots were heavily infected. But there was some rise in infection from the first harvest. This was particularly marked with isolate 6Sh. Some of the one month sample control seedlings were also infected. This infection arose not from contamination of the experimental soil, nor, it is thought, from a wild O. graminis

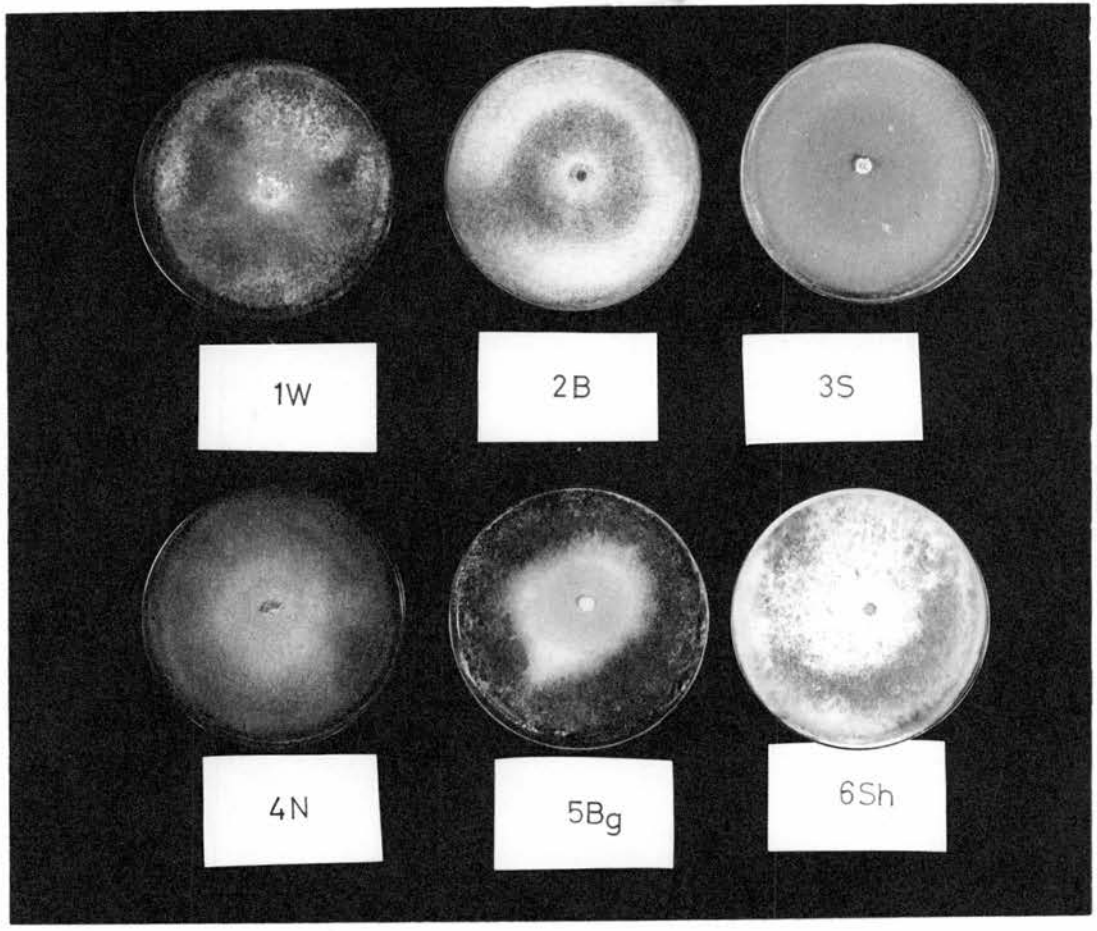
mycelium. After the first sample of seedlings had been dug up, the seed boxes were emptied before refilling with inoculum soil. But they were not washed out but simply scraped free of soil. Minute pieces of infected root must have been left adhering to the base of these boxes, and these pieces of infected root probably infected the next crop of control seedlings and helped to increase the infection of the inoculated seedlings. Unfortunately this was not noticed until after the second month control seedlings had been examined and these showed even greater infection. After this the seed boxes were properly cleaned out and at the four and eight month samples none of the control seedlings showed any signs of the disease.

By the fourth month, infection fell considerably and isolate 3S had disappeared from the inoculum soil. Four months later the other five isolates were only present at a very low level in the inoculum soil. It had originally been intended that a twelve month test should be made, but in view of the low infection of the eight month seedlings this was not undertaken. The proportions in which the inoculum and soil were mixed did not appear to affect the infection of the seedlings; the severity of seedling infection was not proportional to the amount of inoculum added to the soil.

No attempt has been made to differentiate between the morphological characters of the isolates growing in culture, but Figure 3 shows the isolates growing in culture. Padwick (1936)

Figure 3.

Cultures of the six isolates of Ophiobolus graminis growing on potato dextrose agar in plastic Petri dishes.


1W
2B
3S
4N
5Bg
6Sh

found cultural differences between his isolates. The characters of the six isolates varied considerably depending on the cultural medium and were not constant for that medium. Only one isolate always appeared similar in culture, namely 3S. It had a sparse, light coloured mycelium, with few aerial hyphae. White (1942) working with eight single-spore isolates from one ascus found that four of the eight isolates produced a light mycelium in culture and the remaining four a dark coloured mycelium. These characters were constant over a three year period of sub-culturing. This could account for the varying culture colour of the six isolates, as they were not ascospore, but mycelial cultures, and may have been formed from a number of ascospores.

These experiments have failed to show any real differences between five of the isolates, but the sixth, 3S, differed from the others in two respects:-

a) It was not very pathogenic on wheat.

b) It did not survive in soil longer than four months.

Garrett (1956) says that the rapidity of disappearance of O. graminis can be correlated with general microbiological activity of the soil. Also, conditions such as adequate soil moisture, good aeration and high temperatures hasten the disappearance of the fungus from the soil. The conditions in the greenhouse where this experiment was carried out were identical for all the isolates, so neither of Garrett's conclusions can apply to the more rapid

disappearance of isolate 3S. It is possible that this isolate did not colonize the chaff so completely as did the other isolates, or that it was not so tolerant of the conditions to which it was subjected. Henry and McKenzie (1959) think that different isolates of O. graminis may differ not only in pathogenicity, but in ability to overcome the deleterious action of other soil organisms or their products.

The other isolates were highly pathogenic on wheat and were found to survive in soil for up to eight months. Butler (1959) also found that O. graminis could survive in a fertile soil for eight months, but was not recovered in a fertile or infertile soil after 40 weeks. But Semenuik and Henry (1960), using a virulent isolate of the fungus, found that after three weeks the pathogen had declined to near zero, in both sterile and natural soil. This decline was greatest in natural soil, probably because of the presence of other micro-organisms.

Padwick (1936) tested the pathogenicity of his isolates of O. graminis on several varieties of wheat. He found that isolates causing the most severe injury to one wheat variety also caused the most severe injury to the others. This may not hold for different host species. Also different isolates vary in their invasion of different species of grass. Turner (1952) found that the results of pathogenicity tests on eight species of grass showed considerable variation in pathogenicity between different isolates of the same

variety of O. graminis. Using isolates of the fungus from wheat and oats and the eight species of grass, her results show that all the species were more susceptible to the oat isolates.

An experiment was designed to test the pathogenicity of the six O. graminis isolates on three different species of grass, Agropyron repens, Agrostis stolonifera and Holcus lanatus, by comparison with wheat. A. repens and A. stolonifera seedlings were chosen because they are respectively highly susceptible and moderately susceptible to attack by O. graminis. H. lanatus has been mentioned by several workers (Buddin and Garrett 1944, Garrett and Buddin 1947, Brooks 1965 and Walker 1945) as being susceptible to the take-all disease, although it has seldom been used in experimental work. This species was used in this experiment instead of Holcus mollis because the seed has a higher percentage germination and is present in greater quantities in the seed heads. It germinates better, and the seedlings develop more rapidly, making it a very suitable species for experimental work. A. repens rhizome pieces were included because on several occasions they had been found to be as susceptible to take-all infection as are wheat seedlings.

