THE INFLUENCE OF HIGH TEMPERATURE STRESS AND HERBICIDES ON THE SUSCEPTIBILITY OF CREEPING BENTGRASS (*AGROSTIS PALUSTRIS*) TO *CURVULARIA LUNATA*

by

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(ABSTRACT)

The status of *Curvularia lunata* as a primary pathogen of creeping bentgrass (*Agrostis palustris*) has been debated in the literature for many years. The most recent and most complete evidence indicates that this fungus is a weak pathogen, colonizing only senescing tissues. In the present study, four groups of experiments were conducted: 1) The effects of five herbicides on the growth of *C. lunata in vitro* were determined. 2) Acute high temperature stress was evaluated as a predisposing factor in the susceptibility of creeping bentgrass to *C. lunata*. 3) The effects of acute high temperature stress and exposure to five different herbicides on creeping bentgrass were evaluated together and separately. 4) The potential of chronic, moderately high temperature stress and exposure to five herbicides when occurring separately and together to serve as predisposing factors on the susceptibility of creeping bentgrass to *C.*

lunata were evaluated. It was found that effects of the herbicides on the growth of *C. lunata* depend on both the specific herbicide and the concentration. High temperature stress and herbicide exposure increase the colonization of creeping bentgrass by *C. lunata* only to the extent that these treatments produce moribund plant tissue.

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. . . from them I have learned about love. . . the rest of my education pales in comparison.

Give thanks to the Lord, for He is good;
for His lovingkindness is everlasting. (Psalm 136:1)

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INTRODUCTION

Pesticide usage in crop production has increased dramatically in the last three decades. The use of herbicides in particular, has risen at a rate exceeding that of other pesticides. Herbicides have proven to be a cost effective means of increasing the quantity and quality of agricultural yields. It is therefore not surprising to find that management practices for quality turfgrass areas include regular use of herbicides.

Because of the high level of herbicide usage, the effects of herbicides on plant disease has become an area of increasing interest. Herbicides have been shown to have both stimulating and inhibiting influences on plant disease development. These influences may result from alterations in the physiology of the plant pathogens, the host plants, or other organisms which compete with the host or pathogen.

Amenity grasslands serve to enhance the quality of the environment. They function in the preservation of soil by controlling erosion due to wind and water. They are important in the reduction of air pollution, noise, and ambient temperature. Grassy areas reduce dust and mud and do not support vermin or fire. Turfgrasses contribute materially

to increased property values by enhancing the aesthetic beauty of the landscape. They contribute to the mental and physical well being of the population through their recreational usefulness as well as their aesthetics.

Turfgrasses are used on home lawns, public and private institutions, commercial properties, parks, cemeteries, airports, and roadways. Golf courses, tennis courts, bowling greens, and various types of athletic fields are composed of turfgrasses. The management of these areas supports an important industry. In Virginia, for instance, over one billion dollars were spent in 1982 for the management of turfgrass, according to the estimate of a Virginia Department of Agriculture survey (Rowley et al., 1983). A significant portion of this money is spent for the control of weeds and diseases which, if unchecked greatly reduce the usefulness and aesthetic value of the turf.

Herbicide effects on fungal pathogens of turfgrasses include increases or decreases of such processes as germ tube growth rate, mycelial growth, spore formation and spore germination. In addition, herbicides may cause aberrations of germ tube morphology. These observations however, have been obtained from a comparatively small number of studies.

The possible roles of herbicides as mitigating factors in disease development have not been determined for most turfgrass diseases. More specifically, the information available for herbicide side effects on diseases of bentgrasses (Agrostis spp.) is scanty and often of anecdotal nature rather than scientific. Obtaining information on the influence of herbicides on diseases of creeping bentgrass (Agrostis palustris) is desirable because routine maintenance of this grass includes both herbicide applications and disease control practices and because of the high monetary value of stands of this species.

Several species of Curvularia have been reported to be incitants of turfgrass diseases. Curvularia lunata is the most frequently cited species. However, while C. lunata has been given the distinction of being a primary pathogen by some authors, and the disease associated with its colonization of turfgrasses referred to as "Curvularia blight", others maintain that C. lunata is an obligate saprophyte found secondarily associated with necrotic or senescing tissue. Additional data is needed on factors that determine the extent of the pathogenicity of C. lunata to creeping bentgrass.

A study of the effects of herbicides on disease is particularly germane to Curvularia blight. This is true because

management practices make this interaction likely to occur in the field. In addition, herbicide application is known to accelerate senescence in plants and is likely to have a particularly profound effect on a disease that is associated with plant tissues in advanced senescence.

An important factor in the susceptibility of creeping bentgrass to C. lunata appears to be high temperature induced stress. High air temperatures are also important in increasing the sensitivity of creeping bentgrass to herbicides. High air temperatures in conjunction with herbicide applications would seem to be a potent combination in predisposing bentgrass to colonization by C. lunata. Experiments using this combination, then, would be useful in providing further insight into the nature of Curvularia blight.

LITERATURE REVIEW

A. Literature Relating to the Pathogenicity of Curvularia Species to Turfgrasses.

The genus Curvularia was erected by Boedijn in 1933 (Ellis, 1971). It contains a group of phragmosporous dematiaceous hyphomycetes which are recognized by their characteristic conidia which have the following attributes: (Ellis, 1971; Groves & Skolko, 1945)

1. four to six cells (normally)
2. a disproportionately large cell as the third cell from the base (third and fourth cell larger in some cases)
3. more or less strongly curved shape
4. end cells with noticeably lighter pigmentation than the other cells
5. borne spirally or in whorls on simple, often geniculate dematiaceous conidiophores

Many of these fungi are common saprophytes on plant debris and are often isolated from seeds (Ellis, 1971; Groves & Skolko, 1945; McKenzie, 1978). Certain species of Curvularia may be pathogenic to maize and rice (Ellis, 1971; Shurtleff, 1980). Curvularia robusta has been shown to incite a disease

of certain bluestem grasses (Dichanthium spp. and Bothriochloa spp.) which are used as forage in the southern United States (Kilpatrick et al., 1967). Batiki bluegrass, another forage grass (and weed grass), is susceptible to an eyespot disease incited by C. ischaemi (McKenzie, 1981). Certain other weed grasses have also been shown to be parasitized by species of Curvularia (Walker & White, 1979). While the virulence of certain Curvularia species is accepted on these and other hosts, the pathogenicity of various species of Curvularia to turfgrasses has been debated in the literature for a number of years. Some authors consider these fungi to be primary parasites while others regard them as saprophytes or only very weak pathogens which colonize turfgrass tissues only at high air temperatures.

The first reference to Curvularia on turfgrasses is an abstract published in 1941 by Wernham and Kirby. They reported that in hot weather, thinning of stands of 'Metropolitan' creeping bentgrass (Agrostis palustris) and annual bluegrass (Poa annua) was attributable to "a species or perhaps two species of either Helminthosporium or Curvularia." No species designations were given.

Species identifications were first given by Howard et al. (1951) who listed C. geniculata, C. inaequalis, and C. lunata as incitants of "melting out" or "Curvularia mold".

Howard and Davies (1953) isolated C. lunata from creeping bentgrass, annual bluegrass, and velvet bentgrass (A. canina) which exhibited a blotchy yellow discoloration. The condition was given the name "fading out" and C. lunata was assumed to be the incitant. These two reports provided no experimental evidence of pathogenicity. Howard (1953) reported using an isolate of C. lunata from velvet bentgrass to demonstrate pathogenicity on ten kinds of grasses. The report did not include a description of the experimental methods or growing conditions for the plants.

Mower (1961) (Mower and Millar, 1963) conducted the first pathogenicity experiments in which the experimental methods were described. He concluded that "C. lunata must be considered a saprophyte or at best a very weak pathogen" of both common and 'Merion' Kentucky bluegrasses (P. pratensis). He noted that the germ tubes of C. lunata may occasionally enter the substomatal cavities of the leaves but no evidence of infection or colonization was observed in the leaf parenchyma and lesions were not produced in the epidermis. Mower theorized that the fungus would only colonize leaves which were "in a rather advanced stage of senescence."

Bean (1964) also reported inoculating P. pratensis with C. lunata (and/or C. pallescens, the precise species desig-

nation was not determined) and found that few or no lesions were produced. He also reported that growing seedlings in soil infested with this isolate resulted in significantly reduced stands as compared to uninfested controls. Bell (1967) also reported inhibited growth of young plants in infested soil-sand potting mixtures. He tested isolates of C. geniculata, C. inaequalis, C. lunata, and C. pallescens which he had obtained from zoysia lawns exhibiting root and/or crown rot symptoms. The experiments utilized two node length sections of Zoysia japonica stolons transplanted into the infested soil-sand mixtures. Bell (1967) stated that all the species inhibited shoot, root, and stolon growth even though many of the results showed no statistically significant reductions in growth compared to uninfested controls. In addition, compared to Helminthosporium tetramera (Bipolaris tetramera) which was also included in the study, the Curvularia isolates would rate as weak pathogens. Of the four species, Bell considered C. pallescens to be the most aggressive in terms of ability to reduce growth rates of the zoysia.

The reports of Brown et al. (1972) and Falloon (1976) lend the greatest credence to the concept that species of Curvularia can act as primary pathogens on mature turfgrass plants. Brown et al. (1972) obtained isolates of Curvularia species from 415 turfgrass samples, 192 turf seed samples,

and several reference collections. From the 415 turfgrass samples, 132 isolates of C. lunata were obtained. No other species could be isolated from the turf samples. From the 192 seed samples, fifteen isolates of C. lunata, four of C. geniculata, one of C. intermedia, and one of C. protuberata were obtained. A fifth species, C. eragrostidis (C. maculans), was also used in the experiments. In that series of experiments, 153 isolates from those five species, (147 of them were C. lunata) were tested for pathogenicity to three varieties of Kentucky bluegrass (P. pratensis), one variety of red fescue (Festuca rubra), and two varieties of creeping bentgrass (A. palustris).

In each experiment, the plants were clipped 24 hours prior to inoculation with aqueous conidial suspensions and were then kept under plastic covers in the greenhouse for ten days. Four separate experiments were conducted. The first used four isolates of each of the five Curvularia species to test for pathogenicity on 'Delta' and 'Pennstar' Kentucky bluegrass, 'Jamestown' red fescue, and 'Seaside' and 'Penncross' creeping bentgrass at temperatures of 20 to 24 C. In that experiment, no symptoms were detectable after the incubation period of ten days on any of the varieties of grasses using any of the isolates. In the second experiment, the same twenty isolates were tested for pathogenicity to 'Cougar' Kentucky bluegrass at temperatures of 24 to 31 C.

In that experiment, leaf tip dieback was the most common symptom but leafspots and lesions in the crowns were also observed. No statistical treatment of the data was conducted. The third and fourth experiments were conducted at temperatures of 29 to 35 C and used a set of 145 isolates. In the third experiment, pathogenicity to 'Delta' and 'Pennstar' Kentucky bluegrass and 'Jamestown' red fescue was tested while the fourth tested 'Seaside' and 'Penncross' creeping bentgrass.

Brown et al. reported that "Tip dieback or leafspot, or crown lesions were incited by 121 of 145 isolates of Curvularia" in at least one of these experiments on at least one of the host varieties. Tip dieback was again the predominant symptom. Fourteen of the 145 isolates were capable of producing leafspots on at least one of the hosts but never on the bentgrasses. Symptom expression was mild for the isolates from turf samples, an average rating of 2.7 for bluegrasses, 2.8 for bentgrass and 3.4 for red fescue, on a rating scale where "three equals less than 50 percent of leaves exhibit tip lesions 4 mm long." Variability in the pathogenicity of isolates, even of the same species, was high.

The experiments were unreplicated which precludes any statistical treatment of the data to determine significance

of the results. Curvularia was reisolated from samples of symptomatic tissues but no histological observations were made.

A similar experiment was performed by Falloon (1976) in which 11 isolates of C. trifolii were tested for pathogenicity to Poa annua, Agrostis tenuis, A. palustris, and Festuca rubra. Plants were infested with aqueous conidial suspensions, placed in polyethylene bags for 48 hours, removed from the bags, held for 8 more days, and then rated. The plants were held at 28 C in the day and at 15 C at night. This treatment resulted in leaf scorching in the non-infested controls. Red fescue was rated as moderately resistant and annual bluegrass and Colonial bentgrass were susceptible to colonization by C. trifolii. In a second experiment, the isolate most aggressive to annual bluegrass (which was the most susceptible host) was tested for pathogenicity at 15, 20, 25, 30, and 35 C. At 15 and 20 C, symptoms were mild but at 30 and 35 C, leaves were killed and many lesions were reported to occur on the surviving leaf sheaths and blades. The fungus was again reisolated from the symptomatic tissues.

A number of references are made to Curvularia species as pathogens of turfgrasses in various monographs. These books are are important in that they have greatly influenced

the thinking of clinicians and practitioners of turfgrass management. Pirone, Dodge, and Rickett (1960), listed "Fading-Out" as a disease incited by "Curvularia lunata and other species of Curvularia." The description closely follows that of Howard et al. (1951). "Susceptible grasses include bent, blue, fescue, Bermuda, zoysia, centipede, carpet, and bahia" according to Pirone et al. and a positive diagnosis was considered to consist of finding Curvularia spores on grass blades.

Couch (1973) reported being "repeatedly plagued by an inability to produce leaf lesions with various Curvularia isolates when these were tested as primary parasites." He concluded that in terms of pathogenicity to turfgrasses, Curvularia spp. "probably deserve little more credit than the numerous other species of weakly pathogenic fungi that commonly colonize diseased turfgrass." Couch is the only book author who offers any research results of his own.

Vargas (1981) lists fading-out or Helminthosporium-Curvularia complex as a disease incited by Curvularia lunata, C. geniculata, C. intermedia, and C. protuberata. The hosts given are annual bluegrass, Kentucky bluegrass, creeping bentgrass, fine-leaf fescue, Poa trivialis, and Canada bluegrass. Vargas acknowledges opinions from both sides of the controversy as to whether Curvularia species

actively parasitize turfgrasses. He concludes as follows: "While the debate will probably go on forever, the evidence put forth by Brown et al. and Hodges and Madsen leaves little doubt that under proper conditions Curvularia species, either alone or in conjunction with H. sorokinianum, can be important turfgrass pathogens." Smiley (1983) lists eight incitants for "Curvularia Diseases" in his Compendium of Turfgrass Diseases. Muchovej and Couch, (1985) in reviewing the list, point out that of the eight, three (C. eragrostidis, C. penniseti, and C. senegalensis) have never been isolated from turfgrasses. However, Smiley states that these fungi are primary pathogens inciting symptoms including leaf discoloration leading to an indefinate yellow and green dappled pattern, leaf tip dieback, and leaf lesions (lesions occuring on Poa and Festuca spp. but not on Agrostis spp.), crown and leaf sheath dark brown dry rots, and an overall thin and ragged-looking appearance of turf areas in patchs or streaks which may coalesce to affect larger areas. Smiley adds no new turfgrass hosts.

