THE UNIVERSITY OF ALBERTA

STUDIES ON THE PATHOGENICITY AND COMPETITIVE ABILITY OF MONOSPOROUS AND MYCELIAL ISOLATES OF *OPHIOBOLUS GRAMINIS* Sacc.

A THESIS

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ABSTRACT

Monosporous and mycelial isolates of Ophiobolus graminis, from the same source, exhibited wide variations in cultural characteristics when grown on potato sucrose agar. These cultural variations generally did not appear to be related to their pathogenicity or competitive ability.

All isolates studied were found to be highly pathogenic, but some isolates exhibited greater competitive ability than others. In general, the monosporous isolates were better able to compete than were the mycelial isolates.

Individual fungal antagonists, namely, Trichoderma viride and Gliocladium roseum, were found in this study to suppress different isolates of Ophiobolus graminis, in sterilized soil, in much the same way as does the general micropopulation of natural soil. They proved much more active against mycelial isolates of the pathogen than against monosporous isolates. The greater competitive ability of the monosporous isolates would appear to account for this difference. Two other organisms, namely, Bacterium No. 353 and Actinomycete No. 284, were more or less inactive as individual antagonists in sterilized soil.

On potato sucrose agar, neither Trichoderma viride nor Gliocladium roseum was antagonistic to isolates of the pathogen. However, Bacterium No. 353 and Actinomycete No. 284 both inhibited

growth of all isolates on the agar.

As a specific soil amendment, chitin did not induce the suppression of monosporous or mycelial isolates of Ophiobolus graminis, whereas it has been reported to markedly suppress Fusarium pathogens.

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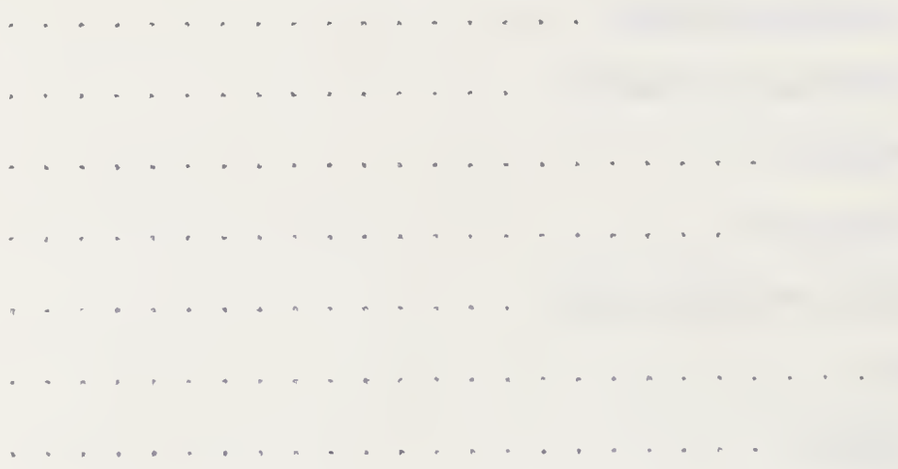

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INTRODUCTION

All plant pathogens, other than the obligate parasites, have two distinct phases in their life cycles. One, the parasitic phase, includes the development of a pathogen on or in the living host plant. The other, the saprophytic phase, is that which occurs in the absence of the host.

In their saprophytic phases, pathogens either remain inactive in the form of resting organs or grow on the tissues of dead host plants or on other available organic materials. Microorganisms, other than pathogens, are also able to utilize such materials as food sources. Hence in nature, pathogens must compete, in their saprophytic phases, with many other organisms until such time as they gain entrance to susceptible living host plants.

An organism which is able to grow successfully in the saprophytic phase, in the presence of other microorganisms, is considered to possess a high "competitive saprophytic ability," (Garrett 11). This expression means "the summation of physiological characters that makes for success in competitive colonization of dead organic substrates." Many of the more specialized pathogens, however, are distinguished by a low degree of competi-

tive saprophytic ability. Garrett considers that an organism with a low competitive saprophytic ability must necessarily have a high pathogenicity, the one being incompatible with the other. While he was referring chiefly to root-infecting fungi, Garrett indicated that his conclusions were applicable to bacterial and fungal pathogens in general.

Pathogenicity is the ability of a pathogen to cause disease. Thus, when a plant and a pathogen come together, pathogenicity may be expressed. However, the production of an infectious disease is not quite so simple. It requires not only that a quantity of the pathogen (inoculum) be brought into contact with a susceptible host, but as well that the environmental factors be favorable for the initiation and subsequent development of the disease. Only under special conditions, therefore, can pathogenicity be expressed. Plant pathogens may operate either above the soil or in the soil. Those functioning in the soil must possess special qualities to be successful in disease production.

The soil is a complex substrate in which a vast and widely varied microbial population exists. This population may include a certain number of plant pathogens, though as a rule, they make up only a small proportion of the total. These soil-borne plant pathogens have been classified into two main groups: the soil inhabitants and the soil invaders. The former are primitive

pathogens in which parasitism is incidental to saprophytism. They are characterized by a high competitive saprophytic ability. The soil invaders, on the other hand, are more highly specialized pathogens, in which parasitism is dominant and saprophytism secondary. Their presence in the soil tends to be limited to certain areas, where local conditions such as may result from the presence of the host plant are favorable for them.

Most soil-borne plant pathogens are soil invaders and a large proportion of these are root-infecting organisms. They are characterized by an expanding parasitic phase on the roots of the living host plants and a declining saprophytic phase after death of the hosts. Root-infecting pathogens are restricted in the saprophytic phase by competition of other soil microorganisms and are generally capable of expressing a high degree of pathogenicity in the parasitic phase.

The ability of a root-infecting pathogen to produce disease must necessarily be determined by the timely association of the following--a susceptible living host, a sufficient quantity of inoculum, and a favorable physical environment. Another factor, peculiar to the soil, also has a marked influence on root disease development, namely the activity of soil microorganisms, other than the pathogen in question. To obtain a true evaluation of the pathogenicity of a given pathogen in the soil, the influence of these other soil microorganisms must be removed. This

is most easily done by sterilization of the soil to kill all the microorganisms present. The desired pathogen and its susceptible host are then introduced into this soil, free from the influence of other microorganisms. A favorable physical environment must then be provided.

It is now suggested that pathogenicity of a root-infecting pathogen is a property which can be fully expressed only under the optimum conditions indicated. The severity of disease in sterilized soil under favorable environmental conditions would seem to be the best measure of the pathogenicity of the root pathogen.

Under natural conditions, in unsterilized soil, a root-infecting pathogen must overcome the influence of antagonistic microorganisms in the soil before it can invade the hosts. Hence, in addition to pathogenicity, the root-infecting pathogen, to be successful in producing disease in natural soil, must possess a competitive ability.

Competitive ability, as it is used in this investigation, is the ability of a root-infecting pathogen to compete successfully with other soil microorganisms or to resist their products in the presence of the living host plant. This ability possibly could be the same as that recognized by Garrett (11), or it might be different, at least in some respects. Garrett, it will be recalled, applied competitive saprophytic ability on non-living organic substrates only, whereas here the influence of the living host roots and the accompanying rhizosphere must also be considered.

In the absence of a susceptible host, it is important that the root-infecting pathogen is able to maintain itself in the soil. Active growth in the soil, at least in the rhizosphere, in proximity to the host roots is necessary for successful parasitism. This necessitates multiplication of the pathogen outside of, and either adjacent to, or on host roots before a successful parasitic association is established. In addition, many fungal root-infecting pathogens, after initial infection, grow along the exteriors of the roots and further infection is accomplished by means of branch hyphae. The external growth of the pathogen is influenced by associated microflora in the rhizosphere and on the root surfaces, and by root excretions. To be successful, the root-infecting pathogen must be able to compete with the other microflora present and utilize or resist the metabolic products of the microflora and the roots. While evidence of beneficial or stimulatory effects of other microorganisms have been reported, they appear, in most cases to be overshadowed by antagonistic or detrimental effects. Thus, many factors, other than, or in addition to those necessary for saprophytic competition, are involved in successful disease production by the root-infecting pathogens.

Ophiobolus graminis Sacc., the pathogen studied in this report, is a root-infecting fungus with an expanding parasitic phase in the living host roots, and a declining saprophytic phase

after death of its hosts. It is classed as a soil invader. That Ophiobolus graminis has poor competitive saprophytic ability has been demonstrated by many workers. Similarly, it has been noted that different isolates of this fungus have exhibited various degrees of pathogenicity. This variation has occurred in isolates from separate sources and has, from the work of others, appeared to be independent of the origin of the isolate, whether from single ascospores or from vegetative mycelia. Thus Padwick (24) and Russell (27), using monosporous and mycelial isolates of Ophiobolus graminis, reported wide variations in the degrees of pathogenicity of their isolates. In his investigations, Russell also noted that all mycelial isolates collected from the same source possessed about the same degree of pathogenicity.

Henry and McKenzie (15) found that all isolates of Ophiobolus graminis, monosporous and mycelial, derived from one sample of diseased wheat, exhibited a fairly uniform, severe pathogenicity. They further reported that the monosporous isolates were more active in natural soil than were the mycelial isolates, as measured by the disease production; they found that the monosporous isolates possessed greater competitive ability when in competition with the general micropopulation of the soil in the presence of living host roots.

PURPOSE

The purpose of this investigation was to compare monosporous and mycelial isolates of *Ophiobolus graminis* from the same source, with respect to pathogenicity and competitive ability, and particularly to determine if they would react in the same way to individual competitive organisms as they react to the soil micropopulation as a whole.

GENERAL MATERIALS AND METHODS

1. ISOLATION OF OPHIOBOLUS GRAMINIS Sacc.

A sample of Thatcher wheat which showed symptoms typical of those caused by the Take-all fungus, Ophiobolus graminis, was received from Cherry Point, Alberta, in August, 1957. This sample bore numerous perithecia containing mature ascospores of this pathogen. It was possible therefore to obtain from this material, not only mycelial isolates, but also single ascospore isolates of this fungus.

Mycelial isolates were obtained from pieces of the infected wheat roots and stems following surface sterilization. The method used here was that developed by Davies (5), who found that the standard method of surface sterilization using mercuric chloride solution inhibited the growth of Ophiobolus graminis. He obtained best results with a silver nitrate solution at a concentration of 1:100.

