This is to certify that the

thesis entitled

STUDIES IN THE ISOLATION, PATHOGENICITY, EPIDEMIOLOGY AND CONTROL OF LEPTOSPHAERIA KORRAE CAUSING NECROTIC RING SPOT OF POA PRATENSIS L. IN MICHIGAN

presented by

MARTHA ELLEN OTTO

has been accepted towards fulfillment
of the requirements for

Masters degree in Plant Pathology

Major professor

Date 2/21/87

O-7639

STUDIES IN THE ISOLATION, PATHOGENICITY, EPIDEMIOLOGY AND CONTROL OF *LEPTOSPHAERIA KORRAE* CAUSING NECROTIC RING SPOT OF *POA PRATENSIS* L. IN MICHIGAN

By

Martha Ellen Otto

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Botany and Plant Pathology

1987

ABSTRACT

STUDIES IN THE ISOLATION, PATHOGENICITY, EPIDEMIOLOGY AND CONTROL OF *LEPTOSPHAERIA KORRAE* CAUSING NECROTIC RING SPOT OF *POA PRATENSIS* L. IN MICHIGAN

by

Martha Ellen Otto

Dark, septate, ectotrophic, runner hyphae have been associated with roots of diseased plants from patches in affected *Poa pratensis* lawns in Michigan. This fungus was identified as *Leptosphaeria korrae* (Walker & Smith), a known incitant of necrotic ring spot of *Poa pratensis*. The pathogenicity of this fungus was demonstrated in growth chamber, greenhouse and field studies.

A host range performed on six grass species showed *P. pratensis* cv. Fylking and Adelphi to be most susceptible, *Agrostis palustris* cv. Penncross and *Lolium perenne* cv. Manhattan to be intermediate, *Festuca arundenacea* cv. K-31, and *Festuca rubra* cv. Pennlawn variable, and the *Cynodon dactylon* cv. tested was resistant.

Patch symptom development was observed in the field throughout the year. Growth chamber studies showed development of symptoms at 15, 20, 24, and 28 C. Symptoms in the field were shown to decrease under prolonged high soil moisture.

The fungicides fenarimol, propiconazol, benomyl, thiophanate, and thiophanate-methyl were effective against this fungus *in vitro*. Iprodione and vinclozalin provided approximately 50% inhibition of mycelial growth, and

triadimefon was not effective. Greenhouse studies showed benomyl, fenarimol, and propiconazol to be effective _in vivo_. Adequate fertility was important in recovery of diseased areas.

Four bacteria and two actinomycetes contained in Lawn Restore, an organic fertilizer, inhibited growth of _L. korrae_ _in vitro_.

Another fertilizer, Green Magic, was significantly more inhibitory to _L. korrae_ _in vitro_ at certain rates than a similar nutrient solution.

ACKNOWLEDGEMENTS

I would like to express my appreciation to my major professor Dr. Joe Vargas for his help and support and to the members of my committee, Dr. Paul Rieke, and Dr. John Lockwood for their guidance throughout my studies.

I would especially like to thank Ron Detweiler for his constant encouragement, and my brother Mark, and my parents without whose support I couldn't have come this far.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

Introduction and Literature Review

In the past few years considerable progress has been made in determining the cause of the disease previously known as *Fusarium* blight of *Poa pratensis* L. (Kentucky bluegrass). *Fusarium* blight, which became widespread in the United States by the 1960's as "improved" *P. pratensis* cultivars became common, was first described by Couch and Bedford (1966). Since their first report a considerable progression of possible incitants and predisposing agents for this disease have been proposed.

Symptomatology

Fusarium blight was characterized by tops of grass plants wilting, turning straw-colored and dying in irregular to distinctly circular patterns. Circular patches of dead grass may occur from six inches to three feet across and often the centers of these patches will become re-colonized by healthy grass tillers or weeds, causing a ring or "frog-eye" appearance. At times these "frogeyes" may expand and become so numerous that they coalesce, leading to a serpentine appearance, or large areas almost completely blighted (Couch & Bedford, 1966). Leaf lesions were also associated with these "frog-eyes", consisting initially of dark green blotches which fade to light green, turning reddish brown to tan, and expanding to the width of the leaf blade (Couch & Bedford, 1966). Foliar blighting was not associated with this disease in all parts of the country however (Endo et al., 1974; Vargas, 1982), and the healthy tuft of grass which caused the term "frog-eye" to be coined was also not consistently present in all areas (Bean, 1966; Cole, 1976; Endo & Colbaugh, 1974).

Etiology

Isolations from diseased root, crown and leaf tissues from patch diseased areas yielded several fungi including Helminthosporium, Pythium, Sclerotinia and Fusarium spp., and within this group Fusarium roseum and F. tricinctum f.sp. poae were isolated most frequently. Alternaria, Curvularia, and Penicillium spp. were also frequently isolated (Couch & Bedford, 1966). Pathogenicity tests with these fungi showed F. tricinctum and F. roseum to produce a high level of foliar blight symptoms on seedlings when placed in moist chambers (Couch & Bedford, 1966). The most typical symptom seen in the field, the patch or frog-eye, was never reproduced, however, leaving the etiology of this disease open to great question.

Subsequent studies implicated a number of factors as possible incitants and predisposing agents of this disease. Relationships were found between moisture stress and high light intensity and disease development (Bean, 1969). Vargas and Laughlin (1972) suggested an interaction between the stunt nematode, Tylenchorhynchus dubius and Fusarium roseum in causing the disease. Goldberg et al. (1974) was not able to show improvement in control with nematicides alone or in combination with fungicides, however. Turgeon and Meyer (1974) showed high spring fertilization to increase the incidence of Fusarium blight on some P. pratensis cultivars and also found a relationship between mowing height and disease that differed with different cultivars.

In the late 1970's serious challenges were made to the original Fusarium blight hypothesis. Smiley & Craven (1977a, 1978, 1979a) found in field studies that iprodione controlled the disease yet amplified numbers of Fusaria in the soil and was ineffective in vitro as a fungistat against F. roseum. He also found that Fusarium blight was least in field studies when the percentage of Fusarium infected plant crowns was highest, and found a positive correlation between the disease and thatch decomposition rate. He suggested that decomposition processes could favor disease by increasing available nutrients, thereby increasing inoculum density, or by releasing phytotoxins, suggesting the possibility of an abiotic cause of Fusarium blight (Smiley et al., 1979a, 1980b).

During the late 1970's evidence began to mount of a dark, septate, fungus associated with the roots and crowns of plants in the "frog-eye" or patch (Sanders et al., 1980; Worf et al., 1982). This fungus was considered Gaeumannomyces-like because of ectotrophic growth habit and pathogenesis (Worf et al., 1982; Jackson, 1984; Smiley et al., 1984b). During this period other researchers in the eastern and northwestern U.S. began to isolate a similar fungus from diseased patches. Through ascospore measurements and growth characteristics they were able to identify it as Leptosphaeria korrae (Jackson, 1984; Chastagner et al., 1984a; Smiley & Craven-Fowler, 1984a).

On potato dextrose agar growth of L. korrae is at first white, gradually darkening in color from the center of the colony. Eventually the aerial mycelium is dark gray and from the underside appears gray to black. The optimal growth for L. korrae occurs at 25 C (4-5 mm per day) (Walker & Smith, 1972). Pseudothecia have not been reported from agar cultures but have been induced on sterile oat grains (Jackson, 1984), and on culms and roots of grass grown in coarse sand (Smiley, personal communication; Otto & Vargas, 1985). Pseudothecia have also been observed rarely in the field (Endo et al., 1985; Jackson, 1984; Chastagner et al., 1984a).

Of these researchers Chastagner et al. (1984), reported an average ascospore size of 135(100-188) x 4-5 um with 7(5-11) septae. Worf's isolates (1986) measured 105-163 x 4-5 um, and Smiley reported (63) 113-138 (158) x (3.3)4-5(5.8) um (Smiley & Craven-Fowler, 1984a). These are somewhat smaller than the measurements listed by the authors, Walker and Smith, (1972) of (120) 140-170 (180) x 4-5 um, with (1-3-6) 7 (15) septae, but fall within the natural range of variation accepted for intensively studied species of ascomycetes (Smiley & Craven-Fowler, 1984a).

In addition to characteristic ascospores, L. korrae also produces unique hyphopodia which consist of dark angular cells in flattened masses beneath which penetration pegs enter into the host root tissue. Penetration pegs may also occur from single lateral and intercalary cells on the mycelium (Walker & Smith, 1972; Smiley et al., 1985e).

Smiley also showed the presence of another pathogen from diseased patches on Poa pratensis, with dark ectotrophic mycelium and simple hyphopodia on roots, which he identified as Phialophora graminicola (Deacon). Both were identified as primary causal agents of the patch disease syndrome of Poa pratensis previously known as Fusarium blight or Fusarium blight syndrome. With these developments Smiley proposed that Fusarium spp. should be recognized as causal agents only of diffusely distributed or irregularly patchy leaf spots and crown and root rots of Poa pratensis. The name summer patch was proposed for the disease caused by P. graminicola, and necrotic ring spot (sensu Worf) for L. korrae-incited diseases (Smiley et al., 1984a,b,c,d).

Also reported in the late 1970's and early 1980's was a cool season patch disease called yellow patch, which was identical in symptomatology to Fusarium blight. Rhizoctonia cerealis was determined to be the cause of this disease (Sanders et al., 1978a; Burpee, 1980; Brown et al., 1983a, b). This may further complicate the epidemiology of patch diseases of turfgrasses since the suspicion exists that this fungus may be a part of a patch disease complex in some areas (Worf et al., 1986; Anonymous, 1983). Although Brown et. al., (1983) reported the pathogenicity of R. cerealis from several sites in Michigan, this researcher has only rarely isolated any Rhizoctonia from patches in Michigan, and these isolates were non-pathogenic to Poa pratensis (Otto, unpublished data).