Shurtleff et al. (1987) state that "Curvularia blight or fading out, caused by five or more species of Curvularia is similar to diseases caused by species of Bipolaris." and that "Curvularia fungi can infect all turfgrasses". These statements are tempered with the observation that most damage

occurs during hot weather when plants are stressed or in an advanced state of senescence.

Beginning in 1972, Hodges published the results of a series of carefully designed and executed experiments. At first, he concluded that C. geniculata was a saprophyte and a secondary invader of diseased turfgrass plants because he found no clear demonstration of pathogenicity in the initial experiments (Hodges, 1972). These experiments utilized standard inoculating procedures on two hosts: A. palustris and P. pratensis. Chlorosis at the cut leaf tips was observed but no blighting or leaf lesions. That conclusion, however, was modified after a second series of experiments (Hodges and Madsen, 1978). In that paper, a disease complex was described in which D. sorokiniana in combination with C. geniculata produced more severe symptoms on P. pratensis together than when either fungus was used as inoculum alone. The increase in disease severity occurred only at 30 C and not at 20, 25, or 35 C. At 30 C it was found that C. geniculata alone could infect leaves but did not cause appreciable symptom development. However, when lesions produced by D. sorokiniana were present, C. geniculata would actively colonize the lesion sites. In conclusion, Hodges and Madsen stated, "it is believed that the controversy surrounding the pathogenic capabilities of C. geniculata should be laid to rest by simply classifying this species as a weak,

high-temperature, primary-leaf pathogen of P. pratensis and possibly other species of Gramineae."

In a final series of experiments (Hodges and Madsen, 1979) reevaluated the results of the earlier experiments by including leaf senescence as a factor in the disease complex. It was found that the synergism described before existed only on the older leaves of the plants. Therefore, the conclusion about the pathogenic potential of C. geniculata to turf was again modified. The final conclusion was that the high temperature enhanced the senescence of older leaves and therefore, the growth of C. geniculata was principly saprophytic and the fungus should be considered "at most an extremely weak primary leaf pathogen."

Madsen and Hodges (1980) published a paper in which the effect of C. geniculata and D. sorokiniana on germinating F. rubra was evaluated. It was found that C. geniculata reduced the rate of seedling emergence but not to the same extent as D. sorokiniana.

Muchovej and Couch (1987, also Muchovej 1984, 1986) carefully considered the physiological age of individual leaves and regions of leaves when studying the infection and colonization of A. palustris by C. lunata. They related their histological observations to the physiological age of

the tissue as measured by a "leaf vigour curve" which was based on chlorophyll a content of the leaves. Using plants that were heat stressed and treated with trichloroacetic acid (to reduce formation of epicuticular waxes), they found that C. lunata was unable to ingress juvenile or mature leaves. With respect to senescing leaves, no ingress was found except in the case of those tissues in a state of "advanced senescence" i. e. necrotic tissue. The inoculated plants in these experiments exhibited symptoms of Curvularia blight even though no mycelium could be demonstated within the plant tissues. In the course of the experiments, it was found that heat stressing leaves caused them to enter into a senescent state sooner than they would have if not stressed. The symptoms they observed suggested to them that the results of earlier investigators may have been due not to infection and colonization of juvenile or mature leaf tissues, but rather due to the rapid discoloration (yellowing) of abundant senescent tissue which had been produced by heat stress. In contrast to uninoculated plants, discoloration of senescent tissue occurred much more rapidly in inoculated plants. Histological examinations combined with special staining techniques showed that C. lunata was unable to breach the cutin of leaves of any physiological age group. This was true when the experimental plants had entirely intact leaves.

In another series of experiments, similar treatments were administered to plants which had their leaf tips cut to simulate mowing injury. In this case, it was found that only leaves which were senescing as opposed to those which were juvenile or mature were susceptible to the ingress of C. lunata mycelium. The leaf tip chlorosis which occurred with non-clipped plants was more striking with clipped plants, occasionally progressing the length of the leaf. An accepted pathogen, Drechslera sorokiniana, was also included in the tests and was inoculated onto creeping bentgrass plants as part of these experiments. In this case, lesions were formed on tissues of all age groups and histological tests showed the formation of appressoria and entry of the fungus into the leaf tissues. They concluded that "C. lunata lacks the ability to infect intact surfaces of juvenile, mature, or senescent leaves." (Muchovej, 1986 p. 77) It was postulated that the uninfected senescent leaves which showed symptoms of Curvularia blight were perhaps detrimentally affected by metabolic byproducts of C. lunata which were able to leach into the plants because of the reduced (or absent) epicuticular wax layer resulting from the trichloroacetic acid treatment. On clipped grass, it was concluded that C. lunata is capable of infecting and colonizing senescing tissue with the resultant symptom of leaf tip chlorosis.

B. Literature Relating to Direct Effects of Herbicides on Fungal Pathogens of Turfgrass *In Vitro*

An extensive amount of literature exists on the effects of herbicides on plant pathogenic fungi *in vitro*. Herbicides can be fungitoxic, inhibitory to mycelial growth, have no effect on mycelial growth, stimulatory to mycelial growth, or affect other aspects of fungal physiology. Physiological effects often vary with concentration. For example, 2,4-D may stimulate or inhibit mycelial growth depending on concentration. While no work has been done with *C. lunata*, itself, literature pertaining specifically to effects of herbicides labeled for use on turfgrasses on the biology of other turfgrass pathogenic fungi is reviewed below.

Karr, Gudauskas, and Dickens (1977, 1979) measured effects of three herbicides on the radial growth of four turfgrass pathogens at three temperatures. The herbicides were placed in agar to produce concentrations of the recommended field use rate, twice the recommended rate, and ten times the recommended rate. Each experiment was run at 18, 26, and 35 C. The herbicides used were benefin, bensulide, and Nortron® (2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulfonate, this herbicide has not yet

been assigned a common name). The fungi tested were Sclerotinia homoeocarpa, Rhizoctonia solani, Drechslera cynodontis, and Pythium aphanidermatum. All concentrations of all three herbicides caused reductions in radial growth of R. solani at 18 and 26 C. These reductions of radial mycelial growth were concentration dependent; increasing concentration resulted in decreased growth. At 35 C, the two highest concentrations of benefin and bensulide inhibited radial growth of R. solani while the lowest concentration of benefin had no effect and the lowest concentration of bensulide stimulated growth. The two lowest rates of Nortron increased growth rate and the highest concentration had no effect on the fungus at 35 C. All three herbicides at all temperatures reduced the growth of S. homoeocarpa except for the two lowest rates of bensulide at 35 C which had no effect. Again, growth was further decreased with increasing herbicide concentrations. Radial growth of D. cynodontis was reduced by all the herbicides at every temperature. In addition, it was observed that the herbicides delayed the formation of conidia by this fungus by about 24 hours at 26 C as compared to non-herbicide treated controls. Increasing herbicide concentration again resulted in reduced growth rates. Radial growth of Pythium aphanidermatum was reduced by bensulide and Nortron at all concentrations at all temperatures. Benefin reduced the growth of P. aphanidermatum except at the two

lowest concentrations at 18 and 35 C where growth was unaffected.

Hodges (1977) observed several growth parameters of Drechslera sorokiniana as influenced by postemergence herbicides. 2,4-D, 2,4,5-T, 2,4,5-TP, dicamba, and MCPP were evaluated at molar rates from 10^{-12} to 10^{-3}. All of these herbicides prevented conidial germination at 10^{-3} M due to plasmolysis of the conidial cells. At 10^{-4} M and lower concentrations the herbicides did not affect germination. In observing the morphology of early germ tube growth, only 2,4,5-T caused anomolous growth patterns and only at the highest concentration tested (10^{-3} M). 2,4,5-T also caused a reduced production of conidia at all concentrations as did MCPP. 2,4-D treatment decreased sporulation at 10^{-3} M but increased sporulation at concentrations of 10^{-4} M to 10^{-8} M and concentrations below that did not produce an effect. 2,4,5-TP treatment had no effect on conidial production at 10^{-12} M and 10^{-11} M; increased conidial production at 10^{-10} M, 10^{-9} M and 10^{-8} M; and decreased conidiation at 10^{-6} M, 10^{-5} M and 10^{-4} M. No effects on conial production were detected when dicamba was tested at concentrations below 10^{-8} M. Concentrations of 10^{-8} M, 10^{-7} M and 10^{-6} M of dicamba increased sporulation while concentrations of 10^{-5} M, 10^{-4} M, and 10^{-3} M decreased spore production. The final growth parameter observed was mycelial growth. 2,4-D did not effect mycelial

growth rate at concentrations from 10^{-12}M to 10^{-7} M. Concentrations from 10^{-6}M to 10^{-3} M of 2,4-D all increased the growth rate of the fungus. No effect on mycelial growth rate was detected with 2,4,5-T used at 10^{-12}M or in the range of 10^{-7} M to 10^{-4} M. A concentration of 10^{-3} M of 2,4,5-T resulted in reduced growth of mycelium and concentrations from 10^{-11} M to 10^{-8} M increased mycelial growth. A similar pattern existed with 2,4,5-TP; no effect on mycelial growth rate was detected at 10^{-12}M or in the range of 10^{-9} M to 10^{-5}M. A concentration of 10^{-4} M of 2,4,5-TP resulted in reduced growth of mycelium and concentrations of 10^{-11} M and 10^{-10} M increased mycelial growth. Dicamba produced increases in mycelial growth rate at the following concentrations: 10^{-12}M, 10^{-11} M, 10^{-10}M, 10^{-9} M, 10^{-8}M, 10^{-6}M, and 10^{-5}M. No effect from dicamba was detected at 10^{-7} M, 10^{-4} M, and 10^{-3} M. With MCPP, all concentrations increased mycelial growth except 10^{-7} M which had no effect and 10^{-3} M which decreased growth.

Hodges performed a similar set of experiments (1981A), this time using four postemergence herbicides. The herbicides benefin, bensulide, DCPA, and siduron were tested at concentrations of 10^{-12}M, 10^{-9} M and 10^{-6} M on the same growth parameters as the last experiment, again using D. sorokiniana. A very low occurrence (less than 3% in every case) of abnormal early germ tube morphology was observed with the high concentrations of bensulide, DCPA, and siduron.

No effect on germ tube morphology was detected with benefin or with the lower rates of the other herbicides. Conidial production was inhibited with all concentrations of benefin and bensulide. Conidial production was increased with all concentrations of siduron. DCPA reduced conidial production at 10^{-9} M and 10^{-6} M and had no effect at 10^{-12} M. Percent conidial germination was reduced with all herbicides at all concentrations. Mycelial growth rate was increased by all concentrations of benefin. It was decreased by all rates of siduron. Bensulide decreased mycelial growth rate at 10^{-6} M and had no effect at the lower two concentrations. DCPA increased growth at 10^{-12} M, had no effect at 10^{-9} M and decreased mycelial growth at 10^{-6} M.

C. Literature Relating to Ancillary Effects of Herbicides on Turfgrass Diseases

The effects of herbicides on non-target crop plants has been a topic of intense research effort. The possibility exists that, in addition to controlling weeds, herbicides may produce deleterious effects on the crop itself. One of these effects is increasing the susceptibility of the crop plants to infection and colonization by pathogens.

The particular research area of side effects of herbicide use on plant diseases is also currently an active one. Herbicides may increase, decrease, or have little effect on disease incidence and severity (Altman and Campbell, 1977). The effects on disease can be attributed to different components of the matrix of disease causality. For instance, among the biotic components of a disease matrix, effects can be noted on the host, the pathogen, and the natural microflora of the host's milieu, any of which alone or in combination could account for stimulation or inhibition of the disease process. A review of the literature concerning the effects of herbicides on turfgrass diseases follows.

For turfgrass hosts in particular, the research which has been conducted on effects of herbicides on disease comprises a rather small body of work, and a portion of that is anecdotal in nature. Turgeon et al. 1974) reported a striking increase in stripe smut (incited by Ustilago striiformis) in an experiment designed to evaluate effects of seven preemergence herbicides on turfgrass quality, clipping yield, root growth, thatch accumulation, and carbohydrate reserves of 'Pennlawn' red fescue, 'Kenblue' Kentucky bluegrass and 'Merion' Kentucky bluegrass. Although no attempt was made to quantify the disease severity, the authors noted that bandane increased the severity of stripe smut

while the other treatments (bensulide, calcium arsenate, benefin, DCPA, turbutol, and siduron) did not.

Karr et al. (1977) conducted a laboratory-based experiment to determine effects of benefin, bensulide, and Nortron on dollar spot, Rhizoctonia blight, and Pythium blight (incited by Sclerotinia homoeocarpa, Rhizoctonia solani, and Pythium aphanidermatum, respectively) on 'Tifdwarf' bermudagrass. Although no statistical treatment of the data was presented, the following results were reported: "Both the field rate and the 3X rate of Nortron® reduced severity of brown patch, and the higher rate also reduced dollar spot and pythium blight. Bensulide had no effect on any disease; however, the severity of brown patch and dollar spot were increased in grass treated with the field rate of benefin."

A similar set of experiments (Karr et al. 1979) using the same diseases and herbicides was later conducted again on bermudagrass, but this time with an additional Pythium blight experiment on annual ryegrass. In this set of experiments, treatments were replicated and statistical analysis was performed. Nortron® again reduced severity of Rhizoctonia blight. Bensulide did not affect the severity of Rhizoctonia blight. The lower rate of benefin increased Rhizoctonia blight severity while the high rate (3X) had no effect. Dollar spot was increased by the 1X rate of benefin

and decreased by the 3X rate of Nortron® while other treatments had no statistically significant effect. On ryegrass, no statistically significant differences occurred among the treatments in the Pythium blight experiment. On bermudagrass however, Nortron® at the 3X rate reduced the Pythium blight severity, while other treatments had no statistically significant effect.

Callahan (1972) reported results from a field experiment where seven herbicides were used for three years at three different rates each spring on 'Penncross' creeping bentgrass turf maintained at putting green height. The precise cause of the loss of turf in this experiment was not determined but it was attributed to a combination of the effects of Pythium blight and phytotoxicity of the herbicides. The concensus was that the herbicides predisposed the bentgrass to greatly increased sensitivity to Pythium blight. Treatments with DCPA, bensulide, benefin, bandane, and turbutol all increased loss of turf compared to non-herbicide treated control plots and tri-calcium arsenate and siduron had no effect. Reduction of root growth was observed to occur in response to many of the herbicide treatments.

Hodges (1978, 1979) determined effects of five postemergent herbicides on the severity of Helminthosporium leaf spot (incited by Drechslera sorokiniana) of 'Newport'

Kentucky bluegrass. The herbicides used were: 2,4-D, 2,4,5-T, 2,4,5-TP, MCPP, and dicamba. These herbicides were applied to the bluegrass by three different methods, (as a soil drench, as a foliar spray, and as droplets containing conidia of the fungus) at these four concentrations: 10^{-12} M, 10^{-9} M, 10^{-6} M and 10^{-3} M (10^{-4} M for 2,4,5-TP which is insoluble at 10^{-3} M). An increase in leaf spot severity occured in each case when droplets of the highest concentration of each herbicide solution containing conidia of the fungus were applied to the leaves. At the three lowest concentrations of 2,4-D, 2,4,5-T, and 2,4,5-TP, a decrease in leaf spots or no statistically significant effect on leaf spots was observed. MCPP and Dicamba caused an increase in leaf spot severity at the three lowest concentrations.

