

A STUDY OF HELMINTHOSPORIUM SATIVUM P. K. & B. AS AN
UNREPORTED PARASITE OF AGROSTIS PALUSTRIS HUDS.

By

WILLIAM KLOMPARENS

A THESIS

Submitted to the School of Graduate Studies of Michigan
State College of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1953

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Symptoms are described for an extremely destructive disease of creeping bent grass, Agrostis palustris Huds. Large areas of turf may turn smoky blue, then chlorotic, followed by the complete destruction of the affected grass plants. Helminthosporium sativum P. K. & B. is the fungus constantly associated with the disease symptoms. Fifty-eight isolations and identifications are listed, with sources ranging from Texas to Ohio. H. sativum, isolated from creeping bent grass, and an isolate from J. J. Christensen, of Minnesota, both were found to cause complete death of established creeping bent grass. These same isolates were compared for pathogenicity to bent grass seedlings and to wheat; the damage caused by all isolates was the same on any one host.

Four attempts to produce the perithecial stage were unsuccessful, using twelve media, ultraviolet light, and varying environmental conditions. The variability of bent grass isolates of H. sativum grown on artificial media corresponded with the variability of this species as reported in the literature. H. sativum isolates from creeping bent grass were found to be stimulated when grown in medium containing small amounts of mercuric and mercurous chloride (10 and 50 $\mu\text{g/ml}$), while the known isolate from Minnesota was not

WILLIAM KLOMPARENS

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stimulated. Nematodes were tested alone and in combination with fungi for pathogenic effects. No effect was found under the conditions of the tests. Dr. G. Thorne, Nematologist, however, felt that Tylenchorhynchus sp. was parasitizing a sample of creeping bent grass sent to him for identification and observation. Curvularia sp. was found to be mildly parasitic to creeping bent grass. Pleosphaerulina sp. was described and shown to be parasitic on creeping bent grass.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Dr. J. R. Vaughn, who, in the capacity of major advisor, was constantly available for discussion of the various phases of this investigation.

Thanks are also due to Dr. Vaughn, Dr. E. S. Beneke, Dr. G. P. Steinbauer, and Dr. W. B. Drew for critically reading this manuscript and providing helpful suggestions as to organization and clarity.

The suggestions and interest shown by Dr. E. A. Andrews, Dr. D. J. DeZeeuw, and Dr. J. Tyson contributed materially to the various phases of this research. Their interest and aid are sincerely appreciated.

The advice and aid so freely given by other members of this department, too numerous to mention, was of considerable help. Sincere thanks are extended to these people.

Last, but far from least, are the thanks due to Dr. Wehmeyer and Dr. Thorne for their valuable contributions to sections of this manuscript. Sections of this work could not have been completed without their assistance.

TABLE OF CONTENTS

	Page
INTRODUCTION	1
LITERATURE REVIEW	3
THE DISEASE	8
MATERIALS AND METHODS	13
Fungus Morphology	13
Pathogenicity Studies	13
Perithecial Production Trials	24
EXPERIMENTAL RESULTS	28
Fungus Morphology	28
Pathogenicity Studies	30
Established grasses	30
Bent grass seedlings	34
Cultural Studies	42
Perithecial production trials	42
Variation due to medium	42
DISCUSSION AND CONCLUSIONS	46
SUMMARY	52
BIBLIOGRAPHY	54
APPENDIX (Plates)	57

INTRODUCTION

There are approximately 15,000 acres of Agrostis palustris Huds. (creeping bent grass) maintained as putting greens on golf courses in the United States, and although figures are lacking, observation leads the author to estimate that there are at least another 15,000 acres in golf course fairways, parks, and lawns. Thirty thousand acres is not in itself an imposing figure, but when the value per acre is set at \$10,000 (12), the 300 million dollar estimated value becomes a considerable investment.

The very high standards for fine turf set up by golf club members, park superintendents, and individual home owners has led, understandably, to conditions favoring diseases caused by fungi. Creeping bent grass, Agrostis palustris, probably has had more unwanted nurturing and care lavished upon it than any of the other turf grasses, such as the colonial and velvet bents, the bluegrasses, and fescues. The high fertility and water levels, close clipping (down to 3/16 inch), and the crushing and scuffing by feet, mowers, tractors, golf clubs, and croquet mallets must contribute to the constant presence of disease problems.

Parasitic organisms affecting turf grasses have, of course, been recognized for many years. The common Sclerotinia homoeocarpa (dollar spot), Pellicularia filamentosa or Rhizoctonia (brown patch), Gloeocercospora sorghi (copper spot), and Fusarium and Typhula sp. causing snow mold have been treated with hundreds of compounds in attempts to eliminate unsightly spots and blotches in order to attain eventually the unmarred green carpet so desired.

In 1949, the failure of these compounds to control an apparently new and extremely destructive disease on the creeping bent grasses stimulated this investigation.

Demonstration of the constant association of a species of Helminthosporium with the diseased condition was relatively easy, but the writer then was beset with the difficulties that have confronted all researchers who have worked with root rots of established grasses. Working with individual seedlings or individual established plants leaves much to be desired, since their natural environment includes large numbers of individuals in close quarters. There is also the necessity of re-creating the predisposing factors of soil and moisture conditions, fertilization, presence of other fungi, close clipping, bruising, and weekly applications of fungicides. These are but a few of the recognized complicating factors that must be evaluated in the following discussion.

LITERATURE REVIEW

In 1923, Drechsler, in an excellent, comprehensive publication, beautifully illustrated twenty-four species of Helminthosporium on various grasses (5). Many of these species were newly described and named. Of this group, only one, H. stenacrum Drechs., was reported to be associated with a withering of creeping bent grass blades of Agrostis stolonifera L. Also in 1923, Drechsler reported that Helminthosporium giganteum Heald and Wolf had been observed as a parasite of creeping bent grass (6). Bent grass parasitism by H. giganteum was again referred to in 1928 on A. stolonifera L. and on A. canina L. (velvet bent) (7), and was further confirmed in 1929 (8).

In 1931, in Connecticut, a species of Helminthosporium was reported to have caused severe damage to creeping bent grass (21). The recently seeded areas were the most severely affected, with the symptoms appearing as if the grass had been matted down with oil. This condition, which occurred again the following year, was favored by hot, damp weather. Dahl, in 1932, again reported parasitism of H. giganteum on creeping and velvet bent grass. Helminthosporium was reported to have caused damage to creeping bent grass in the

Netherlands in 1934 (26). The parasite was also reported to be seed-borne. Infections of lawn grasses by a Helminthosporium which caused a condition described as "black blotch" were reported from South Africa in 1934 (20). This seems to be similar to the condition reported from Connecticut in 1931. A withering effect was reported in 1935 to be due to infection of creeping bent grass by H. erythrospilum Drechs. when this new species was described (9).

In 1941, Helminthosporium sp. were reported on both velvet and creeping bent grass (18). In this general listing, as in most others, no species were noted.

Wernham and Kirby, in 1943 (28), described a Helminthosporium on creeping bent grasses. The disease occurred in warm, humid weather and attacked all grasses under observation. The symptoms exhibited were as follows: bluish cast, darkening, and withering, with the tops dying down to the soil line. Recovery was slow from the undamaged stolons. The general symptoms and the indistinct margins of the diseased areas led to the application of the term "melting-out." Struble (24), in 1943, in describing techniques for disease-control studies, included Colletotrichum in the "melting-out" complex. Review of literature failed to reveal whether or not this organism was ever proven to have been involved in the disease process. At any rate, nothing further has been mentioned with

regard to the Colletotrichum. The "melting-out" was mentioned again in 1949 (16) in a discussion of turf disease control measures.

In conversation with C. C. Wernham in 1951 it was brought out that the disease, as it was described in 1943, more than likely was caused by Curvularia sp. This, however, results in one unexplained question. The Pennsylvania workers stated that velvet bent was the least susceptible to melting-out, in contrast to Howard (14), 1952, who described Curvularia-incited "fading-out" as being very prevalent on velvet bent grass. Observations in the Midwest have shown the Curvularia to be very widespread on the creeping bent grass, but seldom causing extensive damage. Velvet bent is not used to any extent in the Midwest; thus, a comparison of susceptibility with the creeping bents was not possible.

Although Helminthosporium is often mentioned in association with disease of the fine turf grasses, only Drechsler--and Dahl, in one instance--mentioned the species involved. The literature, therefore, loses some of its value, since the exact organisms causing the various symptoms are a matter of conjecture.

It is believed that the fungus involved in the following discussion is Helminthosporium sativum P. K. & B., and the possibility exists that nematodes may also be a factor. Review of the literature here will be limited to that pertinent to this problem.

H. sativum has been investigated more thoroughly than any other species of this genus. Christensen (1, 2, 3) showed that the fungus was extremely variable in morphology, cultural growth, and pathogenicity. His inoculations of three- to four-month-old plants of Agrostis alba L., A. palustris Huds., and A. stolonifera were without success. It has also been shown that although H. sativum is active under semiarid conditions, high humidity and temperature often favor expression of symptoms (4). Elliot, in 1949 (10), presented data indicating a possible reason for the great variation in conidial measurements often present in the literature. Among others, the length of H. sativum spores was shown to be influenced by sugar concentration in the medium. The lower concentrations of sugar in the medium produced the longer spores.

Stevens (23), in 1922, and Sprague (22), in 1950, very completely covered the characteristics of the genus. Stevens' very complete early work was the basis for many subsequent studies, while the latter briefly reviewed literature to date.

The role of nematodes in a root-rot complex was determined by Jenkins in 1948 (15). Four genera of meadow nematodes were found to be furnishing entry points for subsequent infection by fungi and bacteria. There are, of course, well-known examples of nematodes being the primary cause of root lesions and plant damage (11).

The correlation between numbers of nematodes present in the soil in relation to damage was determined for celery in 1952 (19). As expected, the larger numbers caused the most extensive damage.

THE DISEASE

In the summer of 1949, Drs. J. R. Vaughn and E. A. Andrews were asked to investigate an apparently new and destructive disease of creeping bent grass present on several golf courses in Michigan (see Table I). Observation and isolation by Andrews resulted in cultures of Helminthosporium sp. which were turned over to the author for identification and detailed investigations.

The symptoms present in 1949 were apparently dependent upon the extremely hot, humid conditions that were present that particular summer, since subsequent observations indicated that a change in environmental conditions arrested the disease progress. The visible symptoms were a smoky blue cast on areas varying from one to three or four feet in diameter, followed by yellowing and complete killing of grass plants in the affected areas. The irregular, dead areas had more-or-less definite margins, and the appearance of being watersoaked and matted down. There was no new growth from within the most severely affected spots, since root rot is present in the blue and yellow stages.