Distribution and Taxonomy

In 1984 Endo et al. reported L. korrae and three similar but sterile fungi to be a cause of spring dead spot (SDS) of Cynodon dactylon Pers. in the southwestern U.S. Spring dead spot was first described in the U.S. by Wadsworth and Young (1960), but no pathogen was consistently associated with it. L. korrae has yet to be associated with SDS in other parts of the U.S. L. korrae and L. narmari have been associated with SDS in Australia for many years however.

Smith (1965) described a patch disease of several turfgrasses in New South Wales and isolated a long-spored Pleosporaceous fungus which was shown to be capable of reproducing the symptoms of this disease. The fungus was identified as Ophiobolus herpotrichus (Fr.) Sacc.

In 1967 Smith showed that a different species with shorter spores was more frequently associated with this disease. This fungus was identified as a Leptosphaeria sp., later L. narmari. Re-examination of the O. herpotrichus cited by Smith showed it would better be placed in the genus Leptosphaeria and resembles L. korrae (Walker & Smith, 1972).

Another disease of Gramineae, Gaeumannomyces or take-all patch of Agrostis spp. was previously thought to be caused by the fungus Ophiobolus graminis (Sacc.), thus the original name, Ophiobolus patch (Asher & Shipton, 1981). In 1913 Lind listed O. graminis as a synonym for O. herpotrichus (Fr.) Sacc. This confusion shows the close relationship between these fungi and the difficulty in separating them taxonomically.

In 1952 von Arx and Olivier presented conclusive evidence that the take-all fungus did not belong in the genus Ophiobolus Riess. and established for it the new genus Gaeumannomyces (Diaporthales), type species G. graminis (Sacc.) v.Arx & Olivier. Later Walker (1972) established three varieties within this species namely, var. graminis, var. tritici J. Walker, and var. avenae (E.M. Turner)Dennis. The last being an important pathogen on Agrostis spp. in Europe, Australia, and North America. Hornby et. al.(1977) described a new species of Gaeumannomyces, G. cylindrosporus, from rotted roots of wheat and barley colonized by Phialophora radicicola var. graminicola (Deacon, 1974). The authors suspected that G. cylindrosporus may be its perfect state. The relationship between the three graminaceous pathogens is clear, although they are now classified in different orders, Gaeumannomyces (Diaporthales), Leptosphaeria (Pleosporales) in the Ascomycotina, and Phialophora (Hyphomycetales) in the Deuteromycotina (Asher & Shipton, 1981).

Leptosphaeria korrae has now been reported from many eastern as well as central and northwestern states on Poa pratensis L., and in the southwestern U.S. on Cynodon spp. To date the presence of P. graminicola associated with patch disease on Poa pratensis L. has been confirmed only in the eastern U.S. (Smiley & Craven-Fowler, 1984a).

Walker and Smith (1972) have reported L. korrae isolates from grasses of the genera Axonopus and Eremochloa as well as Cynodon. The isolates from Cynodon were also shown to be pathogenic to the cereals, Avena sativa L., Oryza sativa L., and Triticum aestivum L.

Pseudothecia of L. korrae were reported from fungal isolates obtained from Poa pratensis L. , Festuca rubra L., Agrostis spp., and Poa annua L. in New England (Jackson, 1984). Smiley & Craven-Fowler (1985c) have observed that perennial ryegrasses (Lolium spp.) and tall fescues (Festuca arundenacea Schreb.) are fairly resistant while fine fescues (Festuca rubra L.) and bentgrasses (Agrostis spp.) are susceptible. Worf et al. (1986) have reported moderate susceptibility of Poa annua L.; Agrostis palustris Huds. and Poa pratensis L. being about equally affected. Detailed studies including data were not reported.

Epidemiology

In discussing the epidemiology of patch diseases one must distinguish between above ground symptom development and the true infection process which results in colonization and interruption of root function. Smiley reported that *L. korrae* and *P. graminicola* infect roughly circular areas of *P. pratensis* causing stunting of the root mass well before foliar symptoms are expressed (Smiley & Craven-Fowler, 1984a, 1985d). It is difficult to detect the former without destroying the turf so the latter is most commonly dealt with in the literature and is what concerns most turf managers.

The epidemiology of necrotic ring spot (NRS) is still largely speculative and complicated by a range of other organisms which have been implicated in the etiology of patch diseases (Smiley & Craven Fowler, 1984a ; Worf et al., 1986; Smiley, 1983a). Smiley reported that the patch diseases with which *L. korrae* and *P. graminicola* are associated occur under the same cultural and environmental conditions as the *Fusarium* blight syndrome and that the diseases are indistinguishable (Smiley & Craven-Fowler, 1984a, 1983a). He indicates that whereas *P. graminicola* requires high temperature and drought or excess water for symptoms to appear, *L. korrae* may infect over a wider range of temperatures (Smiley & Craven-Fowler, 1985c). Some sources report most NRS disease in the hot summer months, June, July and August, (Worf et al., 1986), others in spring and fall with symptoms disappearing in the summer (Chastagner, 1984b; Jackson, 1984). _Fusarium_ blight was typically associated with drought stress during hot summer months (Bean, 1966, 1969; Fulton & Cole, 1973; Smiley, 1977b; Couch & Bedford, 1966; Funk, 1976; Partyka, 1976; Turgeon, 1976; Vargas, 1981), although it has been linked to alternating wet and dry periods, or heavy rainfall followed by sunny days (Fulton & Cole, 1973; Smiley et al., 1980b). Chastagner associates NRS symptoms with over-watered turf (1984b), while others (Worf et al., 1986; Endo & Colbaugh, 1974) reported symptoms to appear during dry conditions and disappear during wet weather.

In some cases the epidemiology may be complicated by a complex etiology. Smiley has reported isolating _P. graminicola_ and _L. korrae_ as well as other unidentified but similar fungi from a single patch (Smiley et al., 1984b), and the possible involvement of _R. cerealis_ has already been discussed. Thus interactions may occur between pathogens, or different pathogens may incite disease symptoms at the same location at different times. Most researchers agree, however, that patch symptoms most often appear as a direct result of infection by a pathogen and environmental stress due to growth of grasses under environmental conditions to which they are not adapted (Smiley et al., 1981, 1977; Partyka, 1976; Funk, 1976; Turgeon, 1976).

Other stress factors that have been shown to act as predisposing agents of patch diseases under some conditions include arsenate herbicides (Smiley, 1985a; Funk, 1976), high spring nitrogen rates (Turgeon, 1974, 1976), nematodes (Vargas & Laughlin, 1972), anaerobic thatch conditions (Smiley, 1980b), and phytotoxins (Fermanien et al., 1981; Smiley et al., 1985f).

In order to predict a disease epidemic the possibly complex etiology needs to be studied as well as the physical conditions of the site including grass cultivar, soil type, cultural practices, and environmental factors such as soil moisture, thatch and soil oxygen level, soil and air temperature, since the predominant cause for disease development probably varies from site to site and even at different locations within a grass stand.

Management

Mangagement of plant disease is best accomplished by an integrated approach to modify the environment in order to restrict the pathogen's activity by as many factors as possible. Management of fungal plant diseases is probably most commonly attempted through the use of antifungal chemicals. These compounds act by either inhibiting germination, growth and multiplication of the pathogen or by outright killing it. These compounds may be either man made (usually referred to as fungicides), or natural by-products of antagonistic microorganisms. The latter may either be produced in vivo by microooganisms in the vicinity of the pathogen, or produced in vitro and added to the natural system much like synthetic fungicides. Another means of managing plant disease is by cultural practices, especially water and fertility levels. Effects of fungicides and cultural practices have been extensively studied for Fusarium blight and somewhat for necrotic ring spot. The potential for bio-control has been suggested because of the similarity between necrotic ring spot (or Fusarium blight) disappearance and take-all decline in Triticum and Agrostis spp. (Smiley et al., 1984b; Worf et al., 1983, 1985).

In the period from 1966 to 1976 many experiments to control Fusarium blight involved the use of contact fungicides such as mancozeb, thiram, anilizine, difolitan, and chlorothalonil etc. (Bean, 1967, 1969; Couch & Bedford, 1966). Partial control was reported in some cases, but no consistent results were obtained. The introduction of systemic fungicides provided the first reports of consistent control. The benzimidazoles have been widely reported as effective when used as drenches (Cole, 1976; Cutwright & Harrison, 1970; Funk, 1976; Muse, 1971; Vargas & Laughlin, 1971; Turgeon, 1976) against Fusarium blight. However, the incidence of fungal resistance to this group is growing (Vargas, 1981; Cole, 1976; Smiley & Craven, 1977a). In recent work in vitro and in vivo with necrotic ring spot, success with the benzimidazoles, fenarimol, and propiconazol, and variable results with iprodione have been reported. Triadimefon has not been shown to inhibit *L. korrae* *in vitro* or *in vivo* (Chastagner, 1984b; Smiley & Craven-Fowler, 1984d; Worf et al., 1985; Otto & Vargas, 1985). Chemical control of this disease has been spotty at best, probably due in part to the difficulty of getting the chemical into the root zone due to hydrophobic thatch and soil and localized dry spots (Sanders & Cole, 1981). In addition, reports indicate that efficacy of some fungicides, including benomyl and iprodione may be strongly affected by soil type (Sanders & Burpee & Cole, 1978b). Repeated use of fungicide applications to reduce any common soil organism in a perennial crop will not provide a long-term solution to the problem and may only lead to a reliance on this form of management, while possibly causing other non-target effects on the plant and microbial community (Smiley & Craven, 1977a, 1978, 1979a, 1979b).