When applied as a soil drench, every herbicide at each of the concentrations caused an increase in leaf spot severity except 2,4,5-TP at its highest two concentrations, which produced a decrease in leaf spot severity. When plants previously treated with foliar sprays of MCPP, 2,4,5-T, and dicamba were inoculated with D. sorokiniana conidia, an increase in leaf spot severity occured at all herbicide concentrations. 2,4-D treatment also increased leaf spot severity when used at its lowest concentration, but at the three highest concentrations produced no statistically significant effect. 2,4,5-TP treatment increased leaf spot se-

verity at the lowest concentration but decreased disease expression at the highest three concentrations.

Hodges (1980) published the results of a set of experiments similar to those just described except that this time, he took into account the age of the leaves which were being parasitized. This was considered because the chlorotic nature of the lesions found on the bluegrass treated with herbicides was similar to that seen on senescing tissue. In addition, chlorophenoxy herbicides can induce ethylene production in plants and auxin-like herbicides are known to induce leaf senescence in some species. The plants were treated with herbicides either by soil drenching or by foliar spraying. All herbicides were used at 10^{-6} M.

In the control (non-herbicide treated) plants of this experiment, a direct relationship between severity of leaf spots and the age of the inoculated leaves was found. Specifically, the two youngest leaves had identical symptom expression but leaf spotting severity increased from the second to the third to the fourth (the oldest) leaf. None of the herbicides applied in either manner increased the disease expression of the youngest leaf. Both soil and foliar herbicide application always led to increases in symptom expression on older leaves over leaves of the same age on non treated controls, except in the case of 2,4,5-TP. 2,4,5-TP

treatment either decreased symptom expression with respect to controls of the same age or no differences were detected for both spray and soil applications. (This agrees with the results of the previous experiment (Hodges 1979), at lower concentrations however, increases in disease expression occurred.) The trend toward increased symptom severity with increasing age seen in the controls was not always apparent in herbicide treated plants. For instance, 2,4-D increased lesion development on only the oldest leaf of spray treated plants (with no increases from the first to the third leaf) but increases were seen on all three oldest leaves of plants in treated soil. This situation was reversed when dicamba was used, i.e. only the oldest leaf showed increased symptom development when dicamba was used as a soil drench and leaves 2, 3, and 4 exhibited increases when foliar application was used. With 2,4,5-T, MCPP, and dicamba, however, the increase in the severity of symptoms on successively older leaves (as in the controls) was present (including an increase from the youngest leaf to leaf two).

Differences in leaf lesion development on progressively older leaves of herbicide treated plants were qualitative as well as quantitative. The two oldest leaves of herbicide treated plants commonly exhibited lesions with enlarged necrotic areas, extending as far as the leaf margins, with large chlorotic halos and/or chlorotic streaking along the

midvein and connecting lesions. In the most severe cases, the enlarged necrotic areas were accompanied by very severe straw colored chlorosis of the entire leaf. These kinds of symptoms rarely occured on leaf two of herbicide treated plants and never on leaf one. They also were never apparent on any of the leaves of non herbicide treated controls.

In herbicide treated non-infected plants, this kind of chlorosis never occurred. Hodges hypothesized that the auxin-like action of the herbicides promoted senescence of the older leaves which (possibly aided by the action of the phytotoxin helminthosporal) led to the increases in symptom expression.

Hodges (1981B) next initiated an experiment using four different preemergence herbicides not previously tested. Benefin, bensulide, dacthal, and siduron were evaluated for their effects on Helminthosporium leaf spot of 'Newport' Kentucky bluegrass. Each herbicide was evaluated at 10^{-12} M, 10^{-9} M and 10^{-6} M and were applied as soil drenches, or as droplets of herbicide solution containing conidia of D. sorokiniana When these herbicides were applied as droplets containing conidia, leaf spot development was inhibited as a general rule with the two exceptions of bensulide at 10^{-12} M and dacthal at 10^{-6} M where no effect was detected. Responses were herbicide specific when the herbicides were applied as

soil drenches. Leaf spot development was inhibited by benefin at all concentrations and was stimulated by all concentrations of bensulide. Dacthal inhibited leaf spot at 10^{-12} M, had no effect at 10^{-9} M, and stimulated disease expression at 10^{-6} M. Siduron did not effect leaf spot development at 10^{-9} M and 10^{-6} M but was stimulatory at 10^{-12} M.

In series of papers, Madsen and Hodges (1983A, 1983B, 1984) published the results of experiments in which relationships between soil applications of two chlorophenoxy herbicides, the levels of simple sugars and free amino acids in leaf tissues, the age of leaves, and pathogenesis by B. sorokiniana were examined in 'Newport' Kentucky bluegrass. Soil applications of both MCPP and 2,4,5-TP reduced the levels of total soluble sugar in leaves of all ages in test plants. The reduction of total sugar was attributable to drops in sucrose, glucose, and fructose but not raffinose.

The "low sugar" condition has been suggested as a factor which increases the disease; however, there was no difference between sugar levels of the leaves of various ages so it appears levels of sugar were not related to the increase of disease with increasing leaf age. Levels of free amino acids on the other hand decreased with increasing leaf age. However, it was not possible to relate amino acid levels directly to leaf spot development or herbicide usage. In

general, the authors concluded that the interactions that occur with respect to disease in senescing tissues are complex and could not be related to a single factor such as soluble sugars or free amino acid content.

RESEARCH OBJECTIVES

I. To Determine Effects of Five Herbicides on:
   A. The germination of conidia of *Curvularia lunata*
   B. The growth and morphology of the germ tube of *Curvularia lunata*
   C. The radial mycelial growth rate of *Curvularia lunata*
   D. The production of conidia by *Curvularia lunata*

II. To Determine Effects of High Temperature Stress on "Penneagle" Creeping Bentgrass

III. To Determine Effects of Five Herbicides on "Penneagle" Creeping Bentgrass

IV. To Evaluate the Pathogenicity of *Curvularia lunata* to "Penneagle" Creeping Bentgrass

V. To Determine the Effects of Five Herbicides on the Sensitivity of "Penneagle" Creeping Bentgrass to High Temperature Stress

VI. To Determine the Effects of High Temperature Stress on the Predisposition of "Penneagle" Bentgrass to Colonization by Curvularia lunata

VII. To Determine the Effects of Five Herbicides on the Predisposition of "Penneagle" Creeping Bentgrass to Colonization by Curvularia lunata

MATERIALS AND METHODS

To accomplish the objectives, four series of experiments were performed. The procedures are described in four sections accordingly.

I. Determination of Direct Effects of Five Herbicides on Several Aspects of the Growth of *Curvularia lunata*

These ten experiments addressed research objective I.

The fungus isolate used was obtained by plating leaves from 'Penneagle' creeping bentgrass from a golf green at the Virginia Tech Turfgrass Research Center in Blacksburg, Virginia. The isolate was identified as *Curvularia lunata* (Wakker)Boedijn [*Cochliobolus lunatus* Nelson & Haasis] using the illustrated keys of Ellis (1971 and 1976) and Groves and Skolko (1945). This was the same isolate used by Muchovej (1984 and 1986) and Muchovej and Couch (1987). This isolate had been stored lyophilized on wooden sticks in ampules. An ampule was opened to provide fresh inoculum at the begining of each experiment.

The culture medium used was Difco® potato dextrose agar (PDA) (Difco Laboratories, Detroit, Michigan) prepared ac-

cording to the manufacturer's recommendations. Within any one experiment, the same batch of PDA was used. Uniform layering of medium on each plate was obtained by using an autoclaved syringe and an autoclaved filling outfit (Becton, Dickenson and Company, Rutherford, N. J.) to dispense 35 ml of PDA in each Petri dish.

Emulsions of five herbicide formulated products were prepared aseptically in autoclaved distilled water. The individual concentrations of the emulsions were prepared so that amounts of herbicides delivered to the PDA plates could be directly compared to rates recommended for field use. A logarithmic dilution series was prepared aseptically to include these concentrations: 3.16X, 1.00X, 0.316X, 0.100X, 0.0316X, and a control, 0.0X. The 1X concentration contained the recommended field-use rate for weed control on turfgrass and other concentrations are expressed relative to this standard. A one ml aliquot of solution delivered to the agar surface in each plate resulted in the listed fraction of the field rate adjusted for an area the size of a standard 100 X 15 mm disposable Petri plate. Uniform distribution of the solution over the surface of the plate was insured by using a turntable and a sterile glass "hockey stick" to evenly spread the solution. The solutions were allowed to remain on the plates for one to three days before the experiments began in order to allow them to disperse into the agar to the

point that they no longer ran freely over the surface of the plate. Table 1 lists the specific herbicides used in these and all of the following experiments. Table 2 lists the 1X rates of the herbicides used in the *in vitro* studies. Other rates can be calculated by multiplying the factor of the rate in question by the 1X concentration.

Two sets of *in vitro* growth experiments were conducted. One set allowed for the characterization of the radial mycelial growth of *C. lunata* at the five herbicide concentrations and a check which was unexposed to herbicide. These experiments were performed for each of the five herbicides. These radial growth experiments utilized twenty replicates (plates) for each herbicide concentration and twenty for the control. The agar surface was infested by placing a 7 mm diameter plug from a PDA plate on which *C. lunata* was growing, at the center of plate. The plug used for infesting the plates was cut using a flamed cork borer and all plugs were from mycelial mats of 7 to 10 days age. The plates were incubated at 25 C after infestation.

Measurements of radial growth were made with a ruler by measuring to the nearest mm, three diameters (60 degrees apart) of each of the colonies. The 7 mm for the plug was subtracted from the diameters and the resulting figure was divided by 2 to obtain the radius. The mean of the three

Common Name	Chemical Name	Trade Name (Manufacturer)
MSMA	Monosodium Acid Methanearsonate	Daconate 6® (Fermenta)
bensulide	S-(O,O-Diisopropyl phosphorodithioate) ester of N-(2mercaptoethyl) benzenesulfonamide	Betasan 4-E® (Stauffer)
dicamba	Dimethylamine salt of 3, 6-dichloro-o-anisic acid	Banvel 4-S® (Velsicol)
2,4-D	Alkanolamine Salts of 2,4-Dichlorophenoxyacetic acid	Formula 40® (Dow)
mecoprop (MCPP)	Dimethylamine salt of 2-(2-methyl-4-Chlorophenoxy) Propionic Acid	MCPP-D-4® (PBI/Gordon)

Table 1. Names and Manufacturers of the Five Herbicides

Herbicide Common Name	A. I. per 1000 sq ft	F. P. per 1000 sq ft	A. I. per plate	F. P. per plate
MSMA	21.26 g	29.57 ml	1.218 mg	1.694 ul
bensulide	104.13 g	217.22 ml	5.966 mg	12.44 ul
dicamba	0.87 g	7.242 ml	0.050 mg	0.415 ul
2,4-D	13.02 g	27.16 ml	0.746 mg	1.556 ul
mecoprop	13.02 g	27.16 ml	0.746 mg	1.556 ul

Table 2. Active Ingredient and Formulated Product Rates for the 1X Herbicide Concentrations in the In Vitro Growth Experiments

measurements was recorded as the radial growth measurement for that replicate. Measurements were obtained daily for four days. On the fifth day, the colonies in the controls had nearly covered the agar surface in the plates so further measurements were not made.

The other set of *in vitro* growth experiments were designed to allow for observations of the effects of the herbicides on the germination of conidia, germ tube growth and morphology, and the production of conidia of *C. lunata*. In these experiments, the medium was infested by placing one ml of a suspension of *C. lunata* conidia in autoclaved distilled water in each plate. The suspension was prepared by dilution to contain between 100 and 200 conidia per ml. Conidial counts were made using a haemocytometer.

The conidial suspension was dispersed evenly across the surface of the agar by gently tilting and shaking the plates. After this, the plates were incubated at 25 C. Five replicates (individual infested plates) were made for each of the 5 herbicide concentrations as well as for the controls. These experiments were conducted for each of the 5 herbicides. Observations were made of these plates daily for five days after infestation using a stereoscopic dissecting microscope. Observations of conidial germination consisted of determining whether germination took place at all (i.e.

if a germ tube began to form as a bulge on the conidium), whether germination was as extensive as in the controls, and whether germination took place in the same time period as the controls. Observations on germ tube growth and morphology consisted of comparing, with the aid of a microscope, early growth of colonies exposed to the various concentrations of the five herbicides with the unexposed controls. Observations of the production of conidia consisted of determining if conidia were formed at all and if they were formed within the same time period as the controls.

II. Acute High Temperature Stress as a Predisposing Factor in the Susceptibility of 'Penneagle' Creeping Bentgrass to *Curvularia lunata*

This experiment addressed research objectives II, IV, and VI. It was designed to determine effects of pre-inoculation and post-inoculation acute high temperature stress of the suscept ('Penneagle' creeping bentgrass) on altering susceptibilty to infection and colonization by *C. lunata*.

Bentgrass plants were grown from seed in the greenhouse. At eight weeks from the seeding date, the plants had reached the four leaf stage and were then used in the

experiment. The potting medium used was Weblite® (Webster Brick Co., Roanoke, Va.), a heat-expanded shale by-product of brick manufacturing which provides good aeration and drainage and contributes negligibly to the nutrition of the plants. Seeds were sown on Weblite® in 185 ml styrofoam cups (surface area = 34 square cm) which had been punched to allow drainage through the bottoms. The seeds were covered with a very thin layer of Weblite® and the cups were placed in plastic lined flats containing tapwater so that the medium remained moist until the seeds germinated and the roots stabilized the plants in the medium. The seeds used were obtained from a single order of seeds from Wetsel Seed Company (Harrisonburg, Va) and were of the variety 'Penneagle' of creeping bentgrass (Agrostis palustris Huds.). The plants were checked daily and watered as needed. The plants received Peter's 20-20-20 soluble fertilizer once a week at a rate of 273 ppm nitrogen. This nutrition regime results in a medium green color and a moderately fast growth rate. The bentgrass was cut with scissors to a height between one half and three quarters of an inch weekly. A single layer of cheesecloth was suspended four feet above the greenhouse bench to reduce the light and heat absorbed by the plants.

Eight treatment groups were established to evaluate three factors: 1) pre-inoculation acute high temperature stress, 2) post-inoculation acute high temperature stress,

and 3) inoculation with Curvularia lunata. Treatments included all possible combinations of the three factors and are listed in Table 3. The pre-inoculation and post-inoculation high temperature stress treatments consisted of 18 hours of exposure to 38 C (100 F) in a dew chamber occurring immediately prior to or immediately after inoculation with an aqueous (autoclaved distilled water) suspension of C. lunata conidia containing approximately 2500 spores per ml. The foliage of the plants in each container were sprayed with 2 ml of the aqueous spore suspension using a paint sprayer.