Roots and stems of infected wheat plants were cleaned by washing in running tap water for 24 hours. Sections were then cut, each a $\frac{1}{4}$ inch in length, and immersed in a 1:100 aqueous solution of silver nitrate for two minutes, washed in a sterile saturated solution of sodium chloride and finally rinsed in several changes of sterile distilled water. The

sections were then plated on potato dextrose agar. Typical colonies were transferred after three days growth onto potato dextrose agar slants.

Eight mycelial isolates were obtained and labelled: I, II, and IV to IX inclusive.

Monosporous isolates were obtained by plating ascospores from a crushed perithecium in water agar and after two days, cutting out pieces of the agar bearing individual germinating ascospores and transferring these to slants of potato dextrose agar.

Nine monosporous isolates were obtained and labelled 101 to 109 inclusive.

2. SOURCES OF ANTAGONISTS

Trichoderma viride Pers. ex Fries.

This fungus was isolated by Dr. E. W. B. Ward and was maintained as a stock culture in this laboratory.

Gliocladium roseum Bain.

This fungus was taken from a stock culture maintained in this laboratory.

Bacterium No. 353

This unidentified bacterium, known to exhibit antagonism to a wide range of microorganisms, was isolated from a Ghanian soil. It was kindly provided by Mr. L. J. Piening.

Actinomycete No. 284

This actinomycete, known to be antagonistic to a wide range of microorganisms, was also isolated from a Ghanian soil. It too was kindly provided by Mr. L. J. Piening.

3. MAINTENANCE OF CULTURES

Isolates of Ophiobolus graminis were maintained on potato dextrose agar for the first 21 months; thereafter, potato sucrose agar slants were used.

Cultures of the antagonistic organisms were maintained on potato sucrose agar slants.

All cultures were transferred every seven months and were stored in a cold room at 4°C.

4. GLASSWARE

All glassware used in experiments was "Pyrex" brand, and was cleaned in a hot detergent solution (Calgon) and rinsed in tap water, and then in distilled water.

5. STERILIZATION

Dry Glassware

All dry glassware was sterilized in a dry oven at 250°F for 12 hours.

General

For the sterilization of media, etc., a Barnstead steam sterilizer set at 15 pounds pressure (exhaust temperature 248°F.), was used. The period of sterilization varied with the different materials. It is indicated when detailed methods are described elsewhere.

6. PURE CULTURE MATERIALS AND METHODS

Culture Media

i. Potato dextrose agar

The potato dextrose agar used consisted of the following ingredients and was autoclaved for 20 minutes:

15 gm. Difco "Bacto-agar"

20 gm. Dextrose

1000 ml. Potato decoction*

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* Prepared by adding 200 gm. of peeled, sliced potatoes to 1000 ml. of distilled water and cooking for 30 minutes in an autoclave. After straining, the decoction was made up to volume, if necessary, by the addition of distilled water.

ii. Potato sucrose agar

Potato sucrose agar was used in most instances in the preparation of cultures for experiments. Its composition was:

15 gm. Difco "Bacto-agar"

20 gm. Sucrose

1000 ml. Potato decoction

After combination of the ingredients, it was autoclaved for 20 minutes.

Sources of Inoculum** for Experiments

i. Fungi

Ophiobolus graminis, Trichoderma viride, Gliocladium roseum

For all fungi used, mass transfers were made from stock cultures to Petri dishes of potato sucrose agar and allowed to grow for seven days.

Plugs of inoculum (5 mm. diameter) were cut from the peripheries of the colonies and were transferred to other plates of potato sucrose agar and allowed to grow another seven days, before the properties of the isolates were tested. Plugs of inoculum, 5 mm. in diameter, taken from the peripheries of these colonies were then used as inoculum.

** Used in the general sense to indicate propagative material.

ii. Other Microorganisms

Bacterium No. 353, Actinomycete No. 284

Transfers were made from each stock culture to two successive slants of potato sucrose agar, and grown for seven days on each, before the antagonistic properties of the isolates were tested.

7. GREENHOUSE MATERIALS AND METHODS

Certain general procedures which apply throughout this investigation are described here. Any modifications of them are considered with the experiment involved.

Soil Medium

In experiments to test the pathogenicity and competitive ability of isolates of Ophiobolus graminis, it was considered important to provide inoculum which could be added to soil and which would not alter it appreciably. Soil itself might be considered such a medium, but Ophiobolus graminis is a fungus which does not grow readily and multiply in unamended soil. It was therefore decided to compare several media, each consisting of soil plus an organic amendment. The following were chosen for testing after considering the experiences of other workers: soil plus thiamine-biotin, soil glucose-marmite, and soil corn meal.

Ward (31) for instance, had demonstrated in pure culture studies, that the vitamins thiamine and biotin are both necessary for growth of Ophiobolus graminis; and White (34) reported good growth of this fungus on sterilized soil to which had been added a solution containing 0.5% glucose and 0.05% "Marmite"; and in earlier investigations in this laboratory, various types of media were tested as possible substrates for the production of inoculum of Ophiobolus graminis. Soil corn meal was found to be superior to the other media tested, which included sterilized soil and a mixture of boiled barley and oat kernels.

The soil plus thiamine-biotin medium was prepared by adding 18 ml. of a solution containing thiamine and biotin to each 200 ml. Erlenmeyer flask containing 50 gm. air-dried black soil. The amounts of thiamine and biotin added in solution to each flask were equivalent to concentrations of 100 μgm. per kgm. soil, and of 10 μgm. per kgm. soil, respectively.

Soil glucose-marmite medium was prepared by adding 18 ml. of a solution containing 0.5% glucose and 0.05% "Marmite" to each 50 gm. of air-dried black soil in 200 ml. Erlenmeyer flasks.

The soil corn meal medium is described on page 16.

Each medium was autoclaved for three hours.

Growth of isolates of Ophiobolus graminis on the sterilized

soil plus thiamine-biotin was sparse, and even after a period of 10 weeks none of the isolates had completely permeated the medium. Growth of the isolates on the sterilized soil glucose-marmite was somewhat better. The medium was completely permeated after six to seven weeks, but growth was of a sparse, feathery nature. On sterilized soil corn meal, all isolates completely permeated the medium in four to five weeks, and the mycelial growth was dense and the aerial hyphae abundant.

When inoculum from the different media was tested on wheat seedlings in sterilized and natural soils, it was noted that almost no disease was produced by the inoculum grown on the soil plus thiamine-biotin medium. In respect to the other two forms of inoculum, it was noted that that grown on the soil corn meal medium was considerably more effective in disease production than that produced on the soil glucose-marmite medium. In addition, it was observed that the wheat plants grown as checks in soil containing uninoculated soil glucose-marmite medium were somewhat stunted and of a pale green color. In contrast, there appeared to be no adverse effect on the wheat plants by the uninoculated soil corn meal medium.

On the basis of the above results, the soil corn meal medium was selected as the most suitable for the production of inoculum of isolates of Ophiobolus graminis.

i. Preparation

The soil corn meal medium chosen for the greenhouse studies consisted of air-dried black soil from the greenhouse stocks to which was added corn meal at the rate of 10% by weights. This mixture was placed in 200 ml. Erlenmeyer flasks at the rate of 50 gm. per flask. Then distilled water was added at the rate of 20 ml. per flask and the flasks were plugged and autoclaved for three hours at 248°F.

ii. Inoculation procedure

Single agar plugs (5 mm. diameter) of isolates of Ophiobolus graminis were transferred aseptically to the surface of the soil corn meal medium in each flask, and incubated for four to five weeks, by which time the mycelium had completely permeated the substrate.

Similar plugs of Trichoderma viride or Gliocladium roseum were placed on the soil corn meal medium and 0.5 ml. suspensions of Bacterium No. 353 or Actinomycete No. 284 were pipetted onto the medium, when it was necessary to multiply these antagonists for greenhouse experiments. As the growth rates of the antagonistic microorganisms were greater than those of the isolates of Ophiobolus graminis, these were allowed to grow for two to three weeks.

Controls, or check flasks contained no added inoculum but were otherwise identically treated.

All flasks were kept in cupboards at room temperature of about 70°F. for the length of the growing period. By this time, the contents of each flask had formed into a solid mass. Each mass was broken up into a granular state by means of a sterile spatula before it was used as inoculum.

Soil Pots

Clay pots, 6 inches in diameter, were filled with a mixture of moist black soil and sand from greenhouse stocks at a ratio of 3:1. Half the total number of pots prepared was sterilized in a soil sterilizer (241°F.) for eight hours, while the other half was left unsterilized or in a natural state.

The contents of each flask of inoculum, described above, were then added at seed level to a pot of either natural or sterilized soil. Red Bobs wheat (25 seeds per pot) was sown on top of the inoculum, and covered with about an inch of the same soil. The pots were watered and held in a dark chamber at 60°F. until the wheat seedlings had just emerged.

After emergence of the seedlings, the pots were placed in a greenhouse compartment at higher temperatures and were examined when disease symptoms had been fully expressed.

Recording of Data

Disease severity of the wheat seedlings emerging in the

greenhouse pot experiments was recorded. The disease ratings were based upon the number of plants killed to the number of plants emerged, expressed in per cent.

EXPERIMENTAL RESULTS

INFLUENCE OF ARTIFICIAL MEDIA ON THE GROWTH OF OPHIOBOLUS GRAMINIS

Literature Review

After some time on potato dextrose agar, the growth rates of many of the isolates of Ophiobolus graminis were observed to be declining. Hence it seemed desirable to determine if this loss in growth rate was due to the agar medium or to the ageing of the isolates themselves. Therefore growth rates of the same isolates of Ophiobolus graminis were tested on different media.

Potato dextrose agar has been used extensively as an artificial medium for culturing Ophiobolus graminis (Davis 6, Webb and Fellows 33, and Broadfoot 4). Glucose, which is a reducing carbohydrate, is the dextrose sugar used in potato dextrose agar. Several workers have reported, however, on the undesirable effects of steam sterilization of cultural media containing glucose. Thus, Englis and Hanahan (7) found that on autoclaving glucose with phosphates for 30 minutes at 15 pounds pressure, a brown color developed together with a considerable conversion to fructose in the solution. McKeen (21) postulated that autoclaving of the amino acid glycine with a reducing carbohydrate resulted in the formation of a substance which was toxic

or fungistatic to Phytophthora fragariae. However, Ramsay and Lankerford (26) reported that heating of glucose resulted in the production of some compound, believed to be a product of glucose and phosphate, which was stimulatory to the growth of Lactobacillus fermentii.