The earliest symptom is a more-or-less oval spot on the leaves, 3 to 6 mm. long and half as wide. The spots, beginning as

TABLE I

DETERMINATIONS OF THE PRESENCE OF *H. SATIVUM* ON
ATTACHED, DYING, OR DEAD CREEPING
BENT GRASS BLADES¹

Summer	Locality	Occurrences
1949	East Lansing, Michigan	1
1950	East Lansing, Michigan	7
	Brighton, Michigan	5
	Grand Rapids, Michigan	2
	Warren, Michigan	2
	Ann Arbor, Michigan	1
	Detroit, Michigan	4
1951	East Lansing, Michigan	2
	Brighton, Michigan	1
	Ann Arbor, Michigan	1
	Detroit, Michigan	5
	Dallas, Texas	3
	Franklin, Michigan	1
1952	Lansing, Michigan	1
	Chicago, Illinois	2
	Cincinnati, Ohio	1
	Westerville, Ohio	1

TABLE I (Continued)

Summer	Locality	Occurrences
1952 (Cont.)	Birmingham, Michigan	1
	Cleveland, Ohio	1
	Madison, Wisconsin	1
	Franklin, Michigan	1
	Detroit, Michigan	5
	Medinah, Illinois	1
	Park Ridge, Illinois	1
	Niles, Illinois	1
	Worth, Illinois	1
	Orland Park, Illinois	1
	Lisle, Illinois	1
	Rocky River, Ohio	1
	Pontiac, Michigan	1
	Willoughby, Ohio	1
Total		58

Note: In approximately 90% of these determinations, Curvularia sp. was present, while in 30 to 40% Pleosphaerulina sp. was also present.

¹ Many of the samples were received by mail; thus, correlation of disease severity and spore concentration on the sample was not possible. A large number of these are not in culture at present.

minute yellow flecks, soon progress through the spot stage to a general watersoaked-appearing blotch (Plate 4, Appendix). General yellowing is often evident at this point, and roots may be damaged. Platings of roots when the grass is in this yellow stage have led to successful recovery of the organism. Roots, stolons, and aerial parts may all show loss of vigor at the same time, indicating that infection may be general and not necessarily progressing from one organ to another. The reverse may also be true--slight symptoms on aerial parts with root-rotting causing the chlorosis, wilting, and death of aerial parts.

Observations by the author over a period of four summers have indicated that the smoky blue color does not always precede the yellow stage. Slides and tissue platings of dead and dying grass almost invariably show an organism, which subsequently will be identified as Helminthosporium sativum, to be the most prevalent organism. The fungus spores, although present in creeping bent turf at all times, are so numerous in affected areas as to account for some of the dark color.

Other organisms commonly isolated and observed in association with Helminthosporium were Curvularia sp. and Pleosphaerulina sp. Nematodes were also observed in many turf samples. In some instances the nematodes were feeding on blades near or at the soil line

Howard (14) has shown the Curvularia to be parasitic in its own right, and subsequent discussion will show parasitism by Pleosphaerulina, but the exact role of the nematodes in the disease process has not been fully determined.

MATERIALS AND METHODS

Fungus Morphology

Although many fungi were isolated infrequently, Helminthosporium was consistently found, and its identity was determined by comparative measurements, and observations as to color, method of germination, and general morphological characteristics. One hundred spores and sporophores from culture and from nature were measured and observed in order to arrive at the identification described in the section on results.

Limited numbers of measurements of perithecia and ascospores of Pleosphaerulina sp. were made; however, its pathogenicity was well established following Koch's postulates.

Pathogenicity Studies

In January of 1950, spore suspensions derived from the original isolate furnished by E. A. Andrews were sprayed on two-and-one-half-week-old seaside creeping bent plants. Three inoculations at 24-hour intervals were made on this seeded grass.¹ The deep crocks

¹ In the following discussion, where grass from seed was used, all soil for planting was steam sterilized.

were kept covered to raise the humidity. Temperature in the greenhouse ranged from 68° F. to 76° F. Platings of the blades, roots, and crowns were made at five 3-day intervals one month later. Surface sterilization of the plant parts used for isolation was with 1-1000 mercuric chloride and 0.008 percent sodium hypochlorite.

On March 10, 1950, 24-day-old seeded seaside creeping bent grass in flats was given a spore suspension spray on approximately one-third of the area in three of four flats. The flats were placed in a humidity chamber where a relatively constant environment of 88 percent relative humidity and a temperature of 77° F. was maintained. On March 21 and 27, 1950, the above test was repeated, with the only apparent differences being the slight variation in temperature, which, during these trials, was 79° to 84° F.; the humidity, which ranged from 91 to 94 percent; and the fact that two inoculations three days apart were employed.

Eight flats of seaside creeping bent which had been established for two and one-half months were treated as pairs in the following manner on April 4, 1950:

1. Had been seeded in steamed soil and received a spore suspension.
2. Had been seeded in inoculated soil and received no spore suspension.

3. Had been set from stolons in sterile soil and were given a spore suspension.

4. One stolon-set and one seeded into steamed soil and no spore suspension.

One of each of the flats was placed in a separate humidity chamber and held at 86° F. and 90 to 92 percent relative humidity. On August 22 and on August 26, 1950, six flats of well-established Washington creeping bent grass were given spore suspension sprays as follows: one flat, not inoculated; one inoculated with Helminthosporium from bent grass; one received Curvularia sp.; and the last was sprayed with a mixture of the Helminthosporium and Curvularia. In both instances the flats were held in humidity chambers for five days at 90 percent relative humidity and 80° F.

On September 7, 1951, well-established Washington creeping bent was removed from the grass plot on the college farm and brought into the laboratory in flats. Three of five flats were inoculated with a single-spore isolate derived from a previously successful inoculation test; and one flat, with Curvularia sp. Six-inch bell jars were placed over areas of these flats to increase the relative humidity. After three days, when leaf spotting first appeared, individual blades were marked with fragments of thread and left beneath the bell jars for further development of the disease.

On April 1, 1952, seven flats of closely knit turf of the Washington variety were taken from the field and inoculated with a known Helminthosporium sativum received from Dr. J. J. Christensen, three Helminthosporium isolates from creeping bent grass, Pleosphaerulina sp., and an unidentified pycnidial-producing isolate from bent grass. Subsequent to inoculation at opposite ends of the flats, all were held at 90 percent relative humidity and 80° F. for 48 hours. The flats were then held under greenhouse environmental conditions which were approximately 70° F. and 65 percent relative humidity.

From June 30 to July 25, 1952, pairs of flats of a mixture of the Arlington and Congressional varieties of creeping bent grass were given spore suspensions derived from an isolate of Helminthosporium obtained in Indiana. These flats, being out of doors, were subject to the prevailing weather, but were covered the first nine nights of this period by wet paper toweling, which served to increase the relative humidity. The flats were reinoculated every five days during the period of the test.

Since it had often been observed that regrowth into an area of dead bent grass was extremely slow and difficult, the following test was devised to determine possible residual action by toxic metabolic products given off by the causal organism(s). On July 15, 1952, mycelial and spore suspensions, sterile extracts, and sterile-

medium control solutions were applied to plots of Washington creeping bent, replicated three times. The treatment materials were all from 2 percent malt extract solution (Difco). One liter of each of the following was prepared:

1. Mycelium and spore suspension.
2. Sterile¹ extract of No. 1.
3. Pure unused medium.
4. Sterile distilled water.

The replicated plots were marked with golf tees pressed below the surface of the grass, and received the regular mowing and watering of the plot proper. The undiluted solutions were distributed over as small an area in the plot as possible by very slow, direct pouring. A second treatment was applied twelve days later, on July 27.

On August 20, 1952, a test was set up to determine possible correlation between nitrogen fertilization and source of nitrogen with the disease initiation and progress. Four grass varieties--Arlington,

¹ The extract was obtained by filtering and treating the extract for 24 hours with 1 cc. propylene oxide per liter of volume. The materials are first enclosed in a flask with a rubber cork above the cotton plug; the cork is then removed, and the solution allowed to air for 48 hours (with periodic shaking). Treatments 2, 3, and 4 were treated with the propylene oxide to offset any effect that it might have.

Toronto, Congressional, and Washington creeping bents were used.¹ Three fertilizers--ammonium sulfate, Nu Green (urea), Milorganite (organic nitrogen)--and no fertilization were the four treatments. Based upon actual nitrogen content, each of the twelve fertilizer plots received 12 ounces of nitrogen per 1000 square feet from each of the sources, applied in one-foot-wide strips lengthwise on the plot. This was lightly watered in to prevent burning by the inorganic forms of nitrogen. A spore suspension originally isolated from diseased grass from Illinois was applied across the four treatments on each grass variety. A reinoculation across the fertilizer strips was carried out one week later. The second inoculation was with an isolate obtained from diseased grass from Dallas, Texas. The entire plot was watered with a fine mist three or four times a day for the three-week observation period.

Seedling pathogenicity of an isolate (H. 10) derived from the original isolation, H. sativum, and of Curvularia sp. was determined by planting seeds of seaside creeping bent and Yorkwin wheat treated with mercuric chloride and sodium hypochlorite in steamed inoculated soil on November 16, 1952. Rows of twenty-five seeds each, replicated

¹ The author is grateful to Oakland Hills Country Club, Birmingham, Michigan, for use of the plot containing the four grass varieties side by side.

four times, were seeded one day after inoculation of the steamed soil with spores and mycelium of the respective fungi. To facilitate counting of the bent grass seedlings, the following technique suggested by Dr. D. J. deZeeuw was used. The twenty-five bent grass seeds were dipped in 10 percent solution of Methocel (Dow) and placed at intervals on one-fourth-inch strips of personal tissue. Thus, the seeds were firmly fixed and spaced. Upon turning the paper seeds down in the soil, no inhibitory effects on germination were noted, and counts were easily obtained. This test, as well as the next to be described, were preceded by unreplicated preliminary trials which indicated the value of repeating the tests.

Seedling susceptibility was further tested in petri dishes in the laboratory, beginning December 30, 1952. Four turf grasses--Poa annua L. (annual bluegrass); two varieties of Agrostis tenuis Sibth. (colonial bent), Highland and Astoria; and Agrostis palustris Huds. (creeping bent) of seaside variety--were used. The organisms used for inoculation were: H. sativum, isolate H. 10; Curvularia sp.; Pleosphaerulina sp.; Aspergillus niger; and Penicillium notatum. The isolates, singly and in combination, were atomized onto seeds placed in sterile petri dishes containing filter paper. Each dish, containing twenty-five seeds, was replicated four times. Seed-borne contaminations interfered with an earlier test, so all seeds except one series

of the seaside variety were surface disinfested. Seed treatment was the use of mercuric chloride and sodium hypochlorite, as mentioned previously. There was, however, a final rinse in sterile distilled water before seeding. The spore suspensions were standardized to approximately fifty spores per drop and atomized into the dishes at the time of seeding. Counts of seedlings (based upon attainment of one-fourth-inch height) were made for nine consecutive days. When the control plants showed loss of vigor and chlorosis, the experiment was concluded. The plates were held at room temperature near a window for the entire period.