The material for inoculation had been growing for varying lengths of time. The A. repens seedlings had been growing for four weeks, and the rhizome pieces had been planted in soil to shoot also for four weeks. The A. stolonifera and H. lanatus seedlings had

been growing for ten weeks and six weeks respectively. Germination of the A. stolonifera seed was poor and development of those seedlings which did germinate was slow, so that after ten weeks growth the seedlings were only about four centimetres high and had few roots.

Twenty-seven seed boxes were filled to within one centimetre of the top with a soil mixture. This mixture contained four parts of sand to one of soil by volume. Agar inoculum of the six isolates was prepared. The grasses were inoculated using eleven millimetre agar disks of the fungus. There was one box of A. repens seedlings, one box of A. repens rhizome pieces, and one box of H. lanatus seedlings inoculated with each isolate. As there were only a limited number of A. stolonifera seedlings these were inoculated with isolates 2B, 5Bg and 6Sh only. Wheat seeds were inoculated with each of the isolates as controls. All the plants were harvested four weeks later, and examined for the presence of stelar lesions by Method 3. The pathogenicity of the isolates was expressed as the percentage of host roots with one or more stelar lesions. The results are shown in Table 13, page 75.

The wheat seedlings were, in general, most heavily infected by the isolates, followed in descending order by A. repens rhizome pieces, A. repens seedlings, H. lanatus seedlings and A. stolonifera seedlings. The results vary slightly depending on which isolate was

Table 13.
Pathogenicity of six isolates of Ophiobolus graminis on seedlings of Agropyron repens, Holcus lanatus and Agrostis stolonifera, and on rhizome pieces of Agropyron repens, expressed as a percentage of host roots with one or more stelar lesions.

Isolate	A. repens rhizome pieces	A. repens seedlings	H. lanatus seedlings	A. stolonifera seedlings	Wheat seedlings	Mean
1W	44	31	28	-	92	49
2B	47	40	42	25	100	51
3S	23	9	3	-	10	11
4N	47	53	38	-	86	56
5Bg	57	42	30	20	86	47
6Sh	44	29	29	15	71	38
Mean	44	34	28	20	74	

Extension to Table 13. in Appendix C.

used as inoculum, but there was nothing like the variation of infection between the isolates which Turner (1952) found. For example, A. repens rhizome pieces were less susceptible to isolate 4N than were the seedlings of this grass, which is contrary to the results obtained with the other isolates.

This experiment again emphasises the fact that A. stolonifera is less susceptible to O. graminis than is A. repens. It also shows that H. lanatus seedlings are almost as susceptible as are those of A. repens.

The results obtained with isolate 3S are particularly interesting. This isolate was again not very pathogenic on wheat, only ten per cent of the roots being infected. But more than twice this number of roots of A. repens rhizome pieces were infected. The A. repens seedlings were less infected by this isolate than were the wheat seedlings. These results suggest that isolate 3S is much more pathogenic on A. repens rhizome pieces than on any of the other host plants used in this experiment. This result is substantiated by an experiment described in Section 2, page 54, where Table 7 shows that isolate 3S caused more infection to A. repens rhizome pieces than to the wheat seedlings.

In this investigation
Different isolates of O. graminis did vary in their invasion of different species of grass. Although an isolate of this fungus may be only slightly pathogenic on wheat, nevertheless it may cause more infection of A. repens rhizome fragments.

SECTION 4

Susceptibility of clonal material of some cytotypes of Holcus mollis to Ophiobolus graminis.

Holcus mollis is known to exist as a number of cytotypes, (Valentine, 1949). Jones (1958) mentions the existence of four of these groups, the tetraploid (4n = 28), pentaploid (5n = 35), hexaploid (6n = 42) and heptaploid (7n = 49). The pentaploid appears to be by far the most common, and is adaptable to a wide range of conditions. So far the existence of only two heptaploid plants has been recorded. These were both found in Wales by Jones.

The grass spreads almost exclusively by vegetative rhizomes, and little of the seed produced is viable. The tetraploid is probably the most fertile and sets numbers of viable seed under conditions of open pollination. When growing together with diploid Holcus lanatus (2n = 14) crossing may occur and some of the resulting progeny will be triploid. Triploid hybrids are infertile and usually resemble H. mollis in external morphology. They are not generally as vigorous as H. mollis but Carroll and Jones (1962) think that they are capable of maintaining themselves in natural populations. Jones (1958) considers that the hexaploid is produced directly from the tetraploid by unilateral non-reduction. The pentaploid is probably produced from viable non-reduced triploid pollen and a normal tetraploid female H. mollis, and continues to form where H. mollis and H. lanatus come into sexual contact.

Seed of H. mollis is obtainable although in small quantities, and germination is usually uncertain. Difficulties were encountered with the collection of H. mollis seed for experimental work. That collected in the field failed to give adequate germination and most commercial seed merchants do not stock it. But two small samples were kindly provided by Newcastle University, both samples having been collected from the Peak District of Derbyshire. The plants from which the seed was collected were growing together with H. lanatus. After germinating the seed on moist germination pads chromosome counts were made of a random sample of seedling roots, and the chromosome numbers were found to range from 21 - 28. (See Materials and Methods, page 25 for staining and squashing for chromosome counts). The triploids were intraspecific hybrids and the aneuploids of doubtful origin.

Five samples of H. lanatus seed were obtained, one from each of the following places:-

a) Shotover, near Oxford.

b) Botanic Garden, Oxford.

c) Botany Department Experimental Gardens, Leeds University. Two samples, both from plants growing at Bishopdale Beck, Yorkshire.

d) Official Seed Testing Station, Cambridge.

Chromosome counts from a random selection of seedlings grown from

all five of the seed samples showed that they all had fourteen chromosomes and therefore were assumed to be seedlings of Holcus lanatus. (Hubbard, 1954).

An initial experiment was designed to compare the susceptibility of three of the samples of H. lanatus seed, that from Shotover, the Official Seed Testing Station, at Cambridge and the Botanic Garden, Oxford, to Ophiobolus graminis. The seed was germinated in pots of soil in a greenhouse. A chaff inoculum of isolate 1W was used, and the seedlings were six weeks old at the time of inoculation. There were two boxes of wheat seeds inoculated as a susceptible control, and two seed boxes for each of the three samples of seedlings, giving a total of eight seed boxes. One box of each sample of H. lanatus seedlings and one of wheat seedlings were harvested four and five weeks after inoculation. The results are shown in Table 14, page 81, and the disease was estimated by Method 3.

Infection of the seedlings had increased at the fifth week harvest with the exception of the Official Seed Testing Station seedlings where there was a decrease in severity. The increase of infection of the other seedlings was not very marked.

A second experiment was carried out to compare the susceptibility of the two samples of Holcus mollis seed from Newcastle University, and all five samples of Holcus lanatus seed. Germination of the H. mollis seed was poor, so only a limited number of these

Table 14.
Infection by Ophiobolus graminis (isolate 1W) of three samples of Holcus lanatus seedlings compared with wheat seedlings.

Host plant	Number plants in sample	% roots infected
H. lanatus seedlings		
Shotover		
4 week harvest	18	17
5 week harvest	25	28
O.S.T.S.		
4 week harvest	24	23
5 week harvest	23	14
Botanic Garden		
4 week harvest	23	30
5 week harvest	24	41
Wheat seedlings		
4 week harvest	21	67
5 week harvest	23	74

Extension to Table 14 in Appendix D.

seedlings were available, and so the plants were all harvested at one time. Agar inoculum of isolate 2B was prepared and all the seedlings were four weeks old at the time of inoculation. There was a total of eight boxes, one for each of the two samples of H. mollis seedlings, one for each of the five samples of H. lanatus seedlings and one for wheat seed inoculated as a susceptible control. The plants were harvested six weeks later and examined by Method 3. The results are shown in Table 15, page 82.

When examining the seedlings for signs of attack only those roots which were in initial contact with the inoculum disk were

Table 15.
Infection by Ophiobolus graminis (isolate 2B) of samples of Holcus mollis and Holcus lanatus seedlings compared with wheat seedlings.