Webb and Fellows (33) demonstrated that in potato dextrose decoction, Ophiobolus graminis was particularly sensitive to a condition of active alkalinity. In pot experiments, the addition of glucose to sterilized soil did not affect the growth of Ophiobolus graminis along the host plant roots (Garrett 11). Ward and Henry (32) found that, on an artificial liquid medium, growth of Ophiobolus graminis with glucose as the sole carbon source was slightly better than with sucrose as the sole source of carbon, but the difference was not significant.

A second medium tested in the present study was another potato sugar agar, in which sucrose replaced glucose as the sugar.

Corn meal has been used in this laboratory for many years, and more recently by others, as a good nutrient source, when added to the soil, for culturing this fungus. Corn meal agar has also supported good growth of Ophiobolus graminis (Kirby 16, Webb and Fellows 33). Davis (6) also obtained good vegetative growth and perithecial formation of his "New York" strain of this fungus on string bean pod agar. It was decided therefore,

to include both corn meal agar and bean pod agar in the present studies.

Webb and Fellows further found that the optimum reaction for the growth of Ophiobolus graminis varied with the composition of the medium, but that good growth was generally obtained between pH 5.2 and 7.4.

Materials and Methods

Four culture media were selected for study. The composition of two of these, namely, potato dextrose agar (P.D.A.) and potato sucrose agar (P.S.A.), was given earlier. That of the two others, corn meal agar (C.M.A.)* and bean pod agar (B.P.A.)** is given below.

All isolates (nine monosporous and eight mycelial) of Ophiobolus graminis were grown, each replicated four times, on each medium in Petri dishes. Two replicates of each isolate on each medium were placed in a cupboard in complete darkness. The other two replicates of each were placed in continuous light--a diffuse sunlight, supplemented by that from four fluorescent tubes continuously lighted for the duration of the growth period.

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* Corn meal agar—19.0 gm. Difco Bacto dehydrated corn meal agar, were dissolved in 1000 ml. distilled water and sterilized for 20 minutes.

** Bean pod agar--22.5 gm. Difco Bacto dehydrated bean pod agar, were dissolved in 1000 ml. distilled water and sterilized for 20 minutes.

Results

Measurements of colony diameters of each isolate of Ophiobolus graminis on each medium were recorded after eight days of growth, at which time some of the colonies had covered the entire surfaces of the agar plates, (Table I). Each reading is the average of two plates, expressed in centimeters.

The radial growth on agar plates maintained in complete darkness was generally slightly greater than the corresponding growth in continuous light. A distinction could not be made between the growth rates of monosporous and mycelial isolates of Ophiobolus graminis on any of the four media tested. Growth of any isolate was generally greatest, and about equal, on potato sucrose agar and on bean pod agar, and poorest on potato dextrose agar.

After the measurements of the colony diameters had been recorded, all plates were immediately returned to complete darkness or continuous light, where the isolates were allowed to continue growing. After two months the colony in each Petri plate was examined for perithecial formation. However, none was observed, either in those cultures maintained in the dark or in those in continuous light.

From the results of this experiment, it would appear that the decline observed in the growth rates of some isolates of Ophiobolus graminis cultured on potato dextrose agar was due

TABLE I. COMPARATIVE GROWTH OF ISOLATES OF *OPHIOBOLUS GRAMINIS* AFTER EIGHT DAYS ON POTATO DEXTROSE AGAR, POTATO SUCROSE AGAR, CORN MEAL AGAR, AND BEAN POD AGAR

DIAMETERS OF COLONIES IN CENTIMETERS

OPHIOBOLUS GRAMINIS AVERAGES OF TWO PLATES

MONOSPOROUS	CONTINUOUS LIGHT				COMPLETE DARKNESS			
ISOLATE NO.	P.D.A.	P.S.A.	C.M.A.	B.P.A.	P.D.A.	P.S.A.	C.M.A.	B.P.A.
101	2.1	6.9	6.1	6.6	3.0	7.7	6.3	7.5
102	1.2	8.2	8.0	8.2	3.0	9.2	9.1	9.1
103	3.0	5.4	4.2	5.1	4.5	4.8	4.5	4.5
104	2.0	6.3	4.5	6.0	0.9	6.1	5.0	6.2
105	2.5	8.2	6.8	7.9	1.9	8.9	8.6	8.7
106	3.0	7.6	6.3	7.5	4.5	9.3	8.4	9.3
107	3.1	8.2	3.7	8.2	2.9	8.4	5.4	9.2
108	1.7	8.5	7.0	8.1	2.5	9.3	8.2	9.3
109	2.3	6.9	5.0	6.5	3.1	7.2	6.0	6.9

TABLE I (CONTINUED)

DIAMETERS OF COLONIES IN CENTIMETERS

OPHIOBOLUS GRAMINIS — AVERAGES OF TWO PLATES

MYCELIAL	CONTINUOUS LIGHT				COMPLETE DARKNESS			
ISOLATE NO.	P.D.A.	P.S.A.	C.M.A.	B.P.A.	P.D.A.	P.S.A.	C.M.A.	B.P.A.
I	2.5	6.3	3.8	6.1	4.5	6.5	5.0	6.7
II	1.3	6.9	6.2	6.5	1.2	7.7	6.3	7.5
IV	3.5	8.3	5.9	8.4	4.0	9.3	8.3	9.3
V	3.0	8.1	6.2	7.9	4.6	8.7	8.2	8.5
VI	0.8	8.8	7.1	8.5	1.0	9.3	8.5	9.3
VII	0.5*	7.1	6.8	6.2	3.9	7.7	6.2	7.9
VIII	3.5	8.5	6.0	6.3	0.9	8.6	6.3	8.4
IX	3.0	6.1	3.5	5.7	3.0	5.7	5.3	5.8

* No growth was made on either of the two plates.

to the agar medium, rather than to the ageing of the isolates themselves.

Discussion

The above results are generally in direct opposition to the results of several other workers (Kirby 16, Davis 6, Webb and Fellows 33, and Broadfoot 4), who found that potato dextrose agar was as good as, or better than, other media tested, including corn meal agar and bean pod agar, in supporting growth of Ophiobolus graminis. Webb and Fellows, and Broadfoot each used only one isolate of this fungus in their comparisons of media, and for growth experiments maintained their cultures in incubation chambers in darkness. Their results, however, would be comparable to those obtained in this experiment with isolate No. 103 grown on each medium in darkness. While no two isolates of Ophiobolus graminis were equal in the ranges of growth rates over the four media tested, it is entirely possible that had these workers tested more than one isolate in their studies, somewhat different results might have been obtained.

The decline in growth rate of isolates of Ophiobolus graminis on potato dextrose agar was thought to be similar to the effects produced on other organisms by the sterilization of glucose with other materials to form a phytotoxic compound, or coumpounds (Englis and Hanahan 7, McKeen 21).

As a result of the findings of this experiment, all isolates of Ophiobolus graminis in this laboratory were transferred to potato sucrose agar and have since been maintained on this medium, in preference to potato dextrose agar.

CULTURAL CHANGES ON ARTIFICIAL MEDIA AND THEIR EFFECTS ON PATHOGENICITY AND COMPETITIVE ABILITY OF ISOLATES OF *OPHIOBOLUS GRAMINIS*

Literature Review

Garrett (10) reported that "cultures of *Ophiobolus graminis* are not difficult to recognize on the isolation plates, although only mycelial characters are usually available for this purpose; perithecia appear on agar plates rather rarely, and then only after some weeks. Little aerial mycelium is developed; the color of the colony is at first a pale green or buff shade of grey, but later becomes much darker. A useful diagnostic character is the curling back of a majority of the leading hyphae at the margin of the colony."

In a study of the genetics of eight ascospore isolates from one ascus, White (34) observed a segregation into four dark- and four light-colored strains, two of each color developing abundant aerial hyphae. He reported that over a period of three years, the cultural characters and pathogenicity of each isolate remained constant.

Padwick (24) noted that there was a wide variation in the type of growth and pathogenicity between his isolates. He further obtained a saltant of one isolate which was more pathogenic than the original and which was characterized by the

greater production of macrohyphae.

The loss in pathogenicity by isolates of Ophiobolus graminis maintained for long periods in pure culture has received special attention from Russell (27), who conducted pathogenicity tests with a number of isolates at regular intervals over a period of nine years. While the pathogenicity of all isolates tended to decrease with continued artificial culture, some isolates exhibited high pathogenicity after a long period of relatively low pathogenicity. He attempted to alter the pathogenicity of two isolates by maintaining two sets of cultures at two different temperatures, and a third set was passed repeatedly through wheat seedlings under artificial conditions. Neither the variation in temperature of incubation nor the repeated plant passage consistently affected the pathogenicity of either isolate.

In the present studies, an attempt was made to find one or more cultural characters which could be correlated with pathogenicity or competitive ability, or both, among the isolates under observation.

In all cases, observations were made of the isolates of Ophiobolus graminis grown in Petri dishes containing potato sucrose agar. Petri dishes were either maintained in continuous light at room temperature or in incubation chambers in darkness at 15°, 20°, or 25°C.

Results

1. Cultural characteristics, stock cultures.

Observations of the general characters of isolates of Ophiobolus graminis from the stock cultures were made after 10 days of growth, Table II. The "twisting of hyphae" was seen without the aid of a lens, as the curling back of the leading hyphae and was associated with the formation of strands by groups of individual hyphae. The macrohyphae are those referred to by Fellows (8). According to Padwick (24), it is to these macrohyphae that the coloration of the colonies is due. The "plate mycelium" was also seen without the aid of a lens and was found growing along the glass of the Petri plates, away from the line of contact of the glass and the agar. Each was composed of many macrohyphae growing parallel to each other, usually not more than one or two layers in thickness. Cultures produced from these plate mycelia resulted in normal cultures on potato sucrose agar, after an initial lag phase of growth.