Due to the numerous instances in which nematodes were observed in many of the previously described experiments, the following test was set up with hopes that their role in the disease process could be determined. One source of nematodes was obtained from Dr. R. Kiesling, who found them in isolations made from the crown of diseased bean plants. This will be referred to as Nematode 2. A second selection was made from soil in flats of creeping bent which had been held in the greenhouse for four months. This will be referred to as Nematode 1. An attempt was made to isolate in the genus Tylenchorhynchus, since a prepared, identified slide was available for comparison. Dr. Gerald Thorne had previously made the identification from samples of damaged grass sent by the author. In

this selection, the soil in a beaker was washed with slowly running water; the beaker was then tilted for a period of time, after which the supernatant liquid was poured off. The residue was placed in small drops on plates containing 3 percent agar. With the aid of a dissecting microscope, the nematodes could be seen to move well out into clear areas. The individuals were isolated by removing the small circle of the agar on which the nematode was present. This was transferred to either 2 percent malt extract agar plates containing grass clippings or to a rabbit dung-malt extract medium. Either medium was found to be suitable for increasing the numbers of the nematodes. The latter, from the bent grass flats, is referred to as Nematode 1. The identity of these was to be ignored until it was determined whether they caused damage when alone, or if they contributed to the root rotting when placed in combination with various fungi. Treated seaside creeping bent was seeded into 2 x 2 x 4 inch plant bands or into 2 x 2 x 2 inch plant bands for subsequent transplanting into inoculated soil or for spore suspension inoculation.¹ The two band types were used to furnish stands of established grasses of different ages--the deep-band series to be inoculated three weeks

¹ The author is deeply indebted to Dr. E. A. Andrews for suggesting the use of bands as a means of obtaining easily separated units of established grass.

later than the other. The first series was set up on February 1, 1953, and the second on February 20. At the time of inoculation, these grass stands were twenty-four and forty-three days old, respectively. The grass, even in the first series, was well established and tillered. The fungi used alone and in combinations are listed in the following table, which indicates the twenty-two treatments used with each series.

1. Absolute check.
2. Nematode 1.
3. Nematode 2.
4. Nematode 1 + H. sativum.
5. Nematode 2 + H. sativum.
6. Nematode 1 + H. 10.
7. Nematode 2 + H. 10.
8. H. sativum.
9. H. 10.
10. H. sativum + H. 10.
11. Nematode 1 + spore suspension of H. 10.
12. Nematode 2 + spore suspension of H. 10.
13. Nematode 1 + spore suspension of H. sativum.
14. Nematode 2 + spore suspension of H. sativum.
15. Nematode 1 + Nematode 2.

16. Nematode 1 + Nematode 2 + H. sativum + H. 10.
17. Pleosphaerulina sp. spore suspension.
18. Curvularia sp. spore suspension.
19. H. sativum + Pleosphaerulina sp. spore suspensions.
20. H. 10 + Pleosphaerulina sp. spore suspensions.
21. H. 10 spore suspension.
22. H. sativum spore suspension.

In the first ten treatments the fungi and the nematodes were incorporated in the soil previous to transplanting the grass. In the last twelve treatments spore suspensions of the fungi were used, while in the last six, no nematodes were involved. Each of the treatments was replicated three times with each series. The greenhouse temperature and relative humidity were raised by means of escaping steam in a far corner. A hygrothermograph was used to record the temperature and humidity at all times. The high temperature and humidity were maintained for six days, after which cooler, drier conditions were used to determine regrowth from affected grass in the first series. At the time of initiating the second series, the temperature and humidity were again raised for a thirteen-day period. The grass was cut at irregular intervals and the clippings removed. Microscopic examinations and tissue platings of blades and roots were made periodically.

Perithecial Production Trials

Transfers from three single-spore Helminthosporium cultures, H. 7, H. 9, and H. 24, were placed in petri dishes containing 15 cc. each of nine media. Each treatment was replicated four times to furnish one plate for each of the four conditions under which they were held. The conditions used were:

1. Humidity chamber 26° C., 65 percent relative humidity, and constant darkness.
2. As (1), but constant low light (7-1/2 watt bulb at 12 inches).
3. 22° C. incubator (dark).
4. Room temperature and light conditions.

To each of nine lots of grass clippings sterilized by propylene oxide (using the previously described technique) was added 15 cc. of one of the following media:

1. Czapek's¹ sucrose nitrate solution with 30 gm. sucrose and 2 percent agar.
2. Czapek's as above, but only 15 gms. sucrose.

¹ Czapek's sucrose nitrate solution: sodium nitrate, 2 gms.; potassium dibasic phosphate, 1 gm.; potassium chloride, 0.5 gm.; magnesium sulfate, 0.5 gm.; ferrous sulfate, 0.5 gm.; sucrose, 30.0 gms.; 1000 cc. distilled water (plus 17 gms. agar).

3. Medium from M. R. Hatfield composed of 2.0 percent dextrose, 0.5 percent yeast extract, 0.25 percent Bacto-peptone, 1000 cc. distilled water.

4. 2 percent malt extract agar (Difco).

5. Pure 2 percent agar.

6. 2 percent corn meal agar (Difco).

7. Coon's synthetic broth¹ + agar.

8. 2 percent Bacto nutrient agar.

9. 2 percent prune agar (Difco).

The three isolates on the nine media were held under the four conditions for two weeks. They were then treated with ultraviolet light and replaced. The ultraviolet light treatment was one Slimline lamp (2537 Å) at 8 inches for 40 seconds.

A second attempt to produce the perfect stage was initiated on February 5, 1951. Twenty-four 200 cc. screw-cap bottles were used in place of the petri dishes. The bottles were used so that one-half of the series could be held for a long period of time with little likelihood of contamination. The promising isolate H. 7 was chosen for seeding, since it repeatedly produced sclerotial-like bodies in various media.

¹ Coon's synthetic broth: sucrose, 7.2 gms.; dextrose, 3.6 gms.; potassium nitrate, 2.02 gms.; magnesium sulfate, 1.23 gms.; potassium dibasic phosphate, 2.72 gms.; 1000 cc. distilled water.

The medium used was the dextrose, yeast extract, Bacto-peptone medium (No. 3 of the previous test), with the following additions: To one set was added 0.5 gm. of glycine and 0.5 gm. of asparagine; to a second set was added 0.5 gm. of glycine; while the third set received 0.5 gm. of asparagine as an addition. For each bottle, 30 cc. of medium were used. The warm medium was allowed to solidify with the bottles on their sides in order that a large area would be available for growth. After seeding, two bottles of each of the three media were held in the four environmental conditions mentioned in the previous test. These were held for two months, after which one-half were transferred to a refrigerator. The bottles in the refrigerator were held for an additional ten months and then returned to the original environments for three weeks. Those not refrigerated were observed periodically, but received no further treatment.

The cultural growth of eleven single-spore isolates was observed on 2 percent malt extract agar. The comparative growth of these eleven, plus six additional isolates, was also observed on Coon's synthetic medium (with 2 percent agar). Each of the isolates was seeded into the petri dishes, and after seven days the type of cultural growth was recorded. The purpose was to determine if cultural-growth groups established on a natural medium would be

identical or similar to subsequent artificial grouping when grown on a synthetic medium.

Since an earlier in vitro experiment (17) demonstrated that there was an actual stimulation of one Helminthosporium isolate from creeping bent grass by low concentrations of mercurous and mercuric chloride, this test was repeated with two Helminthosporium sp. from bent grass and the H. sativum isolate obtained from Minnesota. The three fungi were seeded into 2 percent malt extract agar containing a mixture of mercurous and mercuric chloride concentrations of 0, 10, 50, 100, 250, and 500 $\mu\text{g/ml}$ and incorporated into the medium by serial dilution. The mercury mixture was not autoclaved with the medium. The replicates of four plates were seeded with 5 mm. disks of five-day-old inoculum and daily growth increments recorded in millimeters. The purpose was to determine whether more of the isolates than the one previously tested would show this stimulation and whether a known isolate of H. sativum would show stimulated growth if small amounts of mercury were incorporated in the medium.

EXPERIMENTAL RESULTS

Fungus Morphology

The measurements of the structures of Helminthosporium that are considered to be of diagnostic value are listed in Table II. Other characteristics, such as spore color and variability, brittle episporium, and germination from one or both terminal cells, were observed to fit very well the description of H. sativum (see Plate I). The spores were found to be slightly wider than H. sativum, which were listed by Sprague (22) as ranging from 15 to 20 μ . The mean of the width of the one hundred measured was 20.50 μ . Variation among spores from the H. sativum isolate from Minnesota and within one culture originally from a single spore are shown in Plate II. Spores on sporophores are pictured in Plate VI.

Of the Pleosphaerulina, Dr. L. E. Wehmeyer, of the University of Michigan, wrote the author as follows:

. . . it has hyaline spores, in saccate asci in small, membranous perithecia, and is found on living leaves, all of which are characteristic of this genus. Last summer, Dr. E. G. Simmons, of Dartmouth College sent me a fragment of Agrostis with, apparently, the same fungus, and just recently Dr. R. P. Korf of Cornell sent me a very similar species on Linguistrium leaves, collected by Dr. Williamson. All three collections have 4-septate spores, running 28-35 x 10-13 μ .

TABLE II

MEASUREMENTS OF ONE HUNDRED CONIDIA OBTAINED FROM
HELMINTHOSPORIUM ISOLATED FROM
CREEPING BENT GRASS

Source	Spore		Sporophore	
	Length	Width	Length	Width
Grass	46-101 μ	17-25 μ	80-191 μ	6-8 μ
	avg. 76.15 μ	avg. 20.50 μ		
2% malt	27-63 μ	17-25 μ		
Extract agar	avg. 52.38 μ	avg. 20.43 μ		
Septations	2-10		2-9	

Helminthosporium sativum: spores, 60-120 x 15-20 μ ; septations, 3-10. Sporophores, 110-150 x 6-8 μ up to 8 septate (22).

There have been a number of species described with very similar spores; i.e., P. briosiana, P. oryzae, P. phaseoli, etc. The genus needs revision, for the species have been described mostly on host differences.

The author's observations as to spore size, color, and septation coincide with those mentioned above. Infection of the creeping bent grass was observed to be either as direct penetration or through stomata. See Plate VII for pictures and camera lucida drawings of perithecia, asci, spores, and infection by germinating ascospores. Plate VII includes a picture of this organism. Seventeen single ascospore isolations resulted in cultures that were apparently identical, and later produced perithecia.

Pathogenicity Studies

Established grasses. The first pathogenicity test in January, 1950, run under ordinary greenhouse conditions, resulted in no apparent damage or symptoms. There was, however, successful recovery of the organism from the various plant parts when surface-sterilized plant parts were plated in nutrient medium.

The flats inoculated on the tenth of March, 1950, but held at an elevated humidity and temperature, resulted in severe killing of large areas of the grass within 96 hours (see Plate III). The symptomatic "blue-stage" was present as the killing progressed. Dead

and dying blades were literally covered with spores and sporophores of Helminthosporium sativum. In the tests repeated on March 21 and 27, however, there were no symptoms, and the organism was not recovered.

The pathogenicity test of April 4, 1950, which was on two-and-one-half-months-old, well-established, stolon- and seed-set plants in sterile and nonsterile soil resulted in slight symptoms. Leaf spotting was evident at various times, but tissue platings showed recovery of the organism from the blades of Treatment Number 1 only. This treatment was inoculation of grass from seed sown in sterile soil. The seeded turf which had been established in infested soil (Treatment 2) and the stolon-set turf (in sterile soil) indicated no disease, and recovery of the organism was not possible. The latter received a spore suspension similar to Treatment 1. Nematodes were observed in slides of root parts of the plants that had been stolon-set in the steamed soil.

The inoculations in August, 1950, resulted in very slight leaf spot or blotch symptoms. Since this turf had been stolon-set, it was not surprising to find nematodes in the slides. Since symptoms were so slight, tissue platings were not attempted. Although the temperature and humidity were high for five days, the test of pathogenicity was considered negative.