All treatments were clipped just before the pre-inoculation high temperature stress treatment. Those plants that were not receiving a high temperature stress treatment were maintained at 24 C (75 F) in a second dew chamber during the same eighteen hour exposure period. The fungus isolate used was the same as that in the in vitro studies described above. The plants were eight weeks from seeding (fifty-two days from emergence) at the time of pre-inoculation temperature treatments. The plants had four expanded leaves at that age. Twelve replicates of each treatment were used. Visual ratings were made immediately after the 18 hour post-inoculation temperature treatments. A rating scale of 0 to 10 was used where 0 is unaffected foliage and 10 is complete necrosis of all foliar tissue.

treatment	pre-inoc temperature	post-inoc temperature	Curvularia inoculum used
1	38 C	38 C	yes
2	38 C	38 C	no
3	38 C	24 C	yes
4	38 C	24 C	no
5	24 C	38 C	yes
6	24 C	38 C	no
7	24 C	24 C	yes
8	24 C	24 C	no

Table 3. Treatments Used in the Pre-inoculation and Post-inoculation Acute High Temperature Stress Experiment

III. Determination of Effects of Five Herbicides and Acute High Temperature Stress on 'Penneagle' Creeping Bentgrass

These five experiments addressed research objectives II, III and V.

The methods and conditions for growth of the bentgrass in these experiments were identical to those in the acute high temperature stress experiment described above. Two temperature regimes and 2 rates of a herbicide as well as non-herbicide treated controls were used in each of 5 experiments, one for each of the 5 herbicides. A high temperature (40 C = 104 F) and a moderate temperature (21 C = 70 F) were used. The herbicides were used at moderate rates (typically the recommended field rate) and higher rates of about 2.5X the moderate rate. The precise rates of the herbicides used in these experiments are given in Table 4 along with the associated fraction of the recommended field use rate. In addition, plants not exposed to a herbicide were included as controls in each experiment. The six treatments which were used in each of the five experiments are summarized in Table 5.

The herbicides were diluted with tap water and applied using a household plant sprayer to bentgrass plants which were 52 days from emergence. The plants had four expanded leaves at that age. Exposure to the temperature regimes began 10 days after the application of the herbicides. The time of exposure to the temperatures was 24 hours. The temperatures were obtained by placing the plants in dew chambers. Each of the five experiments used 90 containers of bentgrass, 15 containers (replicates) per treatment (the MCPP experiment had 13 replicates). The containers were randomly placed in the dew chamber during the exposure to the temperature regimes. Visual ratings were made immediately after the plants were removed from the dew chambers. A rating scale of 0 to 10 was used where 0 is unaffected foliage and 10 is complete necrosis of all foliar tissue.

IV. Herbicide Induced Stress and Chronic High Temperature Stress as a Predisposing Factors in the Susceptibility of 'Penneagle' Creeping Bentgrass to *Curvularia lunata*

These ten experiments addressed research objectives II through VII.

Herbicide Common Name	Moderate Rate F. P. per 1000 sq ft	High Rate F. P. per 1000 sq ft
MSMA	29.57 ml (1.0X)	59.14 ml (2.0X)
bensulide	217.23 ml (1.0X)	543.1 ml (2.5X)
dicamba	7.242 ml (1.0X)	18.10 ml (2.5X)
2,4-D	10.86 ml (0.5X)	27.15 ml (1.25X)
mecoprop	21.72 ml (0.8X)	54.31 ml (2.0X)

Table 4. Herbicide Rates Used in the Acute High Temperature Stress with Herbicide Stress Experiments

Treatment	Herbicide Rate	Temperature
1	herbicide not used	21 C
2	moderate	21 C
3	high	21 C
4	herbicide not used	40 C
5	moderate	40 C
6	high	40 C

Table 5. Treatments Used in Acute High Temperature Stress and Herbicide Experiments

The methods and conditions for growth of the bentgrass and the fungus isolate used in these experiments were identical to those in the acute high temerature stress experiment described above. The herbicides were used at rates similar to those used on bentgrass for weed control. The precise rates are given in Table 6. A separate experiment with each of the 5 herbicides was performed which included the following 4 treatments: 1) a control (untreated grass), 2) grass treated with the herbicide but not inoculated with C. lunata, 3) grass not treated with herbicide but inoculated, and 4) grass treated with the herbicide and inoculated. The procedure for preparing and applying the inoculum was identical to that for the acute high temperature stress experiment described above.

These experiments were performed twice, once using 25 C as the post-inoculation temperature and once using 30 C (chronic high temperature stress) as the post-inoculation temperature. The plants were held at the post-inoculation temperature for 72 hours in a dew chamber. Each treatment was replicated 25 times (except for the 2,4-D experiment which used 23 replicates) using plants which had been clipped and 5 to 8 replicates which had never been cut. The herbicides were applied to plants which were fifty-two days from emergence. Inoculation took place 10 days after the application of the herbicides. The plants were visually

rated immediately after the 72 hour post-inoculation exposure on a 0 to 10 scale where 0 is no effect and 10 is complete necrosis of all foliar tissue.

Herbicide Common Name	Rate of Herbicide F. P. Per 1000 square feet ml	fl. oz.	Percent of Recommended Field Rate
MSMA	29.57	1.00	100
bensulide	217.23	7.35	100
dicamba	7.242	0.24	100
2,4-D	10.86	0.37	50
mecoprop	21.72	0.73	80

Table 6. Herbicide Rates Used to Predispose Bentgrass to Infection and Colonization by C. lunata

RESULTS

The results are reviewed in sections relating to each set of experiments.

I. Effects of Five Herbicides on the Growth of *Curvularia lunata*, *In Vitro*

The results of the experiments relating to effects of the five herbicides on the radial growth rate of *C. lunata* are given in two locations. The data from the fourth day of the *in vitro* radial growth experiment for each herbicide is presented in this section. With each herbicide, this data was subjected to ANOVA and the mean for each of the concentrations was compared with the other concentrations and the control using Duncan's multiple range test (Tables 7 through 11) (Li 1964, SAS Institute Inc. 1985). The probability level used for these procedures was 95 percent ($\alpha = 0.05$). The data from the first three days of each experiment was treated with the same statistical procedures and are found in the appendix (Tables 30 through 44). In addition, photographs of the effects of bensulide and MSMA on radial growth are presented in Figures 1 and 2 respectively. The compar-

isons of the mean radial growths at the various concentrations on the fourth day for each herbicide may be found as follows:

bensulide. . . Table 7

dicamba. . . . Table 8

MCPP . . . . . Table 9

MSMA . . . . . Table 10

2,4-D. . . . . Table 11

The results of the experiments relating to the other aspects of the growth of C. lunata (specifically conidial germination, conidial production, and germ tube and colony morphology) as influenced by the 5 herbicides are presented in this section.

In aspects other than size (i.e. color, shape, configuration of the colony's edge, and texture), gross colony morphology, as observed with the naked eye, was indistiguishable among the treatments of all herbicide experiments.

Conidia were produced in all treatments of all five herbicides. Conidial production took the same amount of time (first observed on the fifth day) for all treatments.

Bensulide Concentration	Mean Radial Growth	Duncan Grouping
0.000 X	24.4 mm	A
0.0316 X	17.8 mm	B
0.100 X	12.3 mm	C
0.316 X	9.5 mm	D
1.000 X	9.2 mm	E
3.160 X	7.3 mm	F

Table 7. Effects of Bensulide on the Radial Growth Rate of C. lunata (20 replicates, α = 0.05, Day Four, Std. Dev. = 0.32 mm)

top row: right to left; control, 0.0316X, 0.10X
bottom row: right to left; 0.316X, 1.00X, 3.16X

Figure 1. *In Vitro* Growth of *C. lunata* on Bensulide Ammended PDA (photograph from day 4)

Dicamba Concentration	Mean Radial Growth	Duncan Grouping
0.000 X	26.2 mm	A
0.100 X	25.9 mm	AB
0.0316 X	25.7 mm	BC
1.000 X	25.5 mm	C
0.316 X	24.8 mm	D
3.160 X	23.6 mm	E

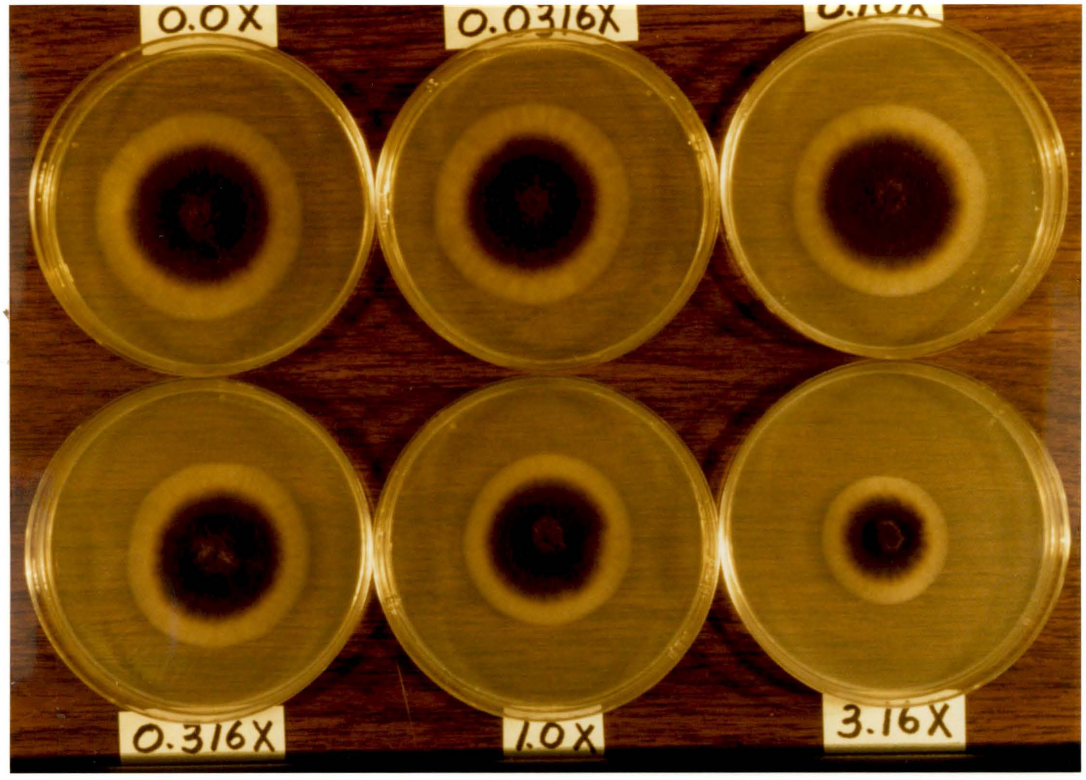
Table 8. Effects of Dicamba on the Radial Growth Rate of Curvularia lunata (20 replicates, α = 0.05, Day Four, Std. Dev. = 0.37 mm)

MCPP Concentration	Mean Radial Growth	Duncan Grouping
0.0316 X	24.1 mm	A
0.100 X	23.1 mm	B
0.000 X	23.1 mm	B
0.316 X	22.2 mm	C
1.000 X	20.2 mm	D
3.160 X	16.5 mm	E

Table 9. Effects of MCPP on the Radial Growth Rate of Curvularia lunata (20 replicates, α = 0.05, Day Four, Std. Dev. = 0.24 mm)

MSMA Concentration	Mean Radial Growth	Duncan Grouping
0.000 X	21.8 mm	A
0.0316 X	21.3 mm	B
0.100 X	20.6 mm	C
1.000 X	18.9 mm	D
0.316 X	18.3 mm	E
3.160 X	13.7 mm	F

Table 10. Effects of MSMA on the Radial Growth Rate of Curvularia lunata (20 replicates, α = 0.05, Day Four, Std. Dev. = 0.32 mm)



MSMA concentrations are given adjacent to each Petri plate in the photograph.

Figure 2. *In Vitro* Growth of *Curvularia lunata* on MSMA Ammended PDA (photograph from day 4)

2,4-D Concentration	Mean Radial Growth	Duncan Grouping
0.100 X	25.0 mm	A
0.0316 X	24.8 mm	AB
0.316 X	24.7 mm	AB
1.000 X	24.5 mm	B
0.000 X	24.0 mm	C
3.160 X	23.3 mm	D

Table 11. Effects of 2,4-D on the Radial Growth Rate of Curvularia lunata (20 replicates, α = 0.05, Day Four, Std. Dev. = 0.26 mm)

Conidial germination was delayed by about one day as compared to controls when MSMA was used at the 3.16 X rate and was slightly delayed (less than one day) at the 1.00 X rate. In addition, the following decreases in conidial germination occurred with MSMA: 15 percent reduction at 3.16 X, 5 percent at 1.00 X, and no reduction of germination at lower concentrations of MSMA. Bensulide at the 3.16 X rate slightly delayed (less than one day) conidial germination. Conidial germination was unaffected by the rest of the herbicide treatments.

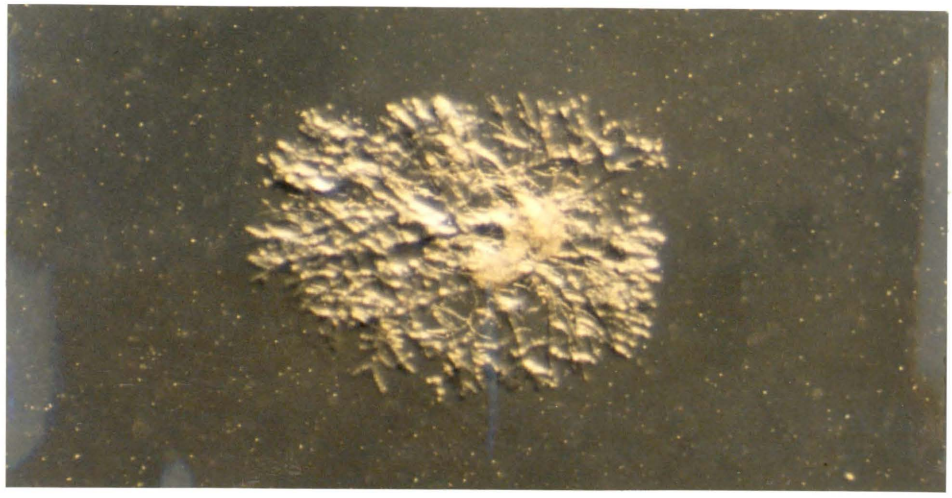
MSMA and bensulide also affected the morphology of the early growth of the colonies (as observed with the aid of a stereoscopic dissecting microscope). When either of these herbicides were exposed to the highest 2 rates, the linear growth of the mycelium was slower than that of the controls. However, branching of the mycelium took place at about the same rate as the controls. Thus, the distance between branches was reduced and it appeared as though the colony branched more frequently even though the total number of branches in a colony from a control plate was probably about the same as for a colony exposed to the herbicides. Photographs of this phenomenon from the bensulide experiment comprise figure 3. The other three herbicides did not influence the pattern of growth in early colony formation.

The production of anomolous germ tubes such as described by Hodges (1978) was not observed with any of the herbicides tested at any of the concentrations tested.