2. Cultural characteristics, continuous cultures.

In the preparation of a greenhouse pot test, isolates of Ophiobolus graminis were grown on potato sucrose agar at room temperature and subcultured every 10 days for a period of 300 days. The pathogenicity test is reported later. The cultural characters of these isolates were noted after 10 days growth of the 30th subculture (Table III). The observations of the

TABLE II CULTURAL CHARACTERISTICS OF ISOLATES OF *OPHIOBOLUS GRAMINIS*

AFTER 10 DAYS GROWTH ON POTATO SUCROSE AGAR

CULTURE SOURCES WERE STOCK CULTURES

O. GRAMINIS ISOLATE NO.	AERIAL MYCELIUM AMOUNT	COLOR	TWISTING OF HYPHAE	MACROHYPHAE	COLONY COLOR	PLATE MYCELIUM
Monosporous						
101	Dense	White	Strong	Very Many	Blue-grey	Few
102	Long, Slender	White	Strong	Very Many	Blue-black	Very Few
103	Sparse, Uneven	Grey	Slight	Few	White	None
104	Dense	Grey	Strong	Very Many	Greenish-grey	Moderate No.
105	Dense	White	Slight	Moderate No.	Greenish-grey	Few
106	Long, Slender	White	Strong	Few	Grey	None
107	Dense	White	Strong	Very Few	Buff	None
108	Sparse, Uneven	White	Strong	Many	Greenish-grey	Very Few
109	Dense	White	Strong	Very Few	Buff	None

TABLE II (CONTINUED)

O. GRAMINIS ISOLATE NO.	AERIAL MYCELIUM AMOUNT	COLOR	TWISTING OF HYPHAE	MACROHYPHAE	COLONY COLOR	PLATE MYCELIUM
Mycelial						
I	Dense	White	Strong	Few	White	None
II	Dense	Grey	Slight	Very Few	Greenish-grey	Few
IV	Long, Slender	White	Strong	Moderate No.	Greyish-green	Moderate No.
V	Sparse, Uneven	White	Strong	Very Few	Greenish-brown	None
VI	Sparse, Uneven	Grey	Strong	Many	Grey	None
VII	Dense	White	Strong	Few	Buff (Creamy)	None
VIII	Long, Slender	White	Slight	Very Few	Buff	None
IX	Sparse, Uneven	Cream	Strong	Few	Yellow	Very Few

TABLE III CULTURAL CHARACTERISTICS OF ISOLATES OF *OPHIOBOLUS GRAMINIS*

AFTER 10 DAYS GROWTH ON POTATO SUCROSE AGAR

CULTURE SOURCES WERE THE 30th CONSECUTIVE SUBCULTURES

O. GRAMINIS ISOLATE NO.	AERIAL MYCELIUM AMOUNT	AERIAL MYCELIUM COLOR	TWISTING OF HYPHAE	MACROHYPHAE	COLONY COLOR	PLATE MYCELIUM
Monosporous						
101	Sparse, Uneven	White	Strong	Very Many	Blue-black	Very Many
102	Sparse, Uneven	Grey	Strong	Very Many	Blue-black	Moderate No.
103	Negligible	---	Strong	None	Buff	Moderate No.
104	Long, Slender	White	Strong	Moderate No.	Greyish-white	Very Few
105	Long, Slender	White	Strong	Moderate No.	*	Very Many
106	Sparse, Uneven	Grey	Strong	Few	**	Very Many
107	Dense	White	Strong	Moderate No.	Yellow	Moderate No.
108***	Dense	White	Strong	Few	Yellowish-brown center Grey outer	Very Few
109	Dense	White	Strong	Very Few	Yellowish-grey	Very Many

* 105--Characterized by a narrow white band, encircling a wide blue-black band. Center area small, white to yellow color. Macrophyphae very pronounced in black band.

** 106--Characterized by a narrow white band, encircling a narrow black band. Center area large, white in color. Macrohyphae very pronounced in narrow black band.

*** 108--Due to growth rate, observations were made on the 20th day of growth.

TABLE III (CONTINUED)

O. GRAMINIS ISOLATE NO.	AERIAL MYCELIUM AMOUNT	AERIAL MYCELIUM COLOR	TWISTING OF HYPHAE	MACROHYPHAE	COLONY COLOR	PLATE MYCELIUM
Mycelial						
I	Negligible	---	Strong	None	Yellow	Few
II	Sparse, Uneven	White	Slight	Very Few	Grey	Very Few
IV	Sparse, Uneven	White	Strong	Many	Greenish-grey	Few
V	Lumpy, Uneven	White	Strong	Moderate No.	Greenish-black center Greyish-white outer	Very Few
VI	Negligible	---	Slight	None	White	None
VII	Sparse, Uneven	White	Strong	Very Many	Mottled White and Brownish-green	Very Many
VIII	Sparse, Uneven	White	Strong	Few	Yellow center Grey outer	Many
IX	Lumpy, Uneven	Yellow	Strong	Few	Yellow	Moderate No.

characters of isolate No. 108 were recorded after 20 days because of its slow growth rate. Many variations in the growth habits of the isolates were observed.

Color reversion was common with most isolates after 150 days of continuous culturing. The phenomenon of color reversion is unexplainable at this time. One example of this type of change is shown in Figure I (Isolate No. 106). The agar surface was completely overgrown. The previous subculture was completely black in color. The change here was towards a white colony color, in over half of the colony. Macrohyphae and the formation of plate mycelium were observed only on the black section of the colony. Subsequent transfers from various locations on this plate did not, in any instance, result in a culture stable as to color or to the presence of macrohyphae. In all cases, subcultures did give colonies exhibiting, in varying proportions, two distinct colony colors; the white, with few or no macrohyphae, and the dark, with a pronounced number of macrohyphae. Further transfers, both by agar plugs and hyphal tips, from the peripheries of the dark and light areas were kept in darkness at the various temperatures stated and under continuous light at room temperature, but neither the temperature nor light had any observable stabilizing effect on the cultures.

Color reversion was not limited to the isolates which had been continuously subcultured. At various times, both during

Figure I. Color reversion, from black to white, of *Ophiobolus graminis* isolate 106, growing on potato sucrose agar.

the 300 day period and after, isolates from the stock cultures were grown on potato sucrose agar in Petri dishes. Color changes were also noted in most of these colonies. Figure II (page 47) shows three plates, each containing an agar plug of isolate No. 105, taken from the same area of a single dark colony. While the plates were from another experiment designed to demonstrate antagonism, the variations in the growth pattern exhibited were striking. That the color changes were not due to the presence of antagonists was demonstrated when hyphal tip and agar plug subcultures were made and all were found to react similarly.

3. Tests on artificial media.

In an attempt to discover the possible mechanism for color changes of the isolates of Ophiobolus graminis, various experiments were carried out on potato sucrose agar. Mycelia of two isolates of the fungus were allowed to grow together on individual plates, maintained either in darkness at various temperatures, or in continuous light at room temperature. In no case did any isolate exhibit antagonism to any other isolate. Similarly, no new isolate was recovered from the growing together of two isolates. Anastomosis was commonly observed, but there was no apparent transfer of characters of one isolate to another. Perithecia were not produced by any of the isolates in artificial culture.

The depth of the agar had no effect upon the colony color and the production of macrohyphae. This was tested in two ways; isolates were grown in Petri dishes containing different amounts of potato sucrose agar, either at a uniform depth throughout the plates or with the agar slanted in the Petri plates. Again the plates were maintained at various temperatures in darkness or in continuous light.

4. Pathogenicity test.

Four monosporous and four mycelial isolates of Ophiobolus graminis from each of two sets of cultures were tested for pathogenicity and competitive ability in natural and sterilized soils. One set was taken from the stock cultures, the other from the 30th consecutive transfer of the continuous cultures. Since the greenhouse temperatures at the time of this experiment were considered to be too high for disease expression, the pots were placed out-of-doors in open frames. The maximum temperatures outside during seedling growth and infection varied from 55° to 95°F.

The results of other experiments have consistently shown that the monosporous and mycelial isolates were similar with respect to pathogenicity as measured by disease severity of wheat seedlings in sterilized soil, and that in natural soil, the monosporous isolates generally caused greater disease than the mycelial isolates. While this same general trend was observed in this experiment, the disease ratings were much less severe.

The temperature was considered the main factor responsible for this over-all low disease severity.

Minor variations in pathogenicity and competitive ability were noted for the individual isolates from the two sources, but there was no consistency in these variations which could be related to the source of the inoculum, or to one or more variations as observed in artificial media.

Discussion

The isolates of Ophiobolus graminis studied on artificial media did not, as Garrett (10) reported, develop a constant colony color, darkening as the colony aged, nor did they conform to the findings of White (34). The monosporous isolates showed extreme variation as to colony color and to the production of macrohyphae and aerial hyphae, whether maintained as stock cultures or as continuous cultures. Variation was also observed with the mycelial isolates. The only characteristic observed to remain common to all isolates over a total period of $4\frac{1}{2}$ years was the tendency of the leading hyphae to curl at the margins of the colonies. This character varied, however, among isolates.

Changes in the general cultural characteristics did occur over a period of time, but the reasons for these are not evident at this time. However, the changes of an isolate on artificial media did not appear to have influenced either its pathogenicity or competitive ability.

THE EFFECT OF INDIVIDUAL ANTAGONISTS ON THE GROWTH, PATHOGENICITY AND COMPETITIVE ABILITY OF MONOSPOROUS AND MYCELIAL ISOLATES OF *OPHIOBOLUS GRAMINIS*

Literature Review

The effect of soil-inhabiting organisms on disease severity due to *Ophiobolus graminis* was studied by Sanford and Broadfoot (29). Their results showed definitely that certain fungi, actinomycetes and bacteria were effective in suppressing what they termed the pathogenicity of *Ophiobolus graminis*.

Henry (13) found that the natural microflora had a marked inhibitive action on the growth of *Helminthosporium sativum* in soil, and reported indications of a similar effect on *Fusarium graminearum*. Bacteria, fungi, and actinomycetes isolated from the soil all showed a suppressive action. A combination of all types proved to be slightly more effective than fungi alone.

In studies on the influence of temperature and soil sterilization on the reaction of wheat seedlings to *Ophiobolus graminis*, Henry (14) found that the disease-temperature curve for this fungus was different in unsterilized soil than in sterilized soil. In unsterilized soil, microbial antagonism to the pathogen had apparently increased with the rise in temperature, resulting in less severe disease.