Little work has been reported with microbial antagonists in biological control of *Fusarium* blight or necrotic ring spot. Similarities have been noted however, between the way in which this disease declines after several years of severe symptoms and the phenomenon of take-all decline in *Triticum* and *Agrostis* spp. (Smiley et al., 1984b; Worf et al., 1983, 1985). Take-all decline has been associated with a build-up of fluorescent *Pseudomonads* and other bacteria on roots and along hyphae on wheat plants infected with *Gaeumannomyces graminis* (Asher & Shipton, 1981). The decline is usually seen after about 5 years of monocropping during which time take-all is active.

The production of antibiotics by bacteria has been proposed as a mechanism of suppression of *Gaeumannomyces* on the root. *In vitro* bioassays in which zones of inhibition between the fungus and bacterium are noted have been employed to test potential for biological disease control (Asher & Shipton, 1981; Cook & Baker, 1974). The relevance of antibiotic production on agar to protection of the plant in field conditions is debatable, however. Several studies have shown no correlation between *in vitro* studies and those done in the greenhouse or field (Sivasithamparam & Parker, 1978; Bowen & Theodorou, 1979; Cook & Baker, 1974; Asher & Shipton, 1981). Reports indicate that antagonism on agar differs greatly with different media, and the relative times of inoculation of fungus and bacterium. It was concluded that if antibiotics are produced in the rhizosphere in sufficient quantities to affect other organisms it is only one of several mechanisms involved in protection against plant pathogens (Asher & Shipton, 1981). Other mechanisms may include competition for nutrients, suitable sites, prior occupancy of such sites, and resistance mechanisms of the host.

With the evidence that natural disease suppression does occur, one must ask if ways exist to exploit this suppression by modifying the environment. Two means that have been attempted are through irrigation and fertility levels. Many sources have linked *Fusarium* blight and necrotic ring spot to drought stress, so management programs should include prevention and relief of this stress. Light midday irrigation is frequently recommended in the heat of the summer to not only eliminate drought stress but provide a cooling effect similar to the common practice of syringing golf greens (Beard, 1973; Vargas, 1981; Turgeon, 1976). Root infecting fungi such as *L. korrae* can cause major damage to the root system by severely reducing water absorption and nutrient uptake. An increased root volume will enable the plants to take up more water and nutrients. Conversely, by increasing the level of nutrients in the soil, less root volume will be needed to meet the needs of the plant. This is especially apparent in marginally fertile soils. The phenomenon by which plants grown in well fertilized soil avoid serious damage though the pathogen may be abundant has been referred to as the "disease escape mechanism" (Garrett, 1956). Although excessive stimulation of plant growth by high amounts of nitrogen in the spring and summer are discouraged (Smiley, 1983; Turgeon, 1974, 1976), adequate balanced fertility is necessary for recuperation of diseased areas, and deficiencies should be avoided (Smiley, 1983).

The objectives of the following studies were to:

1. Characterize and identify the ectotrophic fungi associated with patch symptoms in Michigan.
2. Determine the role of these fungi in this disease.
3. Begin to determine what conditions are necessary for infections to take place and for symptoms to be expressed.
4. Screen fungicidal controls _in vitro_ and _in vivo_ for inhibition to _L. korrae_.
5. Screen microbial antagonists against this fungus.
6. Perform field tests to help determine management strategies.

Chapter 2

Etiology of a Patch Disease of *Poa pratensis* L. in Michigan.

In 1984 dark, ectotrophic hyphae were first observed on the roots and crowns of infected grass plants from diseased patches of *Poa pratensis* in Michigan. The growth habit of these fungi closely resembled that of *Gaeumannomyces graminis*, the cause of take-all of *Agrostis palustris*. This corresponded to a period when researchers in many parts of the country began recognizing the presence of a group of root infecting fungi as possible incitants of what was once thought to be *Fusarium* blight.

MATERIALS AND METHODS

Symptomatology

Patch infected areas of *Poa pratensis* were observed *in situ* for signs and symptoms of disease, and pieces of sod were cut from the edge of such patches and brought to the laboratory for microscopic inspection. Individual plants from these samples were washed, and inspected under a binocular microscope and a compound microscope for any signs and symptoms of disease.

Association and Isolation

Diseased plant tissues were washed under running tap water for several hours, surface sterilized for varying amounts of time in 10% sodium hypochlorite, and diseased tissues or actual fungal mycelium were plated onto potato dextrose agar (PDA, Gibco Laboratories, Madison, WI) amended with 250 mg/l streptomycin and 250 mg/l penicillin, or water agar (Bacto-agar, Difco Laboratories, Detroit, MI). The plates were incubated on a laboratory bench until growth of potential pathogens was observed. Pure cultures of potential pathogens were obtained by transferring hyphal tips to PDA.

Pathogenicity

Inoculation studies were performed with two isolates of L. korrae on P. pratensis L. in growth chambers at several temperatures. Inoculum was prepared by autoclaving 60 ml of untreated Ionia wheat seed with approximately 60 ml of distilled water in 250 ml flasks for 45 minutes on two consecutive days. Agar disks of the respective fungal isolates taken from the edges of actively growing colonies on agar plates were then added. The seeds were incubated for at least 14 days before using.

The isolates used were:

Country Place Condominiums, Novi, MI
Woodland Trails Condominiums, Okemos, MI
New York state isolate, sent by Dr. R.W. Smiley, Cornell University.

To prepare plants for inoculation, 236 ml styrofoam cups were filled to within 1.5 cm from the top with autoclaved, prepared greenhouse soil mix, reported to be 1:1:1 peat:sand:soil. Small clumps of P. pratensis cv. Fylking, approximately 50 plants, were transplanted into the pots from trays of silica sand in which they had been grown for 3-4 weeks in the greenhouse. Three wheat seeds infected with an isolate or three sterile wheat seeds were placed in the root zone of four replicate pots. Pots were transferred to growth chambers at various temperatures and a 12 hour photoperiod. They were then incubated until symptoms appeared (approximately 5-6 weeks). At that point the roots were examined for evidence of fungal invasion and re-isolations were performed. These experiments were repeated several times.

Cultural/Growth Characteristics

Fungal specimens were compared to isolates of *L. korrae* and *P. graminicola* sent by Dr. R.W. Smiley, for such characteristics as mycelial appearance on PDA, growth rate, and pathogenesis.

Four isolates of *L. korrae* from Michigan were used in studies to find relative growth rate at four temperatures. Plates of Difco PDA were inoculated with a standard six mm plug of an *L. korrae* isolate taken from the edge of actively growing cultures. Four replicate plates were placed in growth chambers at four temperatures, 15, 20, 24, and 28 C, and allowed to incubate in the dark. Diameters of growth were measured at three days and nine days, and the difference was divided by six to obtain the growth per day.

Identification

Non-sporulating fungi resembling L. korrae were grown on sterile wheat seed as described in the pathogenicity section. This inoculum was mixed into flats of coarse sand that were seeded with Festuca rubra and placed on trays of water in the greenhouse. These flats were maintained in a saturated condition, and plants were pulled periodically and examined under a binocular microscope for signs of pseudothecia on stem bases and roots, a method suggested by Dr. R.W. Smiley, Cornell University (personal communication). Pots from growth chamber pathogenicity studies were also left saturated after ratings were taken and plants examined for pseudothecial inductions. Pseudothecia found on plant stems and roots were removed nine weeks after inoculation, crushed on a slide and observed under 40x of a compound microscope. At least two mature pseudothecia per isolate were observed and measurements of ten asci and ten ascospores (not of the same ascus) were read using an ocular micrometer.

RESULTS AND DISCUSSION

Symptomatology

The summer and early fall of 1984 brought reports of patch disease development similar to what had previously been described as Fusarium blight or yellow patch. Symptoms appeared as either small, 7-8 cm diameter areas of first wilted, then straw colored turf which spread throughout the season, or as rings of dead grass, 0.1-1 m in diameter (usually in the middle of this range). Sometimes damaged areas displayed a serpentine pattern, with only an occasional discrete patch or ring. No foliar lesions were associated with this disease, although at times a reddening of entire leaf blades in and around patches occurred. Observations of plants collected from the edge of diseased rings or patches showed dark, septate, "runner" mycelium along the outside of root and crown tissue and in advanced cases severe necrosis of root and crown tissues. Hyphopodia and infection pegs fitting the description of those from L. korrae were observed upon examination of root tissue under a compound microscope. Infection usually included invasion of the cortical tissues, and more rarely the vascular tissues of the root and crown.

Association and Isolation

L. korrae was isolated most consistently by washing the grass roots for several hours under running water, surface sterilizing 15-20 seconds in 10% sodium hypochlorite, rinsing in sterile distilled water, and placing mycelium-covered roots on PDA amended with 250 mg penicillin and 250 mg streptomycin per liter. This method eliminated nearly all bacterial growth. Faster growing fungi presented considerable problems since they often overran the plate before the slower growing L. korrae could become established. When fast growing contaminants were not present L. korrae could be observed growing from infected root and rhizome tissue by the fifth day. Our suspect isolates very much resembled the gray, felted mycelium described for growth of L. korrae on PDA (Walker & Smith, 1972; Smiley & Craven-Fowler, 1984a), and looked identical to the isolate that was sent to us by Dr. Smiley.

Approximately 24 samples from P. pratensis lawns exhibiting patch symptoms were examined in the 1984 season. Of these, all showed the characteristic microscopic symptoms on roots and crowns and an L. korrae - like fungus was isolated from three of the samples. The low percentage of samples from which L. korrae - like fungi were isolated may be attributed to the fact that effective isolation procedures were still being developed.

In 1985 approximately 20 samples from different locations were observed. All such samples exhibited dark brown ectotrophic mycelium on the roots and an L. korrae - like fungus was isolated from 10 of these samples.