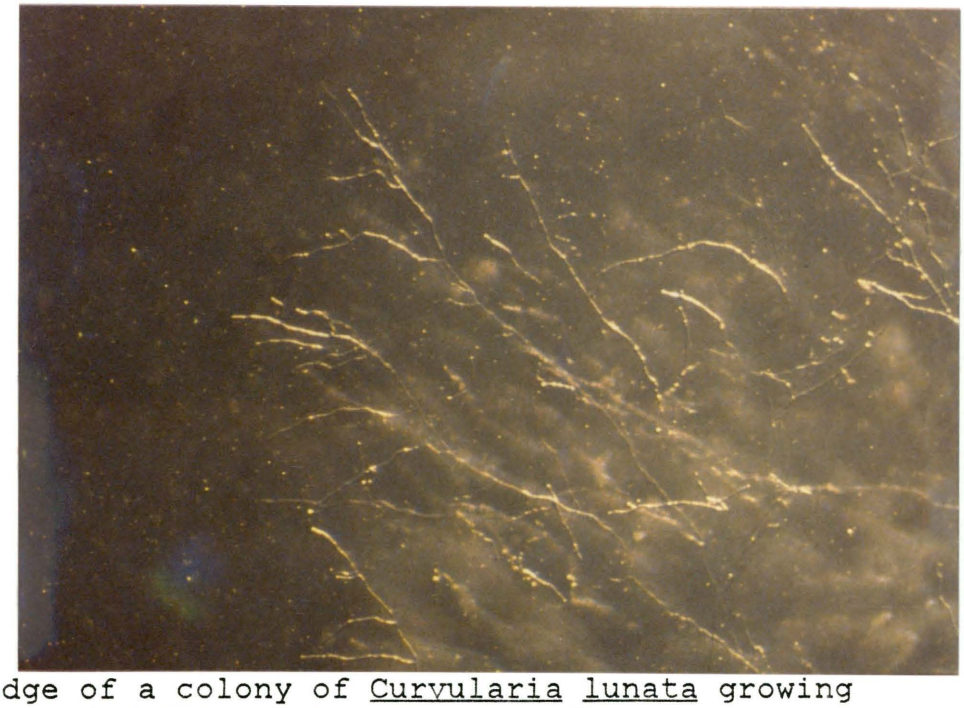
II. Acute High Temperature Stress as a Predisposing Factor in the Susceptibility of 'Penneagle' Creeping Bentgrass to Curvularia lunata

The data from the ratings of symptom severity were subjected to analysis of variance and the means for each of the treatments were compared using Duncan's multiple range test ($\alpha = 0.05$). In addition, each of the the factors (pre-inoculation acute high temperature stress, post-inoculation acute high temperature stress and inoculation with Curvularia lunata conidia) were analysed for the significance of their effect on the bentgrass as evidenced in the ratings. In addition to being analysed individually, all of the possible two-way interactions of these factors were analyzed.

Pre-inoculation acute high temperature stress, post-inoculation acute high temperature stress, and the interaction of these two factors all had highly significant ($\alpha <$ 0.0001) effects on symptom severity as reflected by the ratings. The effects of inoculation with C. lunata and the



A colony of Curvularia lunata growing on PDA amended with 3.16X bensulide.



The edge of a colony of Curvularia lunata growing on unamended PDA at the same magnification.

Figure 3. "Compact" Colony Growth of C. lunata on Bensulide Ammended PDA as Opposed to Growth on Unamended PDA.

interaction of pre-inoculation heatstress with inoculation with C. lunata on disease severity as measured by the ratings were also statistically significant. The effect of the interaction of post-inoculation acute heatstress and inoculation with C. lunata on disease severity was not statistically significant at $\alpha = 0.05$ (but was significant at $\alpha = 0.10$).

The means of the disease severity ratings indicated that pre-inoculation heatstress was the factor which produced the most injury to the bentgrass followed by post-inoculation high temperature stress. An increase in symptom severity due to the colonization of leaves by C. lunata was best demonstrated when inoculation was preceded by acute high temperature stress and not followed by post-inoculation high temperature stress.

The means of the ratings with their respective Duncan's multiple range groupings are given in Table 12. The levels of significance for the various factors in the experiment and the interactions of those factors are given in Table 13.

Bentgrass which was not subjected to acute high temperature stress, whether inoculated or not was symptomless. Symptom expression due to injury from high temperature stress was comprised of wilting of some leaves (especially older

ones) accompanied by general chlorosis. On the inoculated plants exposed to pre-inoculation heatstress but not post-inoculation heatstress, leaf tip chlorosis was visible and examination with the aid of a microscope showed mycelium in this region of the leaves. Mycelium was visible only at cut leaf tips and on the surface of the leaves which were not exposed to acute high temperature stress. Plants exposed to both pre-inoculation and post-inoculation heatstress were severely wilted and chlorotic. Curvularia lunata mycelium was visible in the wilted, dying leaf tissue of the inoculated plants which were exposed to both pre-inoculation and post-inoculation acute high temperature stress. Curvularia lunata was reisolated from surface sterilized leaves (5 seconds in 0.525 percent sodium hypoclorite and immediately rinsed in sterile distilled water) of all inoculated treatments but could not be isolated from uninoculated treatments. A photograph of one of the 12 replicates of this experiment comprises Figure 4.

III. Herbicide Stress and Acute High Temperature Stress

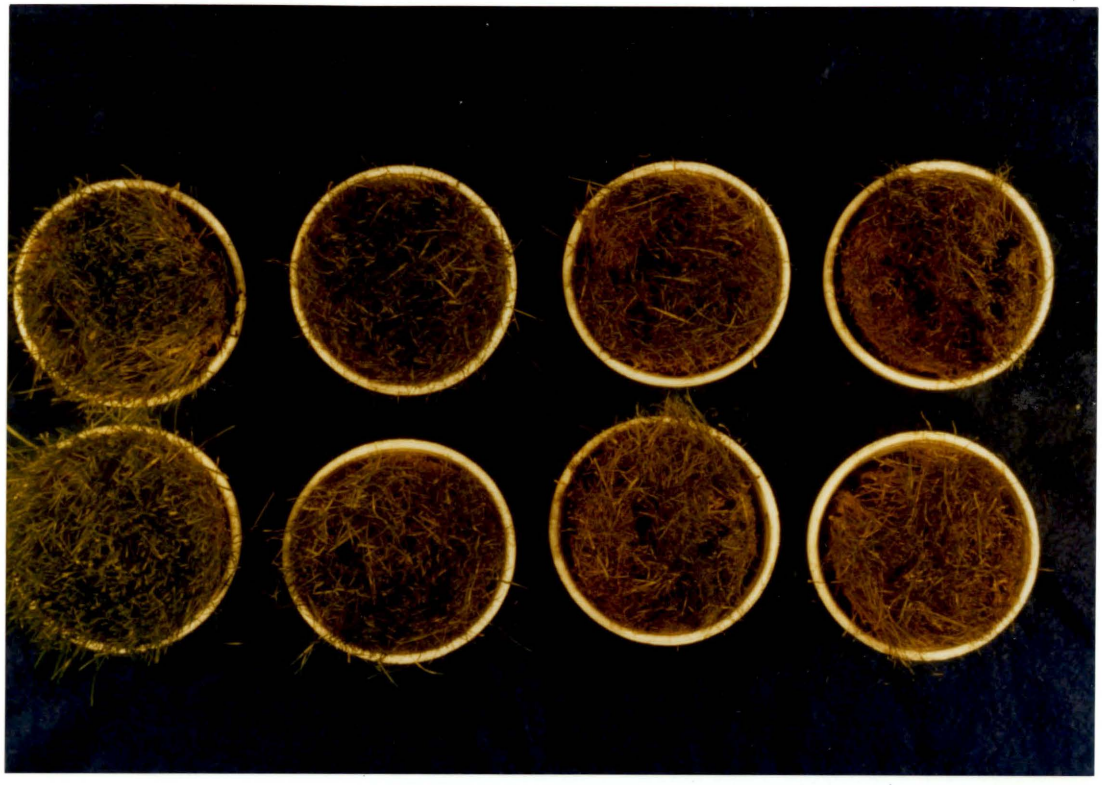
For each of the five experiments, the data from the ratings of symptom severity were subjected to analysis of variance and the means of each treatment were compared using

pre-inoc temperature	post-inoc temperature	C. lunata inoculum used	Symptom Rating (Duncan Group)
38 C	38 C	yes	4.1 (A)
38 C	38 C	no	3.9 (A)
38 C	24 C	yes	3.8 (A)
38 C	24 C	no	2.8 (B)
24 C	38 C	yes	2.1 (C)
24 C	38 C	no	2.0 (C)
24 C	24 C	yes	0.1 (D)
24 C	24 C	no	0.0 (D)

Table 12. Means and Duncan Groups for the Pre-inoculation and Post-inoculation Acute Heatstress Experiment

Source of Variation (Factor or Interaction)	Significance Level of Variation Source (α)
pre-inoculation acute high temperature stress	0.0001
post-inoculation acute high temperature stress	0.0001
effect of Curvularia lunata inoculum	0.0102
preinoculation high temperature stress interaction with post-inoculation high temperature stress	0.0001
preinoculation high temperature stress interaction with Curvularia lunata inoculation	0.0476
postinoculation high temperature stress interaction with Curvularia lunata inoculation	0.0927

Table 13. ANOVA for Acute High Temperature Stress Treatments and Curvularia lunata Inoculation on Bentgrass



Plants in the top row of containers were inoculated with Curvularia lunata conidia while those in the bottom row were not. From left to right, the columns of two containers received: 1) no acute heat stress, 2) post-inoculation acute heat stress, 3) pre-inoculation acute heat stress, 4) both pre-inoculation and post-inoculation heat stress

Figure 4. Bentgrass Plants Exposed to Pre- and Post-inoculation Acute Heat Stress and Inoculated or not Inoculated with C. lunata

Duncan's multiple range test ($\alpha = 0.05$). In addition, for each experiment, the level of significance of the effect of the herbicide factor, the acute heatstress factor, and the interaction of these two factors, on the level of injury to bentgrass as measured by the rating was determined. Tables giving the results of the statistical analyses of the rating data for the five herbicides can be found as follows:

Herbicide	Means with Duncans	Factorial ANOVA
dicamba	Table 14	Table 15
2,4-D	Table 16	Table 17
MSMA	Table 18	Table 19
MCPP	Table 20	Table 21
bensulide	Table 22	Table 23

Acute high temperature stress and exposure to each of the 5 herbicides produced injury in the creeping bentgrass. This is supported by the high level of statistical significance for these factors ($\alpha < 0.0001$ in every case). Interaction between the herbicide treatments and the acute heatstress treatment was statistically significant for dicamba, MSMA, and MCPP but not for bensulide or 2,4-D.

The symptoms of injury from acute heatstress and from herbicide exposure were similar. The symptoms observed were general chlorosis and wilting of leaf tissue, especially of older leaves. The wilted leaf tissue eventually became

necrotic. Photographs showing symptoms for MSMA, MCPP, and bensulide are found in Figures 5, 6, and 7 respectively. In addition, dicamba and 2,4-D caused swellings at the nodes of the bentgrass plants which are shown in figures 8 through 11.

IV. Predisposition of 'Penneagle' Creeping Bentgrass to Colonization by Curvularia lunata due to Chronic High Temperature Stress and Exposure to Herbicides

At a post-inoculation temperature of 25 C, no herbicide treatments increased the susceptibility of the bentgrass to infection and colonization by Curvularia lunata. In addition, the plants which did not receive herbicide treatments were not colonized by C. lunata beyond the cut leaf tip. Neither the herbicide treated nor the non herbicide treated non-clipped plants in these experiments were infected or colonized by C. lunata. These results were confirmed by microscopy and reisolation.

The results of the experimental regimes in which the post-inoculation temperature was 30 C (chronic high temperature stress) and the plants were clipped before inoculation follow.

Factor of Dicamba's Recommended Rate	24 Hour Exposure Temperature	Symptom Rating (Duncan Grouping)
2.5 X	40 C	5.6 (A)
1.0 X	40 C	3.8 (B)
2.5 X	21 C	2.9 (C)
0.0 X	40 C	2.7 (C)
1.0 X	21 C	2.2 (D)
0.0 X	21 C	0.0 (E)

Table 14. Means and Duncan Groups for Dicamba and High Temperature Stress Experiment (α = 0.05, 15 replicates)

Source of Variation (Factor or Interaction)	Significance Level of Variation Source (α)
Treatment with dicamba	0.0001
Acute High Temperature Stress (24 hours at 40 C)	0.0001
Interaction of dicamba Treatment with Acute High Temperature Stress	0.0052

Table 15. Factorial ANOVA for Dicamba and Acute High Temperature Stress Treatments (15 replicates)

Factor of 2,4-D's Recommended Rate	24 Hour Exposure Temperature	Symptom Rating (Duncan Grouping)
1.25 X	40 C	6.4 (A)
1.25 X	21 C	5.3 (B)
0.5 X	40 C	3.9 (C)
0.5 X	21 C	3.2 (D)
0.0 X	40 C	1.9 (E)
0.0 X	21 C	0.3 (F)

Table 16. Means and Duncan Groups for 2,4-D and High Temperature Stress Experiment (α = 0.05, 15 replicates)

Source of Variation (Factor or Interaction)	Significance Level of Variation Source (α)
Treatment with 2,4-D	0.0001
Acute High Temperature Stress (24 hours at 40 C)	0.0001
Interaction of 2,4-D Treatment with Acute High Temperature Stress	0.1639

Table 17. Factorial ANOVA for 2,4-D and Acute High Temperature Stress Treatments (15 replicates)

Factor of MSMA's Recommended Rate	24 Hour Exposure Temperature	Symptom Rating (Duncan Grouping)
2.0 X	40 C	8.9 (A)
2.0 X	21 C	6.9 (B)
1.0 X	40 C	6.1 (C)
1.0 X	21 C	3.4 (D)
0.0 X	40 C	0.8 (E)
0.0 X	21 C	0.3 (E)

Table 18. Means and Duncan Groups for MSMA and High Temperature Stress Experiment (α = 0.05, 15 replicates)

Source of Variation (Factor or Interaction)	Significance Level of Variation Source (α)
Treatment with MSMA	0.0001
Acute High Temperature Stress (24 hours at 40 C)	0.0001
Interaction of MSMA Treatment with Acute High Temperature Stress	0.0001

Table 19. Factorial ANOVA for MSMA and Acute High Temperature Stress Treatments (15 replicates)

left to right, 15 replicates each: 0.0X MSMA, 21 C; 0.0X MSMA, 40 C; 1.0X MSMA, 21 C; 1.0X MSMA, 40 C; 2.0X MSMA, 21 C; 2.0 X MSMA, 40 C.

top row, left to right: 0.0X MSMA, 21 C; 0.0X MSMA 40 C; 1.0X MSMA, 21 C;
bottom row, left to right: 1.0X MSMA, 40 C; 2.0X MSMA, 21 C; 2.0X MSMA, 40 C.