Broadfoot (4), working with cultures of bacteria and fungi, studied their antagonistic and compatible growth relationships towards Ophiobolus graminis on various culture media. He found that many of the organisms which exercised a marked degree of disease control in soil, were not antagonistic on culture media, and many of those exhibiting no effect in soil were antagonistic in culture.

Broadfoot (3) also demonstrated that over a relatively short period of time, the inoculum of Ophiobolus graminis, in steam sterilized soil, was exposed to contamination from the air. This was later confirmed by Ludwig and Henry (19), who added natural soil to sterilized soil. They found that Trichoderma viride soon became dominant in the contaminated sterilized soil and suggested that it may be a major factor in the decline of Ophiobolus graminis in such soil and in natural soil.

Lal (17) isolated large numbers of microorganisms, the most prevalent being Trichoderma viride, from infected wheat roots and attributed the disappearance of Ophiobolus graminis from infested soil to these organisms. He tested their abilities to inhibit this pathogen on cultural media and in soil and found no close correlation between these two activities. Lal further considered that the inhibiting or retarding effect produced by the microorganisms tested was in-

duced by their metabolic products.

The effects of 143 species and strains of fungi and their by-products on Ophiobolus graminis were studied by Slagg and Fellows (30). The production of inhibiting or stimulating by-products by any fungus varied with its stage of growth and the nature of the substrate on which it was cultured. In artificially infested soil, the parasitism of Ophiobolus graminis on wheat roots was definitely lessened by the presence of several common soil fungi.

In earlier experiments with the isolates of Ophiobolus graminis used in this investigation, it was noted (Henry and McKenzie 15) that some were better able to compete in natural soil than others. Generally the monosporous isolates possessed high competitive ability in the presence of the host seedlings in soil containing a normal microbial population. On the other hand, the mycelial isolates generally possessed very low degrees of competitive ability. The competitive ability of an isolate of the pathogen was measured by the severity of disease produced by it in host seedlings in natural soil.

It was therefore decided to test the competitive abilities of different isolates of Ophiobolus graminis, against individual soil saprophytic microorganisms, in order to determine whether the monosporous and mycelial isolates would react in a similar manner with these individual microorganisms as with the soil microflora as a whole.

As to which members of the soil microflora have the greatest influence on Ophiobolus graminis, and how they exert this influence, there have been differences of opinion. However, from the earlier work reviewed above, it would appear that certain fungi may be more important as antagonists of this pathogen in natural soil than either bacteria or actinomycetes.

Four saprophytic soil microorganisms, namely Trichoderma viride, Gliocladium roseum, Bacterium No. 353, and Actinomycete No. 284, were tested as individual antagonists to monosporous and mycelial isolates of Ophiobolus graminis on an artificial medium and in soil. Trichoderma viride and Gliocladium roseum are two saprophytic fungi, commonly isolated from Alberta soils, which have been demonstrated to be antagonistic to Ophiobolus graminis (Ludwig 18) and to a number of other plant pathogens. Bacterium No. 353 and Actinomycete No. 284, both isolated from soil collected in Ghana, are also soil-borne microorganisms, both of which have exhibited wide ranges of antagonism.

a) Influence of Individual Antagonists on the Growth of Monosporous and Mycelial Isolates of Ophiobolus graminis on an Artificial Medium

Materials and Methods

The antagonistic action of Trichoderma viride, Gliocladium

roseum, Bacterium No. 353, and Actinomycete No. 284, was tested separately on nine monosporous and eight mycelial isolates of Ophiobolus graminis in triplicate plates of potato sucrose agar. Each of these antagonists and Ophiobolus graminis were placed about 4.5 cm. apart on opposite sides of an agar plate. An isolate of the pathogen was allowed to grow for three days before the antagonist was added.

Where antagonism was observed, measurements were taken of the least distance between the advancing edge of the colony of the antagonist and that of the colony of the pathogen.

Results

Where Trichoderma viride and Gliocladium roseum were tested on potato sucrose agar as individual antagonists to isolates of Ophiobolus graminis , no measurable antagonistic action was noted. However, it was observed that both saprophytic fungi had higher growth rates than any isolate of the pathogen. These growth rates might lead to the suppression of the pathogen on the basis of competition for limited food sources.

Bacterium No. 353 and Actinomycete No. 284, on the other hand, proved quite antagonistic to all isolates of Ophiobolus graminis on potato sucrose agar, as was evident from the zones of inhibition around the colonies of the antagonists. Measurements of the zones, as given in Table IV, show that the different isolates of Ophiobolus graminis reacted similarly to each

TABLE IV INHIBITIVE EFFECT OF A SINGLE ANTAGONIST (BACTERIUM NO. 353 OR ACTINOMYCETE NO. 284) TOWARDS ISOLATES OF *OPHIOBOLUS GRAMINIS* GROWN ON POTATO SUCROSE AGAR, AFTER FOUR DAYS OF GROWTH

O. GRAMINIS ISOLATE NO.	ZONE OF INHIBITION IN CENTIMETERS AVERAGE OF THREE REPLICATES ANTAGONIST	
	BACTERIUM NO. 353	ACTINOMYCETE NO. 284
Monosporous		
101	1.5	1.4
102	1.1	1.3
103	1.2	1.3
104	1.3	1.3
105	1.4	1.1
106	1.5	1.4
107	1.4	1.3
108	1.9	1.8
109	1.7	1.6
Mycelial		
I	1.2	1.4
II	1.2	1.6
IV	1.3	1.1
V	1.3	1.4
VI	1.6	1.6
VII	1.1	0.9
VIII	1.3	1.2
IX	1.2	1.2

antagonist. Mycelial and monosporous isolates appeared equally susceptible to these antagonistic effects.

The antagonism exhibited by Bacterium No. 353 and Actinomycete No. 284 appeared in each case to be due to one or more diffusible volatile compounds. That the antagonistic factors were diffusible through agar was shown by the circular zones of inhibition between the antagonist and the pathogen (Figure II). Volatility of the factors was indicated by the fact that after a period of time the isolates of Ophiobolus graminis resumed growth. In the presence of the bacterium, growth of the pathogen was inhibited for four to five days, after which time new growth commenced and eventually covered the surface of the bacterial colony. Where antagonism resulted from the actinomycete, the period of inhibition was from eight to ten days, and the new growth covered the remaining agar surface. However, the isolates of the pathogen did not grow over the actinomycete colony.

The presence of either the bacterium or the actinomycete stimulated all isolates of Ophiobolus graminis to produce the black plate mycelium (see page 29) in abundance on the sides of the Petri dishes away from the surface of the agar. In addition, the second growth of the pathogenic isolates, which occurred after inhibition ceased, was of a different nature than the initial growth. In cultures initially possessing a dark colony

color and a moderate number of macrohyphae, the new growth was white in color with the leading hyphae exhibiting a greater tendency to twist and curl. The new growth of those isolates whose initial colonies were white or light grey, was also white, but appeared waxy.

Subcultures from the second phase of growth of all isolates were made. Each was grown, together with a single antagonist as before, or alone on potato sucrose agar plates. In all cases, the subcultures developed the same cultural characteristics as were displayed in the first phase of growth of the original cultures. Those subcultures which were tested with the individual antagonists maintained the same reaction to each antagonist as had the parent colony. The change in colony color and in hyphal tip reaction, as a result of inhibition by the bacterium or the actinomycete did not appear to be associated with an ability to overcome the inhibiting effects of either organism.

b) The Effect of Individual Antagonists on the Pathogenicity and Competitive Ability of Monosporous and Mycelial Isolates of Ophiobolus graminis

Materials and Methods

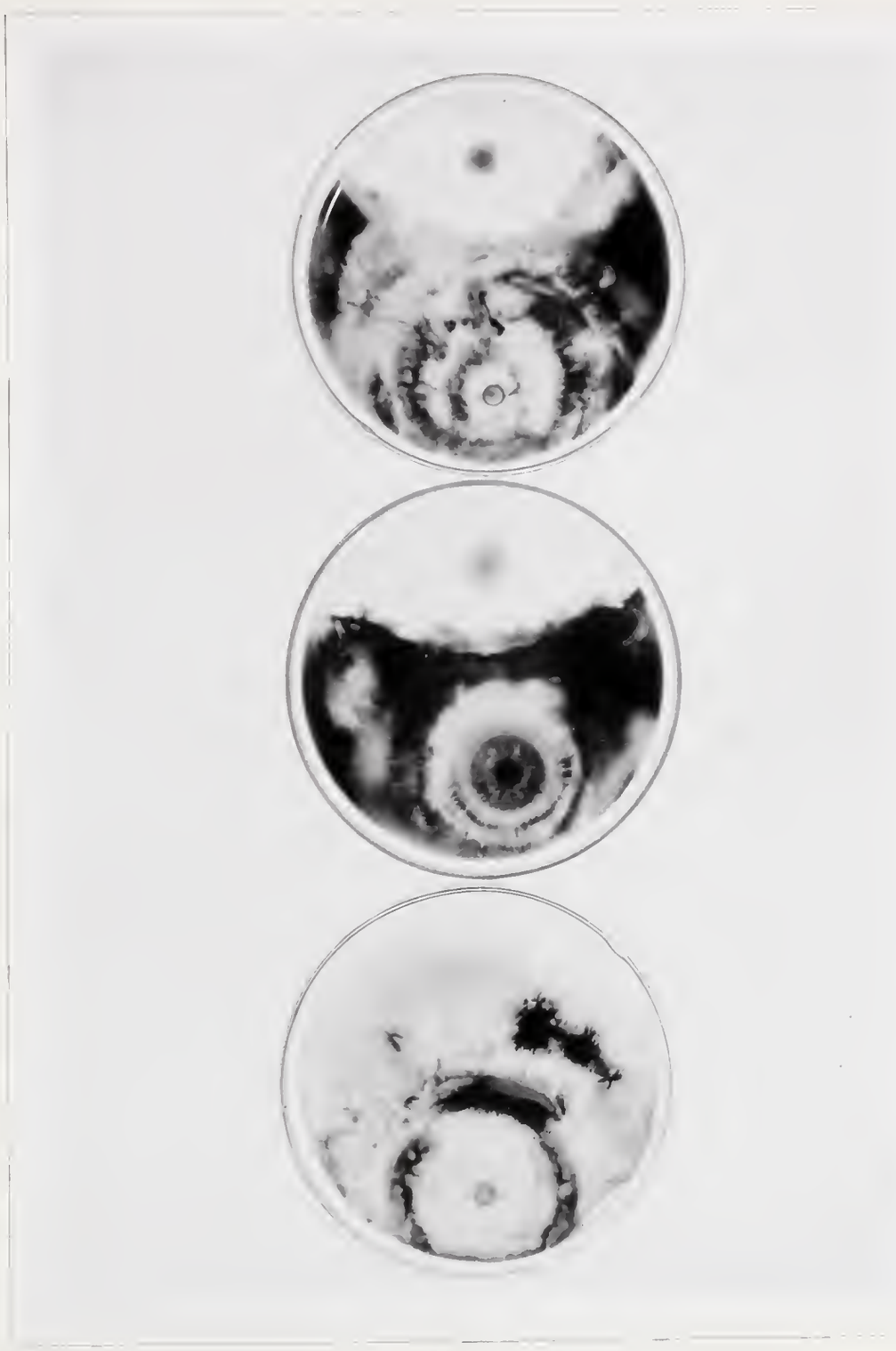
The antagonistic effects of Trichoderma viride,

Figure II. Antagonistic action of Bacterium No. 353 and Actinomycete No. 284 on *Ophiobolus graminis* (isolate 105) on potato sucrose agar.