The flats of grass brought in from the grass plot on September, 1951, which were inoculated with Helminthosporium and Curvularia, and random areas covered with bell jars showed infection from ascospores of Pleosphaerulina. These spores apparently were present in the field, and under the moist conditions were able to penetrate and infect the Washington variety blades. Watersoaked blotches on leaves marked with thread fragments proved to be necrotic areas in which germinating, penetrating ascospores were observed (see Plate VII). Ten platings of leaf blades resulted in recovery of Pleosphaerulina eight times, Helminthosporium three times, and Curvularia once.

The results on the seven flats brought in from the field in April, 1952, and inoculated with Pleosphaerulina, H. sativum, three Helminthosporium isolates from bent grass, and the unidentified pycnidial form are as follows: The grass was damaged most severely by nematodes, although limited penetration by germinated ascospores of the Pleosphaerulina and Helminthosporium conidia was observed. Thousands of nematodes were found on and in all plant parts. Samples of turf from this same source were later sent to Dr. Gerald Thorne, Senior Nematologist, USDA, who in part replied as follows:

The creeping bent turf sample contained hundreds of nemas belonging to the genus Tylenchorhynchus. The species seems to be most closely related to T. magnicaudatus. . . .

if they are feeding on the grass roots - as I am sure they are doing. . . . This type of nema is ecto parasitic and does not usually enter the root tissues, although I have occasionally found them there. . . . Little is known of this group and I can cite no references on them. . . . [See Plate VIII.]

The flats of a mixture of Arlington and Congressional bent grass varieties that were treated out of doors in midsummer (June 30 to July 25, 1952) showed only slight symptoms at any time during this twenty-five-day period, despite the fact that they had been covered with wet paper toweling for nine consecutive nights and inoculated five times during this period. Germinating Helminthosporium conidia produced a slight leaf spotting on July 2, but there was no progression or severe killing at any time.

The extremely heavy inoculation carried out in the test of July 15, 1952, which was set up to determine possible action by toxic metabolic products produced by H. sativum, indicated that mass of inoculum may be a factor in this disease. Although the sterile extracts produced no apparent damage, all of the replicates receiving the mycelium-spore suspension were of a definite yellow color three days after the inoculation. The blades were observed to have been directly penetrated by Helminthosporium germ tubes and apparently were chlorotic due to the mass infections (see Plate IV). Unfortunately, a drop in temperature occurred which probably prevented

disease progression since the yellowing disappeared in 36 hours and did not recur after a second inoculation was made.

The test to determine whether or not the source of nitrogen, or its immediate presence, affected the disease on established grass showed no results. There was a 48-hour period of warm humid weather during the time that the four varieties were being tested, but the rest of the three-week period apparently was not favorable for disease development. The plot had received the same mowing and care as surrounding greens on the Oakland Hills Country Club, but did not have the traffic of players and bruising to which the others were subject. It is of interest to note that, two weeks previous to this trial, sixteen of eighteen greens on the golf course had been attacked by the fungus under discussion.

Bent grass seedlings. The pathogenicity of H. 10, H. sativum, and Curvularia sp. to Yorkwin wheat and to seaside bent in infested soil is indicated in Table III. The actual stand of the wheat was significantly reduced only by the H. 10, but the infection of the wheat plants was high. Based upon evidence of coleoptile and crown lesions, the infection of the wheat by H. 10 and H. sativum was 80 and 65 percent, respectively (see Plate IV). The creeping bent grass stands were significantly reduced by both H. sativum and H. 10. Statistically,

TABLE III

STANDS¹ OF YORKWIN WHEAT AND SEASIDE CREEPING BENT
THIRTEEN DAYS AFTER SEEDING INTO STEAMED,
INFESTED SOIL
(average of four replicates of twenty-five seeds each)

Treatment	Yorkwin Wheat	Severely Dwarfed	Seaside
Control	24.8	0	23.3
<u>H. sativum</u>	23.5	4	11.0**
H. 10	22.0*	6	7.3**
<u>Curvularia</u> sp.	25.0	0	21.0

Least Significant
Difference:

* L.S.D. 0.05	2.19	2.12
** L.S.D. 0.01	3.15	3.05

¹ The actual stand of plants is listed in the table. The infection of the wheat seedlings based upon lesions caused by H. sativum and H. 10 was 65 and 80 percent, respectively (see Plate IV).

there was also reduction of the bent grass stands by the Curvularia sp. This was so slight, however, that it would bear repetition and substantiation.

The seedling susceptibility of the four turf grasses germinated in petri dishes is summarized in Table IV. Since germination of the annual bluegrass was so very low, counts of these seedlings are not included. The significant reduction in stands on the Astoria bent were caused by the H. sativum and by the H. 10. The stand of Astoria seedlings in the Pleosphaerulina sp. treatment was higher than the control at the conclusion of the treatment. This was not significant; however, it was observed that germination in these plates was considerably better during the early days of the trial. The Highland bent grass stand was significantly reduced by H. sativum and H. 10, while Pleosphaerulina sp. and Curvularia sp. significantly increased the stand of this grass.

The seaside creeping bent was also significantly reduced in stand by H. sativum and by H. 10. It is of interest to note, however, that the combinations of these fungi with Curvularia sp. gave a reverse effect. The combinations gave slightly higher but not significantly better stands of seedlings. A significant increase in grass stand was observed with the combination of Curvularia sp. and Pleosphaerulina sp. Neither of these gave this increase alone.

TABLE IV

STANDS OF BENT GRASS SEEDLINGS IN PETRI DISHES
NINE DAYS AFTER SEEDING AND INOCULATION
(average of four replicates of twenty-five seeds each)

Treatment	Grass Variety		
	Astoria	Highland	Seaside
Control (surface disinfested)	21.3	11.0	17.3
H. 10	11.8*	5.3**	13.0**
<u>H. sativum</u>	7.5**	1 **	12.5**
<u>Curvularia</u> <u>sp.</u>	20.0	14.0**	17.5
<u>Pleosphaerulina</u> <u>sp.</u>	22.5	13.0*	18.3
<u>H. sativum</u> + <u>Curvularia</u> <u>sp.</u>			18.5
H. 10 + <u>Curvularia</u> <u>sp.</u>			16.0
<u>Aspergillus</u> <u>niger</u>			17.3
<u>Penicillium</u> <u>notatum</u>			16.8
<u>Curvularia</u> + <u>Pleosphaerulina</u>			20.0**
Untreated seed			14.0**

Least Significant Difference:

* L.S.D. 0.05	9.8	1.99	1.76
** L.S.D. 0.01	13.8	2.79	2.21

There was also a significant decrease in stand with the untreated seaside seed. Many of the untreated seeds apparently were destroyed by an unidentified Phycomycete.

The last test of pathogenicity of the several fungi and the nematodes is presented in Table V and Plate V. It is immediately apparent that the nematodes alone or together were not able to cause noticeable damage to the grass. Treatments 2, 3, and 15 show no evidence of dying grass. Furthermore, under the conditions of the test, they did not contribute to the disease when they were in combinations with the fungal organisms. Pleosphaerulina sp. and Curvularia sp. were but slightly pathogenic to the bent grass. The H. 10 and H. sativum gave evidence of the most parasitic action. This parasitism was much more rapid and severe when spore suspensions were used in contrast to the soil infested with the organism. The one exception was Treatment 9, which resulted in a 60 percent kill in a period of twelve days. The combination of H. sativum and Pleosphaerulina sp. gave evidence of undiminished parasitic action, while this latter fungus in combination with H. 10 was only 50 percent as lethal in Series A. In Series B, little difference was noted. The elevated relative humidity ranged from 63 percent during the day to 100 percent at night, with an average of 89 percent. The elevated temperatures ranged from 68° F. to 105° F. during the day,

KEY TO TREATMENTS IN TABLE V

1. Absolute check.
2. Nematode 1.
3. Nematode 2.
4. Nematode 1 + H. sativum.
5. Nematode 2 + H. sativum.
6. Nematode 1 + H. 10.
7. Nematode 2 + H. 10.
8. H. sativum.
9. H. 10.
10. H. sativum + H. 10.
11. Nematode 1 + spore suspension of H. 10.
12. Nematode 2 + spore suspension of H. 10.
13. Nematode 1 + spore suspension of H. sativum.
14. Nematode 2 + spore suspension of H. sativum.
15. Nematode 1 + Nematode 2.
16. Nematode 1 + Nematode 2 + H. sativum + H. 10.
17. Pleosphaerulina sp. spore suspension.
18. Curvularia sp. spore suspension.
19. H. sativum + Pleosphaerulina sp. spore suspensions.
20. H. 10 + Pleosphaerulina sp. spore suspensions.
21. H. 10 spore suspension.
22. H. sativum spore suspension.

TABLE V

DISEASE RATINGS OF ESTABLISHED SEASIDE BENT GRASS
INOCULATED TWICE, THREE WEEKS APART

Treatment	Series			
	A			B
	Feb. 13	Feb. 27	Mar. 8	Mar. 8
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	1	2+	1-
5	0	1	1-	0
6	0	1	2+	0
7	1	1+	1+	0
8	1	1+	2	0
9	3	3	3	0
10	1	2	1+	0
11	2	3	3	1+
12	2	3	3	2
13	2	3+	3	0
14	0	1	2	1-
15	0	0	0	0
16	3	4	4	1-
17	0	1	1+	0
18	0	0	1-	0
19	2	3+	4	2
20	2	2	2	1+
21	3	4	4	1+
22	3	3	3	1

A = inoculated Feb. 1; B = inoculated Feb. 30; 0 = no damage;
 1 = 0-30% kill; 2 = 30-50% kill; 3 = 50-70% kill; 4 = 70-90% kill;
 5 = 100% kill.

and averaged 87° F. After killing of grass parts was evident, the elevated temperatures and humidity were no longer used. The normal greenhouse temperature and humidity during this period of observation for recovery averaged 81° F. and 64 percent, respectively. No recovery was noted in any case.

The fact that roots were destroyed was substantiated by observations of slides and comparison to roots of control plants. Tissue platings were also made periodically. Recovery of the Curvularia sp. was unsuccessful, as was recovery of Pleosphaerulina sp. when it was in combination with H. sativum and H. 10--Treatments 19 and 20. It was recovered, however, from Treatment 17. Recovery of the Helminthosporium was successful in each case. Both roots and aerial parts were plated. In three isolations (Treatments 11, 12, 16), nematodes were found in the petri dishes. They apparently were in the dead and dying blades or roots, and were unaffected by the mercuric chloride-sodium hypochlorite surface sterilization. Since both blade and aerial parts were plated in the same petri dish and marked as such, the determination as to the source of the freely moving nematode was impossible.

Cultural Studies

Perithecial production trials. The attempts to produce the sexual stage of the Helminthosporium were unsuccessful. None of the twelve media or four conditions was promising enough to warrant further testing. There was no effect from the four environmental conditions nor from holding the flasks for ten months in a refrigerator. The ultraviolet light treatment and the use of nitrogen additives were of no value.

Variation due to medium. Evidence of the variability of the organism is shown in Plate VI. On the 2 percent malt extract agar, eleven original single-spore isolates were arbitrarily separated into eight cultural groups. These varied as to color, amount of aerial growth, rate of growth, production of sclerotial-like bodies, and spore production. However, when these same eleven isolates plus six additional isolates were put on Coon's synthetic agar, only six distinct groups could be distinguished. The comparative grouping in these last six as compared to the grouping on the malt extract is as follows:

1. 5 of 6 together previously.
2. 2 of 3 together previously.
3. 1 of 1 together previously.