Figure 5. Photograph of Acute High Temperature and MSMA Injury to Creeping Bentgrass

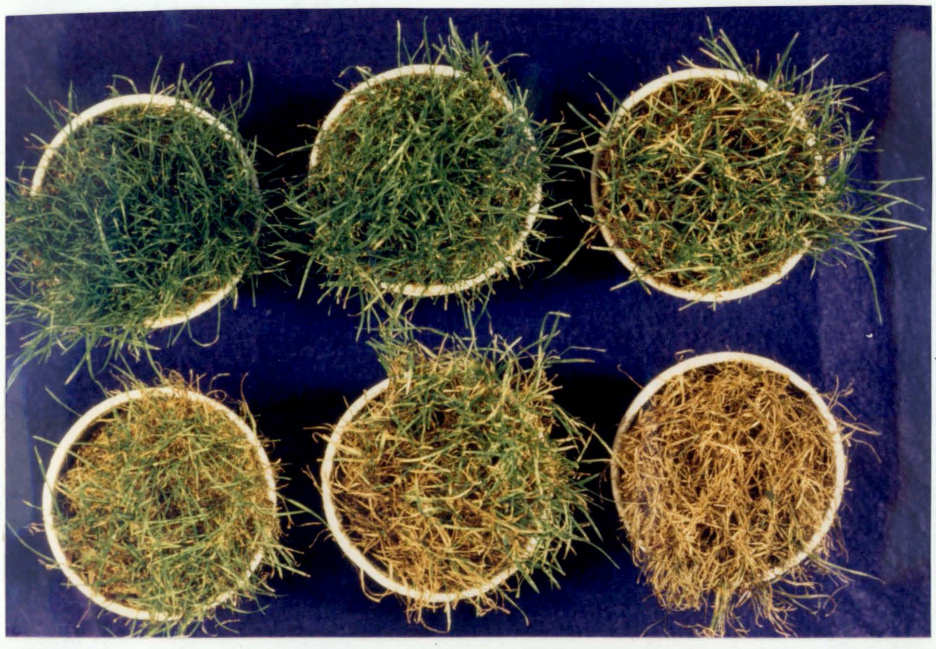
Factor of MCPP's Recommended Rate	24 Hour Exposure Temperature	Symptom Rating (Duncan Grouping)
2.0 X	40 C	8.7 (A)
2.0 X	21 C	3.8 (B)
0.8 X	40 C	3.3 (B)
0.8 X	21 C	2.2 (C)
0.0 X	40 C	1.1 (D)
0.0 X	21 C	0.2 (E)

Table 20. Means and Duncan Groups for MCPP and High Temperature Stress Experiment (α = 0.05, 13 replicates)

Source of Variation (Factor or Interaction)	Significance Level of Variation Source (α)
Treatment with MCPP	0.0001
Acute High Temperature Stress (24 hours at 40 C)	0.0001
Interaction of MCPP Treatment with Acute High Temperature Stress	0.0001

Table 21. Factorial ANOVA for MCPP and Acute High Temperature Stress Treatments (13 replicates)

left to right, 13 replicates each: 0.0X MCPP, 21 C;
0.0X MCPP, 40 C; 0.8X MCPP, 21 C; 0.8X MCPP, 40 C;
2.0X MCPP, 21 C; 2.0 X MCPP, 40 C.



top row, left to right: 0.0X MCPP, 21 C;
0.0X MCPP 40 C; 0.8X MCPP, 21 C;
bottom row, left to right: 0.8X MCPP, 40 C;
2.0X MCPP, 21 C; 2.0X MCPP, 40 C.

Figure 6. Photograph of Acute High Temperature and MCPP Injury to Creeping Bentgrass

Factor of Bensulide Recommended Rate	24 Hour Exposure Temperature	Symptom Rating (Duncan Grouping)
2.5 X	40 C	4.5 (A)
2.5 X	21 C	3.3 (B)
1.0 X	40 C	2.8 (B)
0.0 X	40 C	1.6 (C)
1.0 X	21 C	1.0 (CD)
0.0 X	21 C	0.2 (D)

Table 22. Means and Duncan Groups for Bensulide and High Temperature Stress Experiment (α = 0.05, 15 replicates)

Source of Variation (Factor or Interaction)	Significance Level of Variation Source (α)
Treatment with bensulide	0.0001
Acute High Temperature Stress (24 hours at 40 C)	0.0001
Interaction of bensulide Treatment with Acute High Temperature Stress	0.5195

Table 23. Factorial ANOVA for Bensulide and Acute High Temperature Stress Treatments (15 replicates)

left to right, 15 replicates each: 0.0X bensulide, 21 C;
0.0X bensulide, 40 C; 1.0X bensulide, 21 C;
1.0X bensulide, 40 C; 2.5X bensulide, 21 C;
2.5X bensulide, 40 C.

Figure 7. Photograph of Acute High Temperature and Bensulide Injury to Creeping Bentgrass

Figure 8. Swellings at Nodes of Creeping Bentgrass Due to Exposure to Dicamba.

Figure 9. Swellings at Nodes of Creeping Bentgrass Due to Exposure to Dicamba.

Figure 10. Swellings at Nodes of Creeping Bentgrass Due to Exposure to 2,4-D.

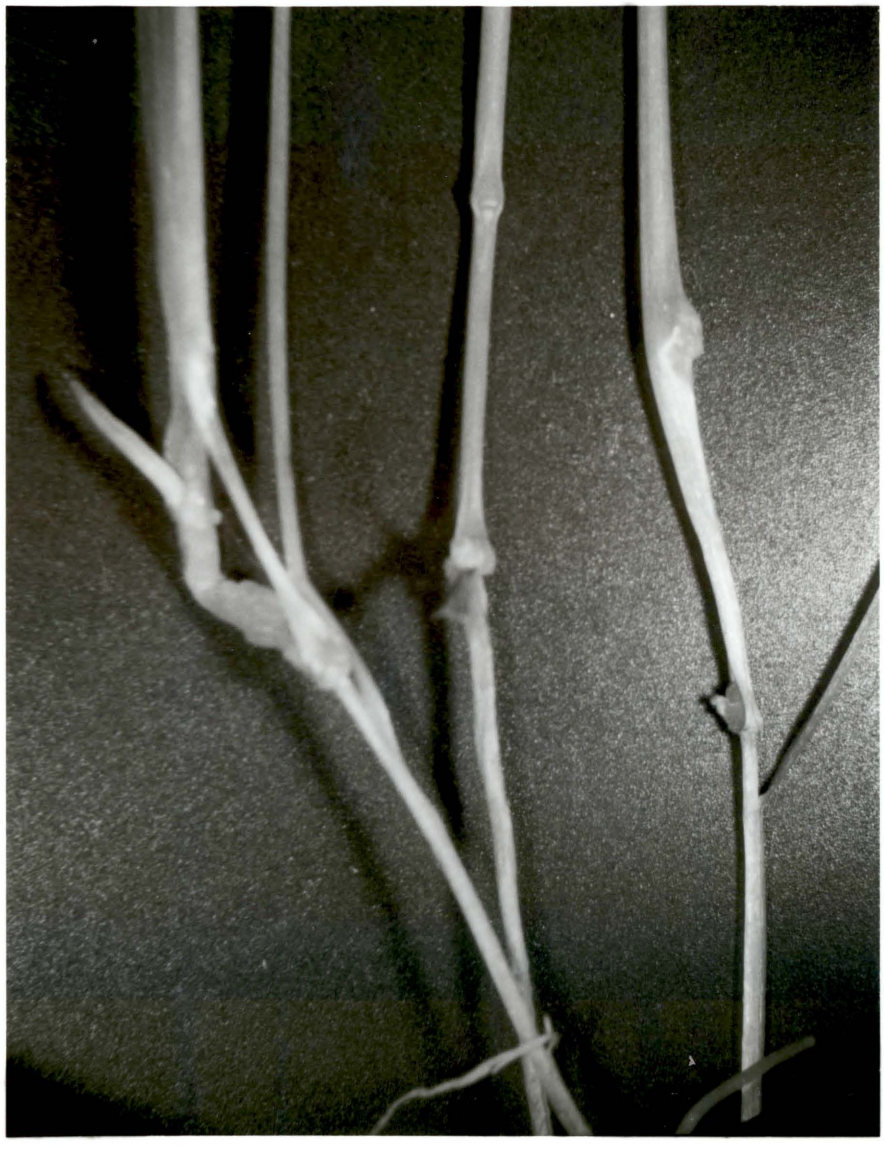


Figure 11. Swellings at Nodes of Creeping Bentgrass Due to Exposure to 2,4-D.

The data from the ratings were subjected to analysis of variance and means of individual treatments were compared using Duncan's multiple range test. In addition, a factorial analysis of variance was performed to determine the significance of herbicide treatments, inoculation with C lunata, and the interaction of these factors on the severity of symptoms as measured by the ratings.

Plants not exposed to herbicides or conidial suspensions but subjected to the chronic high temperature stress (30 C for 72 hours) occasionally showed only very mild chlorosis on older leaves. Plants inoculated but not herbicide treated exhibited leaf tip chlorosis progressing about 2 mm on many leaves. They only rarely showed further chlorosis or necrosis, and then only on the oldest leaves. Examination of these leaves with the aid of a microscope revealed that Curvularia lunata mycelium was in the symptomatic tissues. The fungus was also reisolated from surface disinfested leaves with these symptoms.

With bensulide and MCPP, no significant additional effect over either treatment alone was observed when C. lunata inoculation and the application of the herbicides were combined. With these two herbicides, effects of the herbicide alone were visible as very mild chlorosis and rarely wilting, affecting only older leaves.

For those plants treated with the other herbicides, symptoms were similar but more severe. When bentgrass plants which had been treated with dicamba, 2,4-D and MSMA were later inoculated, a marked increase in severity of symptoms over the herbicide or the fungus inoculum treatments alone occurred. The chlorosis was more extensive (about 5mm) on younger leaves and most older leaves were necrotic over a large portion or all of the leaf length. Again, mycelium was observed in the symptomatic portions of the leaves using a microscope and it was possible to reisolate the fungus from surface disinfested leaves with these symptoms. The differences between inoculated and non inoculated plants in symptom severity were statistically significant for dicamba, MSMA, and 2,4-D treated plants. The results of statistical procedures on the ratings from those treatments are given in the following tables:

Herbicide	Means with Duncans	Factorial ANOVA
dicamba	Table 24	Table 25
MSMA	Table 26	Table 27
2,4-D	Table 28	Table 29

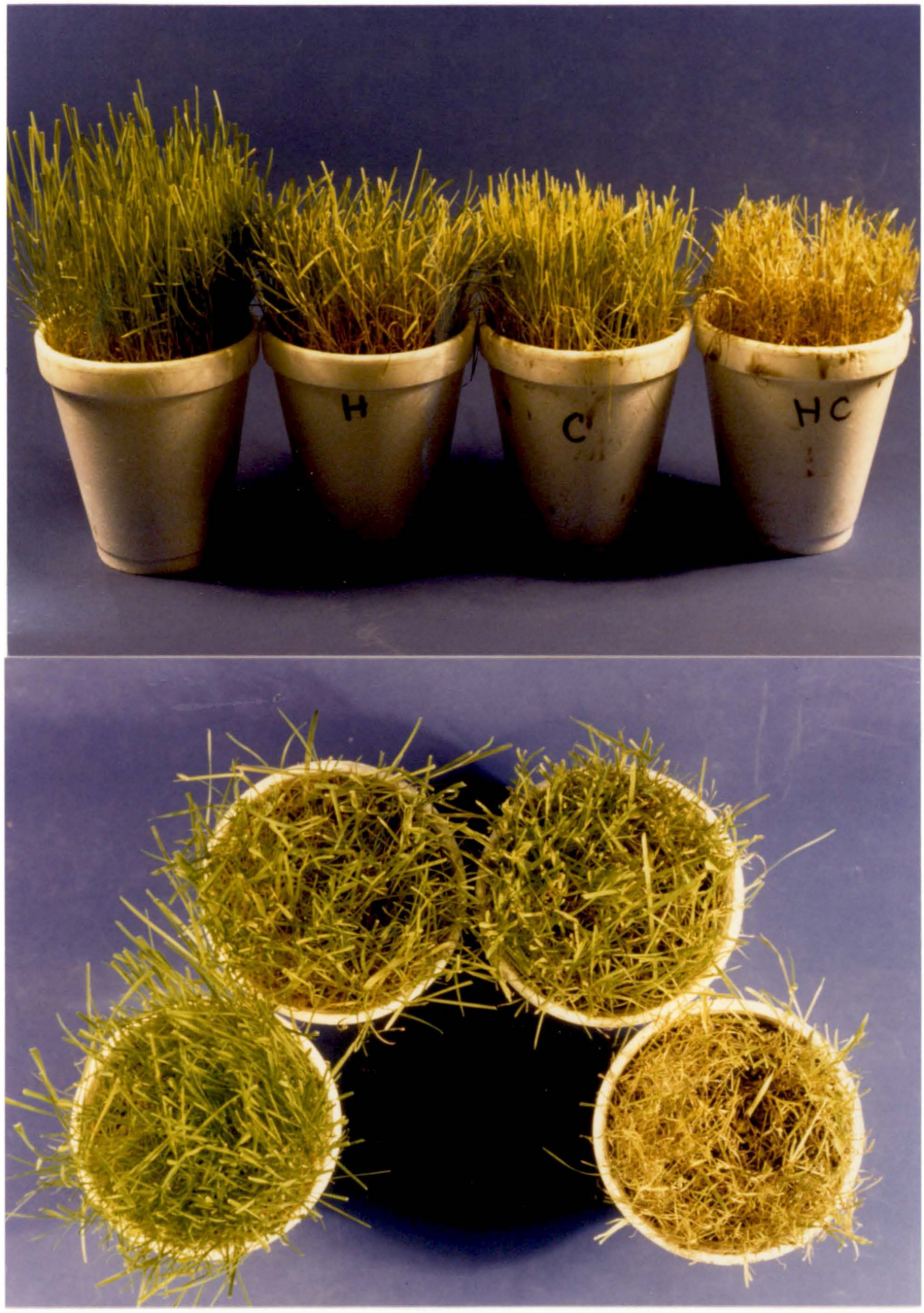
In addition, photographs from each of these experiments are found in Figure 12 (dicamba), Figure 13 (MSMA), and Figure 14 (2,4-D).