Host plant	Number plants in sample	% roots infected
H. mollis seedlings		
Sample 1	23	19
Sample 2	11	2
H. lanatus seedlings		
Shotover	23	42
O.S.T.S.	25	30
Leeds Sample 1	22	20
Leeds Sample 2	24	4
Bot. Gdn.	23	4
Wheat seedlings	21	65

Extension to Table 15 in Appendix D.

examined for stelar lesions. The presence of lesions on roots formed at nodes above the crown was noted but not included in the numerical disease calculation. Only crown and seminal roots were examined for stelar lesions as the seedlings had produced large numbers of adventitious roots above the crown. This was in order that uniformity of disease assessment should be achieved. Infection of all the seedlings was irregular and varied within samples, except where infection was low. There was a great difference in infection of the H. lanatus samples. The Shotover plants were the smallest and the most heavily infected. Although the roots were severely blackened and some roots formed after inoculation were also infected, the shoots appeared green and healthy. By contrast the Leeds Sample 2

Figure 4.

A root tip cell of pentaploid Holcus mollis in the metaphase condition. The thirty-five chromosomes have been stained with aceto-orcein.

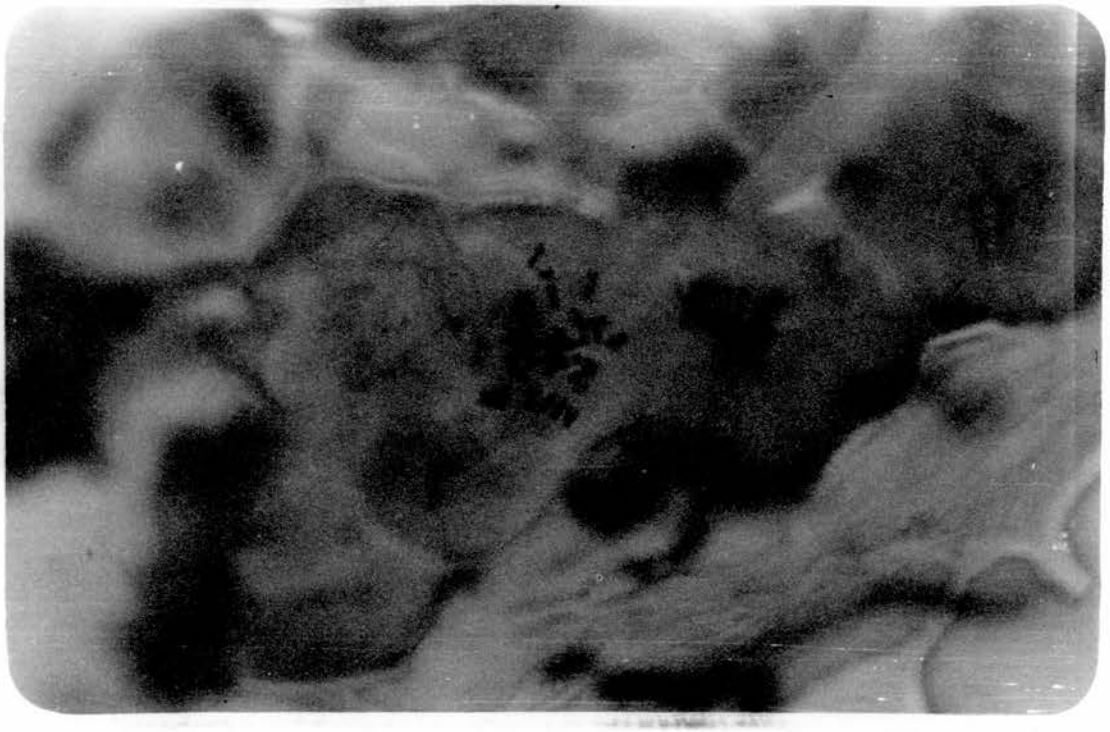


Figure 5.

A root tip cell of tetraploid Holcus mollis in the metaphase condition. The twenty-eight chromosomes have been stained with aceto-orcein.

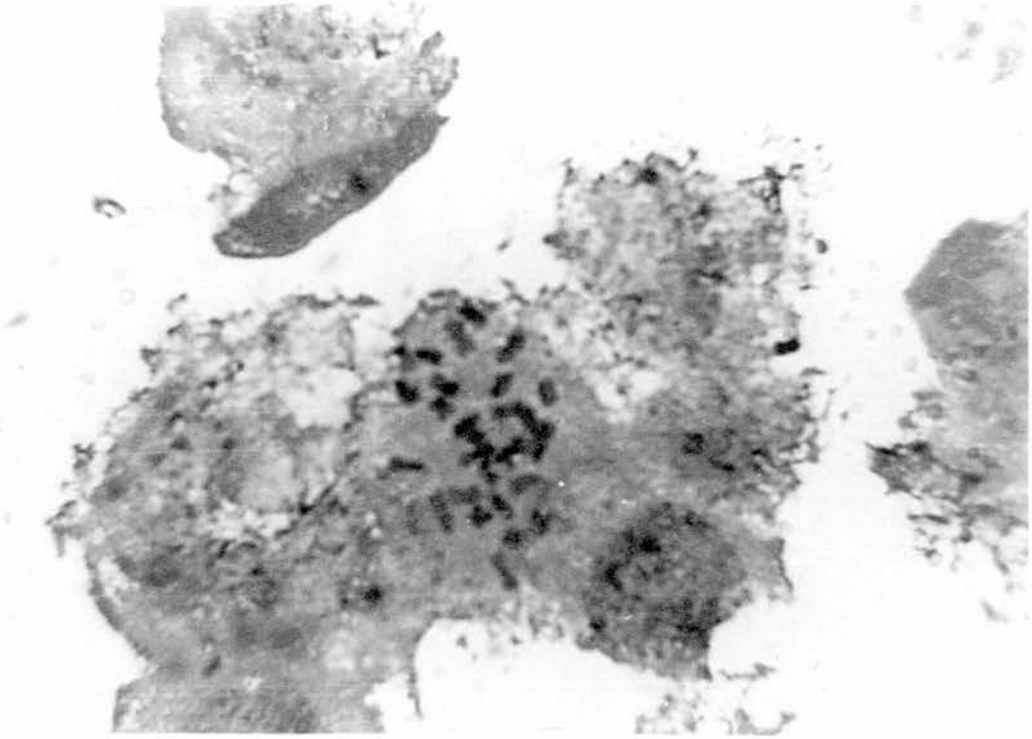
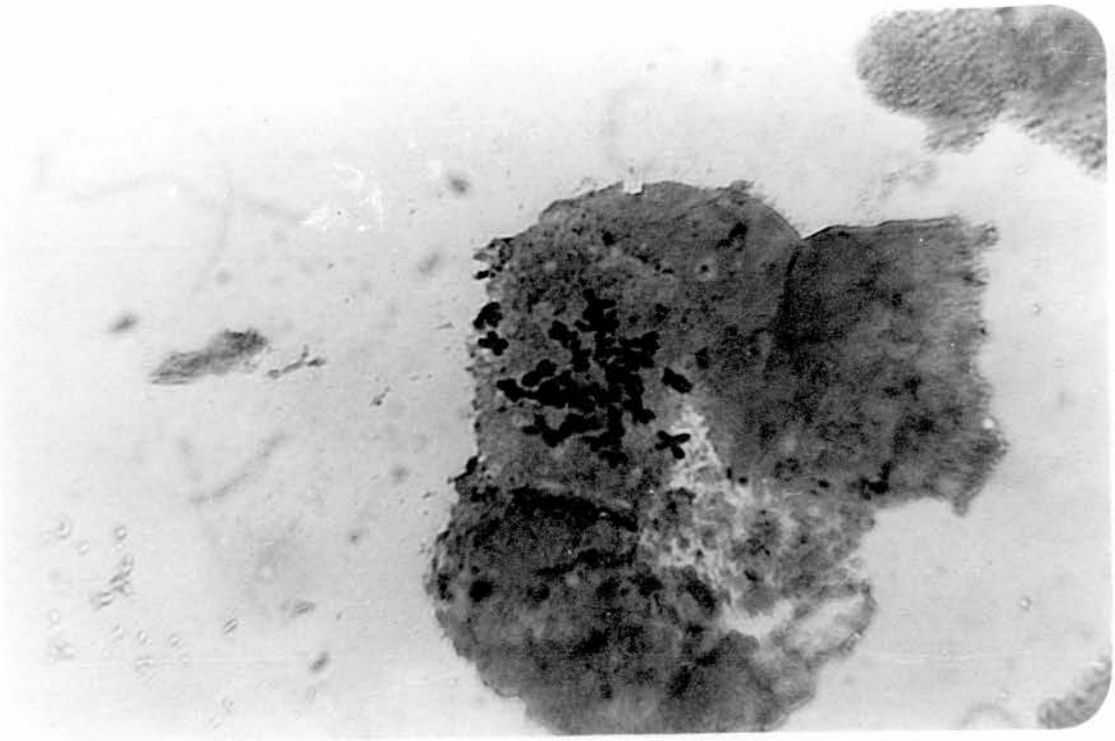


Figure 6.