In 1986, samples from approximately 12 different patch diseased P. pratensis lawns were examined. Of these, all revealed the characterisic dark, ectotrophic mycelium and isolations performed on three of the samples yielded L. korrae - like fungi. Roots from healthy areas revealed no such mycelium on roots.

From 1984-1986 isolations were performed from all sites of field tests. Each site yielded L. korrae or L. korrae - like fungi. Three of these isolates, Novi, Okemos, and HRC, were subsequently identified as L. korrae.

Pathogenicity

Symptoms from pathogenicity studies varied in severity from test to test, but included reddening and thinning of the stand, blackened, necrotic root tissue, and a reduced root mass as compared to the uninoculated control (Table 1). Re-isolations of *L. korrae* were successful in nearly 100 percent of cases from roots showing signs of necrosis and ectotrophic mycelium.

Table 1. Results of preliminary pathogenicity studies with three isolates of *Leptosphaeria korrae* on *Poa pratensis* cv. Fylking at 15, 24, and 28 C[a]

		Symptoms (+,-)[b] Temperature (C)		
Trial	Source of Isolate	15	24	28
1	Novi, MI	+	+	+
2	"	+	+	+
3	"	+	-	
1	Okemos, MI	-	-	-
2	"	+		-
3	"	+	-	
1	Cornell	+	-	+
2	"	-		
3	"	-		

[a]Growth chamber studies, 12 hour photoperiod.
[b](+)Symptoms included necrosis of leaves and roots, some reddening of leaves, and ectotrophic mycelium on roots. (-) indicates no symptoms developed.

From these preliminary results it was concluded that the Novi isolate was capable of producing symptoms at widely varying temperatures on P. pratensis cv. Fylking but that these symptoms were induced with less consistency using the Okemos isolate inoculum. Results with the Cornell University isolate showed nearly as much variability as did the Okemos isolate. Others have reported similar difficulties in working with this fungus (Smiley, personal communication), (Worf et al., 1985).

From growth rate tests on agar, it was clear that these fungal isolates are not necessarily stable in culture even when stored in sterile muck soil. Another possible explanation for lack of symptom development is the use of wheat seed that is not fully colonized by the fungus. Although this did not appear to be a problem, the internal colonization of the wheat seeds was only assumed. Different isolates may vary in their growth rate on artificial media. Invasion of such non-fully colonized wheat seeds by soil micro-organisms upon contact with unsterile soil may result in loss of viability of the inoculum.

Cultural/Growth Characteristics

In culture our isolates closely resembled those described by Walker and Smith (1972). Cultures began light gray to white, darkening from the center of the culture to a dark gray. The underside of the culture blackened as the culture aged. Table 2 lists growth rates for the four isolates used in this study. Differences in growth rate were noted between isolates but for all isolates the fastest growth was at 24 C (2.8-3.12 mm per day (radial)). This is close to the optimum temperature for L. korrae (25 C) (Walker & Smith, 1972; Smiley & Craven-Fowler, 1984a). Although the growth rate is somewhat less than the 4-5 mm per day rate cited in the literature, it is probably within reasonable limits. Problems were sometimes encountered with consistent growth in all replications. At times growth would stop as if a mutation had occurred. In such cases the plate was not figured into the average for growth rate.

Table 2. Average radial growth per day of four Leptosphaeria korrae isolates on potato dextrose agar

Source of Isolate	Average[a] Radial Growth[b] (mm) Temperature (C)[c] 15	20	24	28
Novi	2.2	2.3	3.1	-
Okemos	2.3	2.3	3.1*	-
HRC	2.0	2.3	3.0	-
Holland	1.8	2.0	2.8*	-
Novi	-	1.9*	2.6	0.7*
Okemos	-	1.6*	2.4	-
HRC	-	2.0	2.5*	0.5*
Holland	-	1.6*	2.0*	0.4
Novi	1.1	2.2	-	0.6
Okemos	0.9*	2.4	-	0.5
HRC	2.1	2.3	-	0.4
Holland	0.9*	1.9	-	0.4

[a]Values represent average of four replicates unless (*) indicates one value was discarded.
[b]Calculated from 1/6 of growth over a 6 day period.
[c]Incubated in the dark in growth chambers.

Identification

Flats of F. rubra planted in coarse sand showed reddened leaf blades, and culms and roots blackened with mycelium after four weeks in the greenhouse. At this time none of the plants which were pulled for observation showed signs of pseudothecial formation. Within six weeks stem bases and roots of the Novi isolate, showed black, flask-shaped structures resembling pseudothecia. Upon crushing, long transparent pseudoparaphases were released indicating that the structure was not mature. No such structures were found on the plants infested by the other three isolates before the experiment was terminated. Pseudothecia were also produced on P. pratensis cv. Fylking plants grown in greenhouse soil mix, incubated in a 24 C growth chamber, and kept nearly saturated with water for approximately eight weeks. Three isolates of L. korrae developed pseudothecia on plants that had been incubated for six weeks at 28 C at a 12 hour photoperiod, and transferred to 24 C and 24 hour light for three weeks. Pseudothecia were produced on both P. pratensis cv. Fylking and Adelphi. Measurements of asci and ascospores are listed in Table 3. No significant differences existed for ascospore length or width between the three isolates tested by LSD(.05). Values for these Michigan isolates compare favorably with those reported by Chastagner from Washington state, Smiley from New York, and Worf from Wisconsin. They are somewhat smaller than what Walker and Smith reported but are within reasonable limits.

Table 3. Mean length and width of asci and ascospores of three isolates of _Leptosphaeria korrae_

Isolate		length[a]		width[a]
Novi	asci	(104)121(140)	x	(11)12(15)
	ascospore	(92)122(127)	x	(2.7)3.5(4)
Okemos	asci	(120)132(136)	x	(11)12.5(17)
	ascospore	(93)115(136)	x	4
HRC	asci	(123)136(156)	x	(9)12(17)
	ascospore	(93)122(155)	x	(3)4(5)

[a]Numbers in () indicate high and low values.

Pseudothecial induction was never accomplished with isolates from the Holland or Dearborn site, therefore these isolates could not be positively identified as _L. korrae_.

Smiley and Craven-Fowler (1984a) and Endo et al.(1985) have discussed difficulties in both isolating and identifying _L. korrae_. They also refer to the presence of fungal isolates resembling _L. korrae_ from which pseudothecia were never obtained.

pa

se

th

in

Chapter 3

Pathogenicity/Epidemiology of *L. korrae*

Introduction

The following studies were performed to demonstrate the pathogenicity of several Michigan isolates of *L. korrae* on seven grass cultivars, as well as to attempt to delineate the conditions under which infection may occur *in vitro* and *in vivo*.

Host Range Study for Three Michigan *L. korrae* Isolates

In view of the paucity of information on the host range of *L. korrae*, host range studies were initiated for three Michigan isolates of *L. korrae* at two temperatures.

MATERIALS AND METHODS

Six different grass species were planted from seed and *Cynodon dactylon* was stolonized into a mixture of two parts Turface, (a Montmorillonite clay product of IMC Inc.), and one part steam sterilized greenhouse soil mix in 177 ml styrofoam cups that were filled to within 1.5 cm of the top. The grass species used included:

1. K-31 Tall Fescue (*Festuca arundenacea* Schreb.)
2. Penncross Creeping Bentgrass (*Agrostis palustris* Huds.)
3. Pennlawn Red Fescue (*Festuca rubra* L.)
4. Manhattan Perennial Ryegrass (*Lolium perenne* L.)
5. Bermudagrass (*Cynodon dactylon* (L.) pers.)
6. Adelphi Kentucky Bluegrass (*Poa pratensis* L.)
7. Fylking Kentucky Bluegrass (*Poa pratensis* L.)

Approximately 50 seeds of each species were planted at one point at the center of each cup, and the pots were placed on a tray of water in a plastic bag in the greenhouse until they germinated. The plants were maintained in the greenhouse at a height of two cm for the *Agrostis palustris* cv. Penncross and four cm for all of the other species.

When the plants were approximately one month old and had been trimmed several times, three replicate pots were inoculated with three wheat seeds per pot infested with one of the three isolates of *L. korrae*. Three pots were also treated with a control of sterile wheat seed. At this time the plants were moved to their respective growth chambers at 20 and 28 C. Plants were watered every two days until water drained out of the pots and were incubated on a 12 hour photoperiod. Plants were fertilized twice with 25 ml full strength Hoagland's solution. Ratings were taken on tops for percent necrosis after approximately six weeks, (four weeks for Fylking and Adelphi in trial I). Roots were checked for fungal colonization and re-isolations performed to confirm pathogenicity by L. korrae. Factorial analysis of variance was performed to test for differences in susceptibility between grass species, and to rank the grasses in order of susceptibility to L. korrae infection at two temperatures.

This experiment was performed twice.

RESULTS AND DISCUSSION

Significant differences were noted at the (.05) level for all factors in this experiment, isolate, temperature, and cultivar. Significant differences between isolates of L. korrae were obtained, but all isolates caused significantly more disease than the control.

Cultivar rankings obtained from factorial analysis of variance of two trials at two temperatures are summarized in Table 4. Table 6 shows rankings for cultivars averaged over all other factors.

Slight differences in the rankings for grass species (averaged over isolates), were noted between the 1st and 2nd run of this experiment (Table 4).

At 20 C the main difference between trial I and II was the drop of Adelphi from 2nd most susceptible to 3rd. In trial I Fylking and Adelphi showed severe symptoms approximately two weeks earlier than the other cultivars. Therefore they were rated at that time. Variability among the isolates was observed with Adelphi in trial I, however, accounting for the low average rating (Table 5).

