A STUDY OF THE ETIOLOGY OF SPRING DEAD SPOT

By

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SPRING DEAD SPOT

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INTRODUCTION

Spring dead spot is becoming recognized as the foremost disease problem of bermudagrass (Cynodon dactylon (L.)Pers.) in the United States. This disease is found only on bermudagrass and primarily wherever winters are cold enough to induce dormancy. In fact, there is a marked correlation between disease severity and length of the dormant season. Frederiksen (6) reported spring dead spot to occur along and to the south of a line corresponding to the northernmost areas of bermudagrass adaptation, approximately from central Kansas and Oklahoma through St. Louis, Indianapolis, and Philadelphia and into central New Jersey. Spring dead spot, or a disease strikingly similar to it was found in northern California (19). A.M. Smith (15) reported spring dead spot to be the most damaging disease affecting couch (bermuda) grass in New South Wales, Australia.

Spring dead spot is first noticed in the spring as small, circular areas of dead grass as the surrounding grass breaks dormancy, hence the name (Fig.1). These spots usually contain no living grass. All stems, crowns, stolons, roots, and rhizomes are black and rotted. As the growing season progresses, bermudagrass does not invade these dead areas. The spots remain throughout the summer and into the fall, usually becoming filled with noxious weeds and weed grasses

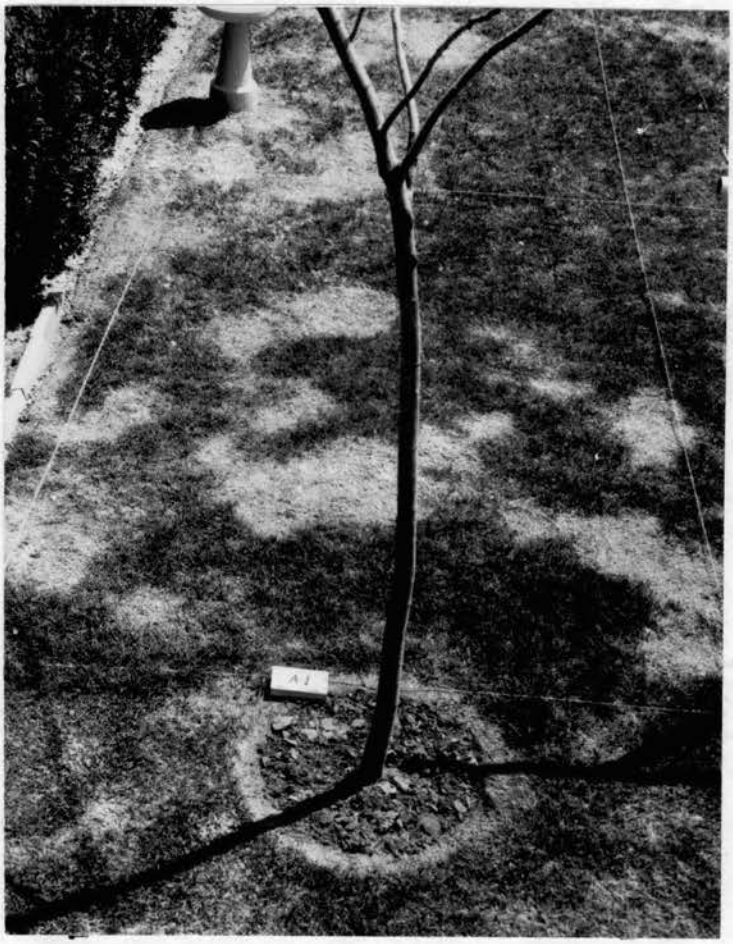

Figure 1. Common bermudagrass lawn affected by spring dead spot

such as crabgrass, henbit, and clover. Crabgrass (Digitaria sanguinalis (L.) Scop.) grows extensively in these spots, seemingly unaffected by the disease, although some crabgrass plants found in spring dead spots in California (19) were infected with Ophiobolus.

The spots do not increase or decrease in size during the summer growing season, although sometimes the spots are covered by runners originating in adjacent healthy turf. These runners do not root in the dead spots. Any roots formed at nodes over the dead spots are quickly attacked and deformed, eventually rotting away completely. During the following winter season when the grass is dormant, dead spots from the previous spring may sometimes be seen in outline through the thin grass covering them, and may also be identified by the weeds present in the spots.

The following spring, dead spots occur in the same position as the year before and often have increased to twice the size of the previous year. The enlargement and coalescense of a number of spots results in large irregular areas of dead grass which may destroy entire lawns or other valuable turf areas. The distribution of spots in any given turf area appears to be random, being unaffected by differences in local terrain or soil type.

Observation and studies of the disease have led to several generalizations concerning its nature. It is apparently active only in the winter season. Longer dormant periods seem generally to produce greater severity. The Circular

appearance of the spots, even on slopes and terraces, indicates that they are not spread by water movement.

This disease seems to be related to the development of thatch that normally occurs with well nourished bermudagrass growth. Thatch may accumulate rapidly, particularly when clippings are not collected, and spring dead spot is found to occur most frequently in areas containing rather heavy thatch accumulations. The disease is not usually found where thatch is removed regularly and thoroughly. Spring dead spot has not been found in newly established turf. It generally is not found until about the third year of growth—long enough for an appreciable thatch accumulation.

The cause of the disease known as spring dead spot has not been found. A species of Helminthosporium has been rather consistantly isolated from spring dead spots in Oklahoma and from rotted roots of runners crossing these dead spots. The same species has also been isolated from similar dead spots in Arkansas, Missouri, and Maryland. It has also been isolated from dead spots of bermudagrass in California, but less frequently than a species of Ophiobolus (19). However, attempts to reproduce the disease by artificial inoculation with these fungi or by the movement of infested soil or grass parts have failed.

The objects of this investigation were to study some of the growth characteristics of the above mentioned fungi, the effects of certain fungicides on their growth, and the relationship of thatch to the growth of bermudagrass itself.

LITERATERATURE REVIEW

Spring dead spot was first noticed in 1954 in Stillwater, Oklahoma, by Wadsworth and Young (17) who reported it as a disease in 1960. In 1961 Wadsworth (18) reported control of spring dead spot with dieldrin, an insecticide, used at a rather high rate as a soil drench. Many other insecticides, fungicides, and herbicides have been tested since that time, but none have given any significant control of spring dead spot. Even dieldrin has not always been effective in control of the disease.

Extensive studies into the cause of spring dead spot have yielded little information. Winter injury, white grubs, and nematodes have occasionally been associated with the disease, but have not been correlated with it (3, 19). A scale insect was found associated with spring dead spot in Arkansas, but Dale and McCoy (4) concluded it was not responsible for the disease. Isolations from bermudagrass affected by spring dead spot in Oklahoma have consistantly yielded only species of Helminthosporium the most common of which has been H. spiciferum (Bain.) Nicot.

H. spiciferum is a small-spored (25-40 X 10-20 μ), three septate Helminthosporium (Fig. 5) first reported by McKinney (10) and described as H. tetramera. At about the same time Drechsler (5) described a similar fungus as H. triseptatum.

These were later consolidated into a single species, H. spiciferum, by Nelson (11) in 1964. Nelson also listed Brachycladium spiciferum as a synonym and stated that the organism was once classified in the genus Curvularia.

Smith (15) reported from New South Wales that Ophiobolus herpotrichus (Fr.) Sacc., isolated from diseased roots and stolons from spring dead spots, would cause severe root and crown rotting of bermudagrass seedlings in the greenhouse. Wadsworth (19) found a species of Ophiobolus in spring dead spots in California. He was able to demonstrate that this species of Ophiobolus, as well as H. spiciferum and H. cynodontis Marig. would grow satisfactorily and cause root and crown rot of bermudagrass at temperatures low enough to be associated with dormancy in bermudagrass. However, no one has yet reported being able to reproduce typical spring dead spot symptoms by field inoculations with these fungi.

The tendency for a spring dead spot to remain for several years in the same location without the re-establishment of bermudagrass in the affected areas suggests the possibility that a toxin might be associated with the disease. Certain species of Helminthosporium are known to produce host specific toxins (9). Wadsworth (19) tested the culture filtrates of H. spiciferum, Ophiobolus sp., H. cynodontis, and a species of Curvularia on seedlings of two varities of oats as well as bermudagrass seedlings. He was, however, not able to find any evidence of phytotoxicity in any of the culture filtrates tested.