A root tip cell of tetraploid Holcus mollis in the metaphase condition. The twenty-eight chromosomes have been stained with aceto-orcein.



and Botanic Garden seedlings were almost completely uninfected and were much larger.

The seedlings of both samples of H. mollis were small compared with the H. lanatus seedlings, but they were producing new uninfected rhizomes. However, the H. mollis Sample 2 seedlings were almost uninfected, while the infection of the H. mollis Sample 1 seedlings was almost ten times greater. Few conclusions can be drawn from the results because owing to poor germination of the seed, there were few plants in the samples, but it does seem that certain samples of seedlings may be more susceptible to the disease than others.

Rhizome material of H. mollis was collected from several places in the field in order that clones of the different cytotypes could be grown. The material was split into individual rhizomes and suitable lengths were washed free of soil and put into shallow dishes of water in a north-facing window. After about five days, adventitious roots, which were produced at the rhizome nodes, were a suitable length for staining and squashing for chromosome counts. (See Figures 4,5 and 6).

Chromosome counts were made of rhizome material from the following places:-

Locality	Cytotype	Chromosome number	Habitat
Nr. Bangor, North Wales	aneuploid	3n + 2 = 23	Woodland
Shotover, Nr. Oxford	tetraploid	4n = 28	Dry woodland
Little Brickhill, Bedfordshire	tetraploid	4n = 28	Barley crop on sandy soil

Locality	Cytotype	Chromosome number	Habitat
Little Brickhill, Bedfordshire	pentaploid	5n = 35	Sandy woodland
Pentland Hills, Nr. Edinburgh	pentaploid	5n = 35	
Hazeley Heath, Berkshire	pentaploid	5n = 35	Badly drained heathland
Woodhall Spa, Lincolnshire	pentaploid	5n = 35	Nr. stream, acid damp soil
Long Mynd, Shropshire	tetraploid	4n = 28	Heathland
Long Mynd, Shropshire	pentaploid	5n = 35	Heathland

Even such a small collection of plants as this shows that the pentaploid is the most common cytotype. Jones (1958) considers that the pentaploid is adaptable to a wider range of conditions than the tetraploid which is generally only found on drier soil. Certainly the soil is sandy and dry at Shotover where the tetraploid grows abundantly.

When the chromosome number of a rhizome was known, it was planted in a seed box of soil in a greenhouse to enable it to spread vegetatively, so that a plentiful supply of rhizomatous clonal material would be available for experimental work. Clones were produced of material from seven localities. (See Figures 7 and 8, and Table 16, page 85).

Sufficient clonal material for experimental work took almost nine months to produce owing to the slow forward growth of the rhizomes. Ovington and Scurfield (1956) found that plagiotropic

Figure 7.

Tetraploid clonal *Holcus mollis*.


S1
S3
S4

Figure 8.

Three pots of pentaploid clonal Holcus mollis, A1, A2 and HH, and one of aneuploid ($3n + 2 = 23$) H. mollis.

A1
A2
HH
W

rhizomes of H. mollis grow outwards from late March to October, with a maximum around June. The clones were started in January, hence the slow rate of rhizome production. The vegetative characters of the clones are given in Table 17, page 86.

Table 16.
Details of clonal material of Holcus mollis.

Serial number	Cytotype	Locality of collection
A1	pentaploid	Pentland Hills
A2	pentaploid	Pentland Hills
HH	pentaploid	Hazeley Heath
S1	tetraploid	Shotover
S3	tetraploid	Shotover
S4	tetraploid	Shotover
W	aneuploid ($3n + 2 = 23$)	Bangor

There was considerable variation in the vegetative characters of the clones, particularly in the hairiness of the plants. Ovington and Scurfield (1956) mention that there is great variation in the hairiness of H. mollis, and they maintain that conditions of shade affect this character. The leaves of plants growing in deep shade are less hairy, and the hairs are permanently reflexed. Those in moderate shade are more hairy. Table 17 shows that the pentaploid material was much more hairy than the tetraploid, and in general the hairs were longer. The tetraploid came from quite deep

Table 17. Vegetative characters of seven clones of Holcus mollis.

VEGETATIVE CHARACTER		PENTAPLOID CLONES 5n = 35			TETRAPLOID CLONES 4n = 28			ANEUPLOID CLONE 3n + 2 = 23
		A1	A2	HH	S1	S3	S4	W
Presence of hairs	Nodes	present	present	present	present	present	present	sometimes present
	Leaves - upper	present	present	present, long	absent	absent	present	sometimes present
	Leaves - lower	absent	present	present	absent	absent	absent	present
	Leaf sheaths	present	present	present	present	absent	absent	present
	Mid-rib	present	absent	present, long	absent	absent	absent	present
Rhizomes		vigorous and tough	vigorous and tough	vigorous, less than A1 or A2	fairly vigorous	fairly vigorous	fairly vigorous	tough, thick Dark purple. Few.
Leaves	Breadth (mm.)	5 - 10	5 - 10	1 - 5	5 - 10	1 - 5	1 - 5	5 - 10
	Colour	bright - dull green	dull green	dull green	bright - dull green	bright - dull green	bright - dull green	bright - dull green
Shoots	Kneed at base	absent	absent	absent	present	present	present	present
Ligule	Form	truncate, serrate	truncate, serrate	pointed serrate	pointed serrate	truncate, serrate	truncate, serrate	truncate, serrate
	Length (mm.)	1 - 5	1 - 2	1 - 5	1 - 5	1 - 2	1 - 2	1 - 5
Mid-rib								
Depression on upper leaf surface		present	present	present	present	present	present	absent
Prominent on lower leaf surface		present	present	present	present	present	present	present
Striping with anthocyanin		basal leaf sheaths dark purple, upper striped	striped sheaths	basal leaf sheaths dark purple, upper striped	striped sheaths	striped sheaths	lower sheaths striped	basal sheaths dark purple, upper striped

shade in an oak woodland area. The HH material was growing in the open away from shade and was particularly hairy, even the leaf mid-ribs were covered with hairs. By comparison with H. mollis, H. lanatus is usually more densely covered with hairs, but Beddows (1961a) says that H. lanatus can vary in hairiness from excessive to an almost glabrous form. Hubbard (1954) differentiates between H. mollis and H. lanatus by the hairiness of the nodes of the former grass. H. lanatus nodes are usually only downy.

The ligule length and shape varied between the clones, as did the breadth of the leaves. There was considerable variation in the colouring of the leaf sheaths with anthocyanin. The aneuploid was very darkly stained. Striping of the leaf sheaths is a characteristic of H. lanatus.

The aneuploid material was particularly interesting as this showed characters common to H. mollis and H. lanatus, although it seemed to resemble H. mollis in external morphology. It was only moderately rhizomatous and much larger than the other clones. The nodes were only sometimes hairy, similar to those of H. lanatus. Some of the flowering characters of the aneuploid were similar to those of H. mollis, for example the awns on the upper lemmas of the aneuploid were straight when dry. Those of H. lanatus are recurved.