At 28 C K-31 proved dramatically more susceptible in trial II than I, and Penncross dropped in susceptibility. F. rubra cv. Pennlawn, showed only intermediate susceptibility in most tests in contrast to Smiley's report (1985c) of susceptibility.

Table 4. Mean[a] percent leaf necrosis[b] of seven grass cultivars caused by inoculation with <u>Leptosphaeria korrae</u>[c] at 20 and 28 C

	20 C				28 C							
	Trial I		Trial II		Trial I		Trial II					
Grass Cultivar	Mean % necrosis		Mean % necrosis		Mean % necrosis		Mean % necrosis		Ave.[x] TI		Ave.[y] TII	
Fylking	81	*	**		90	*	**		86	*	**	
Adelphi	48	*	73	c	78	*	88	a	63	*	80	a
Penncross	64	a[z]	88	a	59	a	53	b	62	a	70	c
Pennlawn	58	b	78	b	49	b	84	a	53	b	81	a
Manhattan	50	c	70	d	48	b	82	a	49	c	76	b
K-31	48	c	60	e	28	c	88	a	38	d	74	b
Bermudagrass	40	d	40	f	31	c	40	c	35	d	40	d
LSD(.05)	4		2		7		7		4		4	

[a]Average of 3 replications and 4 inocula.
[b]Percent leaf necrosis = subjective estimate of % of necrotic leaf tissue/pot.
[c]<u>L. korrae</u> - Novi, Okemos, HRC isolates and sterile wheat seed control.
[x]Ave. TI = Average over 3 replications, 4 inocula, and 2 temperatures for trial I.
[y]Ave. TII = Average over 3 replications, 4 inocula, and 2 temperatures for trial II.
[z]Means followed by the same letter are not significantly different by LSD(.05).
*Fylking and Adelphi were rated at 4 weeks, other cultivars at 6 weeks in trial I. Therefore Fylking and Adelphi were not included in the ANOVA.
**Fylking was not included in Trial II.

Table 5. Average percent necrosis[a] induced on leaf blades of seven grass cultivars by three *Leptosphaeria korrae* isolates at 20 and 28 C

	20 C				28 C			
	Trial I		Trial II		Trial I		Trial II	
Source of Isolate	Mean % necrosis		Mean % necrosis		Mean % necrosis		Mean % necrosis	
Okemos	63	a[x]	73	ab	53	a	72	a
Novi	59	a	71	b	46	b	74	a
HRC	50	b	73	a	45	b	71	a
Check	35	c	52	c	28	c	62	b
LSD(.05)	4		2		6		6	

[a]Percent leaf necrosis = subjective estimate of % of necrotic leaf tissue/pot.

[x]Means followed by the same letter are not significantly different by LSD(.05).

Some variability or inconsistency in the virulence of this pathogen was noted. A factor that has distressed other researchers and has been reported in previous pathogenicity studies by this researcher.

Adelphi has been reported by Smiley and Craven-Fowler (1985c), and Worf et al. (1986) to be relatively tolerant to NRS. In these studies it did rank below the highly susceptible Fylking in susceptibility, but proved to be intermediate to highly susceptible in most tests. Adelphi did appear to be slightly more susceptible at 28 than 20 C. Lolium perenne (perennial ryegrasses), have been reported to be of low susceptibility (Jackson, 1984; Smiley & Craven-Fowler, 1985c), and our results with the cultivar, Manhattan would concur.

Since L. korrae has been reported to be a cause of spring dead spot of Cynodon dactylon, a Cynodon cultivar was included in this study. It was fairly resistant to infection, although a network of mycelium as well as sclerotia similar to Endo's description (1985) were present on the roots, and pseudothecia of the fungus formed on the roots after about six weeks under moist conditions and 28 C temperatures.

Because of variability in virulence of L. korrae, grass cultivar susceptibility varied somewhat between trial I and trial II, making an overall ranking difficult. Temperature had little consistent effect on grass susceptibility.

FIELD INOCULATIONS OF POA PRATENSIS L. CV. FYLKING WITH TWO ISOLATES OF L. KORRAE AND THREE TYPES OF INOCULUM

On May 8, 1985 a block of P. pratensis cv. Fylking at the Hancock Turf Research Center was inoculated with two different Michigan isolates of L. korrae. Disease incidence and progress was monitored over the course of two seasons.

MATERIALS AND METHODS

Three types of inoculum were used in this study, infested wheat seed, agar discs and infected grass plugs. Wheat seed inoculum was prepared as for previous pathogenicity studies. Agar inoculum was prepared by growing the L. korrae isolates on potato dextrose agar. When mycelial growth was nearly across the plate, the agar was cut into thirds and one such piece used for each inoculation site. The infected grass plugs used were from a greenhouse inoculation study performed in February of 1985 in which total kill of the plants did not occur. After the study was completed the plants were stored in the greenhouse until May, when they were transplanted to the Fylking block.

The south end of the Fylking block was measured off into 1.83 x 2.74 m plots. Three replications of each of the following nine treatments were randomly assigned to each plot.

<u>Treatments</u>
1. wheat seed - Isol #1 (Novi)
2. wheat seed - Isol #2 (Okemos)
3. wheat seed - Control
4. PDA disc - Isol #1
5. PDA disc Isol #2
6. PDA disc Control
7. Grass Plug - Isol #1
8. Grass Plug - Isol #2
9. Grass Plug - Control

Three points were inoculated in each plot. A tape was laid down the center of each plot north and south, and a hole was dug with a 2.54 cm diameter soil probe at a point .475 m, 1.372 m and 2.286 m from either the north or south end of the plot. Each plug of soil removed was separated at the interface between soil and thatch, the soil being returned to the hole. The appropriate type of inoculum was then placed into each of the three holes in each plot. Twentyfive wheat seeds were used for those treatments requiring such inoculum. Except for the holes which received grass plugs, the thatch and grass that were originally removed from each hole, were replaced above the inoculum.

In 1985 the plots were monitored weekly for disease development. Rainfall and average daily temperature were recorded throughout the summer. The percentage take of inoculations showing symptoms and the change in size of patches was monitored after patch symptoms started to develop.

In 1986 patch size was monitored bi-monthly starting in late April. A hygrothermograph was used to continuously record air temperature. Tensiometers were placed in the

Fylking block in mid June to measure soil moisture conditions. Evaporation from an open pan of water and rainfall were recorded daily throughout the summer. A disease progress curve was plotted from the patch measurements obtained this season.

RESULTS AND DISCUSSION

1985

By 8/23/85 the first patches started to appear on two plots inoculated with *L. korrae* isolate #1 (Novi) and #2 (Okemos). By 11/15/85 the following results were obtained for each inoculation treatment (Table 6).

Table 6. Percent of inoculation sites showing symptoms of necrotic ring spot on a *Poa Pratensis* cv. Fylking block at the Hancock Turf Research Center

Treatment	11/15/85	4/1/86	9/15/86
1. wheat seed - Isol #1	100	100	100
2. wheat seed - Isol #2	100	100	67
3. wheat seed - Control	0	0	0
4. PDA disc - Isol #1	89	89	89
5. PDA disc Isol #2	100	100	100
6. PDA disc Control	0	0	0
7. Grass Plug - Isol #1	67	22	100
8. Grass Plug - Isol #2	0	0	0
9. Grass Plug - Control	0	0	0

Wheat seed and agar inoculum were more effective than infected grass plugs in inducing necrotic ring spot under field conditions. This may be due to the additional nutritional source available to allow the pathogen to become established. The efficacy of infected grass plugs in causing disease also indicates the possibility for disease transmission through core cultivation, a threat that should be taken into consideration when recommending cultural practices for diseased areas. Spring dead spot has been reported to be transmitted on Cynodon dactylon by turf/soil cores (Pair, 1986). Chastagner (1985) also reported the infectious nature of necrotic ring spot. Further testing should be conducted on the effect of cultivation practices on spread of this disease.

SECONDARY SPREAD OF L. KORRAE IN A NATURALLY INFECTED AND INOCULATED POA PRATENSIS CV. FYLKING STAND

In order to gain insight into the ecology and epidemiology of *L. korrae*, natural infections and artificial inoculations on *P. pratensis* were monitored for disease progress while environmental conditions were recorded.

MATERIALS AND METHODS

In 1985 a block of *P. pratensis* cv. Fylking, 18.29 x 8.23 m, located at the Hancock Turfgrass Research Center at MSU was monitored for disease progress. The block contained several natural infections (patches or rings) at the start of the study. The diameter of these patches was monitored, as well as any new infections that occured throughout the course of the 1985 season.

Diameters of patches were measured weekly from 5/23/85 to 11/15/85. The area occupied by each patch was then approximated and the sum of the diseased areas for each date was divided by the total area of the block to determine the percentage area infected. This value was used as x in the tranformation, $\ln(x/(1-x))$, which was plotted against time. The apparent infection rate was then obtained from the slope of this curve. Average daily temperatures were calculated and recorded by a Reuter Stokes microprocessor. Rainfall was measured at the Hancock Turf Research Center.

In 1986 disease progress was monitored on this same block of grass by measuring the change in diameter of patches resulting from artificial inoculations the previous year. Tensiometers were made from one bar porous ceramic cups (Soil Measurement Systems, 1906 South Espina Street, Suite Six, Las Cruces, NM 88001). Bottoms of the porous cups were wrapped with electrical tape so that only one inch of the ceramic cup was exposed. Six tensiometers were buried within the 18.29 x 8.23 m block of grass in such a way that soil moisture was measured between one and two inch depths. Soil moisture measurements were made daily using a pressure transducer Tensimeter (Soil Moisture Equipment Corp. P.O. Box 30025 Santa Barbara, CA 93105).

Soil moisture was measured daily from 6/17/86 to 11/7/86 and for each date the high and the low value of six determinations were discarded and the other four values were averaged and plotted against time. Missing data were left off the plot. In 1986 daily high and low temperatures were obtained from a hygrothermograph at the plot site and these values averaged to obtain the average daily temperature which was plotted against time.