Considerable evidence exists that the development of thatch in bermudagrass turf may be involved in the spring dead spot disease. Frederiksen (6) reported thatch removal to have no effect on spring dead spot control in St. Louis, Missouri, but his measurements were made on turfgrass areas already severely diseased. Studies at Stillwater, Oklahoma, have indicated that thatch removal may aid in the prevention of spring dead spot. The disease has appeared in experimental turf plots where thatch was allowed to accumulate, but no spring dead spot has yet appeared in the portions of the same plots where thatch accumulation was prevented by vertical mowing each year and by collecting clippings each time the plots were mowed (8).

Several factors may be involved in the apparent correlation between disease incidence and organic matter accumulation: 1) the organic matter may serve as a medium for the growth of certain parasites; 2) organic matter may alter the microbial population of the soil or rhizosphere which may favor certain parasites; 3) soil nutrients may be depleted during microbial decomposition of the added organic residue; 4) the organic residues or their decomposition products may be phytotoxic (2).

Cochrane (2) found that aqueous extracts of clover, ryegrass, and corn residues at concentrations of 1:20 and 1:40 would cause a type of injury to radish roots very similar to root rot. He also found that soybean residues, which were known to reduce strawberry root rot and corn root rot, were

not injurious to radish. In addition, clover residues, which generally increased root rot under field conditions, caused severe damage to radish root cells. He concluded that plant residues may serve to either increase or decrease the incidence of root rot, depending on the residue source and the plants affected. He stated that some root rots may be initiated by the direct toxic action of plant residues so that the activities of soil organisms are secondary and dependent upon an initial injury of chemical origin.

Studies by Patrick, et al. (12, 13, 14, 16) have shown that phytotoxic products are produced during the decomposition of organic matter. They found that injury to the roots of lettuce and spinach seedlings was confined mainly to those parts in direct contact with, or adjacent to, decomposing plant residues in the soil. Only saprophytic organisms were isolated from these lesions. Phytotoxic substances were isolated from the decomposing plant residues which inhibited seed germination and seedling growth, especially root growth. Toxicity levels were found to decrease with increasing periods of decomposition (14). Aqueous extracts of plant residues prepared before decomposition occured or when decomposition was inhibited by heat sterilization, were not toxic (12). Patrick concluded that toxins derived from plant residues or the decomposition of such residues may serve as a primary cause of some root rot diseases predisposing plants to attack by organisms not generally considered pathogenic.

Benedict (1) found that the growth of bromegrass was affected by a substance or substances produced from the dead roots of the same grass. He related this to the field condition in which thick stands of bromegrass become sod-bound and thin out after several years growth. It was his opinion that sod-binding may be at least partially due to the accumulation of growth inhibiting substances in the soil.

Patrick et al. (13) concluded that the formulation of phytotoxic decomposition products was mainly associated with the decay of organic matter under adverse aeration conditions. Low oxygen availability and high moisture levels tended to increase toxin production and activity. Localized pockets of anaerobiosis may be widespread in the soil and in layers or masses of plant residues. Phytotoxic compounds produced under these conditions may, in turn, be broken down by microbial activity, but the rate of inactivation may often be balanced by the rate of production. Thus effective toxin concentrations may be maintained at constant levels for extended periods.

MATERIALS AND METHODS

Isolates of H. spiciferum were obtained from diseased roots and stolons of bermudagrass taken from spring dead spots on the Oklahoma State University campus. Isolations were made during the months of June, August, and December, 1965. Roots and stolons were surface sterilized in 1:3 chlorox in water for a period of 30 seconds to 1½ minutes, depending on size. These plant parts were then placed on potato-dextrose agar (PDA) in petri dishes and held at room temperature. Transfers were made when necessary. The culture of Ophiobolus used in this study was isolated from spring dead spot in California by Wadsworth (19). The species has not been determined and therefore will be referred to only as Ophiobolus sp. in this report.

The rates of growth of H. spiciferum and Ophiobolus sp. were determined by measurements of the radial extension of mycelium growing on PDA. PDA plates were inoculated with vegetative hyphae of the fungus under test and placed in incubators at temperatures of 0°, 10°, 18°, 25°, 30°, 35°, and 40°C. Three to four replications were placed at each temperature and measurements of colony diameters taken at 24 hour intervals to determine the rate of growth. Notes were also taken upon the initiation of sporulation by H. spiciferum.

The effects of changes in temperature on the growth of

H. spiciferum was also tested. One set of culture plates was kept at each temperature as a control while other sets were grown at certain temperatures for a specified period of time, then moved to a different temperature.

The effect of various pH levels on the growth of H. spiciferum and Ophiobolus sp. was tested by growing these fungi on a culture shaker in a basal synthetic medium utilizing three different sources of nitrogen (KNO_3, NH_4NO_3, and L-asparagine). Forty ml of synthetic medium, without dextrose, were placed in 250 ml Erlynmeyer flasks after electrometrically adjusting the pH to the desired value with 2M NaOH and HCl. Each treatment was replicated four times at an initial pH of 3.5, 5.0, 6.5, 8.0, and 9.5. A 10% dextrose solution was autoclaved seperately to prevent carmelization. All flasks were stoppered with cotton plugs and steam sterilized at 15 psi for 30 minutes. After cooling, 10 ml of the sterilized dextrose solution were aseptically added to each flask. Each flask was inoculated by the addition of a suspension of mycelial fragments prepared by grinding mycelium in a Waring blender for 15 seconds. (Appendix II)

H. spiciferum was grown for a period of 5 days in the shaker culture before harvesting while Ophiobolus sp. was grown for 15 days due to a slower growth rate. The cultures were harvested by filtration through a weighed filter paper with a buchner funnel under vacuum. The cultures were then rinsed with distilled water and placed in an oven at 80°C for 48 hours. The papers containing the cultures were

removed from the oven and weighed on a Mettler analytical balance to determine the dry weight. The final pH of each culture filtrate was also measured after harvest.

Tests for pathogenic agents producing symptom expressions similar to spring dead spot involved H. spiciferum, its culture filtrates, Ophiobolus sp., bermuda thatch, and thatch extracts. Surface sterilized bermudagrass cuttings and seedlings were used for assay of symptom severity. Observations were made on root growth, color, and morphology and on total growth of the seedling or cutting.

Mycelia of both Ophiobolus sp. and H. spiciferum were placed in 250 ml Erlynmeyer flasks containing bermudagrass cuttings growing in a liquid nutrient medium. The absence of a carbon source in the medium retarded the growth of the fungi allowing for a more "natural" infection of the bermuda plant. Cultures were placed at room temperature (25-30°C), and at a temperature of 10°C. The progress of infection and subsequent plant growth was observed and recorded.

Culture filtrates of H. spiciferum, prepared as in the pH tests, were poured into petri plates containing cotton discs so that the filtrates would be absorbed into the cotton. Bermuda seed was then sown over the cotton discs and seedling growth evaluated for injury that may have resulted from toxic compounds. The filtrates were both aqueous and ethanolic extracts of the cultures used. The ethanolic extracts were allowed to air dry before sowing the bermudagrass seed. One set of filtrates was treated with dieldrin to

check its effects on any toxicity that might appear. Culture filtrates were also tested on bermudagrass cuttings growing in nutrient medium in 250 ml Erlynmeyer flasks.

Although thatch as it exists in the field is composed of many plant parts, in this study only leaf and stem clippings were used. This type of thatch and extracts from it were used in both laboratory and greenhouse tests of bermudagrass toxicity caused by either the organic matter itself or its decomposition products. The clippings used were collected from both U-3 and Sunturf varieties of bermudagrass.

Thatch extracts were prepared by adding 25 g dry bermuda clippings to 500 ml water. The mixture was then steam sterilized for 20 minutes at 15 psi. The extract was then filtered free of bermuda clippings and referred to a a 1:20 dilution of dry matter to water. Other dilutions were then made in 250 cc bottles using a sterile nutrient solution as the diluent. Each bottle was plugged with cotton after two surface sterilized bermuda cuttings had been placed in each. Observations were taken on root growth, color, and morphology as well as leaf and stem growth.

A greenhouse test of bermuda thatch toxicity was carried out by a modification of the method used by Benedict (1) in evaluating the phytotoxicity of bromegrass roots. Fifteen four-inch clay pots were filled with sand and sterile bermuda thatch so that each pot contained 1.5-2.0% organic matter by weight. The pots were half filled with sand, then the bermuda thatch was added and covered with another inch of sand.

Five other pots were filled with sand only to serve as controls. Each pot was planted with two Kaw wheat seedlings, three bermudagrass sprigs, and one green foxtail (Setaria viridis (L.) Beauv.) seedling. There were four treatments of five replications each. In one treatment the moisture content of the soil was varied and in another the moisture content was held constant. A third treatment was also held at a constant moisture level and in addition received three applications of 50 ml of a 1:5000 dieldrin solution per pot. The final treatment was the check planted in pure sand and held at a constant water level. The temperature varied from 19-29°C with an average of 24°C.