An experiment was designed to compare the susceptibility of vegetative pieces of the seven clones to Ophiobolus graminis. The

six isolates of O. graminis were used to see if there was any difference in their invasion of the clones. Rhizomes of each of the clones were dug up, washed completely free of soil, and cut into single nodal pieces. Each piece was two centimetres long and the node was central. Rhizome pieces from each of the clones were planted in boxes of soil to enable them to produce roots and a shoot. Four weeks later all the pieces were dug up and washed free of soil.

All the material selected for this experiment was as similar as possible, reducing cutting variation between the vegetative fragments of a clone to a minimum. Each rhizome piece had a shoot about four centimetres high, and adventitious roots. Any variations were noted. Agar inoculum of potato dextrose agar plus one per cent malt was used, the disks being eleven millimetres in diameter. Seed boxes were filled to within one centimetre of the top with soil containing four parts of loam soil to one part of fine sand by volume. Shooting rhizome pieces of six of the clones were inoculated with each of the six O. graminis isolates. A limited number of the aneuploid rhizome pieces had produced shoots, so this material was inoculated with isolates 2B, 3S and 4N only. Wheat seeds were inoculated with each of the six isolates as susceptible controls. All 45 seed boxes were filled with the soil mixture, randomised, and left in a greenhouse at about 21° C.. Four weeks later all the plants were harvested, and the amount of disease was estimated by Method 3. The results are shown in Table 18.

Table 18.
Infection of clones of Holcus mollis by six isolates of Ophiobolus graminis, calculated as the percentage of primary adventitious roots arising from the rhizome piece with one or more stelar lesions.

Isolate	Wheat seedlings	Holcus mollis clones							Mean
		A1	A2	HH	S1	S3	S4	W	
1W	43	20	22	18	21	34	42	-	29
2B	51	25	32	15	27	18	23	44	29
3S	14	16	15	14	21	20	24	41	21
4N	10	20	32	24	18	48	42	42	29
5Bg	2	52	58	12	21	48	56	-	36
6Sh	58	49	49	40	49	55	54	-	51
Mean	30	30	35	21	26	37	40	42	

Extension to Table 18 in Appendix D.

Only the primary adventitious roots produced from the rhizome were examined for stelar lesions. Infection of secondary roots arising from the primary roots was ignored. Lesions on roots which were produced at nodes up the stem above the rhizome piece during the four week inoculation period were noted, but not used in the numerical disease calculation. The disease was calculated as the percentage of primary roots arising from the rhizome piece with one or more stelar lesions.

The results show great variation, and there is little pattern to be found, although infection with 6Sh was consistently high.

3S produced little infection of any of the clones, with the exception of the aneuploid which by comparison was heavily infected. The infection of the wheat seedlings was unusually low, particularly of those seedlings inoculated with 5Bg and 4N. These results were unexpected, as these isolates are generally highly pathogenic on wheat.

An analysis of variance carried out on the results in Table 18 showed that they were statistically significant. (Appendix F). In view of this a t-test was carried out with respect to the HH clone and this clone was found to be significantly less susceptible to attack by the O. graminis isolates, at the P.05 level, than clones S4, S3 and A2. This clone was not significantly less susceptible than clones S1 and A1 at the P.05 level. Clone S1 was found to be significantly less susceptible to the disease at the P.05 level than clone S4. The results can be divided into two groups which cut across the cytotypes. A1, HH and S1, which were not statistically different from each other, form one group, and S3, S4 and A2, which were more susceptible to O. graminis than the clones in the first group, form the other. There is no clear indication that the tetraploid and pentaploid differ in their susceptibility to O. graminis, but there is a difference within the cytotypes.

Although there are only limited results available, there is strong evidence from Table 18 that the aneuploid clone was much more

susceptible to O. graminis than the other clones. The results with this clone inoculated with isolate 3S are particularly high by comparison with the other clones inoculated with this isolate.

The results of this experiment show that some of the Holcus mollis clonal material was less susceptible to the disease than others. This would suggest that it may be that this resistance may be genetically determined.

CONCLUSIONS

The following conclusions can be drawn from this work:-

1. Holcus mollis rhizome pieces were extremely susceptible to attack by Ophiobolus graminis.

2. Agropyron repens rhizome pieces were also extremely susceptible to attack by O. graminis, but Agrostis stolonifera stolon pieces were only moderately susceptible.

3. H. mollis and A. repens rhizome lengths, and A. stolonifera stolon lengths, were susceptible to attack by this fungus.

4. Holcus spp. and A. stolonifera seedlings were more resistant to take-all than the vegetative fragments of these grasses. However, although these seedlings showed little stelar infection, they supported large numbers of runner hyphae on their roots which could be a danger to succeeding cereal crops.

5. Of the three grass weeds tested, A. stolonifera was found to be the most resistant to take-all.

6. The position of A. repens and A. stolonifera rhizome or stolon nodes in relation to the terminal growing point was found to be important in their susceptibility to attack by O. graminis. The older vegetative fragments were more heavily attacked by the fungus.

7. The age of seedlings of A. repens over the range of nine to eleven weeks, and of H. mollis over the range of two to six weeks at inoculation, did not affect their susceptibility to take-all. Size may be a more important factor, as the smaller A. repens

seedlings were less heavily attacked, although they were older.

8. There was a difference in the infection of the same host material caused by different isolates of O. graminis. New root production by the host plant, as well as initial host resistance and a possible difference in the growth rates of the take-all isolates may be significant factors with regard to infection.

9. The validity of five of the six O. graminis isolates used in this work was determined by measurements of ascospore length. The sixth isolate (1W) did not produce ascospores, but was considered to be Ophiobolus graminis as its cultural and infection characteristics were similar to those of the other five isolates.

10. Measurements of the daily increase of colony diameter by the six isolates divided them into two groups,

a) Those which had an initial twenty-four hour lag phase before growth commenced and

b) Those which had no appreciable lag phase.

11. Pathogenicity tests showed that five of the isolates were highly pathogenic on wheat. The sixth (3S) was only moderately pathogenic on wheat. Re-isolation from infected wheat roots had little effect on the pathogenicity of the isolates.

12. Five of the isolates persisted as chaffed inoculum in unsterile soil in a heated greenhouse for up to eight months. The sixth (3S) had almost disappeared from the soil after four months.

13. The severity of attack of O. graminis on A. repens, H. lanatus, A. stolonifera and wheat seedlings, and on A. repens rhizome pieces, was found to vary according to the isolate used as inoculum. One isolate (3S) caused twice as much infection of A. repens rhizome pieces as of wheat seedlings. Wheat seedlings were found to be the most susceptible to all the isolates followed by A. repens rhizome pieces, A. repens seedlings, H. lanatus seedlings and A. stolonifera seedlings.

14. Samples of H. lanatus seed, obtained from different parts of the British Isles, were found to differ in their susceptibility to O. graminis. Two samples of seedlings of H. mollis, which were triploid and aneuploid, also differed in their susceptibility.

15. Seven clones of three different cytotypes of vegetative H. mollis, three pentaploid, three tetraploid and one aneuploid, obtained from different localities and habitats, were found to vary considerably with respect to some of their vegetative characters.

16. These clones were found to differ in their susceptibility to O. graminis depending on the isolate used as inoculum. The results of an experiment with the seven clones inoculated with the six O. graminis isolates showed that the clones could be divided into two groups depending on their susceptibility to the fungus.

a) Those which did not differ statistically from each other and

b) Those which were more susceptible to O. graminis than the

clones in the first group. The grouping cut across the cytotypes, two pentaploid clones and one tetraploid being included in the first group, and one pentaploid and two tetraploid clones in the second.

17. The pathogenicity of the six O. graminis isolates was found to vary with different experiments. In the final experiment with the seven H. mollis clones, one isolate was found to be statistically significantly more pathogenic on the clones than any of the other five isolates. In this experiment therefore the isolates could be divided into two groups,

a) Containing five isolates which were less pathogenic and

b) Containing the remaining isolate (6Sh) which was more pathogenic on all the H. mollis clones.

However, one isolate in the first group was found to be statistically only slightly less pathogenic than the isolate in the second group.

APPENDICES

APPENDIX A.