Soil samples were taken in October of 1986 with a 2.54 cm soil probe to a depth of five cm. Twenty samples were taken and the soil and thatch were divided and analyzed separately for pH and nutrient deficiencies.

RESULTS AND DISCUSSION

The slope of these disease progress curves (Figures 1 and 2) represent the apparent infection rate (r) (Table 7), a parameter that helps describe and predict the progression of epidemics. This parameter measures the pooled effects of inoculum production, dispersal, and infectivity (Huisman, 1982).

Table 7. Apparent infection rate[a] for *Leptosphaeria korrae*[b] on *Poa pratensis* cv. Fylking[c] during three periods in 1985

time period	app. infection rate(r)
5/24-9/13	.007566
9/13-10/11	.009738
10/11-11/8	.003851

[a]From slope of disease progress curve.
[b]Natural infections.
[c]At the Hancock Turf Research Center.

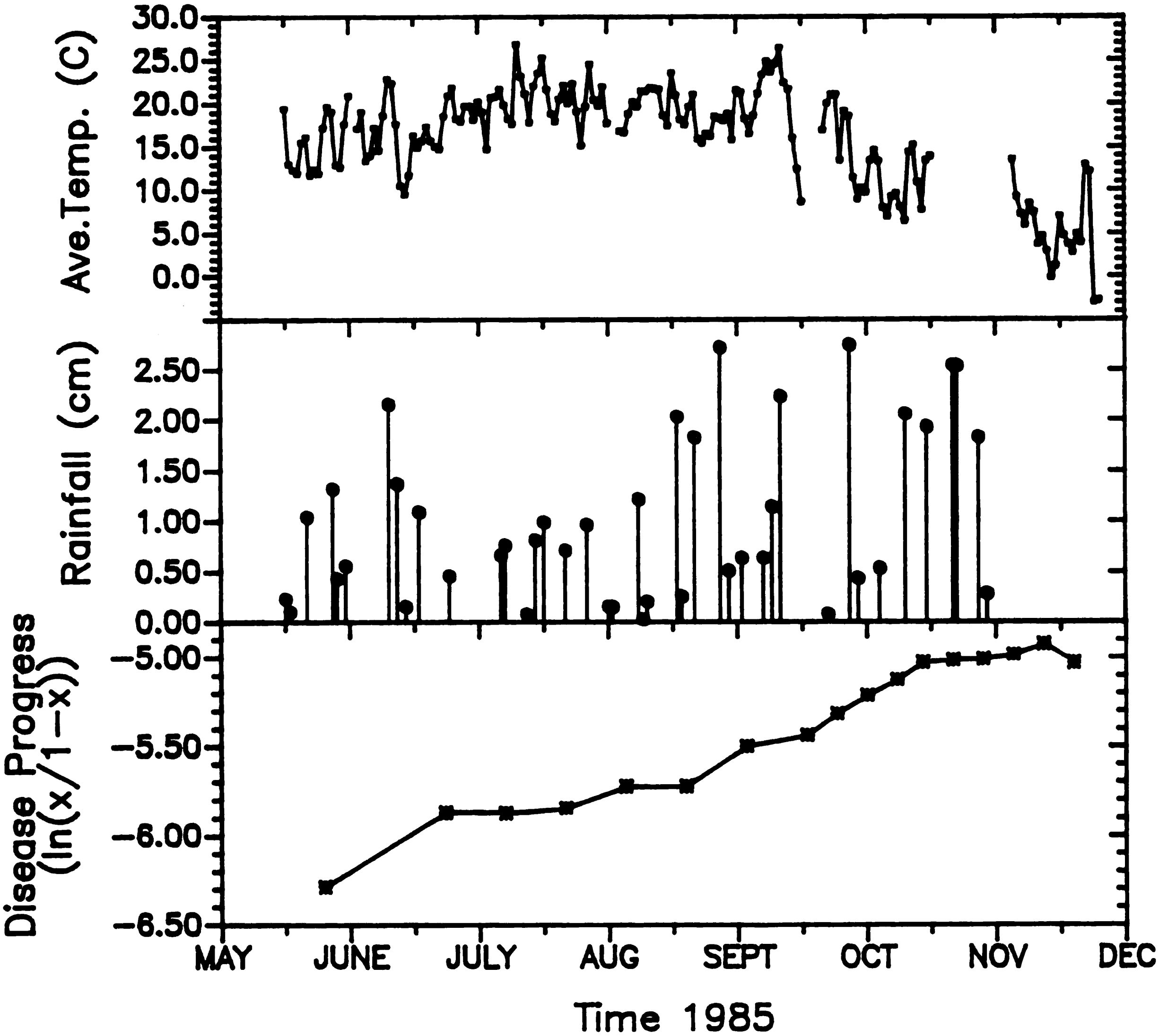
In 1985 a steady increase in disease symptoms was observed throughout the summer (Figure 1). A slight steepening of the disease progress curve was noted between September 13 and October 11, with a leveling off observed between October 11 and November 8. Although soil water potential was not measured in 1985, no great fluctuations in disease symptoms occurred throughout the season and the rainfall was distributed fairly evenly as well. Since irrigation was only applied at wilt, rainfall was the major contributor to soil moisture. Some evidence was observed that nitrogen played a part in disease severity since slight retardation in disease progress was noted following the July, August and September fertilizer applications.

Table 8. Nitrogen applications[a] in 1985 and 1986 at the Hancock Turf Research Center

1985	1986
June 11,	June 27
July 15	Aug 21
Aug 15	Sept 14
	Nov 4

[a]24 kg/ha of 46-0-0.

Figure 1. Necrotic ring spot progress, daily rainfall and average daily temperature for a block of <u>Poa pratensis</u> cv. Fylking at the Hancock Turf Research Center in 1985.



30.0
25.0
20.0
15.0
10.0
5.0
0.0
Ave.Temp. (C)
2.50
2.00
1.50
1.00
0.50
0.00
Rainfall (cm)
-5.00
-5.50
-6.00
-6.50
Disease Progress (ln(x/1-x))
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
DEC
Time 1985

Figure 1

In 1986 the most dramatic decreases in disease were noted during periods of prolonged very moist soil conditions (Figure 2). This occurred from mid July to early August, due to heavy irrigation and mid September to mid October due to heavy rainfall. From these results it appears that soil moisture at least in the absence of other environmental deficiencies is a major contributor to the transience of the disease symptoms, if not actual development of the disease. This agrees with findings of Worf (1986), who reported that heavy rains interrupted symptom development.

No direct relationship was noted in either 1985 or 1986 between average daily temperature and disease progress except that the greatest diseased area was present during mid to late October when the average daily temperature was generally less than 15 C. The period of sharpest disease progress in 1986 was after October 1st and that period corresponded to a sharp dropoff in average daily temperature, as well as little rainfall and a decrease in soil water potential (Figure 2).

Figure 2. Necrotic ring spot progress, soil moisture, weekly rainfall and average daily temperature for a block of *Poa pratensis* cv. Fylking at the Hancock Turf Research Center in 1986.