In a similar greenhouse test, different sources of thatch or organic matter were used. These consisted of sterile bermuda thatch, unsterile bermuda thatch, sterile wheat straw, and sterile bentgrass clippings. A fifth treatment consisted of pots of pure sand in which bermuda cuttings had been rooted and a layer of bermuda thatch placed over the surface of the sand. Five replications (pots) were used in each treatment and each pot contained 3 bermuda cuttings, and one foxtail seedling.

Fifteen chemicals were evaluated in the laboratory for their fungicidal or fungistatic effects on the growth, sporulation, and spore germination of H. spiciferum, and upon the growth of Ophiobolus sp. A stock solution of each chemical was prepared containing one part active ingredient in 100 parts aqueous solution. Other concentrations used were

made from these stock solutions. Each test was prepared by adding 40 ml of the synthetic medium, adjusted to pH 6.5, to a 250 ml Erlynmeyer flask which was stoppered with cotton. A 10% dextrose solution was made and steam sterilized seperately as described previously. After sterilization, 10 ml of the dextrose solution were added to each flask along with the desired amount of 1% chemical solution. Each flask was then seeded with 1 cc of a mycelial suspension and placed on a culture shaker at 25°C. Two replications were used. The harvest and dry weight measurement of each culture was accomplished as previously described. (Appendix I)

The effect of various chemicals on the germination of conidiospores of H. spiciferum was determined by preparing a 1:50,000 dilution of each chemical in water agar. Twenty ml of the water agar containing the chemical were added to each petri dish, which was then seeded with spores by inverting a culture of H. spiciferum over the test dish and tapping gently so that the spores would fall onto the agar surface. The plates were then held in the dark at a temperature of 25°C. Observations were made once each hour until germination was observed. Records were made of the percent germination, time of germination, and of any morphological differences noted in the germinating spores. The percent germination was determined by counting the number of germinated and non-germinated spores seen in 5 randomly selected microscope fields. Spores were considered to be germinated when the germ tubes reached a length equal to that of the parent spore.

Two home lawns severely affected with spring dead spot were used to evaluate eight chemical treatments for disease control over a period of two years. Each yard was mapped and divided into 15 plots of 100 square feet each. Five treatments were made per yard with each treatment replicated three times in a randomized design. The chemicals were applied with an Ortho hose-on applicator and then drenched into the soil by watering.

Each spring after dormancy was broken the lawns were mapped and the spring dead spots plotted on a grid so that the square feet of affected turf could be determined. This method also gave an accurate record of the size and location of each spot for comparison from year to year. A determination of the diseased area in each plot was made by the use of tracing paper with a constant weight per unit area. A tracing was made of the grid of each plot and the tracing paper showing the affected areas were cut out and weighed. Multiplying the weight of the tracing paper showing the dead spots from each plot by the unit weight of the paper then gave a measurement of the affected turf area.

RESULTS

Temperature effects on growth

The optimum temperature for mycelial growth of H. spiciferum was found to vary with the age of the culture. During the first 3 days of growth the optimum appeared to be near 35 C, but with cultures older than 3 days the optimum temperature was 30C (Fig. 2). Temperatures above 30C caused a rather sharp drop in the mycelial growth rate. Below 30C the growth rate was gradually reduced until 0C where no growth occurred.

Sporulation of H. spiciferum was initiated within 48 hours of inoculation on PDA at 30C and 35C. The initiation of sporulation required progressively more time as the temperature was reduced, requiring 6 days at 10C. No sporulation occurred at 40C and the colonies were very dark in color, whereas the vegetative colonies at the lower temperatures were white (Fig. 4). There appeared to be no correlation between size of the vegetative colony and the initiation of sporulation.

Similar experiments indicated that an abrupt temperature change had little or no effect on the subsequent growth of H. spiciferum (Fig. 6). For example cultures growing at 10C when moved to 30C would immediately begin to grow at the same rate as cultures held continuously at 30C with no

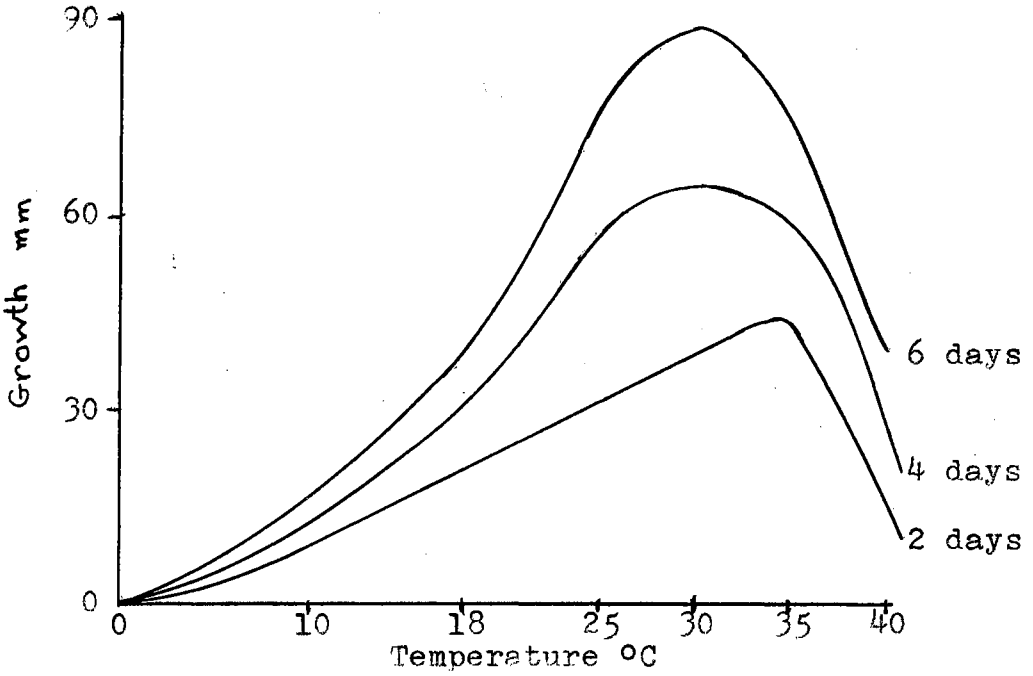

Figure 2. Growth curves of Helminthosporium spiciferum on PDA after 2, 4, and 6 days

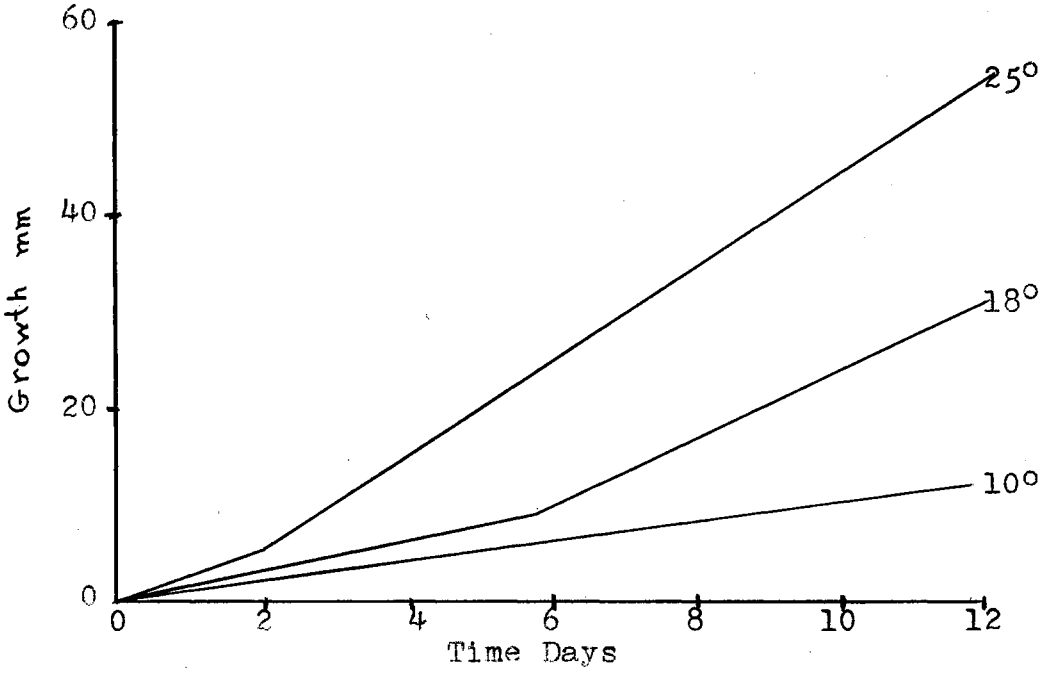

Figure 3. Rate of growth of Ophiobolus sp. at temperatures of 10, 18, and 25 degrees Centigrade