Top -- Bacterium No. 353 (see upper part of plate) and *Ophiobolus graminis* 105

Middle -- Actinomycete No. 284 (see upper part of plate) and *Ophiobolus graminis* 105

Bottom -- *Ophiobolus graminis* 105 with no added antagonist.



Gliocladium roseum, Bacterium No. 353 and Actinomycete No. 284 on four monosporous isolates (102, 103, 106, and 108) and four mycelial isolates (I, IV, V, and VII) of Ophiobolus graminis were tested in soil. The inoculum of each organism was grown on soil corn meal medium in flasks.

For this experiment, 100 gm. of soil corn meal medium was added at seed level to each pot of soil, instead of the usual 50 gm. lots. In the antagonistic comparisons, the medium consisted of the contents of one flask (50 gm.) of an isolate of Ophiobolus graminis plus 50 gm. of an antagonist, or the uninoculated control. The medium for the check pots contained either, 50 gm. of an antagonist plus the contents of one control flask, or 100 gm. of uninoculated control.

The experiment was conducted in both natural and sterilized soils. The wheat seedlings in the soil pots were allowed to emerge in a dark chamber at 60°F. Soon after emergence, the pots were transferred to the greenhouse or placed outside.

Disease severity shown by the wheat seedlings in the inoculated pots was finally used as a measure of the inhibitive effect of the antagonists.

Due to its size, this experiment was divided into two series, with Trichoderma viride and Gliocladium roseum being tested in one, and Bacterium No. 353 and Actinomycete No. 284

in the other. In each series there were four replicates of each treatment in both sterilized soil and in natural soil.

Results

1. Trichoderma viride and Gliocladium roseum.

The greenhouse temperature was maintained at 65°F. for the duration of this series.

The results, which are shown in Table V, are the averages of four replicates. Each figure represents the proportion of plants killed to the number of plants emerged, expressed in percent. There is one exception; the figure for Ophiobolus graminis VII with Gliocladium roseum in natural soil represents the average of three replicates. The germination in the fourth pot produced only two seedling plants from 25 seeds and the results were discarded.

In sterilized soil, the addition of either Trichoderma viride or Gliocladium roseum resulted in general in a decrease in the disease severity and especially in that produced by the mycelial isolates of the pathogen. The overall effects appeared similar to those of the natural soil microflora on the same isolates of the pathogen.

The addition of Gliocladium roseum to individual monosporous isolates of Ophiobolus graminis produced an antagonistic effect which on the average was greater than that of the natural microflora as a whole. When Trichoderma viride was used as the

TABLE V COMPARATIVE INHIBITION OF MONOSPOROUS AND MYCELIAL ISOLATES OF *OPHIOBOLUS GRAMINIS* BY *TRICHODERMA VIRIDE* OR *GLIOCLADIUM ROSEUM* AS INDIVIDUAL ANTAGONISTS IN SOIL

	PERCENTAGE OF EMERGED WHEAT SEEDLINGS KILLED					
O. GRAMINIS	STERILIZED SOIL			NATURAL SOIL		
ISOLATE NO.	*T. VIRIDE*	*G. ROSEUM*	CONTROL	*T. VIRIDE*	*G. ROSEUM*	CONTROL
102	83.3	74.0	100.0	72.8	68.9	95.8
103	95.6	69.5	96.0	76.6	93.2	83.0
106	95.6	95.8	100.0	60.7	91.6	96.5
108	91.9	64.9	99.0	53.8	33.4	84.4
I	6.2	10.8	91.5	3.1	18.7	18.5
IV	53.1	57.1	74.0	34.3	42.3	58.8
V	88.0	75.0	100.0	37.6	34.5	62.9
VII	18.4	19.8	87.9	15.4	5.9*	19.2
Control	0.0	0.0	0.0	0.0	0.0	0.0

* Average of three replicates.

antagonist, the effect was similar, but not quite so pronounced. The results showed the effect of Trichoderma viride to be slightly greater than, or nearly equal to the effects by the microflora of the natural soil on the isolates of the pathogen.

Addition of either of these fungal antagonists singly to the natural soil, under the conditions of this experiment, caused a further decrease in the severity of disease over that produced by the natural soil microflora alone.

No disease or inhibition of the wheat seedlings was observed in the check pots, due either to the antagonist or to the addition of uninoculated soil corn meal medium.

2. Bacterium No. 353 and Actinomycete No. 284.

When the series using Bacterium No. 353 and Actinomycete No. 284 was started, the greenhouse temperature was considered too high for satisfactory disease development, so the pots in this series were placed out-of-doors in an open frame where the maximum temperatures during seedling growth and infection varied from 60° to 95°F.

The results from this series are presented in Table VI. Here each figure represents the average percent death of seedlings in four replicates. Striking differences in pathogenicity as measured in sterilized soil, were observed between the individual isolates of Ophiobolus graminis.

While the temperature must have had some effect on the

TABLE VI COMPARATIVE INHIBITION OF MONOSPOROUS AND MYCELIAL ISOLATES OF *OPHIOBOLUS GRAMINIS* BY BACTERIUM NO. 353 OR ACTINOMYCETE NO. 284 AS INDIVIDUAL ANTAGONISTS IN SOIL

	PERCENTAGE OF EMERGED WHEAT SEEDLINGS KILLED					
O. GRAMINIS	STERILIZED SOIL			NATURAL SOIL		
ISOLATE NO.	BACTERIUM NO. 353	ACTINOMYCETE NO. 284	CONTROL	BACTERIUM NO. 353	ACTINOMYCETE NO. 284	CONTROL
102	79.0	80.0	88.0	50.2	43.9	74.5
103	32.5	37.6	40.0	22.5	28.2	28.4
106	58.8	53.4	61.5	38.0	49.1	55.8
108	23.1	25.4	31.2	30.9	19.2	30.4
I	38.0	23.1	53.0	14.8	19.6	19.5
IV	16.4	22.5	27.2	19.1	15.0	20.5
V	35.4	28.8	38.0	15.6	12.1	16.2
VII	58.9	44.9	92.0	19.7	18.7	29.6
Control	0.0	0.0	0.0	0.0	0.0	0.0

growth of the isolates, it is interesting to note that isolate No. 108, which had been the most pathogenic isolate in earlier tests, was one of the least pathogenic in this series. The growth rate of this isolate on potato sucrose agar had also decreased considerably prior to its use in this series. This is in agreement with Garrett's observation (9) that a deterioration in pathogenicity of Ophiobolus graminis tended to be correlated with a noticeable decrease in its growth rate.

The trend, with regard to pathogenicity and competitive ability of the monosporous and mycelial isolates was again observed in this series, although the disease was much less severe. Generally the monosporous and mycelial isolates caused a similar amount of damage to wheat seedlings in sterilized soil. In natural soil, the monosporous isolates produced higher disease ratings than did the mycelial isolates. However, exceptions were noted.

Both Bacterium No. 353 and Actinomycete No. 284, added with individual isolates of Ophiobolus graminis in sterilized soil, slightly reduced the severity of disease in wheat seedlings, but not to the same extent as did the microflora of the natural soil.

The antagonistic effect of Bacterium No. 353 on the mycelial isolates of Ophiobolus graminis in sterilized or natural soil appeared to be slightly less than that produced

by Actinomycete No. 284. Added with the monosporous isolates, the effect by the bacterium was about the same as that by the actinomycete. However, the antagonism of both organisms varied with the isolate of the pathogen.

No seedling death occurred in the check pots, either in sterilized soil or in natural soi. The antagonists alone did not appear to affect the growth of the wheat seedlings.

Discussion

While neither fungal antagonist, Trichoderma viride nor Gliocladium roseum, was antagonistic to isolates of Ophiobolus graminis in artificial culture on potato sucrose agar, both greatly inhibited the same isolates of the pathogen in sterilized soil, as measured by their ability to attack wheat seedlings. On the other hand, both Bacterium No. 353 and Actinomycete No. 284 inhibited, at least temporarily, the growth of all isolates of Ophiobolus graminis on potato sucrose agar, but only very slightly reduced the ability of the pathogen to produce disease in soil. These results support the work of Broadfoot (4) and Lal (17), who reported no close correlation between the activities of certain microorganisms to antagonize Ophiobolus graminis on cultural media and in soil.

No direct comparisons can be made from the above results

between the two series of the relative effects of the antagonists in soils. The series in which the antagonism of the two saprophytic fungi was tested on the isolates of Ophiobolus graminis, was carried out in the early spring, in the greenhouse where the temperature was controlled at 65°F. The second series, involving Bacterium No. 353 and Actinomycete No. 284, was conducted later in the summer, at which time the greenhouse temperatures approached and exceeded 100°F. As a result, the pots of the second series were placed outside where the temperature and thus the moisture content of the soils tended to fluctuate widely. The variability of these environmental factors naturally affected the results of this series. Due to these unfavorable conditions, the results of the second series were considered to be inconclusive.

The results of the first series demonstrated that Trichoderma viride and Gliocladium roseum were individually antagonistic to all isolates of Ophiobolus graminis. Further, each was more antagonistic to the mycelial isolates than to the monosporous isolates. This antagonistic reaction by either soil saprophytic fungus appeared to be due to a competition for available organic material in the sterilized soil, rather than to the production of antibiotics by the saprophytes. Competition for available food sources was indicated by the fact that the microflora of natural soil inhibited the pathogen to much the same extent as did either

antagonist in sterilized soil.