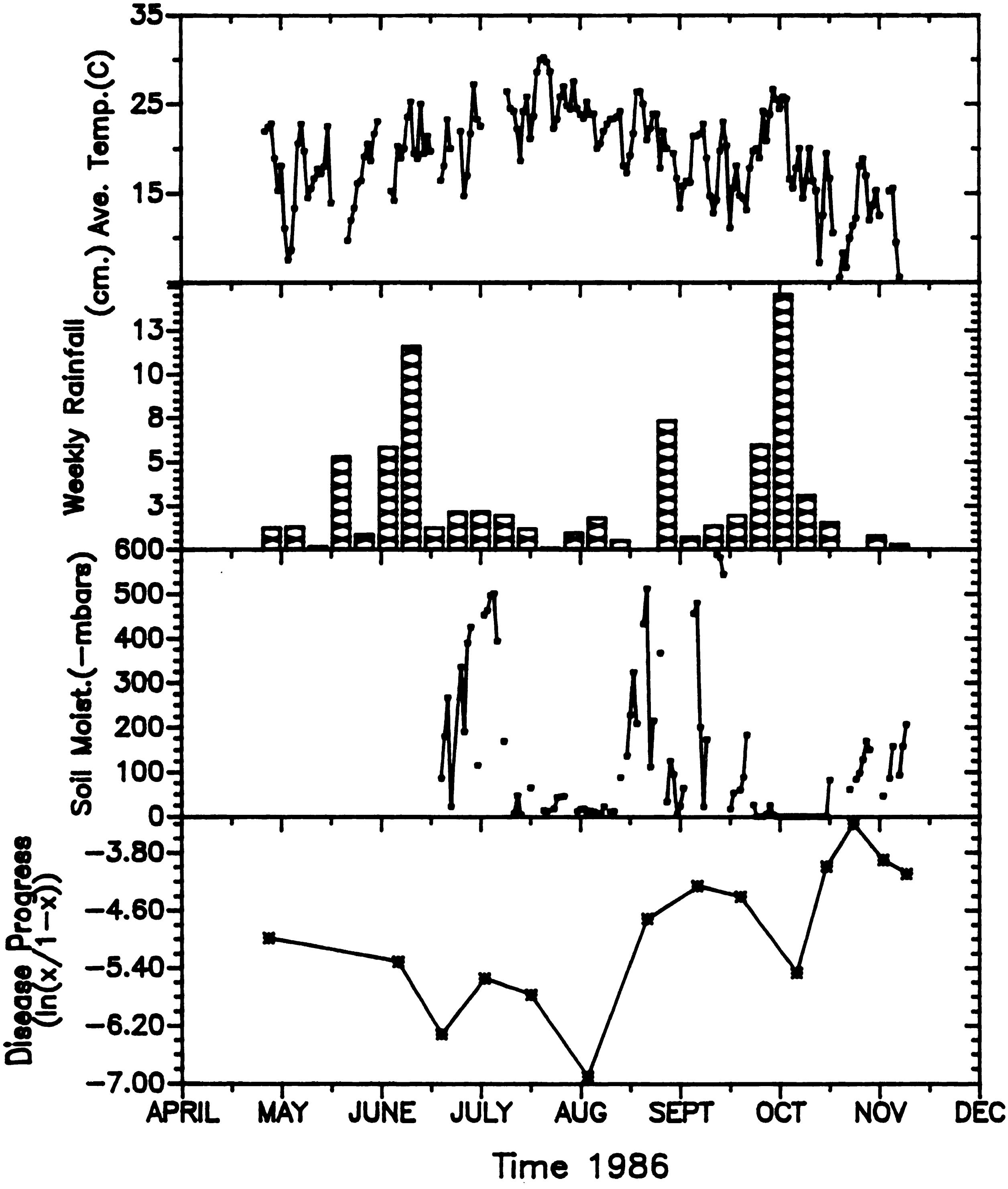

35
25
15
(cm.) Ave. Temp.(C)
13
10
8
5
3
Weekly Rainfall
600
500
400
300
200
100
0
Soil Moist.(—mbars)
-3.80
-4.60
-5.40
-6.20
-7.00
Disease Progress (ln(x/1—x))
APRIL
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
DEC
Time 1986

Figure 2

Thatch and soil fertility analyses performed in October 1986 showed the P levels in both layers as well as the K level in the soil to be low (Table 9). One effect nutrients e.g. N, P and K have on plant growth is stimulation of root production which often enables a plant to escape severe damage by root pathogens.

Table 9. 1986 Soil Tests[a] _Poa_ _pratensis_ cv. Fylking
Hancock Turf Research Center

	pH	P	K	Ca	Mg
			(kg/ha)		
thatch	7.0	40	376	4056	713
soil	7.4	24	92	2131	440

[a]21 samples to 5 cm depth, thatch and soil separated. Analyzed at the Michigan State University Soil Testing Lab.

Another factor that should be considered in this study is the use of the very susceptible cultivar, Fylking. Less susceptible cultivars may not have shown as great a fluctuation in disease response to changes in soil moisture as was observed on this cultivar at this location.

Environmental conditions, including soil pH, nutritional levels, soil moisture levels, temperature, as well as pathogen population all play a part in disease epidemics. Since _L._ _korrae_ is known to be present in many stands even where no disease symptoms are apparent above ground (Smiley & Craven-Fowler, 1984a, 1985c, 1985d), environmental conditions which increase the stress on the host plant and/or enhance the ability of the fungus to infect are the parameters that need to be measured in order to predict an outbreak of symptoms.

In 1986 the greatest factor affecting disease progress appeared to be soil moisture, with disease symptoms increasing as the soil dried and disappearing when the moisture increased to saturation. Data from 1985 do not disagree with these results, although soil water potential was not measured that year.

Further studies at more locations are needed to determine the nature of the relationship between soil water potential and necrotic ring spot. In addition, controlled studies in the laboratory and growth chamber should be implemented to determine how different water potentials affect growth of *L. korrae* on agar, and pathogenicity *in vivo*. Oxygen level of the thatch layer, especially during and after periods of saturation is another factor that should be monitored.

Effects of Irrigation on Turf Quality and Necrotic Ring Spot Development on *Poa Pratensis* cv.Baron/Bristol/Victa.

In 1986 an irrigation study was established on blocks of *P. pratensis* cv. Baron/Bristol/Victa which had been inoculated in late November 1985 and May 1986 with combinations of *Phialophora graminicola* and *Leptosphaeria korrae*, the causal fungi of summer patch and necrotic ring spot (NRS) respectively. The purpose of this study was to test the effects of three different irrigation regimes on patch disease development and general turf quality.

MATERIALS AND METHODS

This inoculation study was designed as a 3x3x12 factorial with 3 irrigation regimes, 3 types of sod and 12 different combinations of test organisms including controls. The irrigation blocks were inoculated with different combinations of four test organisms on two occasions, November 26-27, 1985 and May 5-6, 1986. Wheat seed inoculum was used in all cases. The test organism treatments included:

1. *L. korrae* - isolate #1 (Novi)
2. *L. korrae* - isolate #3 (HRC)
3. P. graminicola - isol. #1 (Walnut Hills)
4. *P. graminicola* - isol. #2 (Orchard lake)
5. 1 + 2
6. 1 + 3
7. 1 + 4
8. 2 + 3
9. 2 + 4
10. 3 + 4

11 & 12 = controls

The three irrigation regimes begun July 5 included, 0.25 cm applied daily at noon, 80% of pan evaporation applied twice weekly, and no irrigation. Within each irrigation block are three types of sod which were established in the fall of 1984. These include mineral soil sod, muck soil sod, and seeded.

Tensiometers were placed, two per sod block as described in the previous section in late June 1986. Soil moisture readings were taken daily to check the distribution of the irrigation treatments.

RESULTS AND DISCUSSION

As of 8/15/86 the non-irrigated blocks showed an obvious reduction in turf quality and density. Several of the thinned areas were sampled and isolations were performed from necrotic root tissue. *L. korrae* was obtained from a small percentage of isolations (approximately five percent). The possibility exists therefore that these generalized symptoms may be due in part to pathogen attack.

Of the three types of sod, the seeded had the best quality rating followed by the muck sod. The mineral soil sod blocks had the thinnest plant density of the three, and in the non-irrigated blocks, showed signs of dying out similar in symptoms to a patch disease. As of November 15, no symptoms of NRS or summer patch were noted at any of the artificial inoculation sites.

The diffuseness of the symptoms that did appear, and the general lack of symptoms where *L. korrae* inoculum was placed may be due in part to the use of a blend of *Poa pratensis* containing three, only moderately susceptible cultivars.

Chapter 4

Studies in the Management of _L. korrae_.

Introduction

No chemicals were registered for necrotic ring spot management at the initiation of this study. Presently only two fungicides, iprodione and fenarimol, are registered for NRS, but several other systemic fungicides are registered for _Fusarium_ blight. In view of this and the lack of consistent chemical control methods for this disease, several fungicides were screened _in vitro_ against _L. korrae_. Studies were also performed _in vivo_ in the greenhouse and the field to further test these chemicals. In addition, several microorganisms contained in Lawn Restore fertilizer, (a product of Ringer Corp. that proved efficacious in previous field tests (Ross & Vargas, 1984), were screened for antagonism _in vitro_ to _L. korrae_. Green Magic fertilizer (Agro-Chem Inc), another product which gave good preliminary results, was used in an _in vitro_ bioassay to test for anti-fungal activity. Both Lawn Restore and Green Magic underwent further field tests including different timings in an effort to develop a management program for necrotic ring spot.

In Vitro Fungicide Bioassay of Four *L. korrae* Isolates

In order to determine the effect of several antifungal compounds on the mycelial growth of four Michigan *L. korrae* isolates, *in vitro* fungicide bioassays were performed.

MATERIALS AND METHODS

Eight fungicides were tested in this study, including three benzimidazoles, benomyl, thiophanate, and thiophanate-methyl, two dicarboximides: vinclozalin and iprodione, and the sterol inhibitors, propiconazol, fenarimol and triadimefon.

Fungicides were suspended in sterile distilled water at a rate of 10,000 ug ai/ml and aliquots were pipetted directly into partially cooled, autoclaved PDA to give concentrations of 1, 10, and 100 ug ai/ml. Approximately 20 ml of agar was poured into each sterile 15 x 100 mm petri dish. Six mm diameter agar plugs were taken from the margins of actively growing fungal colonies, inverted and transferred to the center of each of four replicate plates of each fungicide concentration, and to four non-amended PDA plates. The plates were incubated on the laboratory bench, and fungal colony growth was measured along the same diameter at 7, 10, and 14 days after inoculation. The 14 day diameters were chosen for factorial analysis of variance because of good differentiation between the fungicide treatments. LSD's were calculated for each isolate. This experiment was performed twice.

RESULTS AND DISCUSSION

Table 10. Diameters of growth[a] (mm) of *Leptosphaeria korrae*[b] on agar amended with 1, 10, or 100 ug/ml of eight fungicides

	Diameter of Growth (mm)		
Treatment	1 ug/ml	10 ug/ml	100 ug/ml
Fenarimol	6.5 A[z]	0.0 A	0.0 A
Propiconazol	26.5 B	0.8 A	0.0 A
Iprodione	45.8 C	21.0 B	26.2 B
Benomyl	65.5 D	0.0 A	0.0 A
Thiophanate	67.0 D	4.0 A	0.0 A
Vinclozalin	67.0 D	39.8 C	29.2 B
Triadimefon	68.2 D	67.0 D	53.0 C
Th-methyl	68.8 D	5.8 A	0.0 A
PDA	71.0 D	71.0 D	71.0 D

LSD(.05)=4.61
LSD(.01)=6.28

[z]All treatments followed by the same letter are not significantly different at the (.01) level by LSD.
[a]Diameters measured at 14 days.
[b]Isolate #1 (Novi).

Results of the two trials of this experiment were nearly identical so results of trial 1 are summarized in Table 10 and Figures 3-5. Analysis of variance of the means for each isolate showed no significant differences between the four isolates. Therefore the results for Isolate #1 (Novi) are given. This isolate was obtained from a site where extensive field trials have been conducted by our laboratory for the past four years.

A general ranking based on significance at (P=.01) showed fenarimol to be the most inhibitory at the 1 ug/ml rate, followed by propiconazol, and then by iprodione which was significantly more inhibitory than all the rest.