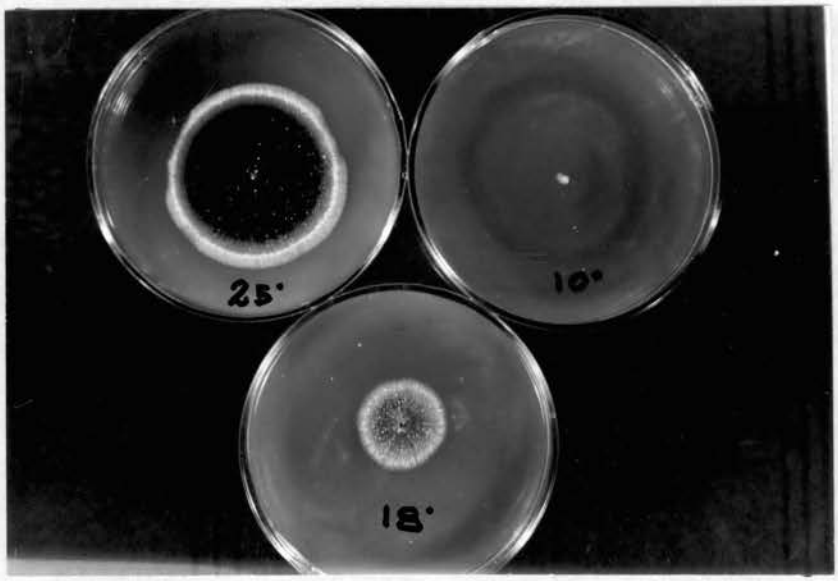

Figure 4. H. spiciferum grown on PDA at temperatures of 10, 18, and 25 degrees C for three days

Figure 5. H. spiciferum spores and mycelium 450X

variation induced by the former temperature.

The growth of Ophiobolus sp. (Fig. 7) was found to be most rapid at a temperature of 25C. No growth was observed at 30C or above and none at 0C (Fig. 3). Ophiobolus sp. grew much more slowly than H. spiciferum, taking 20 or more days to cover a 90 mm petri dish at optimum temperature. H. spiciferum produced the same size of culture in six days at its optimum temperature.

pH Effects on Growth

H. spiciferum grew readily in a liquid synthetic medium over a wide range of pH (Fig. 8). The best growth, as measured by the weight of the mycelial colony, was attained at pH 5.5 and pH 8.0, but a drop in growth was noted at pH 6.5. Sporulation was heaviest between pH 6.5 and 7.5. Ophiobolus sp. also grew over a wide range of pH. Growth increased steadily as the pH increased with a maximum at approximately pH 7.5.

Varying nitrogen source in the medium influenced the growth of both fungi. H. spiciferum attained its best growth with L-asparagine, but good growth was also produced with NH_4NO_3 and KNO_3 at the higher pH levels (Table I). When NH_4NO_3 was used the final pH of the medium was lowered significantly. Conversely, when KNO_3 was used the final pH was raised. L-asparagine had little effect on the final pH.

The growth of Ophiobolus sp. was tested using L-asparagine as a source of nitrogen at four initial pH levels,

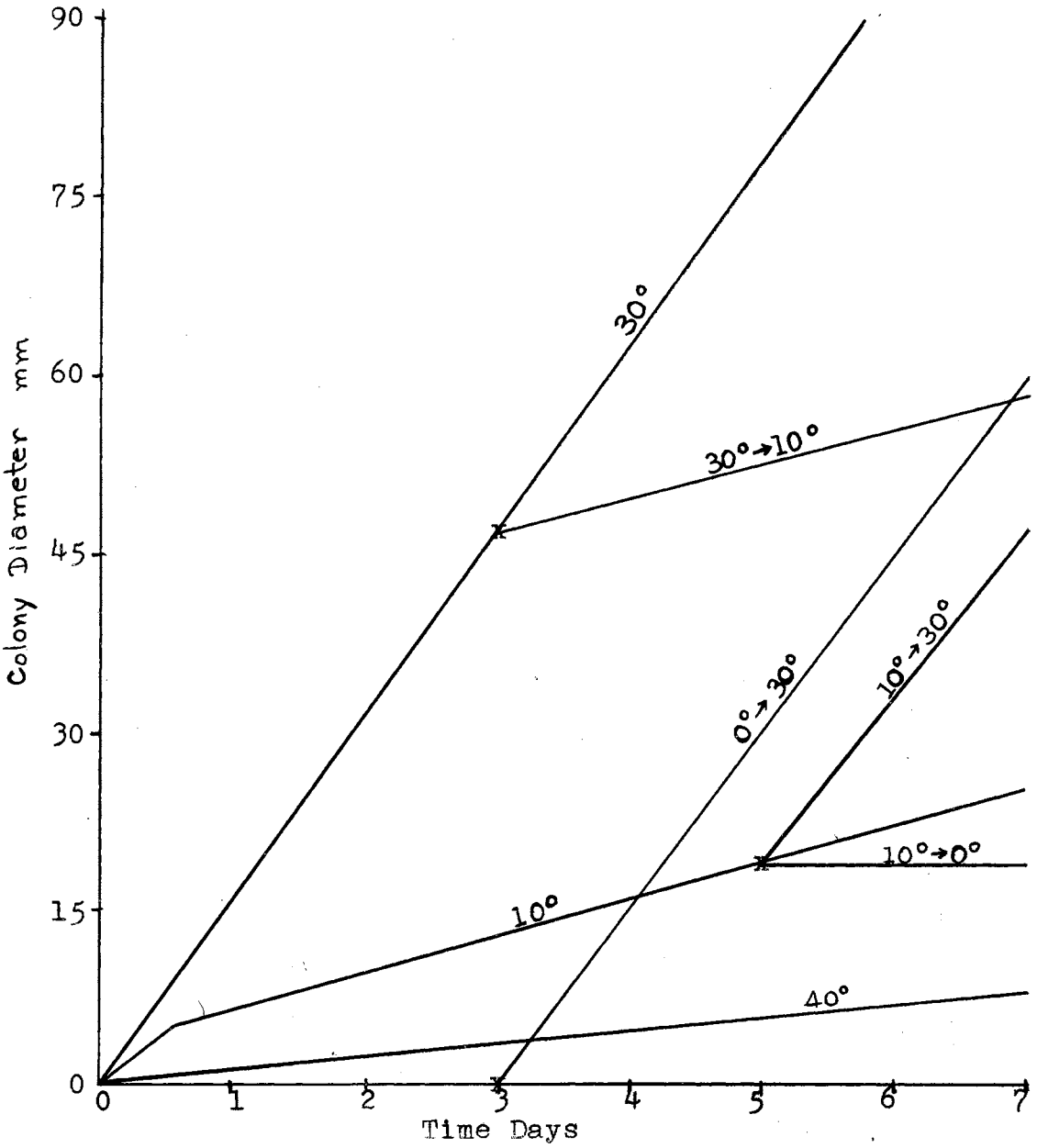

Figure 6. Effect of temperature changes on the rate of growth of H. spicif-erum as measured by the slope of the rate of growth line, x equals temperature change

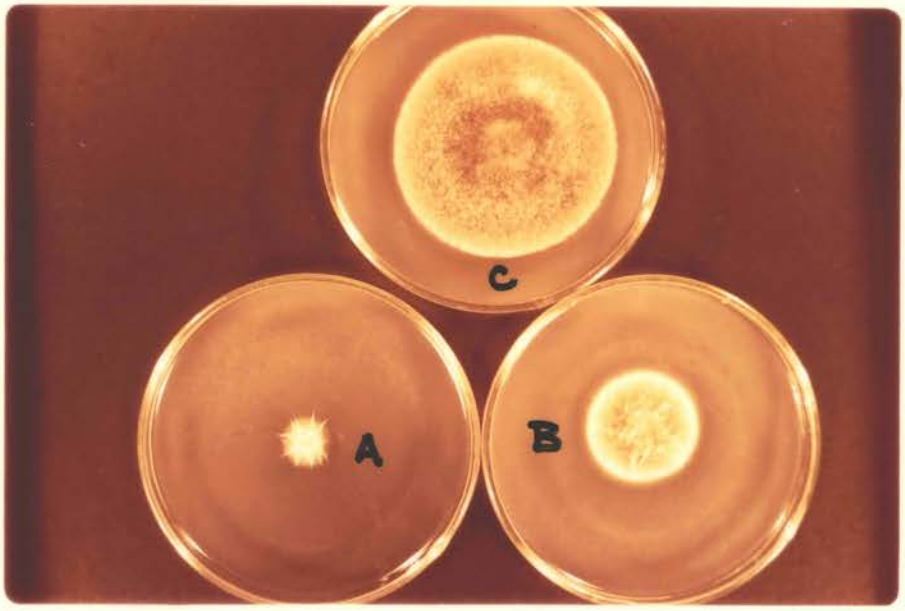

Figure 7. Ophiobolus sp. grown on PDA at temperatures of A, 10°; B, 18°; and C, 25°C for a period of 12 days