Description of Treatments	Symptom Rating	Duncan Groups
72 hours at 30 C post-inoculation temp + 1X Dicamba + Curvularia lunata	2.8	A
72 hours at 30 C post-inoculation temp + Curvularia lunata	1.3	B
72 hours at 30 C post-inoculation temp + 1X Dicamba	0.9	C
72 hours at 30 C post-inoculation temp	0.2	D

Table 24. Mean Symptom Rating of Bentgrass Exposed to Chronic High Temperature Stress and Dicamba and/or C. lunata

Source of Variation (Factor or Interaction)	Significance Level of Variation Source (α)
Treatment with 1X dicamba	0.0001
Inoculation with C. lunata	0.0001
Interaction of dicamba treatment with Inoculation with C. lunata	0.0134

Table 25. Factorial ANOVA for Treatment With Dicamba and Curvularia lunata Inoculation on Creeping Bentgrass



left to right, both photographs: 72 hours at 30 C,
72 hours at 30 C + 1X dicamba, 72 hours at 30 C + inoculum
72 hours at 30 C + 1X dicamba + C. lunata inoculum

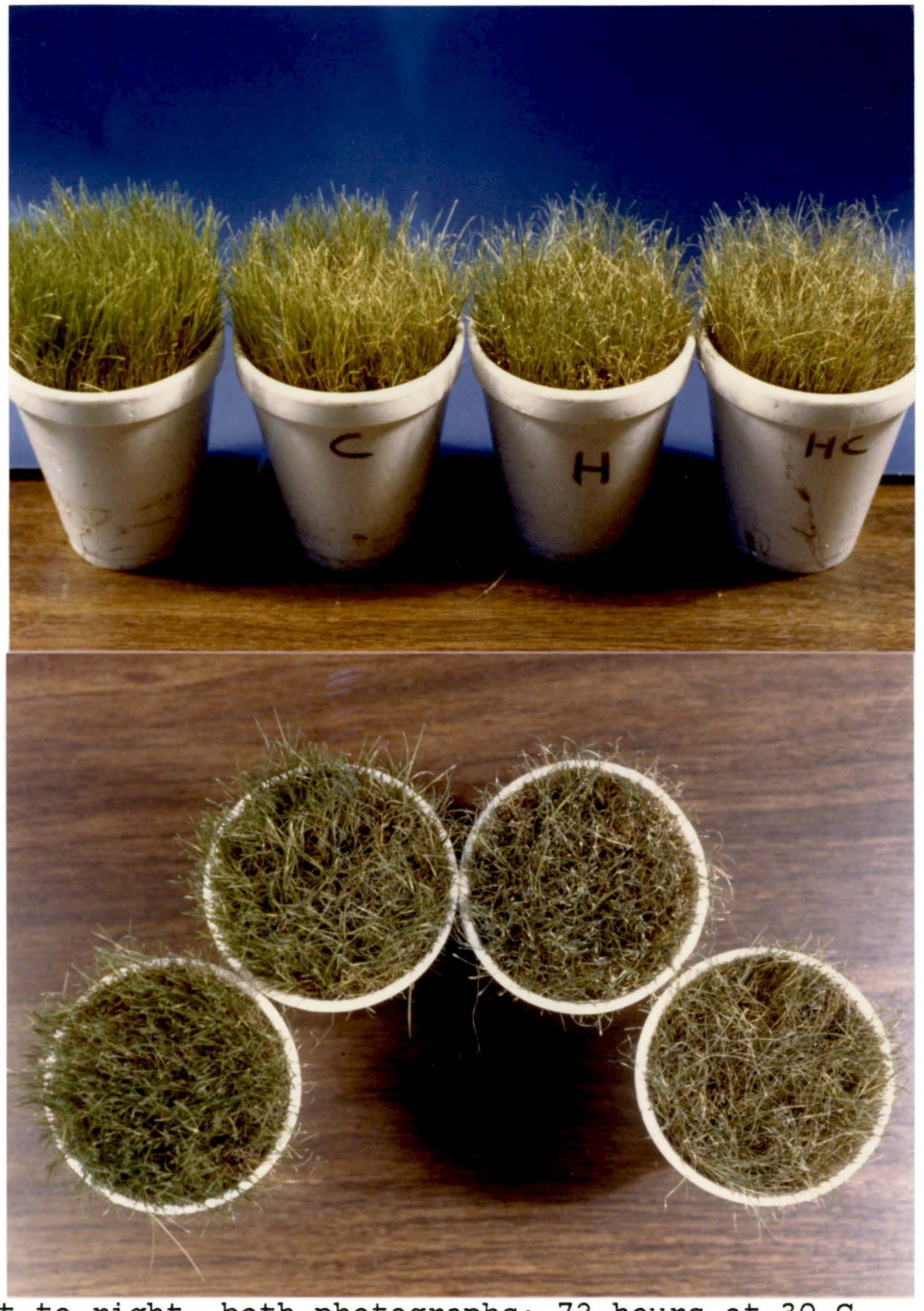
Figure 12. Photograph Showing Effects of Curvularia lunata and Dicamba on Bentgrass, In Vivo

Description of Treatments	Symptom Rating	Duncan Groups
72 hours at 30 C post-inoculation temp + 1X MSMA + Curvularia lunata	3.3	A
72 hours at 30 C post-inoculation temp + 1X MSMA	2.6	B
72 hours at 30 C post-inoculation temp + Curvularia lunata	2.4	B
72 hours at 30 C post-inoculation temp	0.7	C

Table 26. Mean Symptom Rating of Bentgrass Exposed to Chronic High Temperature Stress and MSMA and/or C. lunata

Source of Variation (Factor or Interaction)	Significance Level of Variation Source (α)
Treatment with 1X MSMA	0.0001
Inoculation with C. lunata	0.0001
Interaction of MSMA treatment with Inoculation with C. lunata	0.0042

Table 27. Factorial ANOVA for Treatment With MSMA and Curvularia lunata Inoculation on Creeping Bentgrass



left to right, both photographs: 72 hours at 30 C,
72 hours at 30 C + inoculum, 72 hours at 30 C + 1X MSMA
72 hours at 30 C + 1X MSMA + Curvularia lunata inoculum

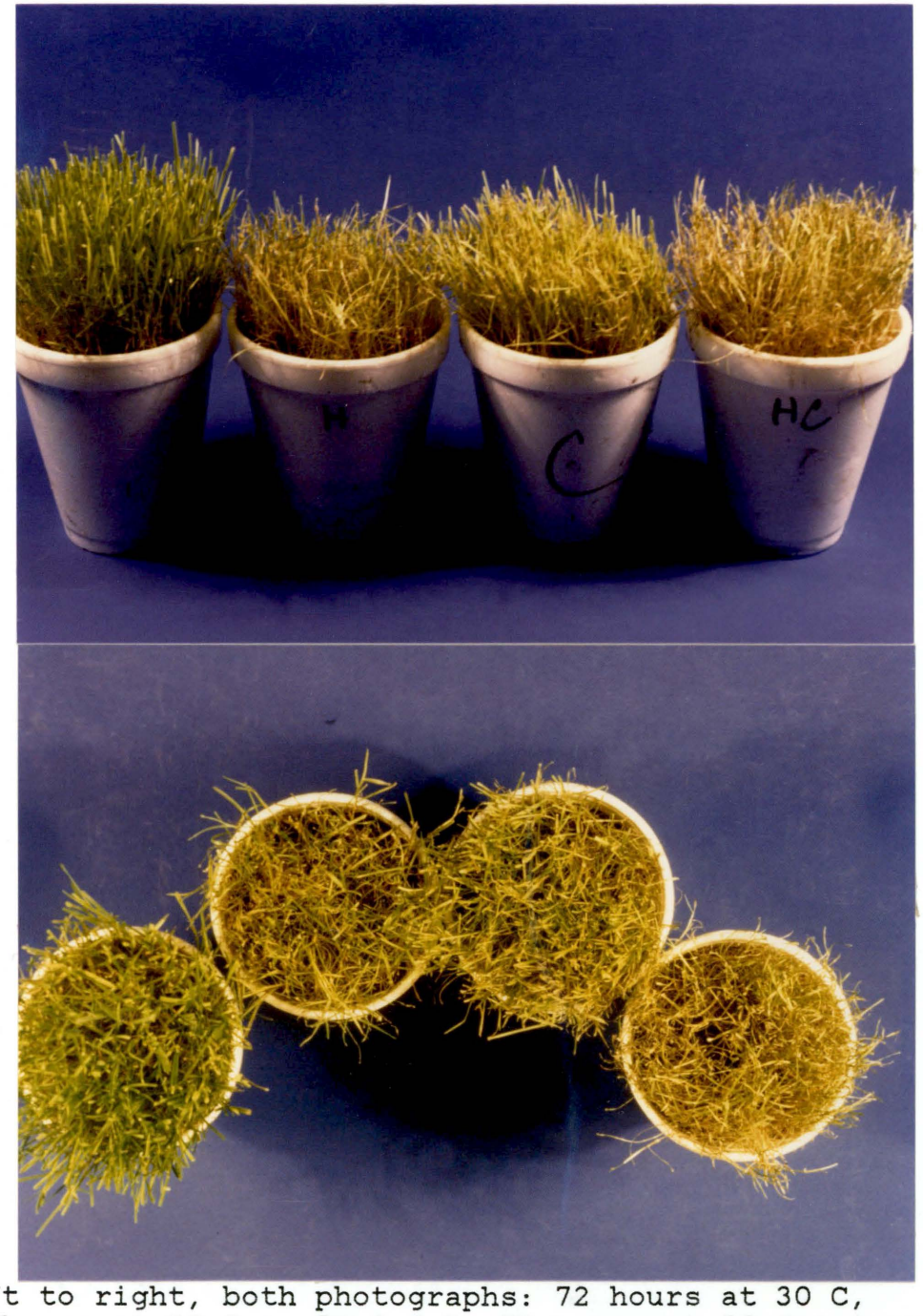
Figure 13. Photograph Showing Effects of Curvularia lunata and MSMA on Bentgrass, In Vivo

Description of Treatments	Symptom Rating	Duncan Groups
72 hours at 30 C post-inoculation temp + 2,4-D + Curvularia lunata	5.0	A
72 hours at 30 C post-inoculation temp + Curvularia lunata	2.5	B
72 hours at 30 C post-inoculation temp + 2,4-D	2.2	B
72 hours at 30 C post-inoculation temp	1.4	C

Table 28. Mean Symptom Rating of Bentgrass Exposed to Chronic High Temperature Stress and 2,4-D and/or C. lunata

Source of Variation (Factor or Interaction)	Significance Level of Variation Source ( )
Treatment with 2,4-D	0.0001
Inoculation with C. lunata	0.0001
Interaction of 2,4-D treatment with Inoculation with C. lunata	0.0007

Table 29. Factorial ANOVA for Treatment With 2,4-D and Curvularia lunata Inoculation on Creeping Bentgrass



left to right, both photographs: 72 hours at 30 C, 72 hours at 30 C + .5X 2,4-D, 72 hours at 30 C + inoculum 72 hours at 30 C + .5X 2,4-D + Curvularia lunata inoculum

Figure 14. Photograph Showing Symptoms of Curvularia lunata and 2,4-D on Bentgrass, In Vivo

DISCUSSION

The discussion is divided into sections according to the groups of experiments in the materials and methods and the results portions of the thesis.

I. Effects of Five Herbicides on the Growth of *Curvularia lunata* In Vitro

Curvularia lunata conidia were able to germinate on the amended agar with any of the 5 herbicides tested at all of the concentrations tested. Hodges (1977, 1981A) reported similar results with *B. sorokiniana*. The plasmolysis of conidia he observed occurred only at concentrations much higher than those used in these experiments. The slight reduction in percent germination with MSMA treatments at the 3.16 X and 1.0 X rates (the highest rates) cannot be directly compared with other work since this herbicide has not been previously tested for effects on fungi. However, the results obtained are similar to those of Hodges (1981B) with other herbicides. Hodges detected a reduction of conidial germination of *B. sorokiniana* exposed to bensulide but no such response was detected in these experiments with *C. lunata*. This may reflect a difference in the fungi or his

experimental procedure may have been more sensitive for this parameter. However, the delay in germination of conidia at the highest two concentrations of MSMA and at the highest concentration of bensulide is similar to the results of Hodges (1981) and Karr et al (1977, 1979) with fungi they treated with bensulide.

That none of the herbicides at any of the concentrations tested prevented the formation of conidia agrees well with Hodges (1977, 1981A) and Karr et al (1977, 1979). None of the herbicides at any of the concentrations tested caused changes of germ tube morphology. This agrees with Hodges (1977, 1981A) results with 2,4-D, MCPP, and dicamba (MSMA has never been tested in this regard) with B. sorokiniana. Hodges (1981A) found germ tube abnormalities with bensulide treatments, but only at very low frequencies at the highest concentration. Again, this may reflect a difference in the fungi or that his experimental procedure may have been more sensitive for this parameter. No changes in gross colony morphology were observed in these experiments or in those of other investigators with these herbicides (Karr et al 1977, 1979; Hodges 1977, 1981A).

Bensulide and MSMA treatments at the highest two concentrations produced colonies which appeared "compacted" when observed with a microscope due to their slower growth rates.

MSMA has not been previously tested in this way. While other investigators have not observed this particular growth parameter in their bensulide experiments, this result is in agreement with their results on mycelial growth rates (Karr et al 1977, 1979; Hodges 1981A).

In the radial growth rate experiments, the degree of inhibition and/or stimulation of growth was concentration dependent for each of the herbicides. It is common, with a given chemical, to find some concentrations are too low to affect fungal growth, while other concentrations, over a given range, stimulate growth, and finally, higher concentrations are inhibitory to growth or toxic (Hodges 1977, 1981A; Altman and Campbell 1977). The reductions in growth obtained with bensulide on C. lunata are similar to the results of Hodges (1981A) with B. sorokiniana and Karr et al (1977, 1979) with S. homoeocarpa, D. cynodontis, and P. aphanidermatum. The response of R. solani to bensulide (Karr et al 1979) is different however, in that an increase in growth was found with this fungus.

The reductions in the radial growth rate of C. lunata caused by MSMA at the concentrations tested provide new information since others have not worked with this herbicide. The response of C. lunata to dicamba (a slight reduction in growth rate) is different from that of B. sorokiniana which

is unaffected or increases growth rate in response to dicamba. 2,4-D exerted a slight influence on the radial growth rate of C. lunata at the concentrations tested consisting of small increases in growth at low concentrations and a small decrease at the highest concentration. This pattern is similar to that obtained by Hodges (1979) with B. sorokiniana. MCPP causes a moderate reduction of the radial growth rate of C. lunata at the higher concentrations tested and an increase at the lowest concentration tested which is again similar to the results Hodges (1979) observed for MCPP with B. sorokiniana.

The effects of the herbicides on the fungus in vitro may not be used to predict what their effect on the disease will be in vivo because their effects on the suscept must also be evaluated. Information from in vitro tests, however, may help in evaluating results from in vivo tests because they relate to one (of the many) factors involved in disease development.

II. Acute High Temperature Stress as a Predisposing Factor in the Susceptibility of 'Penneagle' Creeping Bentgrass to Curvularia lunata

In this experiment, pre-inoculation acute high temperature stress ranked as the most important factor influencing the health of the bentgrass followed by post-inoculation heatstress. An increase in symptom severity due to colonization by C. lunata was statistically significant when inoculation was preceded by acute high temperature stress and when not masked by the effect of post-inoculation high temperature stress. This illustrates the importance of high temperature stress as a predisposing influence in the causation of Curvularia blight. Since no statistically significant effect from C. lunata is apparent in non high temperature stressed plants, it is evident that the acute high temperature stress predisposed the bentgrass to colonization by the fungus. A similar conclusion is reached by Muchovej (1984, 1986) and Muchovej and Couch (1987). Heatstress causes the rapid senescence of older leaves and it is this tissue that is colonized.