In a natural soil, a normal mixed micropopulation is present, and includes many fungi, bacteria, and actinomycetes. A number of these microorganisms have produced antibiotics under artificial conditions. It has been generally assumed that the same microorganisms are able to, and do in fact, produce these substances in localized areas in the soil. However, the antibiotic producers must compete with other soil microorganisms, some of which may also produce antibiotics, with the consequence that there may be a cross-antagonism, with a wide variety of antibiotic substances produced and very limited in quantity.

Trichoderma viride produces, under certain conditions, strong antifungal antibiotics. In sterilized soil to which Trichoderma viride has been added, it might be expected that the severe inhibition of Ophiobolus graminis would result through antibiosis. However, because of high antibiotic production in the absence of other soil saprophytes, the inhibition in sterilized soil would be expected to be much greater than inhibition by the microflora of natural soil, where cross-antagonism would be exhibited. Under the conditions of this experiment, Trichoderma viride did inhibit the isolates of Ophiobolus graminis, but the reduction in the disease severity by the saprophytic fungus on the isolates was similar to, and

not much greater than the reduction by the natural microflora of the soil. The addition of the antagonist to natural soil further reduced the severity of disease. The same conclusions applied when Gliocladium roseum was used as the antagonist.

Because the effect of a single antagonist in sterilized soil was so similar to that of the natural soil microflora, it seems probable that similar mechanisms of action may have been operating in the two cases.

Brian (1, 2) indicated that, in view of the present experimental evidence, the production of antibiotics is not a likely cause of widespread soil fungistasis. Griffin (12) noted that the general fungistatic effect of natural soil may have resulted in part from the general saprophytic activities of the soil microflora, and further that toxic metabolites, other than specific antibiotic substances, may have been involved. The general saprophytic activities of the soil microflora are dependent on competition for food sources. Thus, if the mechanisms of action of the single antagonists to Ophiobolus graminis in this experiment are similar to those of the soil microflora, then competition for available food sources between the antagonist and the isolates of the pathogen is indicated, rather than inhibition by antibiotic substances produced by the antagonists.

In this investigation, competition for available

organic matter was continuous until the death of the wheat plants. The monosporous isolates of the pathogen appeared better able to compete than did the mycelial isolates.

EFFECT OF CHITIN ON THE SUPPRESSIVE ACTION OF INDIVIDUAL ANTAGONISTS AND THE NATURAL MICROFLORA OF THE SOIL ON MONOSPOROUS AND MYCELIAL ISOLATES OF *OPHIOBOLUS GRAMINIS*

Literature Review

The addition of specific substrates to natural soil, in the form of amendments, may selectively stimulate a segment of the microbial population that suppresses, directly or indirectly, a given soil-borne pathogen. In 1926, Sanford (28) suggested that the control of common scab of potatoes might be effected in this way. Since that time, microbial associates of root pathogens have been recognized to have a marked influence on these disease organisms, and a great amount of work has been carried out on this type of ecological approach on the control of soil-borne diseases.

Recently, Mitchell and Alexander (22, 23) obtained control of certain Fusarium diseases of beans and radishes by the addition of chitin, a naturally occurring polymer of acetyl glucosamine, as a specific soil amendment. They found that chitin stimulated growth of actinomycetes and lytic bacteria, which were antagonistic toward these soil-borne pathogens.

Because the chitin amendment was found to markedly reduce the severity of these Fusarium root diseases, it was decided to determine if it might act similarly on the Take-all disease,

caused by Ophiobolus graminis. In particular, the principal objective was to ascertain if the addition of chitin to the soil would enhance the antagonistic action of individual antagonists and of the natural soil microflora on Ophiobolus graminis.

Materials and Methods

The influence of chitin on the antagonistic effects of the four saprophytic microorganisms, Trichoderma viride, Gliocladium roseum, Bacterium No. 353 and Actinomycete No. 284, and of the natural microflora of the soil was tested on two monosporous isolates, 106 and 108, and two mycelial isolates, V and VII, of Ophiobolus graminis. Chitin was added to the soil pots in two of the three series at rates of 400 lb. per acre and 200 lb. per acre. The third series contained no added chitin. Each treatment was composed of two replicate pots, each sown with 25 seeds of Red Bobs wheat.

The saprophytic organisms and the isolates of Ophiobolus graminis were all grown on corn meal medium in 50 gm. lots in Erlenmeyer flasks. Uninoculated soil corn meal in 50 gm. lots was used as a control. Each soil pot received 100 gm. of inoculated or control medium. In the antagonistic comparisons, the medium added consisted of 50 gm. of an isolate of Ophiobolus graminis plus either 50 gm. of one of the antagonists or 50 gm. of the control. The medium added to the check pots consisted

of 50 gm. of an antagonist plus a similar amount of the control, or 100 gm. of the control.

Where chitin was used, it was finely ground and added at seed level to each pot, just below the added medium. This experiment was carried out in sterilized soil and in natural soil. After seeding, all pots were watered and held in a dark chamber at 60°F. until the wheat seedlings had emerged. The pots were then moved into a greenhouse compartment where the temperature was maintained at 65°F. for the duration of the experiment.

Results

Disease severity of the wheat seedlings in the inoculated pots was again used as a measure of the effects of the treatments on the pathogen. The results are presented in Table VII, in such a way as to compare the effects of the addition of chitin on the antagonism of the individual soil saprophytic organisms and the natural soil microflora on the isolates of Ophiobolus graminis. Each figure is the average obtained from two pots, expressing the percent of the emerged wheat seedlings killed by Ophiobolus graminis.

Under the conditions of this experiment, chitin had no effect on the antagonistic action of any of the antagonists on the isolates of the pathogen in sterilized soil. Moreover,

TABLE VII EFFECT OF CHITIN ON THE INHIBITION OF MONOSPOROUS AND MYCELIAL ISOLATES OF *OPHIOBOLUS GRAMINIS* BY INDIVIDUAL ANTAGONISTS IN SOIL

ANTAGONIST	*O. GRAMINIS* ISOLATE NO.	PERCENTAGE OF EMERGED WHEAT SEEDLINGS KILLED					
		STERILIZED SOIL			NATURAL SOIL		
		CHITIN 400 lb. per acre	CHITIN 200 lb. per acre	NO CHITIN	CHITIN 400 lb. per acre	CHITIN 200 lb. per acre	NO CHITIN
a) *TRICHODERMA VIRIDE*	106	85.7	89.2	97.9	77.1	86.8	78.0
	108	95.0	93.8	69.9	65.0	63.2	60.0
	V	55.5	65.0	46.5	4.8	2.4	7.6
	VII	10.4	8.3	6.3	2.7	2.4	3.0
	Control	0.0	0.0	0.0	0.0	0.0	0.0
b) *GLIOCLADIUM ROSEUM*	106	63.2	58.4	67.6	75.5	73.2	72.4
	108	59.2	65.8	42.9	40.8	32.4	47.6
	V	12.0	6.1	19.2	3.0	4.2	2.4
	VII	4.1	2.0	0.0	3.0	2.5	0.0
	Control	0.0	0.0	0.0	0.0	0.0	0.0

TABLE VII (CONTINUED)

ANTAGONIST	O. GRAMINIS ISOLATE NO.	PERCENTAGE OF EMERGED WHEAT SEEDLINGS KILLED					
		STERILIZED SOIL			NATURAL SOIL		
		CHITIN 400 lb. per acre	CHITIN 200 lb. per acre	NO CHITIN	CHITIN 400 lb. per acre	CHITIN 200 lb. per acre	NO CHITIN
c) BACTERIUM NO. 353	106	100.0	100.0	100.0	83.6	79.6	94.6
	108	96.9	95.9	97.9	81.2	82.8	82.8
	V	32.4	28.4	42.8	8.9	11.9	4.8
	VII	8.5	4.4	15.0	10.0	5.8	11.3
	Control	0.0	0.0	0.0	0.0	0.0	0.0
d) ACTINOMYCETE NO. 284	106	98.0	96.0	100.0	79.2	82.2	73.3
	108	72.2	73.9	77.1	71.0	59.5	71.0
	V	40.5	37.5	37.8	13.4	12.0	6.7
	VII	7.0	4.1	17.1	7.1	4.8	7.0
	Control	0.0	0.0	0.0	0.0	0.0	0.0

TABLE VII (CONTINUED)

ANTAGONIST	O. GRAMINIS ISOLATE NO.	PERCENTAGE OF EMERGED WHEAT SEEDLINGS KILLED					
		STERILIZED SOIL			NATURAL SOIL		
		CHITIN 400 lb. per acre	CHITIN 200 lb. per acre	NO CHITIN	CHITIN 400 lb. per acre	CHITIN 200 lb. per acre	NO CHITIN
e) NONE	106	97.9	97.9	100.0	93.9	92.8	95.4
	108	84.0	85.7	82.0	85.7	83.5	69.9
	V	56.0	57.2	48.6	23.4	21.4	19.4
	VII	11.3	12.8	10.4	2.5	3.0	0.0
	Control	0.0	0.0	0.0	0.0	0.0	0.0

chitin added to natural soil apparently did not modify the normal microbial population of such soil in such a way as to increase its suppressive action on any of the isolates of Ophiobolus graminis.

Neither level of chitin (400 lb. per acre or 200 lb. per acre) had any consistent suppressive effect on the isolates of Ophiobolus graminis themselves, as is shown by the results in sterilized soil, Table VII, Part e.

In the check pots, neither the addition of chitin nor soil corn meal nor both appeared to influence the growth of the wheat seedlings.

In Table VIII, the results of the antagonistic reactions of the individual soil saprophytes to the isolates of Ophiobolus graminis in the non-amended series were taken from the complete results of Table VII. While only two replicates of each treatment were used in this experiment, these results allow a direct comparison of the effects of the individual antagonists and supplement the results of the previous experiment. Here again, both Trichoderma viride and Gliocladium roseum inhibited the isolates of Ophiobolus graminis in much the same manner as the natural microflora of the soil inhibited the same isolates.