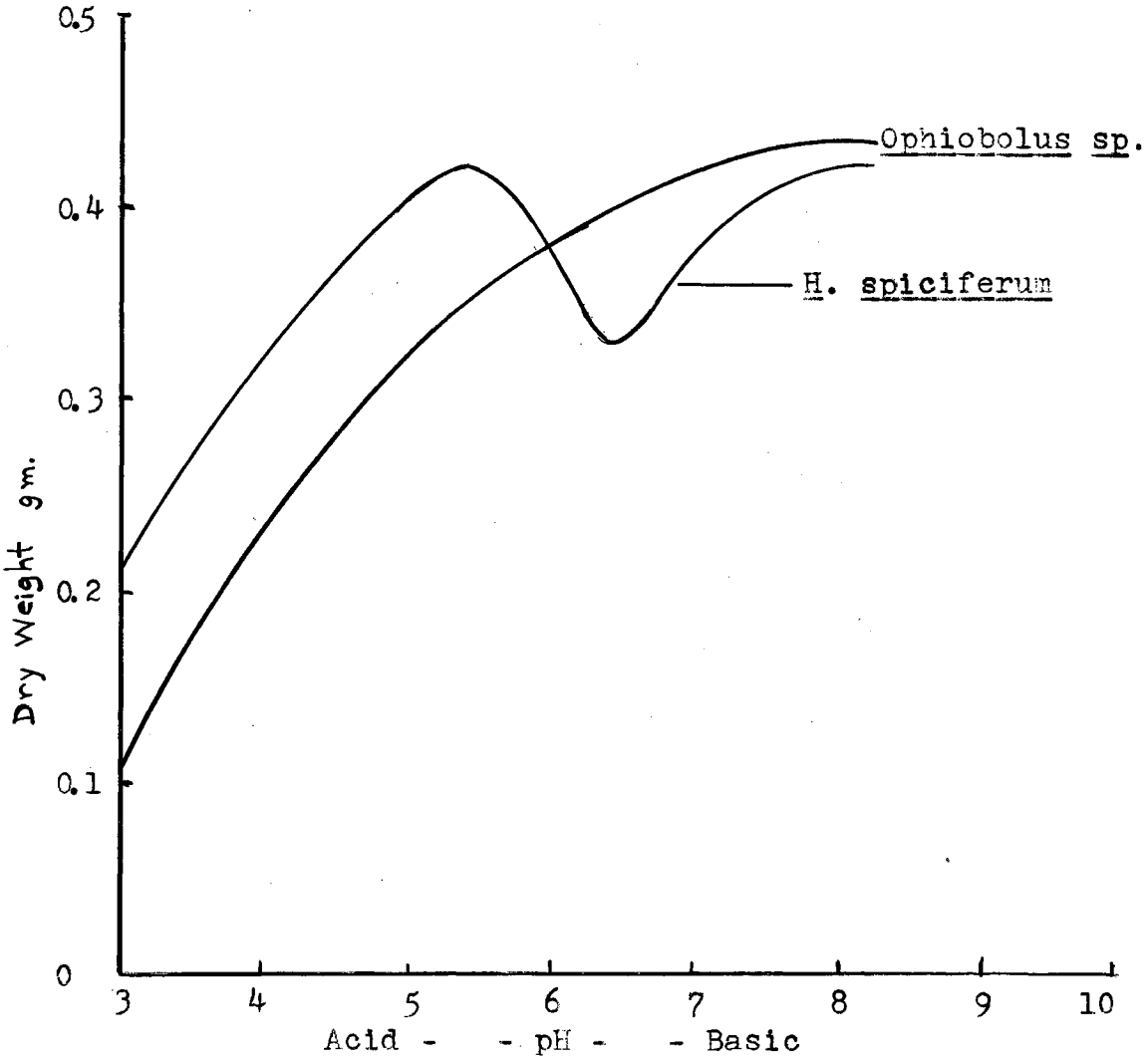

Figure 8. pH-growth curves for H. spiciferum and Ophiobolus sp. grown on synthetic medium at 25°C

but with NH_4NO_3 and KNO_3 at only one pH level (Table II). The growth of this fungus on L-asparagine was good at all pH levels except the most acid (pH 3.5). At pH 6.5, growth on KNO_3 was good, but at the same pH growth was rather poor with NH_4NO_3 as the nitrogen source. The effect of nitrogen source upon the final pH of the medium was approximately the same with Ophiobolus sp. as with H. spiciferum.

Culture Filtrates

A test was made for toxic substances produced by H. spiciferum cultures growing on bermuda decoction broth and on bermuda clippings at 3 temperatures. Ethanol extracts from these cultures were placed on cotton pads in deep petri dishes and allowed to air dry. When the pads were dry 20 ml sterile distilled water were added to each dish and 20 bermuda-grass seeds were sown across the moist cotton pads. Only 15% of this seed germinated, but definite inhibition of root growth of the seeds that did germinate was noted. The data are presented in Table III.

Another test was made in which ethanolic extracts were made of H. spiciferum cultures grown at 25C on bermuda clippings, bermuda clippings and sand, and a synthetic medium. In addition, an ethanol extract was made from sterilized bermudagrass clippings and a water extract was made of a culture of the fungus grown on synthetic medium. The treatments were prepared as before, allowing the extract to air dry on cotton pads in petri dishes. When the pads were dry half of the cotton pads were wet

TABLE I

GROWTH OF H. SPICIFERUM AT VARIOUS pH LEVELS AND WITH THREE NITROGEN SOURCES

Nitrogen Source —	Asparagine	NH_4NO_3	KNO_3
Initial pH	Average dry weight in grams		
3.5	.2634	.2122	.2103
5.0	.4125	.2048	.2938
6.5	.3232	.2031	.3586
8.0	.4074	.3757	.3551
9.5	.4191	.3700	----
	Final pH of medium		
3.5	3.5	2.2	6.9
5.0	6.0	2.2	7.3
6.5	6.5	2.2	7.8
8.0	7.5	2.5	7.9
9.5	7.8	5.6	---

TABLE II

GROWTH OF OPHIOBOLUS SP. AT VARIOUS pH LEVELS AND WITH 3 NITROGEN SOURCES AT pH 6.5 ONLY

Nitrogen Source ——	Asparagine	NH_4NO_3	KNO_3
Initial pH	Average dry weight in grams		
3.5	.1887	----	----
5.0	.3422	----	----
6.5	.3920	.1264	.3038
8.0	.4297	----	----
	Final pH of medium		
3.5	4.4	--	--
5.0	4.8	--	--
6.5	5.7	2.8	8.1
8.0	6.4	--	--

TABLE III

INHIBITION OF ROOT GROWTH OF BERMUDAGRASS SEEDLINGS BY ETHANOL EXTRACTS FROM CULTURES OF *H. SPICIFERUM* GROWN ON BERMUDAGRASS DECOCTION BROTH AND ON BERMUDA CLIPPINGS AT DIFFERENT TEMPERATURES

Treatment	Root Length	Remarks
1) Broth 25C	0 mm	2 plants dead
2) Clippings 25C	5-8 mm	roots off white color
3) Broth 30C	15 mm	"
4) Clippings 30C	15 mm	"
5) Broth 10C	5-10 mm	"
6) Clippings 10C	10 mm	"
7) Check (distilled water)	30 mm	roots long, white, vigorous

with a 1:5000 dieldrin solution and the other half with sterile distilled water. The data are given in Table IV. The extracts from cultures grown on bermuda clippings and on bermuda clippings plus sand were quite inhibitory both to germination and to root growth, while the extract of H. spiciferum grown on the synthetic medium had no inhibitory effect. The dieldrin solution did not alter root growth appreciably, but did seem to increase the percent of germination. The extract of the sterile bermuda clippings did not seem to affect germination, but did have a pronounced inhibitory effect on root growth. Both germination and root growth were affected by the water extract of the H. spiciferum culture growing on the synthetic medium. It is possible, however, that this extract may have contained some of the living organism.

After the ethanol extracts were made for the previous experiments, the culture material, consisting of both fungus and medium, was wet with sterile nutrient solution, placed in sterile 250 ml flasks and two surface sterilized bermuda cuttings placed in each flask. In addition, similar cuttings were added to flasks containing viable mycelium of H. spiciferum and to flasks of sterile nutrient solution. Representative flasks of each of the latter treatments were placed at temperatures of 25C and 10C and, in addition, flasks of viable H. spiciferum and of the sterile nutrient solution were alternated from 25C to 10C every 24 hours. These cuttings were allowed to grow for

6 weeks and were then evaluated and photographed.