4. 2 of 3 together previously.
5. 0 of 2 together previously.
6. 0 of 2 together previously.

Although there is a tendency toward grouping on both media, some isolates are apt to be culturally quite different when the medium is changed. At one time, eight single spores were taken from a single dish and allowed to grow separately. Seven of the eight were identical in cultural characteristics when grown on the same medium, while the other showed slight variation. This agrees with results of Christensen (2, 3) and Stevens (23). The entire test shows the extreme variability which may be encountered when attempting to describe this fungus on an artificial medium. The extreme variation mentioned by Sprague (22) and Christensen (2, 3) was easily reproduced in these studies.

A difference in sensitivity to low concentrations of mercury was demonstrated by the repetition of an earlier test (17). Two isolates from bent grass H. 10 and H. 19 were stimulated by 10 and 50 $\mu\text{g}/\text{ml}$ in the medium, while the H. sativum from Minnesota showed no stimulation at these concentrations (see Table VI). The H. 19, which was the test isolate used previously, was stimulated to the greatest extent, but not as markedly as in the previous trial. Plate

TABLE VI

DIAMETER OF FIVE-DAY-OLD COLONIES OF HELMINTHO-
SPORIUM SATIVUM AND OF TWO ISOLATES OBTAINED
FROM CREEPING BENT GRASS

(measurements in mm. from replicated plates of 2 percent malt
extract agar containing varying amounts of mercury)

Isolate	μ g/ml of Mercurous and Mercuric Chloride Mixture ¹						Least Sig- nif. Dif.	
	0.0	10.0	50.0	100.0	250.0	500.0	*.05	**0.01
<u>H. sativum</u>	59.3	60.8	55.3	47.8	33.0	15.0	2.06	2.86
H. 10	55.0	59.0*	57.8	36.8	14.8	11.5	2.88	3.98
H. 19	44.5	56.0*	55.8*	45.3	17.8	11.3	5.09	7.03

¹ Based upon active ingredients contained in Calo-clor,
Mallinckrodt Co.

X shows the growth on plates covering the range of mercury concentrations employed.

DISCUSSION AND CONCLUSIONS

Although the conidia of the isolates from creeping bent grass may be slightly wider than the figures given for H. sativum, and the stimulation by low concentrations of mercury occurred only with the bent grass isolates, there are important characteristics so nearly identical that the author believes the fungi are of the same species.

The reaction on the wheat was identical--both isolates caused the characteristic stem lesions, as shown in Plate IV. The parasitic action on bent grass seedlings and killing of established grass was nearly identical. Culturally, some of the bent grass isolates closely resemble the H. sativum growth on the same medium. And culturally, the variation reported for H. sativum is easily obtained. Cultures vary greatly in ability to produce conidia, rate of growth, color of the colony, and production of aerial mycelium. These variations may occur with changes in temperature and humidity, or change of medium. The color, method of germination of the spores, and morphology of the sporophores is also the same for selected isolates of Helminthosporium when they are on the same medium and under identical environmental conditions.

The Helminthosporium from bent grass is therefore considered to be H. sativum, as previously stated. There are slight differences in degree of severity of attack on the Colonial and creeping bents, and in tolerance to mercury, but these are not considered adequate for species differentiation. Plate II indicates the variability among spores from the same petri dish and similarities between the known H. sativum and an isolate from creeping bent grass. Also, Plate IX illustrates the growth in culture of three Helminthosporium isolates.

The many failures in the attempts to produce severe killing on established stands of creeping bent grass may be partially explained to be due to the impossibility of controlling weather conditions. The artificial inoculations under controlled environmental conditions did result in killing of established grass. However, some of these were not entirely satisfactory.

The conclusion to be drawn is that H. sativum can be parasitic on creeping bent seedlings and on established stands of this grass. The rapidity of progression and severity of the disease as it occurs in nature, however, has only once been satisfactorily repeated (see Plate III).

There is the possibility that large masses of inoculum are necessary. There is also the possibility that nematodes play an

important part in furnishing entry points, if not actually contributing to the destruction of root and aerial parts by parasitic action. The possible action of Curvularia sp. and Pleosphaerulina sp. in conjunction with each other and with H. sativum should not be overlooked; however, in tests of these combinations, no evidence of combined parasitic action was noted.

The possibility still exists that high nitrogen may be a factor in the disease progress. Fertilization of chlorotic areas has been a common practice on golf courses. An examination of these yellow areas has often indicated a high percentage of infection from H. sativum. There, of course, was no corrective reaction from the nitrogen, and the observations were that destruction of grass was more rapid following the fertilizer application.

Although limited research was done on Pleosphaerulina and on nematodes, both were established as parasites of creeping bent grass in their own right.

Plate VII indicates the morphology and method of penetration by the Pleosphaerulina, while the correspondence with Dr. Thorne establishes the identity of the parasitic nematodes of the genus Tylenchorhynchus (see Plate VIII). Since no damage was caused by the nematodes in the one trial, either the wrong species was used,

the number introduced into the soil was too small, or the nematodes are not disease producers alone.

The tolerance of the bent grass isolates of Helminthosporium to low concentrations of mercury may be a partial explanation as to the apparent sudden outbreak of this new disease on bent grass. Mercury compounds have for the last thirty years been the main components of standard fungicide sprays. The possibility arises that selection and mutation by the fungus may have resulted in these tolerant strains increasing in numbers to the point where sudden infections could occur. It has been observed that only extremely high concentrations of the inorganic mercuries have any noticeable effect on Helminthosporium. Such concentrations probably would cause as much damage to the grass by toxicity as the fungus would in its parasitism.

However, it is probably nearer the truth to say that the disease has been present for years, but completely overlooked, due to the almost complete lack of work on fine-turf diseases by plant pathologists. Routine fungicide testing has been practiced to some extent, but thorough, complete investigations into the disease problems of ornamental grasses is nearly nonexistent. Within the last few years, considerable interest has been generated, and there are

now approximately ten plant pathologists in this country and abroad who are engaged in part-time turf disease control studies.

A present, the only control known is applications of the antibiotic fungicide Acti-dione (17, 27). In an unpublished report this material was also shown to keep the numbers of conidia in established grass at a minimum. Spore counts were made from samples obtained from plots of seaside bent grass receiving twelve different fungicidal sprays. Reduction in numbers of viable spores was observed only in the Acti-dione-sprayed plots. From the observations so far made, the value of preventative spraying cannot be over-emphasized, since mass establishment of the fungus seems to be a prerequisite to severe disease.

Future work should include extensive tests on established field plots using H. sativum, alone and in combination with Curvularia sp., Pleosphaerulina sp., and Tylenchorhynchus sp. The various treatments should include a study of the masses of the different inocula necessary to incite disease. Preliminary sprays to reduce the amount of fungus already present in the soil should be employed. The spray residues could be leached with water just previous to the first inoculation. The environment should be controlled insofar as possible.

The various isolates should also be separated into biologic forms for subsequent testing for pathogenicity. Christensen (1, 2) has shown that the biologic forms obtained from culture studies do vary greatly in pathogenicity. Since all isolates have not been tested, it is possible that the most pathogenic form was never used for inoculation.

A testing program could be set up to determine if H. sativum can increase its tolerance to inorganic mercury, and if this tolerance is correlated with increased pathogenicity to creeping bent grass. The biologic forms could be transferred to medium containing increasing amounts of mercury and tested periodically on established grass, or on the seedlings for a more rapid, analyzable determination.

A difficult, but perhaps necessary, study of the predisposing factors should be undertaken. Soil pH, flora, fauna, and fertility level may influence the disease. Certain previous environmental conditions may be necessary. Normal traffic and resulting injury to the grass may be a factor, as may be the insecticide applications previously used.

SUMMARY

1. An apparently new disease of Agrostis palustris Huds. (creeping bent grass) is described.
2. The fungal organism constantly associated with the disease is identified as Helminthosporium sativum P. K. & B.
3. Fifty-eight isolations and their areas of origin are listed.
4. The Helminthosporium is found to cause complete death of established creeping bent grass plants.
5. The bent grass isolates and a known H. sativum are shown to cause identical damage on established bent grass, bent grass seedlings, and Yorkwin wheat.
6. Twelve media and four environmental conditions are used in an attempt to induce production of the perithecial stage. No sexual stage was obtained.
7. The variability of seventeen bent grass isolates was observed on two different media. Both medium and environment were found to contribute to this variation.
8. H. sativum and bent grass isolates are compared as to stimulation when grown in medium containing inorganic mercurials.

The Helminthosporium isolates from bent grass were found to be stimulated by low concentrations of mercury.

9. Nematodes (Tylenchorhynchus sp.) are found to be parasitic on creeping bent grass.

10. Curvularia sp. is found to be mildly parasitic on creeping bent grass.

11. Pleosphaerulina sp. is shown to be an apparently unreported parasite of creeping bent grass.

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APPENDIX

PLATE I

Camera lucida drawings of Helminthosporium sativum conidia obtained from creeping bent grass. The shorter, germinating spores from artificial medium--the longer spores scraped from infected grass. See Table II for measurements. Approximately 500X.