III. Effects on 'Penneagle' Creeping Bentgrass of Acute High Temperature Stress and Exposure to Five Herbicides

Acute high temperature stress alone produced chlorosis and wilting not evident in plants not exposed to acute high temperature stress. This point is clear in the work of Muchovej and Couch (Muchovej, 1984, 1986, Muchovej and Couch,1987) but was not given enough consideration in the work of Howard (1953), Brown et al (1972), and Falloon (1976). In addition, herbicides used at moderate rates alone were sufficient to produce statistically significant increases in symptom severity. Increased herbicide concentrations produced greater symptom severity. When herbicide exposure is combined with acute heatstress, further increases in symptom severity occur. These further increases can be simply additive effects of the two individual factors as was found with bensulide and 2,4-D. However, with dicamba, MSMA, and MCPP, the increase resulted from the indivdual factors and an interaction between the high temperature stress and the herbicide factors which could be interpreted to suggest that the herbicides predispose bentgrass to greater injury from acute heatstress. Such effects of herbicide induced stress can lead to important changes in disease susceptibility as suggested by Altman and Campbell (1977), Callahan

(1972), Hodges (1978, 1979, 1980, 1981B, 1984), and Karr et al (1979).

The swellings at the nodes of bentgrass plants exposed to both rates of dicamba and 2,4-D are most likely due to hypertrophy and/or hyperplasia of the meristematic cells which are localized in that region of grass plants due to the auxin-like effects of these herbicides. These meristematic cells would be expected to be particularly sensitive to growth hormone analogs.

IV. Herbicide Exposure and Chronic High Temperature Stress as Predisposing Factors in the Colonization of 'Penneagle' Creeping Bentgrass by C. lunata

When herbicide treated or untreated bentgrass was inoculated with spores of C. lunata and then exposed to a temperature of 25 C for 72 hours, no colonization by the fungus was evident beyond the cut leaf tip. This result parallels the findings in the acute heatstress portion of the present research as well as the work of Muchovej and Couch (1987). This temperature regime does not promote an increase in colonization because it does not increase the amount of

senescent leaf tissue. Effects due to colonization by Curvularia lunata also were not evident in unclipped bentgrass. This agrees with the findings of Muchovej and Couch (1987) that C. lunata cannot infect intact leaf surfaces but can infect through wound sites.

The fact that there is no statistically significant additional effect (or interaction) due to C. lunata in bentgrass which is treated with MCPP or bensulide and then exposed to a temperature of 30 C for 72 hours indicates that with a mild, chronic high temperature stress regime, these herbicides do not significantly increase the amount of senescent tissue. When creeping bentgrass was treated with 2,4-D, MSMA, or dicamba, and exposed to chronic high temperature stress (72 hours at 30 C), additional increases in symptom severity occured in C. lunata inoculated plants. In these treatments, the effect of colonization of tissue which had become senescent in response to herbicide stress was evident.

V. Discussion of Results from the Experiments as they Relate to the Management of Creeping Bentgrass

The occurrence of high temperature stress similar to that experienced by plants in these experiments as well as the use of these herbicides at rates comparable to those used in these experiments is the rule in bentgrass culture. In addition, Curvularia lunata is a ubiquitous fungus and its conidia are likely to be present on the phylloplane at all times. Therefore, the kinds of interactions with acute heatstress, herbicide stress, and combined chronic heatstress and herbicide exposure with Curvularia blight which have been investigated are certain to occur each growing season. These experiments show that a stressing situation which produces newly senescent tissue is necessary for Curvularia blight to have any real impact on a stand of bentgrass.

The senescent tissue produced by stress, in the absence of C. lunata will become necrotic rather slowly. The fungus, if present however, will colonize the senescing tissues causing them to become necrotic rapidly and thus have a visible impact on the grass. Therefore, any control measures exercised for Curvularia blight, will not actually save healthy grass but would be desirable in order to preserve the "cosmetic" appearance of the stand. The timing of such a

spray should be after mowing (to help prevent infection of new wound sites) and at the onset of an anticipated stress period such as herbicide application or severe high temperature stress.

CONCLUSIONS

Conidia of Curvularia lunata are able to germinate on agar amended with any of the five herbicides used in the present study at all of the concentrations tested. MSMA at 3.16 X and 1.0 X rates (the highest rates) cause a slight decrease in percent germination. Germination of conidia is slightly delayed at 3.16 X and 1.00 X rates of MSMA and at a rate of 3.16 X bensulide. None of the herbicides at any of the concentrations tested prevent the formation of conidia or caused changes in germ tube morphology. No changes in gross colony morphology are produced by the herbicides. Bensulide and MSMA at the 3.16 X and 1.00 X concentrations produce colonies which appeared "compacted" when observed with a microscope due to their slower growth rates.

Dicamba and 2,4-D produced mild effects on the radial growth rate of C. lunata at concentrations up to 3.16 X. MCPP and MSMA caused large reductions in the radial growth rate of C. lunata at the higher concentrations tested (0.316 X, 1.0 X, and 3.16 X). Bensulide caused profound reductions in the radial growth rate of C. lunata at all the concentrations tested (0.0316 X to 3.16 X).

In testing effects of herbicides at moderate rates and at high rates alone and in combination with acute heatstress the following are concluded: 1) Increasing herbicide concentrations from moderate to high rates leads to increases in the severity of chlorosis and wilting of the treated creeping bentgrass. 2) Acute high temperature stress alone and herbicides used at moderate rates alone are sufficient to produce statistically significant increases in symptom expression over non treated controls. 3) When herbicide exposure is combined with acute high temperature stress, further increases in symptom severity occur.

Chlorosis and wilting are symptoms of acute heatstress, herbicide stress, and the combination of these factors. Swellings at the nodes of bentgrass plants are produced in response to 2,4-D and dicamba, probably due to auxin-like activity on the meristematic cells.

Acute heatstress predisposes creeping bentgrass to colonization by Curvularia lunata. This can be demonstrated when the effect of colonization by C. lunata on pre-inoculation high temperature stressed bentgrass is not masked by the effect of post-inoculation high temperature stress.

Effects due to colonization by Curvularia lunata are not evident in unclipped bentgrass; a wound site is necessary for infection.

Herbicide treated or untreated bentgrass is not colonized beyond the point of the cut leaf tips by C. lunata when it is exposed to a temperature of 25 C for for a period of 72 hours after inoculation because the stresses produced by these treatments are not severe enough to produce newly senescent tissues. There is no significant additional effect due to C. lunata colonization of bentgrass which is treated with MCPP or bensulide and then exposed to a temperature of 30 C for 72 hours for the same reason. When creeping bentgrass is treated with 2,4-D, MSMA, or dicamba, and exposed to chronic heatstress (72 hours at 30 C), additional increases in symptom expression occur in C. lunata inoculated plants because newly senescent tissue is produced.

SUMMARY

Two differing opinions concerning the nature of Curvularia blight of creeping bentgrass are expressed in the literature. One theory holds that Curvularia lunata is a primary pathogen of creeping bentgrass, inciting a common hot weather disease. The other opinion is that C. lunata is a very weak pathogen and only colonizes bentgrass foliar tissues in advanced senescence. Experiments were performed to evaluate the effects of stress from high temperature and herbicide exposure, both of which can lead to premature senescence of foliar tissue and both of which are normal occurences in bentgrass culture. The herbicides evaluated (2,4-D, MCPP, MSMA, bensulide, and dicamba) are among the few which are labeled for use on bentgrass in Virginia. Effects of these herbicides on certain aspects of the growth of C. lunata, in vitro were also evaluated.

In general, when Curvularia lunata is exposed to these herbicides in vitro at concentrations lower than those used in the field for weed control, they had negligible effects on growth parameters other than radial mycelial growth rate. Dicamba and 2,4-D produced mild effects on the radial growth rate of C. lunata at the concentrations tested. MCPP and MSMA cause a moderate reductions in the radial growth rate

of C. lunata at the higher concentrations tested. Bensulide caused profound reductions in the radial growth rate of C. lunata at the concentrations tested.

These herbicides alone were shown to produce visible evidence of stress to creeping bentgrass. In addition, acute heatstress alone was shown to produce visible evidence of stress to bentgrass. The herbicides predisposed the bentgrass to further injury from heatstress.

Curvularia lunata did not produce visible symptoms on unclipped bentgrass or symptoms beyond the first two millimeters of the clipped leaf tips on nonstressed but clipped bentgrass. Acute high temperature stress predisposed creeping bentgrass to colonization by Curvularia lunata. 2,4-D, MSMA, and dicamba, in combination with chronic high temperature stress (72 hours at 30 C) predisposed bentgrass to colonization by the fungus.

The results indicate that stress which produces moribund tissue in creeping bentgrass allows Curvularia lunata to colonize that tissue as long as a wound site infection court is provided, thus confirming the view that the fungus is a weak primary parasite but an active colonizer of senescing tissues.

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APPENDIX

The results of the first three days radial growth measurements for the five herbicides studied (In Vitro experiments, see Results section I) are summarized below with the concentration effects compared using Duncan's multiple range test. The probability level is 95 percent ($\alpha = 0.05$) for each procedure.

Bensulide Concentration	Mean Radial Growth	Duncan Grouping
0.000 X	3.2 mm	A
0.0316 X	2.9 mm	B
0.100 X	2.0 mm	C
1.000 X	1.4 mm	D
0.316 X	1.4 mm	D
3.160 X	1.2 mm	E

Table 30. Effects of Bensulide on the Radial Growth Rate of Curvularia lunata (20 Replicates, Day One)

Bensulide Concentration	Mean Radial Growth	Duncan Grouping
0.000 X	9.1 mm	A
0.0316 X	7.0 mm	B
0.100 X	4.7 mm	C
0.316 X	3.6 mm	D
1.000 X	3.2 mm	E
3.160 X	2.4 mm	F

Table 31. Effects of Bensulide on the Radial Growth Rate of Curvularia lunata (20 Replicates, Day Two)

Bensulide Concentration	Mean Radial Growth	Duncan Grouping
0.000 X	16.2 mm	A
0.0316 X	12.0 mm	B
0.100 X	8.3 mm	C
0.316 X	6.3 mm	D
1.000 X	6.0 mm	D
3.160 X	4.4 mm	E

Table 32. Effects of Bensulide on the Radial Growth Rate of Curvularia lunata (20 Replicates, Day Three)

Dicamba Concentration	Mean Radial Growth	Duncan Grouping
0.100 X	5.4 mm	A
0.000 X	5.3 mm	A
0.0316 X	5.1 mm	B
0.316 X	5.1 mm	B
1.000 X	5.0 mm	B
3.160 X	4.5 mm	C

Table 33. Effects of Dicamba on the Radial Growth Rate of Curvularia lunata (20 Replicates, Day One)

Dicamba Concentration	Mean Radial Growth	Duncan Grouping
0.100 X	10.2 mm	A
0.316 X	10.1 mm	AB
0.000 X	10.0 mm	AB
0.0316 X	9.9 mm	B
1.000 X	9.9 mm	B
3.160 X	9.5 mm	C

Table 34. Effects of Dicamba on the Radial Growth Rate of Curvularia lunata (20 Replicates, Day Two)

Dicamba Concentration	Mean Radial Growth	Duncan Grouping
0.000 X	17.8 mm	A
0.100 X	17.6 mm	AB
1.000 X	17.4 mm	BC
0.0316 X	17.2 mm	CD
0.316 X	17.0 mm	D
3.160 X	16.0 mm	E

Table 35. Effects of Dicamba on the Radial Growth Rate of Curvularia lunata (20 Replicates, Day Three)

MCPP Concentration	Mean Radial Growth	Duncan Grouping
0.0316 X	4.8 mm	A
0.100 X	4.5 mm	B
0.316 X	4.2 mm	C
0.000 X	4.1 mm	C
1.000 X	4.1 mm	C
3.160 X	3.1 mm	D

Table 36. Effects of MCPP on the Radial Growth Rate of Curvularia lunata (20 Replicates, Day One)

MCPP Concentration	Mean Radial Growth	Duncan Grouping
0.0316 X	12.5 mm	A
0.100 X	11.6 mm	B
0.316 X	11.2 mm	C
0.000 X	10.7 mm	D
1.000 X	9.9 mm	E
3.160 X	7.8 mm	F

Table 37. Effects of MCPP on the Radial Growth Rate of Curvularia lunata (20 Replicates, Day Two)

MCPP Concentration	Mean Radial Growth	Duncan Grouping
0.0316 X	17.4 mm	A
0.100 X	16.9 mm	B
0.000 X	16.1 mm	C
0.316 X	16.1 mm	C
1.000 X	14.9 mm	D
3.160 X	11.7 mm	E

Table 38. Effects of MCPP on the Radial Growth Rate of Curvularia lunata (20 Replicates, Day Three)

MSMA Concentration	Mean Radial Growth	Duncan Grouping
0.0316 X	2.2 mm	A
0.000 X	2.1 mm	AB
0.100 X	2.0 mm	B
0.316 X	1.4 mm	C
1.000 X	1.4 mm	C
3.160 X	0.9 mm	D

Table 39. Effects of MSMA on the Radial Growth Rate of Curvularia lunata (20 Replicates, Day One)

MSMA Concentration	Mean Radial Growth	Duncan Grouping
0.000 X	9.4 mm	A
0.0316 X	9.1 mm	B
0.100 X	8.8 mm	C
1.000 X	8.2 mm	D
0.316 X	7.5 mm	E
3.160 X	5.3 mm	F

Table 40. Effects of MSMA on the Radial Growth Rate of Curvularia lunata (20 Replicates, Day Two)

MSMA Concentration	Mean Radial Growth	Duncan Grouping
0.000 X	15.8 mm	A
0.0316 X	15.0 mm	B
0.100 X	14.1 mm	C
1.000 X	12.8 mm	D
0.316 X	12.1 mm	E
3.160 X	8.7 mm	F

Table 41. Effects of MSMA on the Radial Growth Rate of Curvularia lunata (20 Replicates, Day Three)

2,4-D Concentration	Mean Radial Growth	Duncan Grouping
0.000 X	5.0 mm	A
0.0316 X	4.9 mm	AB
3.160 X	4.7 mm	BC
0.316 X	4.7 mm	BC
1.000 X	4.5 mm	C
0.100 X	4.2 mm	D

Table 42. Effects of 2,4-D on the Radial Growth Rate of Curvularia lunata (20 Replicates, Day One)

2,4-D Concentration	Mean Radial Growth	Duncan Grouping
0.100 X	10.6 mm	A
0.0316 X	10.5 mm	AB
1.000 X	10.5 mm	AB
0.000 X	10.4 mm	ABC
0.316 X	10.3 mm	BC
3.160 X	10.2 mm	C

Table 43. Effects of 2,4-D on the Radial Growth Rate of Curvularia lunata (20 Replicates, Day Two)

2,4-D Concentration	Mean Radial Growth	Duncan Grouping
0.100 X	19.9 mm	A
1.000 X	19.8 mm	AB
0.316 X	19.6 mm	AB
0.0316 X	19.5 mm	B
0.000 X	18.9 mm	C
3.160 X	18.6 mm	C

Table 44. Effects of 2,4-D on the Radial Growth Rate of Curvularia lunata (20 Replicates, Day Three)

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