Potato dextrose agar

1,000ml. potato stock

20g. glucose

15g. 'Oxoid' Ionagar

If desired, 1% yeast and / or 1% malt extract

Potato stock was made by boiling 200g. of potato in 500ml. of demineralized water, and after filtering through cotton wool, making the volume up to one litre. The glucose and Ionagar were added to the stock and the yeast or malt extract if used. The solution was then sterilized in an autoclave for 20 minutes at 20lb. pressure per square inch.

Oatmeal agar

70g. oatmeal

1,000ml. tap water

15g. 'Oxoid' Ionagar

The oatmeal and water were steamed together for two hours, filtered through cotton wool and made up to one litre with tap water. The Ionagar was added and the solution sterilized in an autoclave for 20 minutes at 20lb. pressure per square inch.

Lilley and Barnett's agar

10g. carbon source

2g. Asparagine

1g. KH_2PO_4

0.5g. $MgSO_4 7H_2O$

0.2g. Fe^{+++}

0.2g. Zn^{++}

0.1mg. Mn^{++}

5µg. Biotin

100µg. Thiamine

20g. agar

1,000ml. distilled water

The solution was sterilized in an autoclave for 20 minutes at 20lb. pressure per square inch.

APPENDIX B.

Extension to Table 7, Section 2, page 54.

Host plant		Average number roots per plant	Average number infected roots per plant	Runner hyphae only
Isolate 5Bg.				
A. repens seedlings				
Age at time of inoc.	9 weeks old	7.2	4.2	-
	10 weeks old	7.8	4.6	-
	11 weeks old	7.1	3.1	-
A. repens rhizome pieces				
	"Old"	7.4	5.8	-
	"Young"	7.3	5.0	-
Wheat seedlings		4.4	3.2	-
Isolate 3S.				
A. repens seedlings				
Age at time of inoc.	9 weeks old	6.8	2.2	-
	10 weeks old	6.5	2.8	-
	11 weeks old	6.6	1.9	-
A. repens rhizome pieces				
	"Old"	7.3	4.1	-
	"Young"	7.7	3.4	-
Wheat seedlings		4.5	1.6	-

Extension to Table 8, Section 2, page 57.

Host plant	Average number roots per plant	Average number infected roots per plant	Runner hyphae only
A. repens seedlings			
Age at 5 weeks old	7.5	5.4	-
time of 6 weeks old	6.6	5.3	-
inoc. 7 weeks old	6.7	5.4	-
A. repens rhizome pieces			
4 weeks shooting	9.2	8.5	-
5 weeks shooting	7.7	7.4	-
Wheat seedlings	5.7	4.5	-

Extension to Table 9, Section 2, page 59.

Host plant	Average number roots per plant	Average number infected roots per plant	Runner hyphae only
Isolate 4N			
A. repens seedlings			
4 week harvest	6.3	4.2	-
5 week harvest	7.1	6.2	-
6 week harvest	6.1	3.7	-
Wheat seedlings			
4 week harvest	4.7	1.6	-
5 week harvest	4.5	2.6	-
6 week harvest	5.0	3.4	1
Isolate 5Bg			
A. repens seedlings			
4 week harvest	7.0	5.5	-
5 week harvest	5.6	3.8	-
6 week harvest	5.4	4.4	-
Wheat seedlings			
4 week harvest	4.2	2.8	-
5 week harvest	4.1	3.7	-
6 week harvest	4.9	3.4	-

APPENDIX C.

Extension to Table 11, Section 3, page 65.

Isolate	Number plants in sample			Average number roots per plant			Average number infected roots per plant		
Experiment	1	2	3	1	2	3	1	2	3
1W	26	26	21	3.9	4.2	4.4	3.8	1.9	3.5
Reisolate 1W	-	25	21	-	4.6	5.6	-	1.0	3.5
2B	23	28	16	3.6	4.2	4.2	3.3	0.1	3.8
Reisolate 2B	-	29	19	-	4.7	4.4	-	1.7	3.7
3S	22	28	17	3.2	4.0	5.6	1.6	0.3	3.4
Reisolate 3S	-	25	18	-	4.8	5.2	-	1.8	3.5
4N	24	29	17	3.4	4.7	5.0	3.4	2.2	4.5
Reisolate 4N	-	27	15	-	4.5	4.6	-	2.0	3.7
5Bg	24	27	20	3.8	4.0	4.3	3.8	0.7	3.7
Reisolate 5Bg	-	28	18	-	5.3	5.0	-	1.6	3.1
6Sh	22	29	20	3.6	3.5	4.5	3.4	0.9	3.6
Reisolate 6Sh	-	25	21	-	3.9.	4.9	-	0.9	4.0

Extension to Table 12, Section 3, page 68.

Isolate	Proportion inoculum to soil	Number plants in sample					Average number of roots per plant					Average number of infected roots per plant				
		Months from inoculation					Months from inoculation					Months from inoculation				
		0	1	2	4	8	0	1	2	4	8	0	1	2	4	8
1W	1 : 5	24	26	26	27	25	3.2	4.4	4.4	3.3	5.2	3.0	3.3	2.6	1.1	0.1
	1 : 10	22	28	28	26	26	3.3	4.1	3.9	3.7	5.4	2.4	2.9	2.1	0.7	0.0
	1 : 15	29	26	25	26	23	3.4	4.4	3.6	3.8	**5.9**	2.3	2.8	1.7	0.6	0.0
2B	1 : 5	28	25	26	28	26	3.7	3.8	3.9	3.6	5.0	2.8	2.7	3.0	1.5	0.4
	1 : 10	22	27	24	29	25	3.2	4.2	4.0	4.1	5.6	2.3	2.7	1.9	0.4	0.4
	1 : 15	22	27	28	26	29	3.7	4.3	4.3	4.4	5.9	1.4	2.9	2.4	0.2	0.0
3S	1 : 5	24	25	26	31	31	3.4	3.8	3.1	3.5	6.6	1.4	1.2	0.5	0.0	0.0
	1 : 10	25	26	25	28	26	2.7	3.1	3.5	3.8	5.8	1.9	0.4	0.5	0.0	0.0
	1 : 15	24	32	25	26	26	3.0	3.4	3.0	3.5	4.6	1.5	0.3	0.2	0.0	0.0
4N	1 : 5	26	26	23	29	27	3.7	3.4	3.1	3.5	5.0	2.4	1.8	1.7	0.4	0.0
	1 : 10	25	27	24	28	24	4.2	3.3	3.0	**3.8**	5.0	2.8	1.9	1.6	0.6	0.3
	1 : 15	25	24	25	28	30	4.1	3.3	3.2	3.6	5.0	2.6	2.0	1.0	0.2	0.1
5Bg	1 : 5	25	27	24	26	28	2.6	3.6	3.4	4.3	5.5	1.9	3.2	2.3	1.6	0.3
	1 : 10	28	21	27	27	30	3.5	4.1	3.9	4.2	5.2	2.5	2.7	2.5	1.6	0.0
	1 : 15	24	28	27	27	27	3.4	4.3	3.4	4.1	5.4	2.1	2.7	2.4	1.3	0.1
6Sh	1 : 5	25	28	24	25	27	3.6	3.5	5.2	4.6	5.6	2.0	2.6	3.3	1.8	0.1
	1 : 10	21	25	29	25	29	3.6	4.3	3.9	3.4	6.0	1.9	3.3	2.3	1.2	0.1
	1 : 15	19	28	25	26	27	3.6	4.5	4.0	3.7	5.3	2.0	3.0	**2.6**	1.2	0.1

Extension to Table 13, Section 3, page 75.