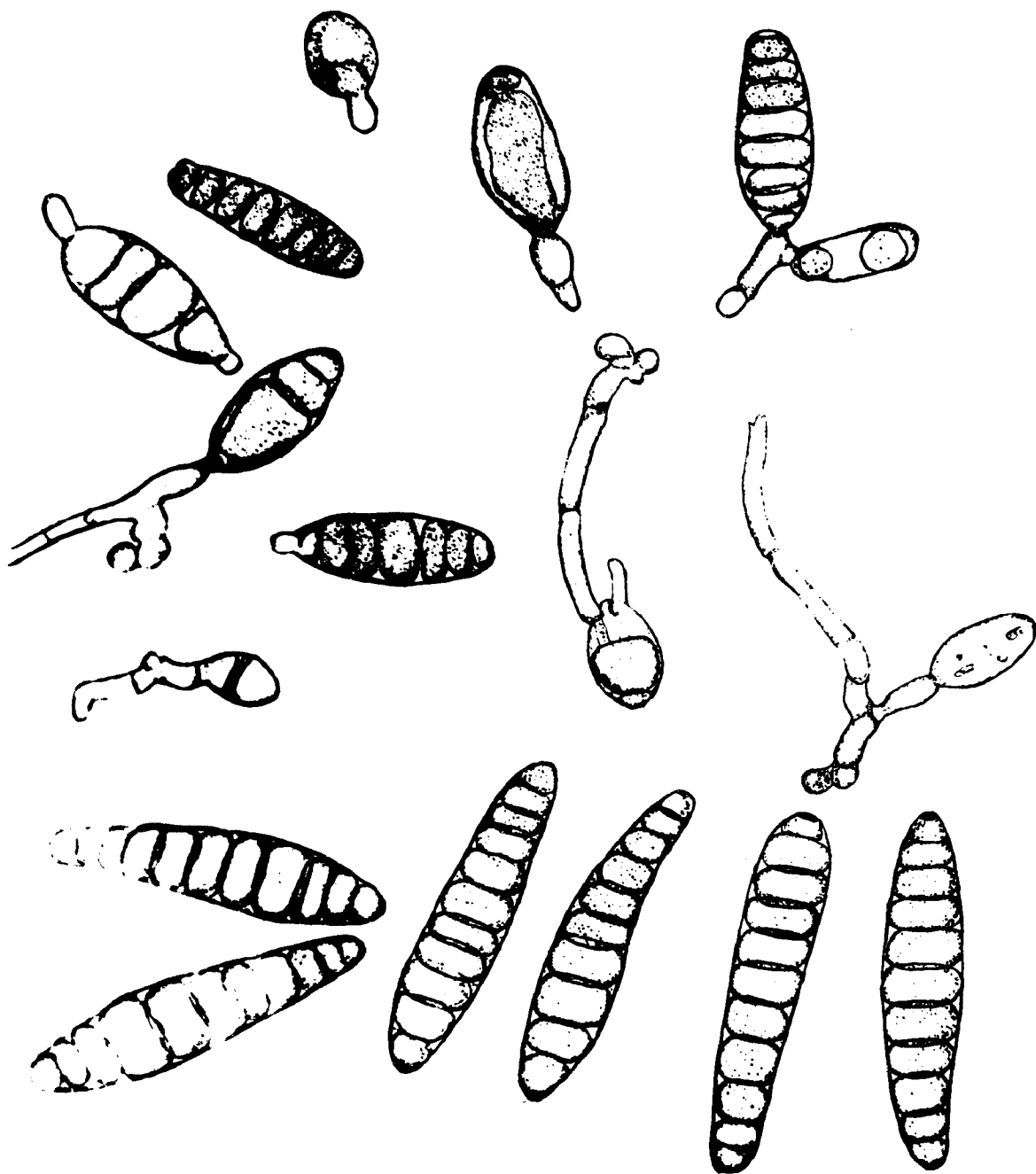


PLATE I

PLATE II

A, B, C, D. Conidia of H. sativum obtained from creeping bent grass.

E, F. Conidia from H. sativum isolate received from J. J. Christensen,
Minnesota.

Note the lighter color of immature conidia and variability in spore shapes. Photographs from spores produced on 2 percent malt extract agar. Approximately 450X.



PLATE II

PLATE III

- A. Flats of Washington creeping bent grass grown in the greenhouse. Left flat inoculated with H. sativum conidia three days previously. Flat on the right sprayed with sterile distilled water. Both held in humidity chamber (Plate VIII) at 77° C. and 90 percent relative humidity.
- B. Close-up of infected grass in Figure A three weeks later. Complete killing was evident, since no regrowth occurred.



PLATE III

PLATE IV

- A. Yorkwin wheat seedlings from pathogenicity test.
8. From soil infested with H. 10 (creeping bent grass isolate).
 7. Control.
 6. From soil infested with H. sativum from Minnesota.
- B. Washington variety creeping bent grass with small oval spots and larger blotches from infection by H. sativum.



A



B

PLATE IV

PLATE V

Seaside creeping bent grass established for twenty-four days in plant bands, then transferred to pots for inoculation. Photographed sixteen days after inoculation. Selected treatments as follows:

- A. Spore suspension of H. 10 sprayed on grass growing in steamed soil.
- B. Spore suspension of H. sativum sprayed on grass growing in steamed soil.
- C. Spore suspensions of H. 10 plus H. sativum sprayed on grass established in steamed soil infested with Nematodes 1 and 2.
- D. Established grass growing in steamed soil infested with H. 10.
- E. Control.
- F. Established grass growing in steamed soil infested with Nematode 1 and Isolate H. 10.

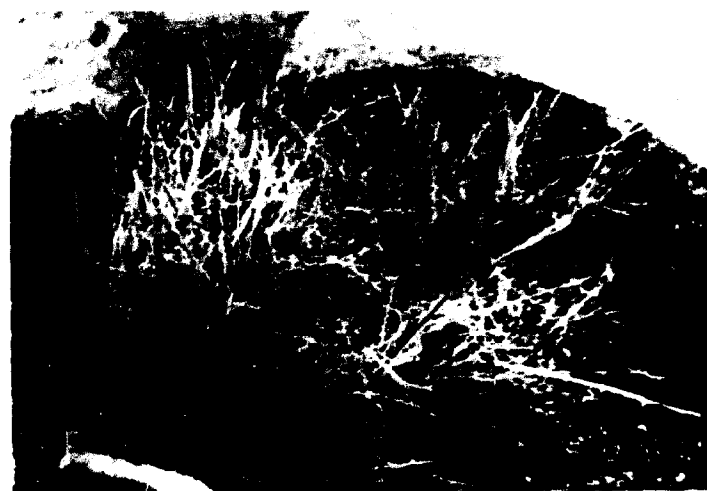
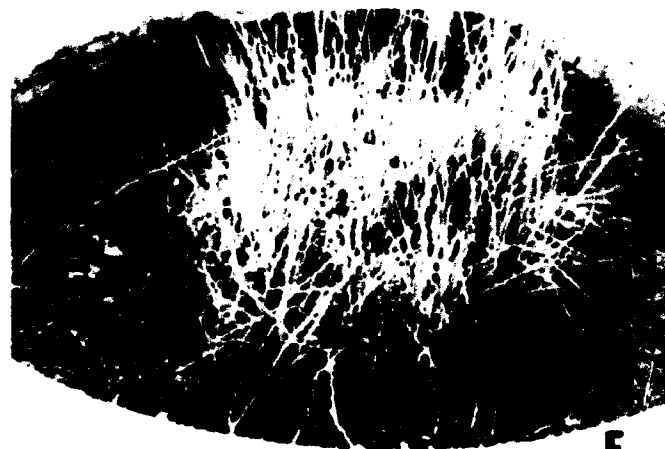
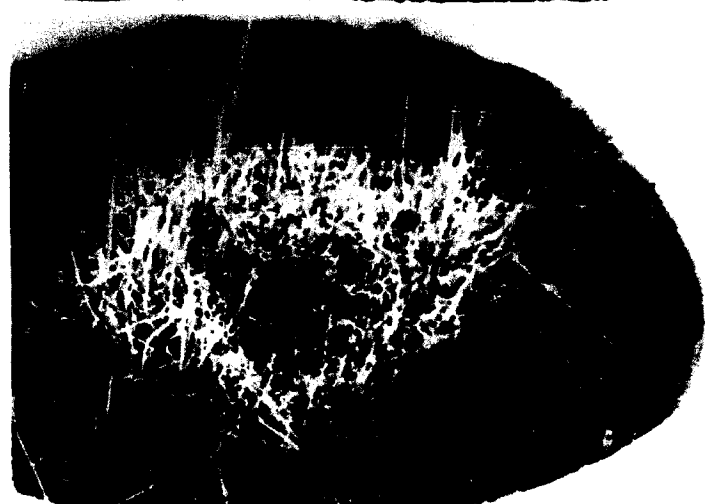
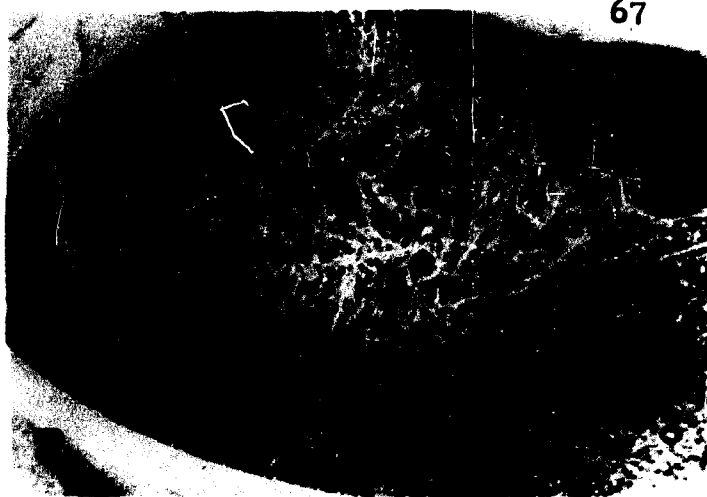
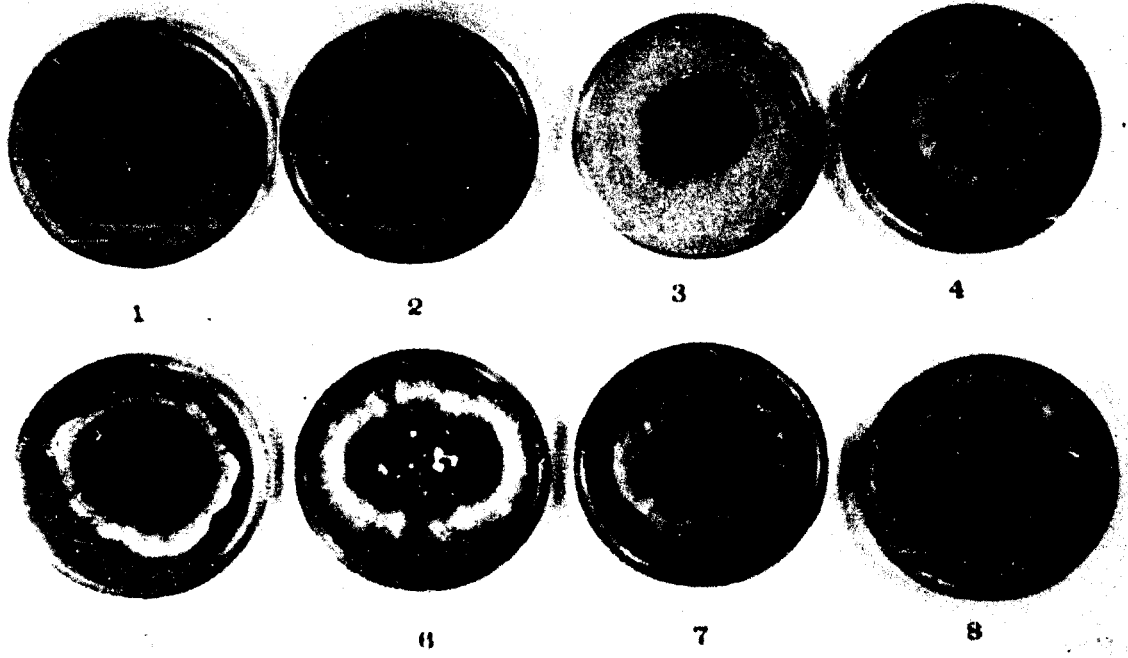


PLATE V

PLATE VI

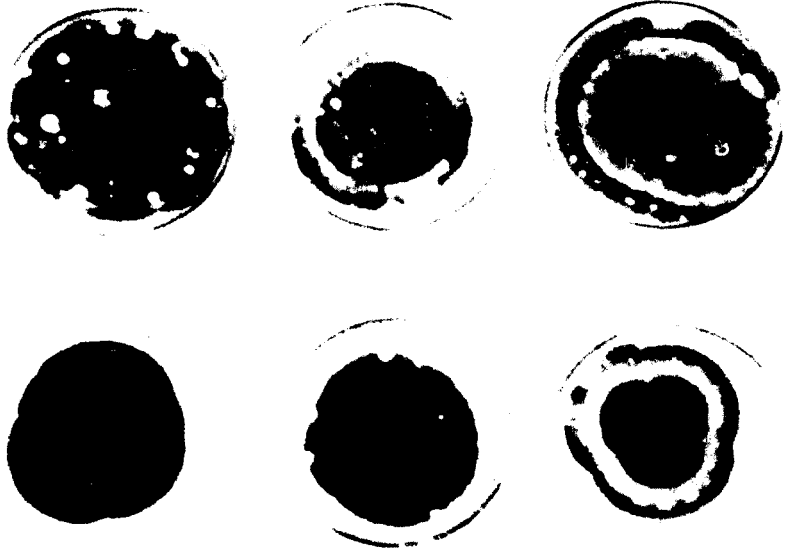
- A. Helminthosporium isolates from creeping bent grass grown on 2 percent malt extract agar. Eight artificial groupings from an original eleven isolates.
- B. Six artificial groupings of the isolates used in A grown on Coon's synthetic medium. The eleven isolates from A and six additional single-spore cultures resulted in only six groups on this medium.
- C. Immature Helminthosporium sativum conidium on a sporophore (1500X).
- D, E. Helminthosporium sativum sporophores and mature spore (260X).