The association of Bacterium No. 353 or Actinomycete No. 284 with the individual isolates of the pathogen did not appreciably influence their disease-producing abilities, either

TABLE VIII

COMPARATIVE INHIBITION OF MONOSPOROUS AND MYCELIAL ISOLATES OF *OPHIOBOLUS GRAMINIS* BY INDIVIDUAL ANTAGONISTS IN SOIL

	PERCENTAGE OF EMERGED WHEAT SEEDLINGS KILLED				
O. GRAMINIS ISOLATE NO.	*TRICHODERMA VIRIDE*	*GLIOCLADIUM ROSEUM*	BACTERIUM NO. 353	ACTINOMYCETE NO. 284	CONTROL
			STERILIZED SOIL		
106	97.9	67.6	100.0	100.0	100.0
108	69.9	42.9	97.9	77.1	82.0
V	46.5	19.2	42.8	37.8	48.6
VII	6.3	0.0	15.0	17.1	10.4
			NATURAL SOIL		
106	78.0	72.4	94.6	73.5	95.4
108	60.0	47.6	82.8	71.0	69.9
V	7.6	2.4	4.8	6.7	19.4
VII	3.0	0.0	11.3	7.0	0.0

in sterilized or natural soils.

Under the conditions of this experiment, Gliocladium roseum was generally more antagonistic to the isolates of Ophiobolus graminis than was Trichoderma viride.

Discussion

While chitin was found to provide effective control of certain Fusarium diseases, (22, 23), it did not have any particular effect upon the Take-all disease in the present investigation.

Chitin stimulates the growth of lytic bacteria and actinomycetes. It was this segment of the population of soil microorganisms which was stimulated and which was active in the control of Fusarium sp., according to Mitchell and Alexander (23). They considered that by chitin amendment, a means may have been obtained of specifically selecting antibiotic-synthesizing species. However, Piening (25), working with Helminthosporium sativum and the effect of soil amendments, noted a closer relationship between the total numbers of actinomycetes and the disease severity than between the numbers of antagonistic actinomycetes and the disease severity. Such findings would seem to support the hypothesis that competition rather than a direct antibiotic action may be mainly responsible for the suppression of this pathogen in amended soils.

In the present experiments, neither the bacterium nor the actinomycete used had an effect on the disease severity of the isolates of Ophiobolus graminis. If chitin did stimulate a segment of the actinomycete and bacterial population in the natural soil under the conditions of this experiment, few, if any, of these species appeared able to suppress, directly or indirectly, the isolates of Ophiobolus graminis.

Further, chitin did not appear to affect either of the saprophytic fungi, Trichoderma viride or Gliocladium roseum, both which have been found to cause a considerable reduction in the disease severity by the isolates of Ophiobolus graminis.

DISCUSSION AND CONCLUSIONS

The investigations reported here were initiated for the purpose of comparing monosporous and mycelial isolates of Ophiobolus graminis with respect to pathogenicity and competitive ability. A special objective was to determine the comparative reaction of these to individual antagonists and to the general microflora of the soil. It was further planned to search for correlations between cultural characteristics and the pathogenicity and competitive ability of these isolates. The effect of the addition of one particular amendment, namely chitin, to the soil on the pathogen was also studied in some detail. The main conclusions drawn are as follows.

On artificial media, the isolates of Ophiobolus graminis displayed a wide variety of cultural characteristics, most of which could not be correlated either with pathogenicity or competitive ability. The variations observed occurred both between and within the isolates over a period of time. The most striking change was colony color, which depended upon the ability of the colony to produce macrohyphae. This ability, or its lack, did not appear to be due to temperature or light conditions, nor to the amount of agar present. The production of macrohyphae varied in a single isolate even in the same colony. Other cultural characters which were observed to vary were the amount and color of the aerial hyphae, the twisting of the

advancing hyphae, and the production of plate mycelia. The growth rate of an isolate of the fungus on potato sucrose agar was one character which did appear to be correlated with pathogenicity.

The growth rate of isolate No. 108 at one point dropped to about half of its original rate. At that time, its pathogenicity to wheat seedlings was the lowest of the monosporous isolates, whereas it originally was one of the most pathogenic. Towards the end of these investigations, the growth rate on potato sucrose agar increased and it again became one of the most pathogenic isolates.

The pathogenicity of all isolates of Ophiobolus graminis tested, was, as determined in sterilized soil, high and fairly uniform. However, the competitive ability of the monosporous isolates proved much greater than that of the mycelial isolates, a finding confirming that previously reported by Henry and McKenzie (15).

The competitive ability of the isolates was demonstrated in natural soil, where they were in competition with the general micropopulation of such soil, and in sterilized soil where a single saprophytic microorganism was present. The ability of the isolates of Ophiobolus graminis to compete in the soil was similar, under the conditions of these experiments, whether the competition was supplied by the micropopulation of the soil as

a whole, or by either of two individual saprophytic fungi, namely Trichoderma viride and Gliocladium roseum. Of the four saprophytes tested, Trichoderma viride and Gliocladium roseum were active in competition with the isolates of Ophiobolus graminis, but Bacterium No. 353 and Actinomycete No. 284 were more or less inactive.

The mechanism conferring competitive ability on a particular isolate is unknown, but it is probably associated with the genetical makeup of that isolate.

Using Aspergillus glaucus, Mather and Jinks, (20) found that differentiation and ageing of the cells involved characteristic changes of the cytoplasm. They further noted that selection could be used to produce changes of character which sprang wholly from alterations in the cytoplasmic content, and if propagation was carried out for several generations by hyphal tips only, the fungus eventually lost its capability for sexual reproduction and finally it became progressively difficult for the hyphae to maintain full growth. They noted that envigoration resulted from passage through the sexual stage and the formation of ascospores.

Should competitive ability and pathogenicity, in the case of Ophiobolus graminis, be associated with the cytoplasmic makeup of this fungus, it might explain some of the differences found between the isolates. The monosporous isolates, derived from single ascospores, possessed high pathogenicity and high competitive ability. The mycelial isolates of Ophiobolus graminis, on

the other hand, possessed high pathogenicity but low competitive ability, and were vegetative in nature and probably not pure cultures when originally isolated. On this basis, it would appear that competitive ability might be associated with the cytoplasm and that its loss in the mycelial isolates was due to alterations in the cytoplasmic content. This might have resulted through continuous vegetative propagation or possibly through anastomosis.

In nature, the mycelium of Ophiobolus graminis is probably a mixture of several strains, each being modified through anastomosis with one another. This mycelium either has not passed or is not able to pass through the sexual stage. Isolates derived from this source might, therefore, be expected to lack vigor and hence not possess very high degrees of competitive ability.

The revival of vigor, by means of restandardization of the cytoplasm, could have resulted from passage through the sexual stage. The cytoplasm of the monosporous isolates could thus have been envigorated. The single ascospore cultures were isolated and maintained as pure cultures, before anastomosis and contamination of the cytoplasm with other strains could have occurred, so that the abilities of these isolates to compete was not modified or lost.

Loss in pathogenicity by isolates of Ophiobolus graminis

maintained for nine years in pure culture was noted by Russell (27). Similarly, loss in pathogenicity over a period of time was noted in the present investigation. In the last experiment, isolate VII, for instance, caused only light disease in wheat seedlings in sterilized soil, whereas it was severely pathogenic in earlier experiments. Isolate VII appears to have lost its pathogenicity over the last $4\frac{1}{2}$ years, as a result of continuous vegetative culturing. It would therefore appear that pathogenicity could also be associated with, or affected by cytoplasmic changes due to vegetative propagation.

At present, proof for these suppositions requires suitable tests, using monosporous, conidial and vegetative propagations. Ascospores of Ophiobolus graminis are not produced readily in artificial culture and conidial production has never been demonstrated. Hence, before the importance of the cytoplasm towards the control of pathogenicity and competitive ability in Ophiobolus graminis may be determined, further investigation is necessary with conidial-producing Ascomycetes, such as Aspergillus glaucus, to determine suitable tests which do not require the use of conidia.

Trichoderma viride and Gliocladium roseum were both able to reduce the disease-producing ability of all isolates of Ophiobolus graminis in greenhouse experiments. The addition of chitin as an organic amendment did not alter the competitive

effects of these saprophytic fungi towards isolates of the pathogen. Chitin is known to influence a segment of the soil micropopulation which includes actinomycetes and lytic bacteria. This segment of the micropopulation did not, however, under the conditions of this investigation, have an effect on the disease-producing ability of the isolates of Ophiobolus graminis. Hence, successful control of Take-all caused by Ophiobolus graminis through the incorporation of organic amendments will probably depend on the use of a soil amendment which will stimulate other segments of the micropopulation, possibly ones which include Trichoderma viride and Gliocladium roseum.

SUMMARY

1. Potato sucrose agar was found to be superior to potato dextrose agar for use in the artificial culture of isolates of Ophiobolus graminis.

2. Striking differences and changes were noted in cultural characteristics on potato sucrose agar of all isolates of the pathogen.

3. Variation in cultural characteristics of an isolate was not correlated with a change in the pathogenicity or competitive ability of that isolate. A reduction in the growth rate of an isolate on potato sucrose agar, however, did appear to be associated with a loss of pathogenicity of that isolate.

4. While all isolates of Ophiobolus graminis studied exhibited high degrees of pathogenicity, monosporous isolates, derived from single ascospores, generally showed high degrees of competitive ability, whereas mycelial isolates were generally poor competitors.

5. The comparative effects of the individual fungal antagonists, namely, Trichoderma viride and Gliocladium roseum, in sterilized soil proved to be similar to the effects of the natural soil micropopulation as a whole on the monosporous and mycelial isolates of Ophiobolus graminis. However, neither proved antagonistic to isolates of the pathogen on potato sucrose agar.

6. Monosporous isolates of Ophiobolus graminis proved more resistant to the action of either fungal antagonist than did the mycelial isolates, a relationship reported by Henry and McKenzie (15) for the soil micropopulation as a whole.

7. Bacterium No. 353 and Actinomycete No. 284 both inhibited, at least temporarily, growth of all isolates of the pathogen on potato sucrose agar, but were more or less inactive in the soil.

8. Chitin, as a specific soil amendment, did not appear to suppress, directly or indirectly, the disease-producing abilities of monosporous or mycelial isolates of Ophiobolus graminis, whereas it has been reported by Mitchell and Alexander (23) to inactivate certain Fusarium pathogens.

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