Isolate	Host plant	Number plants in sample	Average number roots per plant	Average number infected roots per plant
1W	A. repens seedlings	18	3.5	1.1
	A. repens rhizome pieces	18	6.4	2.8
	H. lanatus seedlings	20	11.4	3.2
	A. stolonifera seedlings	-	-	-
	Wheat seedlings	13	3.6	3.3
2B	A. repens seedlings	15	4.7	1.9
	A. repens rhizome pieces	18	6.8	3.2
	H. lanatus seedlings	17	10.1	4.2
	A. stolonifera seedlings	16	7.9	2.0
	Wheat seedlings	13	4.2	4.2
3S	A. repens seedlings	13	2.1	0.2
	A. repens rhizome pieces	17	5.2	1.2
	H. lanatus seedlings	18	8.7	0.3
	A. stolonifera seedlings	-	-	-
	Wheat seedlings	15	3.0	0.3
4N	A. repens seedlings	14	3.4	1.8
	A. repens rhizome pieces	17	4.7	2.2
	H. lanatus seedlings	17	11.8	4.5
	A. stolonifera seedlings	-	-	-
	Wheat seedlings	16	3.5	3.0
5Bg	A. repens seedlings	16	4.5	1.9
	A. repens rhizome pieces	16	6.1	3.5
	H. lanatus seedlings	16	10.0	3.0
	A. stolonifera seedlings	12	8.0	1.6
	Wheat seedlings	9	3.6	3.1
6Sh	A. repens seedlings	16	3.5	1.0
	A. repens rhizome pieces	18	6.2	2.7
	H. lanatus seedlings	17	12.0	3.5
	A. stolonifera seedlings	7	5.9	0.9
	Wheat seedlings	7	3.4	2.4

APPENDIX D.

Extension to Table 14, Section 4, page 81.

Host plant	Average number roots per plant	Average number infected roots per plant	Runner hyphae only
H. lanatus seedlings			
Shotover			
4 week harvest	7.2	1.2	-
5 week harvest	7.4	2.1	-
O.S.T.S.			
4 week harvest	6.4	1.5	-
5 week harvest	6.3	0.9	-
Botanic Garden			
4 week harvest	8.3	2.5	-
5 week harvest	7.0	2.9	-
Wheat seedlings			
4 week harvest	5.1	3.4	-
5 week harvest	4.6	3.4	-

Extension to Table 15, Section 4, page 82.

Host plant	Average number roots per plant	Average number infected roots per plant	Runner hyphae only
H. mollis seedlings			
Sample 1	4.7	0.9	-
Sample 2	5.0	0.1	1
H. lanatus seedlings			
Shotover	5.7	2.4	-
O.S.T.S.	5.0	1.5	-
Leeds Sample 1	5.0	1.0	-
Leeds Sample 2	5.3	0.2	-
Bot. Gdn.	5.3	0.2	-
Wheat seedlings	5.8	3.8	-

Extension to Table 18, Section 4, page 89.

Host plant	Isolate 1W	2B	3S	4N	5Bg	6Sh
PENTAPLOID CLONES 5n = 35						
A1						
Number plants in sample	23	24	24	23	24	23
Average number roots per plant	5.5	6.5	6.4	6.1	4.4	6.1
Average number inf. roots per plant	1.1	1.6	1.0	1.2	2.3	3.0
A2						
Number plants in sample	24	24	24	24	24	24
Average number roots per plant	6.0	6.9	8.4	7.8	7.8	6.8
Average number inf. roots per plant	1.3	2.2	1.3	2.5	4.5	3.3
HH						
Number plants in sample	23	23	24	24	12	12
Average number roots per plant	7.4	6.6	6.6	7.4	6.5	7.5
Average number inf. roots per plant	1.3	1.0	0.9	1.8	0.8	3.0
TETRAPLOID CLONES 4n = 28						
S1						
Number plants in sample	20	24	23	23	24	24
Average number roots per plant	7.5	6.7	6.3	6.8	5.8	6.3
Average number inf. roots per plant	1.6	1.8	1.3	1.2	1.2	3.1
S3						
Number plants in sample	24	24	23	24	24	24
Average number roots per plant	5.9	6.2	4.9	5.2	5.2	5.5
Average number inf. roots per plant	2.0	1.1	1.0	2.5	2.5	3.0
S4						
Number plants in sample	23	24	24	24	23	23
Average number roots per plant	5.7	6.0	5.8	5.5	5.9	5.9
Average number inf. roots per plant	2.4	1.4	1.4	2.3	3.3	3.2
ANEUPLOID CLONE 3n + 2 = 23						
W						
Number plants in sample	-	7	8	8	-	-
Average number roots per plant	-	7.7	4.9	3.8	-	-
Average number inf. roots per plant	-	3.4	2.0	1.6	-	-

Extension to Table 18 continued.

Host plant	Isolate					
	1W	2B	3S	4N	5Bg	6Sh
Wheat seedlings						
Number plants in sample	18	21	19	18	17	19
Average number roots per plant	5.3	4.7	4.3	4.2	5.0	4.7
Average number inf. roots per plant	2.3	2.4	0.6	0.4	0.1	2.7

APPENDIX E.

Mono-bromonaphthalene

Add five drops of mono-bromonaphthalene to 50ml. of tap water. Leave to stand in a dark stoppered bottle. Shake vigorously before use.

Aceto-orcein (1%) (Ritter, 1958)

1g. orcein

45ml. glacial acetic acid

55ml. distilled water

Heat the acetic acid in an Erlenmayer flask until moderately warm. Remove from the flame and add the orcein, shaking rapidly. The distilled water must be added immediately if the dye is to dissolve. Place the stoppered flask in cold running water, shaking occasionally until cold. Shake the flask again well, and transfer to a cork-stoppered narrow-necked bottle. Stand overnight and filter as used. The staining properties improve with age.

Aceto-orcein (2%)

2g. orcein

45ml. glacial acetic acid

55ml. distilled water

Prepare as above. To every nine parts of two per cent aceto-orcein solution add one part of one Normal hydrochloric acid.

APPENDIX F.

The calculations in the Analysis of variance on the results presented in Table 18, page 89, were done by Professor A.K. Weaver, Royal Military College of Science, Shrivenham.

Analysis of variance.

The results of the wheat seedlings (control) were omitted.

a) Between individual elements.

$$n = 42$$

$$\Sigma x = 1432.1$$

$$\Sigma x^2 = 58764.2$$

$$(\Sigma x)^2 = 2050910.4$$

$$\frac{(\Sigma x)^2}{n} = 48831.2$$

$$\Sigma x^2 - \frac{(\Sigma x)^2}{n} = 9933.0$$

b) Between Holcus mollis clones.

$$n = 7$$

$$\Sigma x = 1432.1$$

$$\Sigma x^2 = 312839.6$$

$$\frac{(\Sigma x)^2}{n} = 292987.2$$

$$\Sigma x^2 - \frac{(\Sigma x)^2}{n} = 19852.4$$

$$\frac{1}{6}\left(\Sigma x^2 - \frac{(\Sigma x)^2}{n}\right) = 3308.4$$

c) Between Ophiobolus graminis isolates.

$$n = 6$$

$$\Sigma x = 1432.1$$

$$\Sigma x^2 = 374206.6$$

$$\frac{(\Sigma x)^2}{n} = 341818.4$$

$$\Sigma x^2 - \frac{(\Sigma x)^2}{n} = 32388.2$$

$$\frac{1}{7}\left(\Sigma x^2 - \frac{(\Sigma x)^2}{n}\right) = 4626.9$$

	Degrees of freedom	Sum of squares	Mean square
Between clones	6	3308.7	551.5
Between isolates	5	4626.9	925.4
Residual	27	1997.4	74.0
Total	38	9933.0	

Tests of significance.

Between clones $r = \frac{551.5}{74.0} = 7.5$

$n_1 = 6, \quad n_2 = 27, \quad r\ 5\% \simeq 2.5$

This result is highly significant.

Between isolates $r = \frac{925.4}{74.0} = 12.5$

$n_1 = 5, \quad n_2 = 27, \quad r\ 5\% \simeq 2.6$

This result is also highly significant.

t-tests.