A



E



B

PLATE VI

PLATE VII

- A. Perithecia of Pleosphaerulina sp. on creeping bent grass (250X).
- B. Close-up of A (350X).
- C. Camera lucida drawings of germinating Pleosphaerulina sp. ascospores. Penetration of creeping bent grass leaf blades is shown by the arrows. Careful focusing showed direct penetration as well as entrance through stomata (750X).

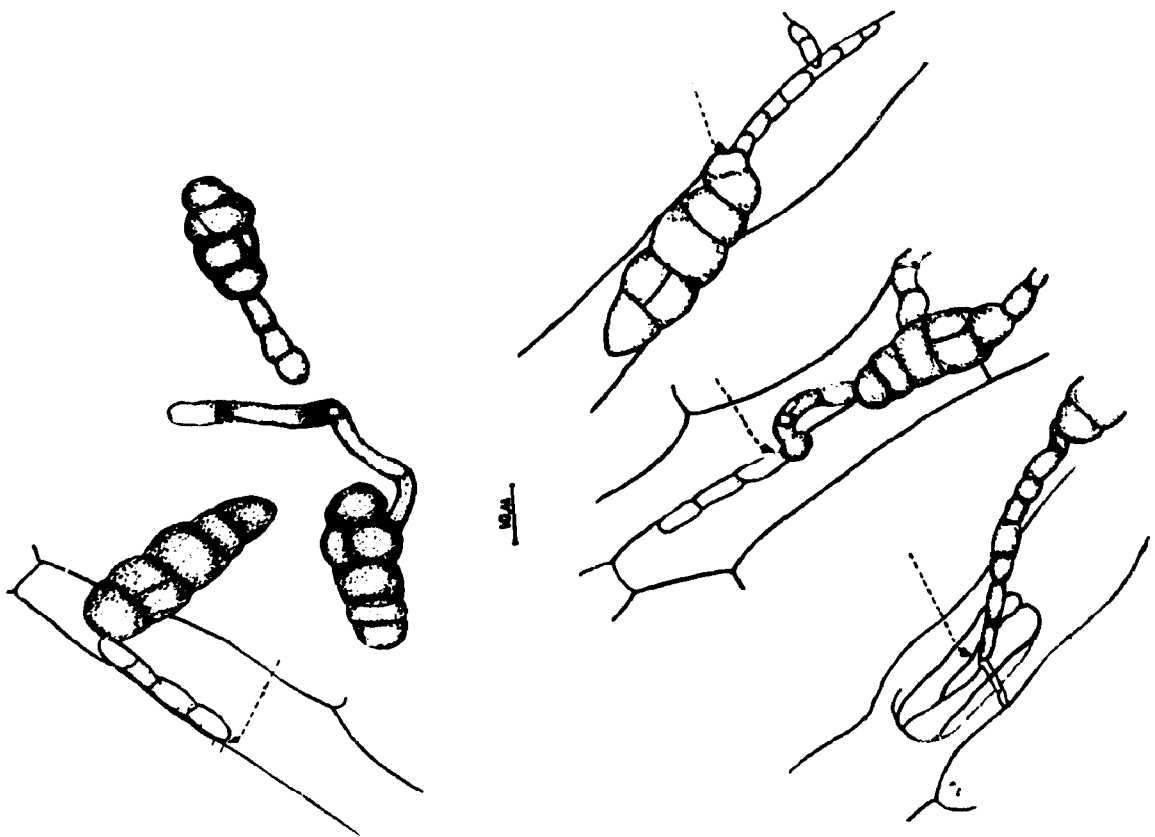
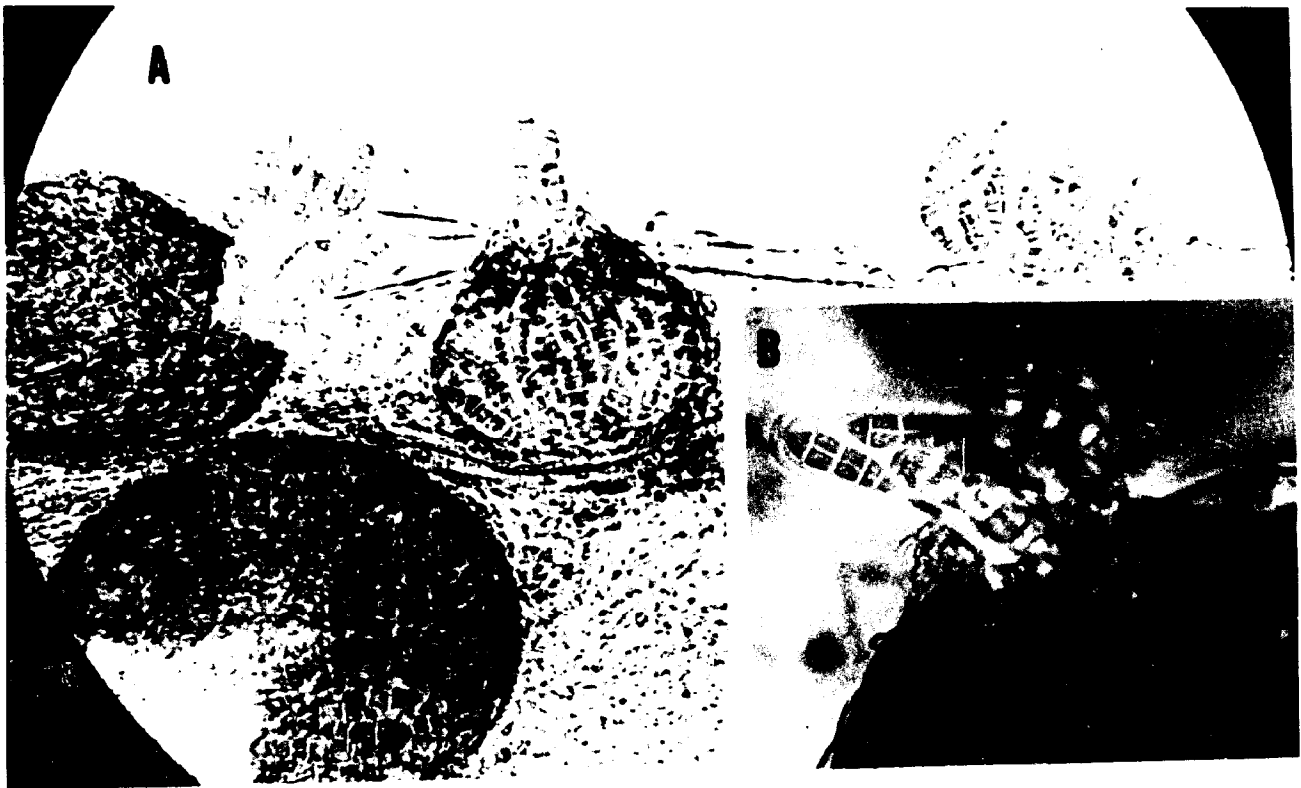


PLATE VII

PLATE VIII

- A. Humidity chamber used for early pathogenicity tests containing plugs of infected grass for future isolation and identification work.
- B, C. Photographs of Tylenchorhynchus sp. from creeping bent grass. Mounts of the nemas prepared and identified by Dr. Gerald Thorne.