The data are presented in Table V. It is evident that the inhibition of bermudagrass root and shoot growth was pronounced only where bermuda clippings were used in preparation of the extracts of filtrates, regardless of whether H. spiciferum was included or not (Fig. 9). Plants in flasks containing viable cultures of H. spiciferum had rather vigorous roots, but less branching than plants in the sterile nutrient medium.

TABLE IV

INHIBITION OF BERMUDAGRASS GERMINATION AND ROOT GROWTH BY EXTRACTS FROM BERMUDAGRASS CLIPPINGS, AND FROM CULTURES OF H. SPICIFERUM GROWING ON BERMUDA CLIPPINGS, BERMUDA CLIPPINGS AND SAND, AND ON A SYNTHETIC MEDIUM

Extract From:	Extract Not Treated		Extract Dieldrin Treated	
	Root Length mm	% Germination	Root Length mm	% Germination
H. spiciferum on clippings	0	0	0	13
H. spiciferum on clippings and sand	0	5	0.6	8
H. spiciferum on synthetic medium	15.5	21	9.7	9
Bermudagrass clippings	5.3	17	4.9	53
Check (distilled water)	18.8	13	18.2	27
Water extract of H. spiciferum on synthetic medium	8.9	5	8.3	17

TABLE V

INHIBITION OF ROOT AND TOP GROWTH OF BERMUDAGRASS SEEDLINGS ON FILTRATE RESIDUES FROM ETHANOLIC EXTRACTS OF H. SPICIFERUM ON BERMUDA CLIPPINGS, BERMUDA CLIPPINGS AND SAND, AND SYNTHETIC MEDIUM AS COMPARED TO VIABLE CULTURES OF H. SPICIFERUM ON A NUTRIENT MEDIUM AT DIFFERENT TEMPERATURES

Treatment	Avg. Root length mm	Avg. Top Growth in	Remarks
H. spiciferum on clippings	2-3	0	Roots stunted, brown
H. spiciferum on clippings plus sand	2-3	0	Roots stunted, brown
H. spiciferum on synthetic medium	100+	6	Roots thin, white
Bermudagrass clippings (sterile)	0	0	Stolon living, no evident growth
H. spiciferum filtrate residue (water extract)	100+	5	Roots vigorous, white
Viable H. spiciferum in nutrient medium 10C	25	0	Shoot dormant, roots brown
Viable H. spiciferum in nutrient medium 25C	100+	8	Roots vigorous, but with lesions
Nutrient solution Check 10C	10-15	½	Nearly dormant, roots white
Nutrient solution Check 25C	100+	10	Roots white, vigorous
Viable H. spiciferum in nutrient solution alternating 10-25C	50	1½	Shoot almost dormant, roots white with lesions
Nutrient solution Check alternating 10-25C	10	½	Shoot almost dormant, roots white

Figure 9. Bermudagrass cuttings grown for a period of six weeks in A, sterile bermuda thatch plus nutrient solution; B, viable H. spiciferum plus nutrient solution; C, pure nutrient solution

Figure 10. H. spiciferum infected rootlet of bermudagrass sprig grown in nutrient solution, wholemount at 100 X from plant in figure 9 "B"

The presence of viable cultures of H. spiciferum was always associated with lesions on the roots (Figs. 9 and 10), but did not cause complete necrosis or appreciable stunting in this study. Temperature had little effect, except that growth was appreciably reduced at the low temperature (10C) regardless of whether the fungus was present or not.

Bermuda Clipping Toxicity

In order to more fully investigate the toxic properties found in bermudagrass clippings, a study was made with an aqueous extract of bermuda clippings on live bermudagrass cuttings. Steam sterilization of 25 g dry bermuda clippings in 500 ml water gave a 1:20 extract from which further dilutions were made. Each dilution was placed in a 250 cc bottle which contained 2 bermudagrass stem cuttings. Dieldrin was added to one bottle containing a 1:1000 dilution of the extract so that a 1:10,000 dieldrin solution resulted. The bottles were then plugged with cotton.

Observations of root and shoot growth were made after 12 days and the data are given in Table VI. Extreme inhibition of bermudagrass root and shoot formation and growth was apparent, especially in dilutions up to 1:10,000. Roots that did form grew from nodes above the liquid level and were discolored. The root tips were often necrotic and very few lateral roots were formed. The check plants had long, white, much branched roots that grew from nodes both

TABLE VI

INHIBITION OF ROOT AND SHOOT GROWTH OF BERMUDAGRASS BY VARIOUS DILUTIONS OF HOT-WATER EXTRACTS OF BERMUDAGRASS CLIPPINGS

Treatment	Root length mm	Shoot growth in	Root Description
Check Nutrient solution	60	4	white, branched, from nodes above and below liquid level
Hot water extracts			
1:20	1	0	initiated but no growth, brown, necrotic
1:100	25	0	from node above liquid level, brown and necrotic, do not touch liquid
1:1000	5	0	from node at water level, but thin and brown
1:10,000	15	0	from node above surface, but thin and brown
1:200,000	60	4	from both nodes, mostly white, but with some discoloration
1:100 plus 1:10,000 dieldrin	20	0	from node above surface only

above and below the liquid surface. A dilution of dieldrin at 1:10,000 did not alter the toxicity of the bermudagrass clipping extract.

A similar test was then made, but with more dilutions of dieldrin mixed with the extract dilutions. This test was evaluated after 15 days and the results are recorded in Table VII. As before, the nutrient solution check had long white roots with much branching while the higher concentrations of clipping extract (1:1000 and higher) resulted in discolored, stunted root growth. At lower concentrations, the roots resembled those of the check plants except for a small amount of discoloration. The dieldrin treatments indicated that this material may also be phytotoxic to bermudagrass roots at concentrations as low as 1-5 ppm. At 100 ppm the grass roots were killed.

A final test was made to determine the effect of resterilization of the hot-water clipping extract on root growth. Bermudagrass clipping extract was diluted to 1:100 and 1:1000 in nutrient solution and steam sterilized. These dilutions were compared with similar dilutions that had not been sterilized a second time. In addition, a portion of the stock extract solution was centrifuged at 8000 g twice for ten minutes, and 1:100 and 1:1000 dilutions of this stock were used for comparison. The pellet derived from the centrifugation was resuspended in an equal volume of water to make a stock from which corresponding dilutions were made. The results are given in

TABLE VII

INHIBITION OF ROOT AND SHOOT GROWTH OF BERMUDAGRASS GROWN IN VARIOUS DILUTIONS OF WATER EXTRACTS FROM BERMUDAGRASS CLIPPINGS, IN VARIOUS DILUTIONS OF DIELDRIN, AND COMBINATIONS OF EXTRACTS AND DIELDRIN

Treatment	Root Length mm	Shoot Growth in	Root Description
Check Nutrient Solution	50	4	Long white, vigorous
Water Extract Dilutions			
1:20	5	0	Stunted, brown
1:100	10	0	Short, brown
1:1000	40	3	Long, slightly discolored
1:100,000	50	4	Long, white, vigorous, some discoloration
Dieldrin Dilutions			
1:10,000	0	0	Roots and stolons dead
1:100,000	25	2	Some discoloration
1:1,000,000	45	2½	Some root discoloration
Extracts and Dieldrin			
1:1000 extract + 1:10,000 dieldrin	0	0	Roots and stolons dead
1:1000 extract + 1:100,000 dieldrin	45	3½	Long, branched, white

TABLE VIII

EFFECT OF RESTERILIZATION AND CENTRIFUGATION OF HOT-WATER EXTRACTS OF BERMUDAGRASS CLIPPINGS ON THE INHIBITION OF ROOT AND SHOOT GROWTH OF BERMUDAGRASS CUTTINGS

Treatment	Root Length mm	Shoot Growth in	Root Description
Sterile hot-water extract			
1:100	50	3	Fairly vigorous, branched, brown colored.
1:1000	65	3	
Unsterile hot-water extract			
1:100	5	0	Stunted, brown.
1:1000	25	½	Slow growing, brown.
Centrifugate of extract			
1:100	30	2½	Very fine, brown.
1:1000	50	3	
Centrifuged pellet			
1:100	45	2½	Long, vigorous, some discoloration.
1:1000	60	3	
Check (nutrient solution)	70	4	Long, white, much branched.
1:100			
1:1000			

Table IX. Both resterilization and centrifugation reduced the amount of root damage when compared to the plain hot water extract at the same dilution.