Using the HH and S3 *Holcus mollis* clones:-

		A HH	B S3
Number of observations		18 15 14 24 12 40	34 18 20 48 48 55
		$nA = 6$	$nB = 6$
		$\Sigma A = 123$	$\Sigma B = 223$
		$\bar{A} = 21$	$\bar{B} = 37$
		$\Sigma(A^2) = 3065$	$\Sigma(B^2) = 9513$
Common factor	$\frac{(\Sigma y)^2}{n}$,	$CFA = 2521$	$CFB = 8288$
Sum of squares	$\Sigma y^2 - CF$,	$SSA = 544$	$SSB = 1225$
Degrees of freedom	$n - 1$,	$DFA = 5$	$DFB = 5$
Variance	$\frac{SSA}{DFA}$,	$VA = 109$	$VB = 245$

$$\text{Common variance } Vcom = \frac{SSA + SSB}{DFA + DFB} = 176.9$$

$$\text{Standard error } SEd = \sqrt{\frac{Vcom}{nA} + \frac{Vcom}{nB}} = 5.5$$

$$t\text{-ratio} = \frac{\bar{A} - \bar{B}}{SEd} = 2.9$$

Tabulated t for P.05 and DFA + DFB degrees of freedom is 2.23. This is less than the calculated t-ratio, therefore the means are significantly different at the 5% level.

Tests with the other clones and isolates were carried out similarly.

BIBLIOGRAPHY

BEDDOWS, A.R. (1961a) Biological flora of the British Isles. *Holcus lanatus* L. J. Ecol. 49, 421 - 430.

BROOKS, D.H. (1965) Wild and cultivated grasses as carriers of the take-all fungus (*Ophiobolus graminis*). Ann. appl. Biol. 55, 307 - 316.

BROOKS, D.H. (1965a) Root infection by ascospores of *Ophiobolus graminis* as a factor in epidemiology of the take-all disease. Trans Brit. Mycol. Soc. 48, 237 - 248.

BUDDIN, W. and GARRETT, S.D. (1944) Take-all of cereals in 1943. J. Minist. Agric. 51, 108 - 110.

BUSSMANN, B. (1936) Untersuchungen uber die Virulenz von *Ophiobolus graminis* Sacc.. Phytopath. Z. 9, 571 - 581.

BUTLER, F.C. (1959) Saprophytic behaviour of some cereal root-rot fungi. 4. Saprophytic survival in soils of high and low fertility. Ann. appl. Biol. 47, 28 - 36.

CARROLL, C.P. and JONES, K. (1962) Cytotaxonomic studies in *Holcus*. 3. A morphological study of the triploid F_1 hybrid between *Holcus lanatus* and *Holcus mollis* L. New Phytol. 61, 72 - 84.

DAVIES, F.R. (1935) Superiority of silver nitrate over mercuric chloride for surface sterilization in the isolation of Ophiobolus graminis. Canad. J. Res. C13, 168 - 173.

FENTON, E. Wyllie (1948) Some notes on Holcus mollis L. Ann. appl. Biol. 35, 290 - 291.

GARRETT, S.D. (1936) Soil conditions and the take-all disease of wheat. Ann. appl. Biol. 23, 667 - 699.

GARRETT, S.D. (1941) Soil conditions and the take-all disease of wheat. 7. Survival of Ophiobolus graminis on the roots of different grasses. Ann. appl. Biol. 28, 325

GARRETT, S.D. (1950) Ecology of the root inhabiting fungi. Biol. Rev. 25, 220 - 254.

GARRETT, S.D. (1956) Biology of root inhabiting fungi. Cambridge University Press.

GARRETT, S.D. and BUDDIN, W. (1947) Control of take-all under the Chamberlain system of intensive barley growing. J. Minist. Agric. 54, 425 - 426.

GOTTLIEB, D., VOLOVSKY de HERNANDEZ, D., and ROGERS, M. (1958) The resistance of various grasses to Ophiobolus graminis. Plant Dis. Rept. 42, 26 - 29.

GRAINGER, J. (1935) Studies on plant root systems. Naturalist no. 943, 169.

HENRY, A.W. and McKENZIE, A.R. (1959) Note on the comparative activity of monosporous and mycelial isolates of Ophiobolus graminis Sacc. from the same source, as wheat pathogens in natural and sterilized soil. Canad. J. Pl. Sci. 39, 405 - 407.

HUBBARD, C.E. (1954) Grasses. A guide to their structure, identification, use and distribution in the British Isles. Penguin Books, Middlesex.

JONES, K. (1958) Cytotaxonomic studies in Holcus mollis L. 1. The chromosome complex in Holcus mollis L. New Phytol. 57, 191 - 210.

LILLEY, V.G. and BARNETT, H.C. (1947) The influence of pH and certain growth factors on mycelial growth and perithecial formation by Sordaria fimicola. Amer. J. Bot. 34, 131 - 138.

MANN, Harold H. and BARNES, S.T.W. (1947) The competition between barley and certain weeds under controlled conditions. 12. Competition with Holcus mollis. Ann. appl. Biol. 34, 252 - 266.

O'MARA, J.G. (1948) Acetic acid methods for chromosome studies at prophase and metaphase in meristems. Stain Tech. 23, 201 - 204.

OVINGTON, J.D. and SCURFIELD, G. (1956) Biological flora of the British Isles L.C. (Ed. 2) No. 2147. Holcus mollis L. J. Ecol. 44, 272 - 280.

PADWICK, G.W. (1935) Influence of wild and cultivated plants on the multiplication, survival and spread of cereal foot-rotting fungi in the soil. Canad. J. Res. 12, 575 - 589.

PADWICK, G.W. (1936) Biologic strains of Ophiobolus graminis Sacc. Ann. appl. Biol. 23, 45 - 56.

PADWICK, G.W. and HENRY, A.W. (1933) The relation of species of Agropyron and certain other grasses to the foot-rot problem of wheat in Alberta. Canad. J. Res. 8, 349 - 363.

PALMER, J.H. (1958) Studies in the behaviour of the rhizome of Agropyron repens (L) Beauv.. 1. Seasonal development and growth of the parent plant and rhizome. New Phytol. 57, 145 - 159.

RATTENBURY, J.A. (1956) A rapid method for permanent aceto-carmine squash preparations. Nature, 177, 1185 - 1186.

RITTER, Hilda B. (1958) A simple permanent aceto-orcein stain for free cells, cultures and homogenates. Stain Tech. 33, 167 - 170.

ROBINSON, R.K. (1963) A study of some aspects of the biology of certain root-infecting fungi. D. Phil. thesis, Oxford University.

ROBINSON, R.K. and LUCAS, R.L. (1963) The use of isotopically labelled mycelium to investigate the host range and rate of spread of Ophiobolus graminis. New Phytol. 62, 50 - 52.

RUSSELL, R.C. (1934) Studies of take-all and its causal organism, Ophiobolus graminis Sacc. Dom. Canad. Dept. Agric. Bull. N.S. 170.

SALLANS, B.J. (1965) Root rots of cereals 3. Bot. Rev. 31, 505 - 536.

SEMENUIK, G. and HENRY, A.W. (1960) Relative decline of Ophiobolus graminis, Helminthosporium sativum, and Fusarium culmorum in the soil. Canad. J. Pl. Sci. 40, 288 - 294.

THOMAS, D. (1966) Unpublished communication.

TJIO, Joe Hin, and LEVAN, Albert. (1950) The use of oxyquinoline in chromosome analysis. Annales de la Estacion Experimental de Aula Dei. 2, 21 - 64.

TURNER, Elizabeth M. (1940) Ophiobolus graminis Sacc. var avenae var N., as the cause of take-all or white-heads of oats in Wales. Trans. Brit. Mycol. Soc. 24, 269 - 281.

TURNER, Elizabeth M. (1952) The nature of the resistance of oats to the take-all fungus. J. exp. Bot. 4, 264 - 271.

VALENTINE, D.H. (1949) The units of Experimental Taxonomy. Acta Biotheoretica. 9, 75 - 88.

WALKER, Alan G. (1945) Rhizomatous grass weeds and Ophiobolus graminis Sacc.. Ann. appl. Biol. 32, 177 - 178.

WEHRLE, Valerie, and OGILVIE, L. (1955) Effect of ley grasses and the carry-over of take-all. Plant Path. 4, 111 - 113.

WESTE, Gretna, and THROWER, L.B. (1963) Production of perithecia and micro-conidia in culture by Ophiobolus graminis. Phytopath. 53, 354

WHITE, N.H. (1942) The genetics of Ophiobolus graminis Sacc. 1. Heritable variations for culture colour and pathogenicity. J. Coun. sci. industr. Res. Aust. 15, 118 - 124.