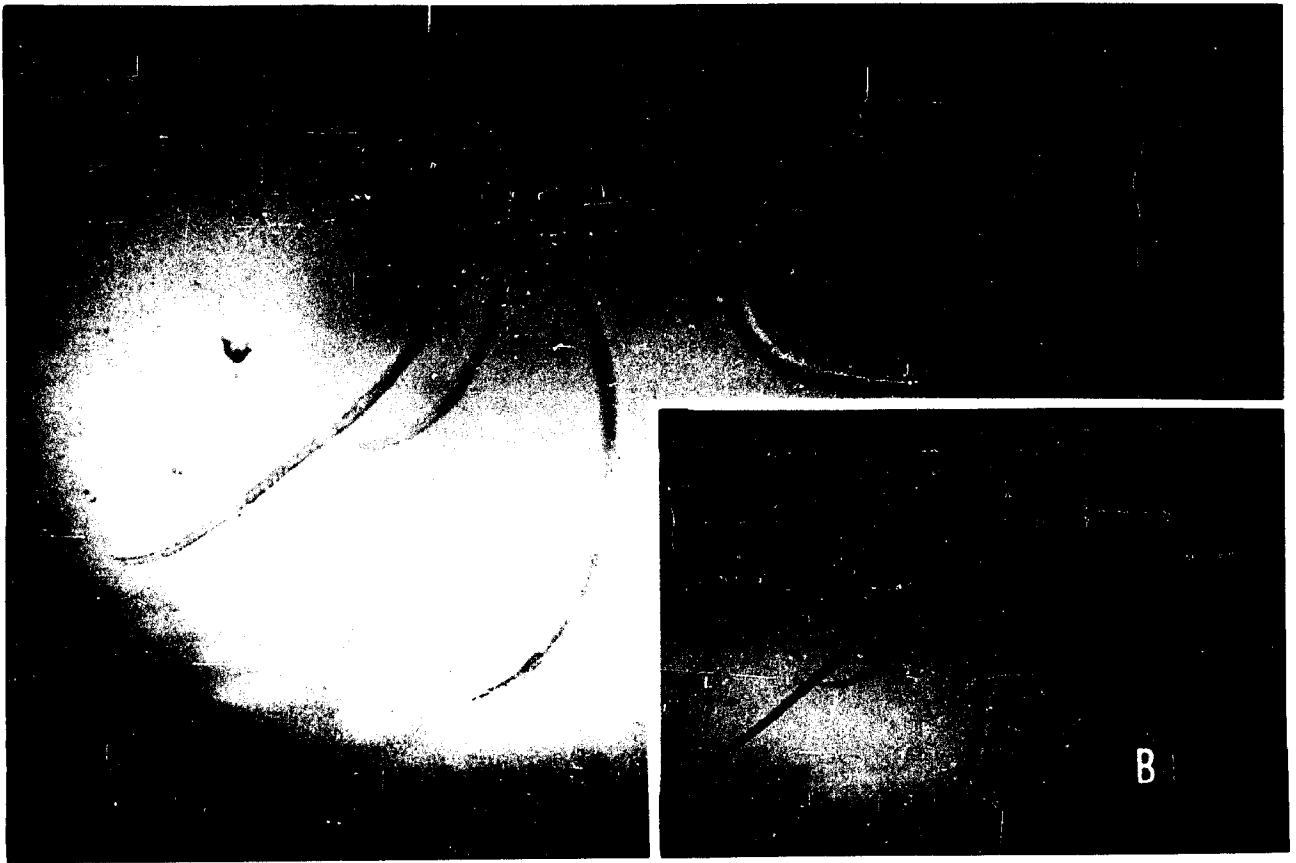
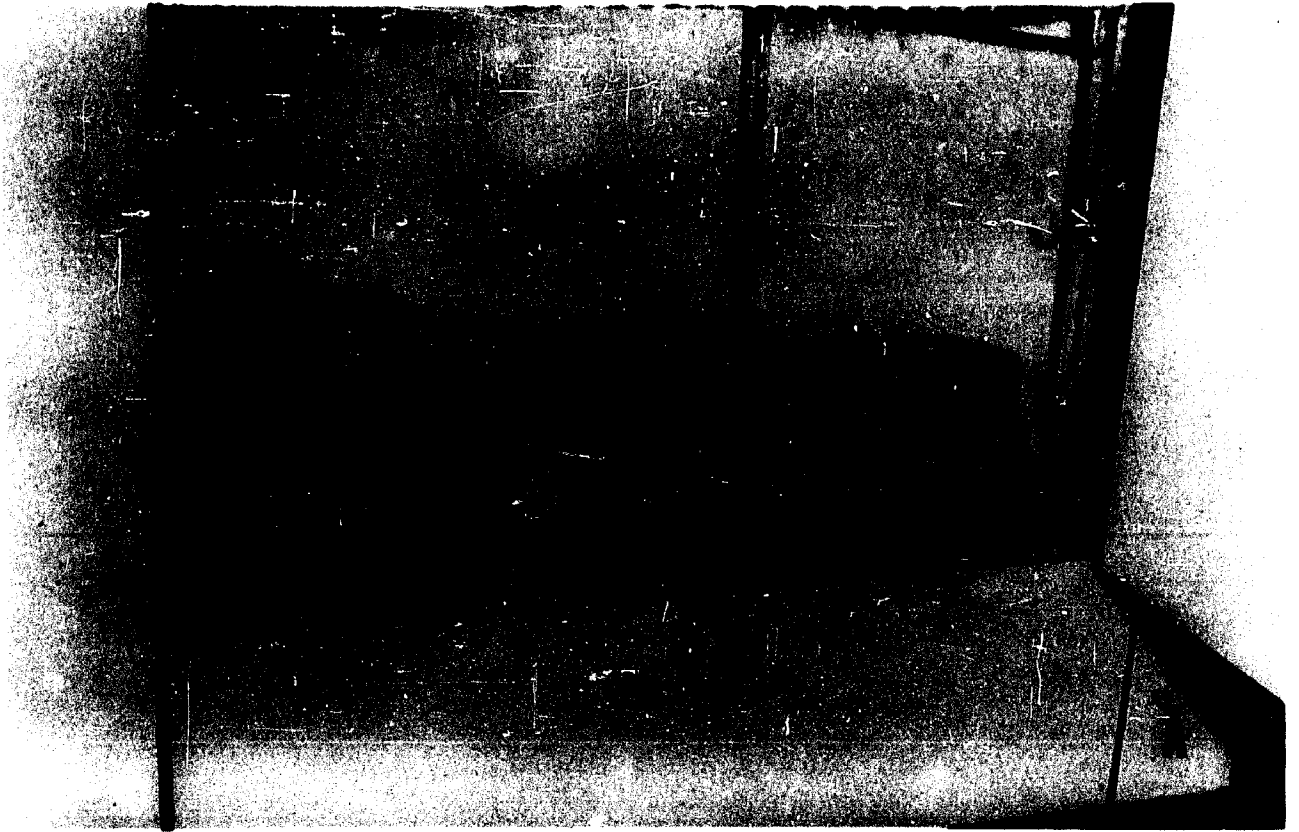


PLATE VIII

PLATE IX

A. Cultural characteristics of some of the fungi used in this study growing on Czapek's synthetic medium.

0. Pleosphaerulina sp.

1. Curvularia sp.

2. H. sativum.

3. H. 10 (creeping bent grass isolate).

B. Cultural characteristics of Helminthosporium sp. growing on 2 percent malt extract agar.

Top left: H. 19 (creeping bent isolate).

Top right: H. 10 (creeping bent isolate).

Bottom: H. sativum, Minnesota.

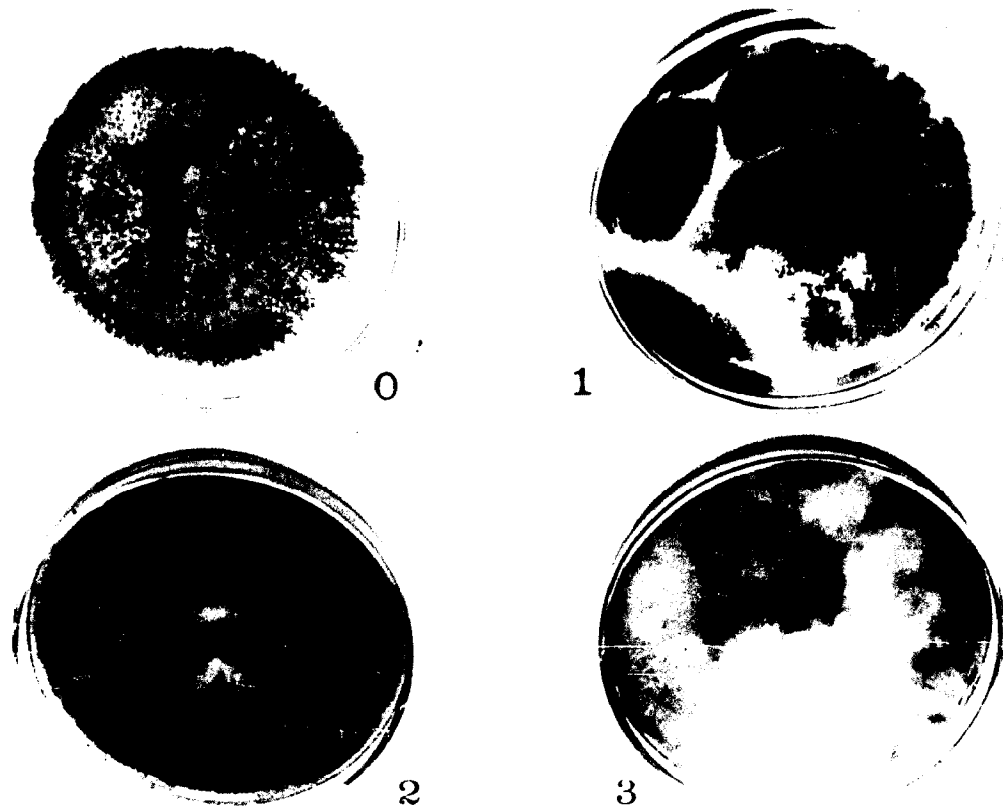
**A****B**

PLATE IX

PLATE X

Simulation of the growth of two Helminthosporium isolates from bent grass when grown in 2 percent malt extract agar containing small amounts of inorganic mercury.

A. Isolate H. 10.

To left: Control.

Top right: 10 $\mu\text{g/ml}$ mercury.

Bottom left: 50 $\mu\text{g/ml}$ mercury.

Bottom right: 100 $\mu\text{g/ml}$ mercury.

B. Isolate H. 19.

Top left: Control.

Top right: 10 $\mu\text{g/ml}$ mercury.

Bottom left: 50 $\mu\text{g/ml}$ mercury.

Bottom right: 100 $\mu\text{g/ml}$ mercury.

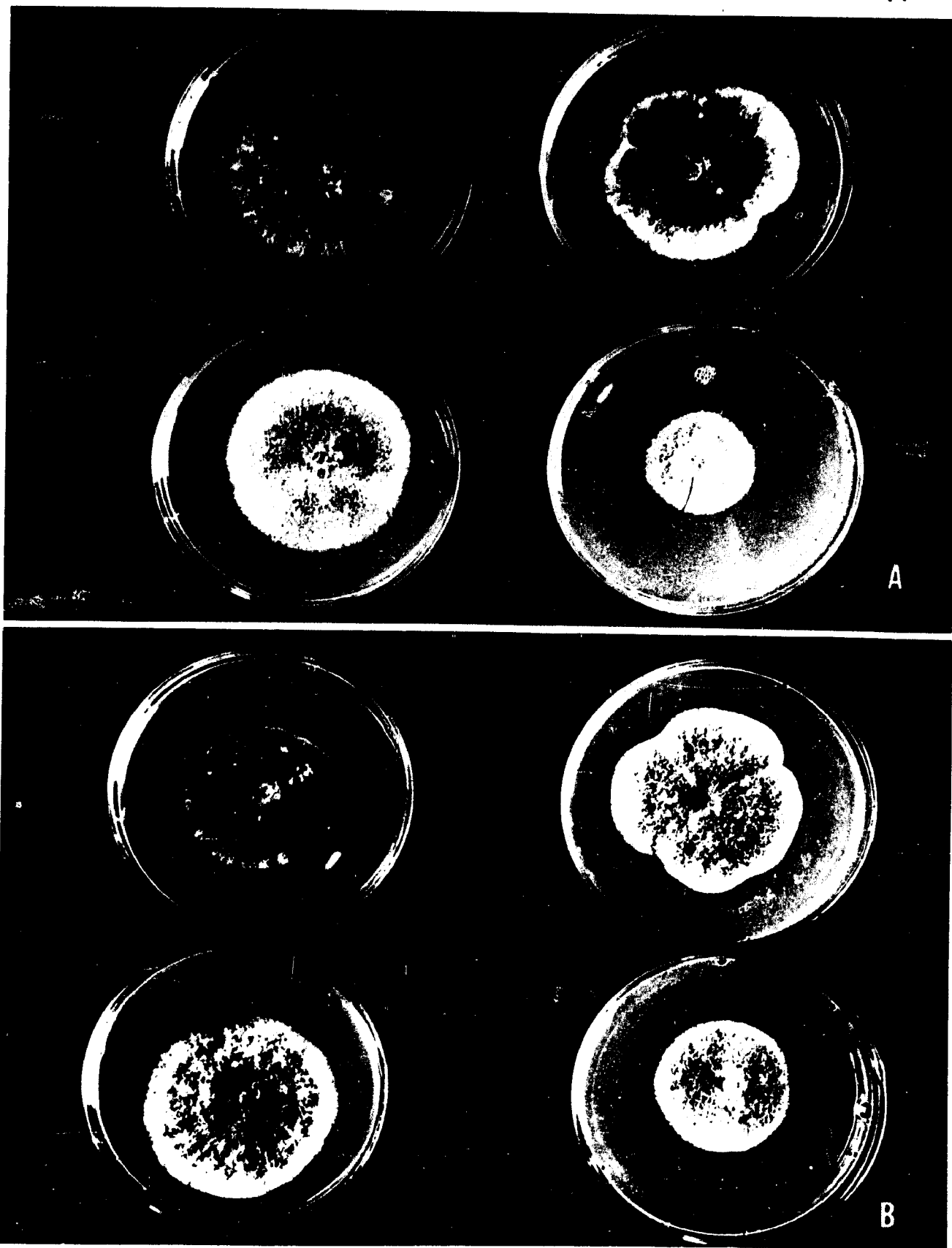


PLATE X