Greenhouse Thatch Test

In greenhouse tests where sterile bermuda clippings were added to pots of pure unsterilized builders sand in which bermuda sprigs and wheat and foxtail seedlings were planted, root stunting and discoloration was found after a period of two weeks (Table IX). Lateral roots were only discolored brown stubs, yet the amount of clippings added constituted only 2% of the sand-clipping mixture by weight. The control pots of pure sand had healthy bermudagrass, wheat and foxtail plants with long white roots and prolific lateral root formation. One series of pots containing the bermuda clippings was allowed to dry to the point of incipient wilting. When this was done, only the foxtail seedlings recovered turgidity with the addition of water. Three additions of 50 ml of a 1:5000 solution of dieldrin was added to another series of pots containing the bermudagrass clippings with little apparent effect. The average temperature in the pots during this study was approximately 24C.

Another test was made in which sterile wheat straw, and sterile bentgrass clippings were used as well as sterile bermuda clippings placed both in and on the surface of the sand. This test was made later in the spring than the previous study and greenhouse temperatures were higher,

TABLE IX

GREENHOUSE THATCH STUDIES

Treatment	Root Development
Pure Sand:	Long, white, and vigorous, much branching, and many lateral roots.
Sand plus 2% bermudagrass clippings	
a) Kept moist:	Brown, stunted, few lateral roots.
b) Dried to wilting:	Brown, stunted, and necrotic, except roots of foxtail were not necrotic.
c) Dieldrin treated, 3 applications of 50 ml of 1:5000 concentration	Brown, stunted, few lateral roots.

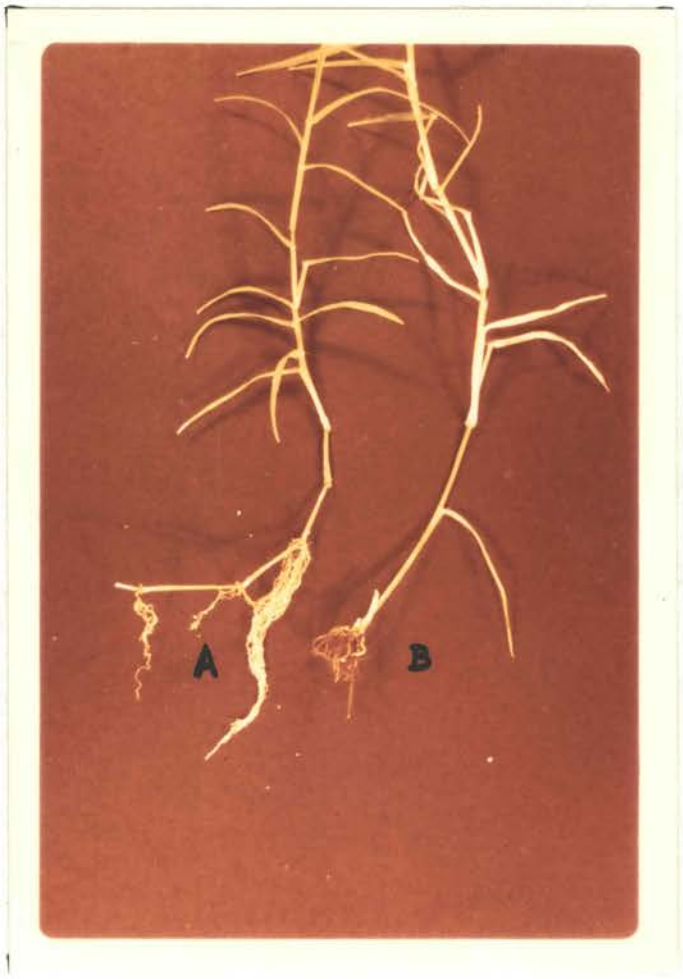

Figure 11. Bermudagrass sprigs from the greenhouse thatch test A, control grown in pure sand; B, plant grown in sand containing 2% thatch by weight and kept at a constant moisture level

averaging over 30C in the pots. Very little effect was noted in this higher temperature range. Consequently, another test involving only bermudagrass clippings mixed with sand (2% by weight) and a pure sand control was made in a light room at a constant temperature of 10C. Both bermudagrass cuttings and wheat seedlings were planted in the test pots. Severe root inhibition and necrosis was observed on the bermudagrass within two weeks. Many of the bermuda plants were dead, while the wheat seedlings exhibited only a small amount of discoloration, and were actively growing.

Laboratory Evaluations of Chemicals

Chemical effects on the growth, *in vitro*, of *H. spiciferum* and *Ophiobolus sp.* are summarized in Table X. Each chemical treatment was replicated twice and tested at concentrations of one part chemical to 5000 and to 50,000 parts of the culture medium. Fifteen chemicals were tested and Phenmad, Copper 8-hydroxyquinolinate, Panogen, and BOS were the only chemicals which completely restricted the growth of both fungi at both concentrations tested.

Since dieldrin had been reported to exert some control of spring dead spot, a test of this material was made (Table XI). It was found to reduce the amount of growth of both fungi progressively as the concentration in the medium increased. At the highest concentration tested (1:5000) growth of *H. spiciferum* was reduced approximately 50 percent and the growth of *Ophiobolus sp.* was reduced about

TABLE X

EFFECT OF VARIOUS CHEMICALS ON THE GROWTH OF *H. SPICIFERUM* AND *OPHIOBOLUS SP.* IN CULTURE

Treatment	dry weight grams		
	H. spiciferum		*Ophiobolus sp.*
Concen.	1:5000	1:50,000	1:50,000
Check	.3912	.4462	.0828
Dieldrin	Tr	.4095	.0168
Phenmad	0	0	0
Cu 8- hydroxy-quinolinate	0	0	0
Cd 8- hydroxy-quinolinate	Tr	.0206	.0369
GB-65	0	.4150	.0711
Zytron	.1845	.3570	.1029
Panogen	0	0	0
Thiramad	0	.0413	0
Sevin	.1735	.4214	.0636
Ethion	.1938	.3988	.0389
Chlordane	.1732	.3601	.0829
Heptachlor	.1410	.2144	.0129
Fore	0	.4644	0
BOS	0	0	0
Daconil 2787	0	.0405	0

TABLE XI

EFFECT OF VARYING CONCENTRATIONS OF DIELDRIN ON THE GROWTH OF H. SPICIFERUM AND OPHIOBOLUS SP. IN CULTURE

Treatment (dieldrin conc.)	Growth (average dry weight) H. spiciferum	Ophiobolus sp.
None (check)	.2096 g	.0856 g
1:500,000	.1704 g	.0633 g
1:50,000	.1191 g	.0472 g
1:5,000	.1311 g	.0235 g

TABLE XII

EFFECT OF VARIOUS CHEMICALS ON THE GERMINATION OF CONIDIOSPORES OF H. SPICIFERUM

Treatment	Percent Germination in No. Hours After Inoculation						
	3	5	7	24	72	120	168
Check	50	94	98	98	98	98	98
Phenmad	0	0	0	0	0	0	0
Dieldrin	Tr	79	87	95	98	98	98
Cu 8-hydroxy-quinolinate	Tr	3	3	4	5	5	5
Cd 8-hydroxy-quinolinate	Tr	87	66	75	90	90	90
GB-65	0	0	0	0	24	25	25
Zytron	Tr	98	99	100	100	100	100
Panogen	0	0	0	0	0	0	0
Thiramad	0	0	0	0	0	0	0
Sevin	0	0	0	0	0	2	2
Ethion	0	0	0	98	100	100	100
Chlordane	Tr	20	53	100	100	100	100
Heptachlor	50	96	97	100	100	100	100
Fore	0	0	0	0	0	0	0
BOS	Tr	1	7	11	7	8	8
Daconil 2787	0	0	0	0	0	0	0

75 percent.

The same fifteen chemicals mentioned above were used to test the germination of conidiospores of H. spiciferum. The methods used to conduct the test are described elsewhere in this paper. The data are given in Table XII. Phenmad, Panogen, Thiramad, Fore, and Daconil 2787 completely inhibited germination. Several other materials delayed or reduced germination or both, but none of these materials have been effective in control of spring dead spot in the field.

Lawn Treatments

Eight chemical treatments were used in an attempt to control spring dead spot on two common bermudagrass lawns. These data are given in Table XIII. The area of diseased turf decreased over the two year period 1965-1967 in the control areas of both lawns. However, some affected turf was observed in all of the treatments except where dieldrin was used. At the end of the test, no diseased turf was found in these dieldrin treated plots. The plots receiving Phenmad, BOS, GB-65, and Copper 8-hydroxyquinolinate also seemed to be better than the check, or at least the disease was reduced faster in these plots than in the check.

TABLE XIII

THE EFFECT OF CERTAIN CHEMICALS ON THE CONTROL OF SPRING DEAD SPOT IN TWO LAWNS IN STILLWATER, OKLAHOMA

Treatment	Rate per 1000 ft^2	Application Dates	Diseased Area ft^2 1965	1966	1967
Lawn A					
Cd 8-hydroxy-quinolinate	32 oz	Oct 15	45.4	22.7	29.7
Cd 8-hydroxy-quinolinate	15 oz 25 oz	Sept 30 Oct 15	67.8	19.8	26.6
GB-65	32 oz	Oct 15	50.9	19.3	13.9
Cu 8-hydroxy-quinolinate	15 oz 25 oz	Sept 30 Oct 15	75.5	20.9	16.5
Check	--	--	123.6	56.6	103.6
Lawn B					
Zytron	64 oz	Oct 15	50.0	37.3	25.6
Dieldrin	22 oz	Oct 15	21.2	6.0	-0-
BOS 50w	16 oz	Oct 15	22.9	27.7	16.3
Phenmad	6 oz	Oct 15	24.1	18.7	5.7
Check	--	--	19.3	24.2	10.5

DISCUSSION

The cause of spring dead spot has been a matter of conjecture for many years. H. spiciferum and Ophiobolus sp. have been suggested as possible pathogens by Wadsworth (19). However, his investigations and those of Gudauskas (7), as well as this study, indicated that these fungi, although capable of producing root and crown rot of bermudagrass under controlled conditions, will not produce the normal spring dead spot disease syndrome.

The growth of H. spiciferum and Ophiobolus sp. was inhibited in varying degrees by certain of the fifteen chemicals tested in the laboratory. No correlation could be found between laboratory control of the two fungi and field evaluations of the same chemicals for spring dead spot control. For example, both fungi were found to grow on a medium that contained concentrations of dieldrin that would kill bermudagrass cuttings in laboratory tests, yet dieldrin has been the only chemical to give fairly consistant control of spring dead spot in field studies. On the other hand, certain chemicals which have shown no control of the disease in the field have given complete fungal inhibition in laboratory tests.

That this disease may have one or more predisposing factors is indicated by: 1) the apparent inability of any

chemical to give complete control of spring dead spot; 2) the inhibitory effects of certain of these chemicals on the growth of H. spiciferum and Ophiobolus sp. in culture; 3) the inability to reproduce the normal disease syndrome with the generally weak parasitism of the fungi isolated most frequently from diseased areas; and 4) the severity and persistence of the disease.

The toxicity of bermudagrass clippings or thatch itself to bermudagrass reported in this study, and the apparent relationship of the spring dead spot disease to thatch buildup in nature, may give a clue to the cause of the disease. A great deal of evidence has accumulated in support of the idea that thatch or its decomposition products may be a predisposing factor in the development of this disease.

Bermudagrass thatch as it accumulates in nature, is composed of a dense, tightly packed layer of stolons, roots, leaf debris, and silt deposited between the soil surface and the clipping level of the grass. Unless removed, this thatch will accumulate each year, and at a greater rate in better kept turf areas that are regularly mowed and fertilized. Such areas are attacked earlier and with greater severity than areas receiving less care.

Spring dead spot has never appeared in newly established turf areas before the third year of growth - long enough for an appreciable thatch accumulation under high fertility-moisture maintenance. Also, the failure of spring dead

spot to develop in areas where all thatch was removed by annual vertical mowing supports the theory that thatch is directly related to the development of the disease.

The production of toxic decomposition products from organic residue was related by Patrick (13) to cool temperatures, poor aeration, and high water content of the soil containing the residues. The density of the thatch layer could very conceivably contain localized pockets of anerobiosis, especially in spots that have been wet for a period of time. The cool temperatures in the thatch layer during the winter and early spring could also contribute to the production of toxic products during the microbial breakdown process.

The sudden appearance of spring dead spot in the spring and its apparent inactivity during the summer would indicate a cool temperature relation to toxin production if this is a factor in the disease. That toxic materials may be produced and be effective at lower temperatures has been substantiated in greenhouse tests in which sterile bermudagrass clippings caused inhibition of bermudagrass root growth when temperatures averaged 20-25C. Growth chamber assay of the toxicity of bermudagrass clippings indicated an even more severe effect at 10C. However, when a greenhouse test was made later in the spring, very little toxicity appeared and it was noted that the soil temperatures at this time averaged over 30C. Temperatures recorded in the thatch layer of a Sunturf Bermudagrass plot

in Stillwater, Oklahoma, averaged 28C or higher during the growing season, while during most of the winter or dormant season the temperature of the thatch layer remained near 5-6C.

The phytotoxins produced during organic decomposition were reported by Toussoun and Patrick (16) to greatly increase the susceptability of bean to root rot by pathogenic organisms. It is conceivable, then, that toxin production during the winter could predispose the grass to attack by the fungi found associated with spring dead spot and therefore account for the enlargement of the spots each spring. Both H. spiciferum and Ophiobolus sp. grow well on bermudagrass thatch, and their presence, together with other organisms that might be parasitic under these conditions, in turf areas weakened by toxic materials originating in thatch or its decomposition products may well induce the disease known as spring dead spot.

SUMMARY

1. a) H. spiciferum made the greatest radial growth of mycelium at a temperature of 30C. The growth rate was unaffected by sudden temperature changes, adjusting to the new temperature regime almost immediately. Sporulation occurred most rapidly at a temperature of 35C.
   b) Ophiobolus sp. made the greatest growth at 25C. No sporulation occurred at any temperature.

2. a) H. spiciferum and Ophiobolus sp. were found to grow well over a wide range of pH, the optimum being 5.0 to 8.0 and 5.0 to 7.0 respectively.
   b) Both fungi grew well on three sources of nitrogen, L-asparagine, KNO_3, and NH_4NO_3. When NH_4NO_3 was used in the medium both fungi utilized the NH_4^+ ion in preference to the NO_3^- ion.

3. Ethanol extracts of culture filtrates of H. spiciferum were not toxic to Bermuda grass.

4. H. spiciferum was able to infect bermudagrass cuttings, but at warm temperatures (25C) damage to the host was less extensive.

5. Severe inhibition of growth of Bermuda grass roots was caused by decomposing Bermuda grass thatch under

laboratory and greenhouse conditions.

6. Certain chemicals were tested for their effect on the growth of H. spiciferum and Ophiobolus sp. in culture.
   a) Dieldrin was found to be fungistatic, less growth occurring with increasing concentrations of the chemical.
   b) Phenmad, Cu 8-hydroxyquinolinate, Panogen, and BOS allowed no growth of either fungus.
   c) Other chemicals tested gave varying degrees of fungal inhibition.
   d) Phenmad, Panogen, Thiram, Fore, and Daconil 2787 completely inhibited germination of spores of H. spiciferum. A few chemicals delayed germination or reduced the amount of germination, while others, including dieldrin, had no apparent effect.

7. Eight chemicals were used as drenches for spring dead spot control in two lawns infected with spring dead spot. Only dieldrin gave any significant degree of control.

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APPENDIX I

CHEMICAL SOURCES

Chemical	Company or Corporation
Dieldrin 18.7%	Shell Chemical Co.
Phenmad 10%	Mallenckrodt Chemical Co.
Thriamad 75%	"
Zytron 35%	E. I. duPont de Nemours
Panogen 2.2%	Velsicol Chemical Co.
Chlordane 72%	"
Heptachlor 2E	"
Daconil-2787 75%	Diamond Alkali Co.
Fore 80%	Rohm and Haas
Cu 8-hydroxyquinolinate 21%	Metalsalts Chemical Co.
Cd 8-hydroxyquinolinate	"
GB-65	"
B O S 20%	"
Ethion 25%	Niagara Chemical Co.

APPENDIX II

MEDIA

1) Potato-Dextrose Agar (PDA)

17 g Agar

10 g Dehydrated Potatoes

10 g Dextrose

Distilled water to 1000 ml

Dissolve agar, strain, adjust pH, autoclave.

2) Bermuda Decoction Broth

50 g Dry Bermuda Clippings

10 g Dextrose

Distilled water to 1000 ml

Cook, strain, steam sterilize

3) Basal Synthetic Medium *

20 g Dextrose

2 g KNO_3

1 g KH_2PO_4

0.5 g $MgSO_4 \cdot 7H_2O$

2 ml Minor Elements Solution

1 ml Thiamine Solution(100 ug/ml)

1 ml Biotin Solution (100 ug/ml)

Autoclave dextrose seperately

* Lilly and Barnett. 1951. Physiology of the Fungi. McGraw Hill.

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MASTER OF SCIENCE

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