At the 10 ug/ml rate iprodione fell behind the benzimidazoles and fenarimol and propiconazol. Triadimefon remained ineffective at inhibiting fungal growth.

At 100 ug/ml fenarimol, propiconazol, benomyl, thiophanate, and thiophanate-methyl were able to completely inhibit mycelial growth. Iprodione and vinclozalin both provided approximately 50% reduction in growth and triadimefon only slightly inhibited fungal growth.

The effect of rate on fungicide efficacy was most apparent among the benzimidazoles (Figure 3). Thiophanate, thiophanate-methyl, and benomyl were all highly effective at the 10 and 100 ug/ml rate but were not effective at 1 ug/ml. The dicarboximides, vinclozalin and iprodione gave similar dose-response curves. Iprodione significantly inhibited L. korrae growth at 1, 10, and 100 ug/ml, while vinclozalin was significantly better than the control only at 10 and 100 ug/ml (Figure 4). Of the sterol inhibitors, fenarimol and propiconazol were highly inhibitory at all three rates, and triadimefon was significantly different than the control only at the 100 ug/ml rate (Figure 5).

Results of this study coincide with reports of in vitro control of L. korrae reported in the literature (Chastagner, 1984b; Smiley & Craven-Fowler, 1984d; Worf et al., 1985).

Figure 3. Effects of three benzimidazole fungicides on growth of *Leptosphaeria* *korrae* *in* *vitro*

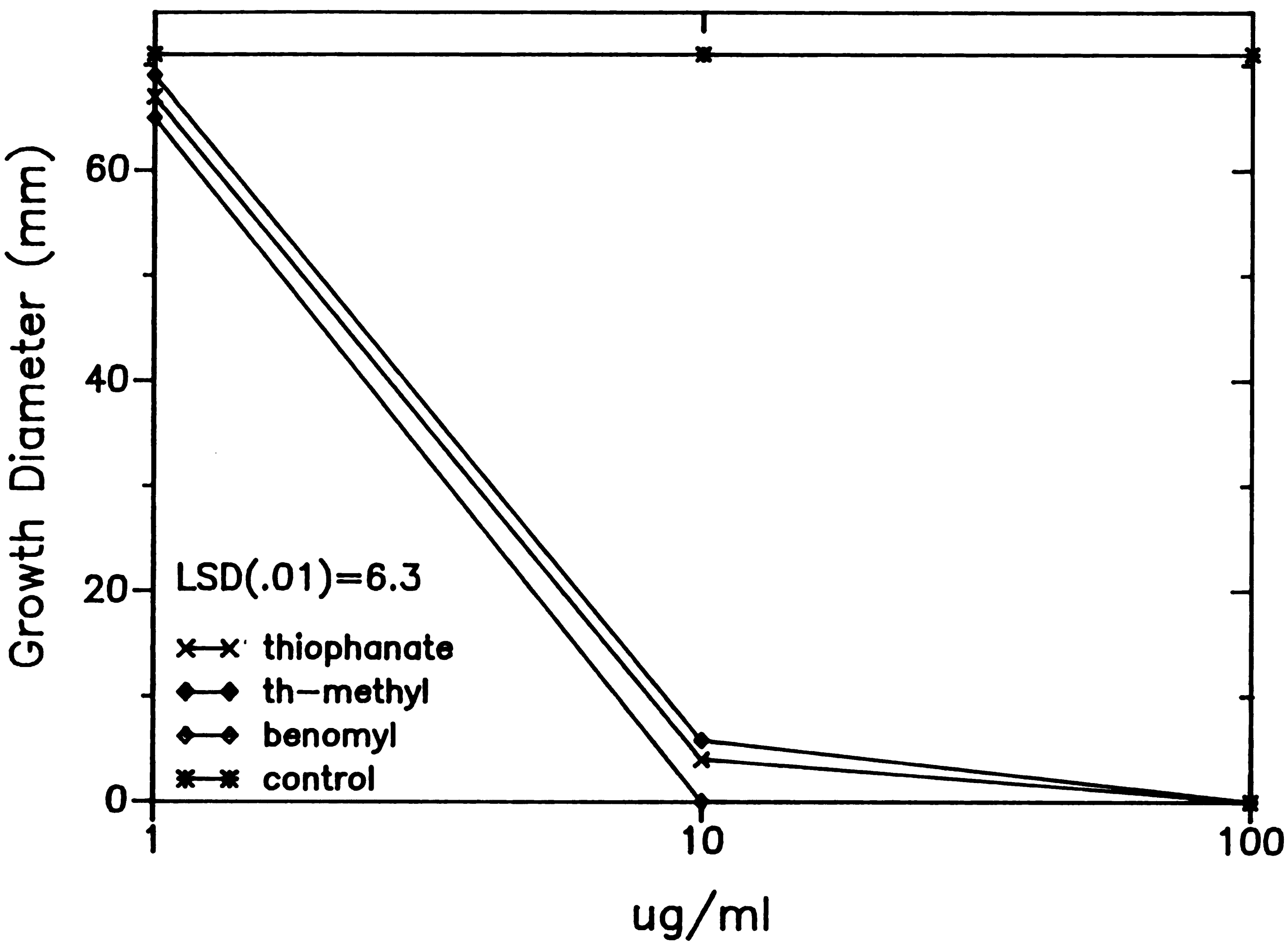

Growth Diameter (mm)
60
40
20
0
1
10
100
ug/ml
LSD(.01)=6.3
thiophanate
th-methyl
benomyl
control

Figure 3

Figure 4. Effects of two dicarboximide fungicides on growth of *Leptosphaeria korrae in vitro*

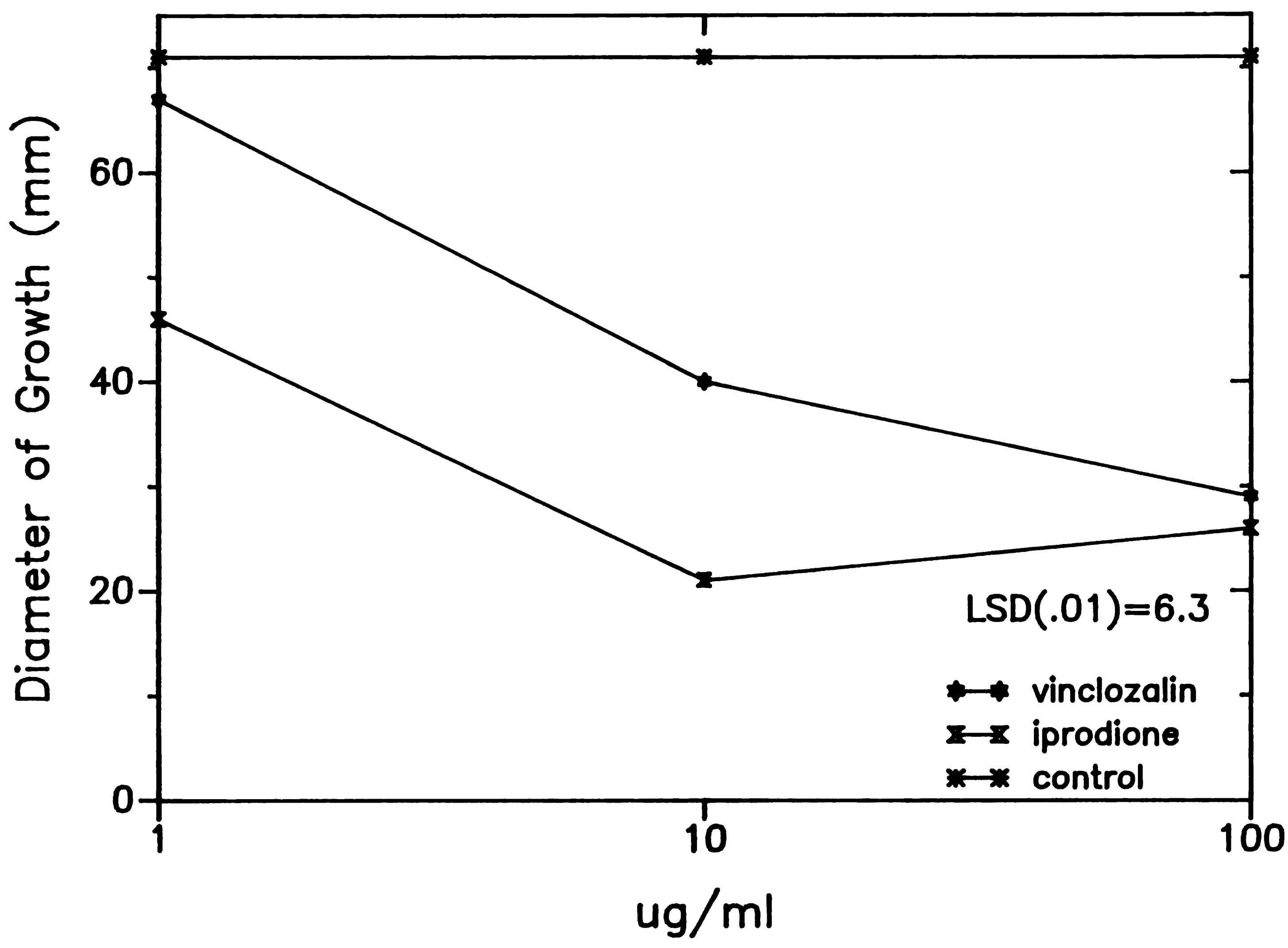

Diameter of Growth (mm)
60
40
20
0
1
10
100
ug/ml
LSD(.01)=6.3
vinclozalin
iprodione
control

Figure 4

Figure 5. Effects of three sterol inhibiting fungicides on growth of *Leptosphaeria korrae in vitro*

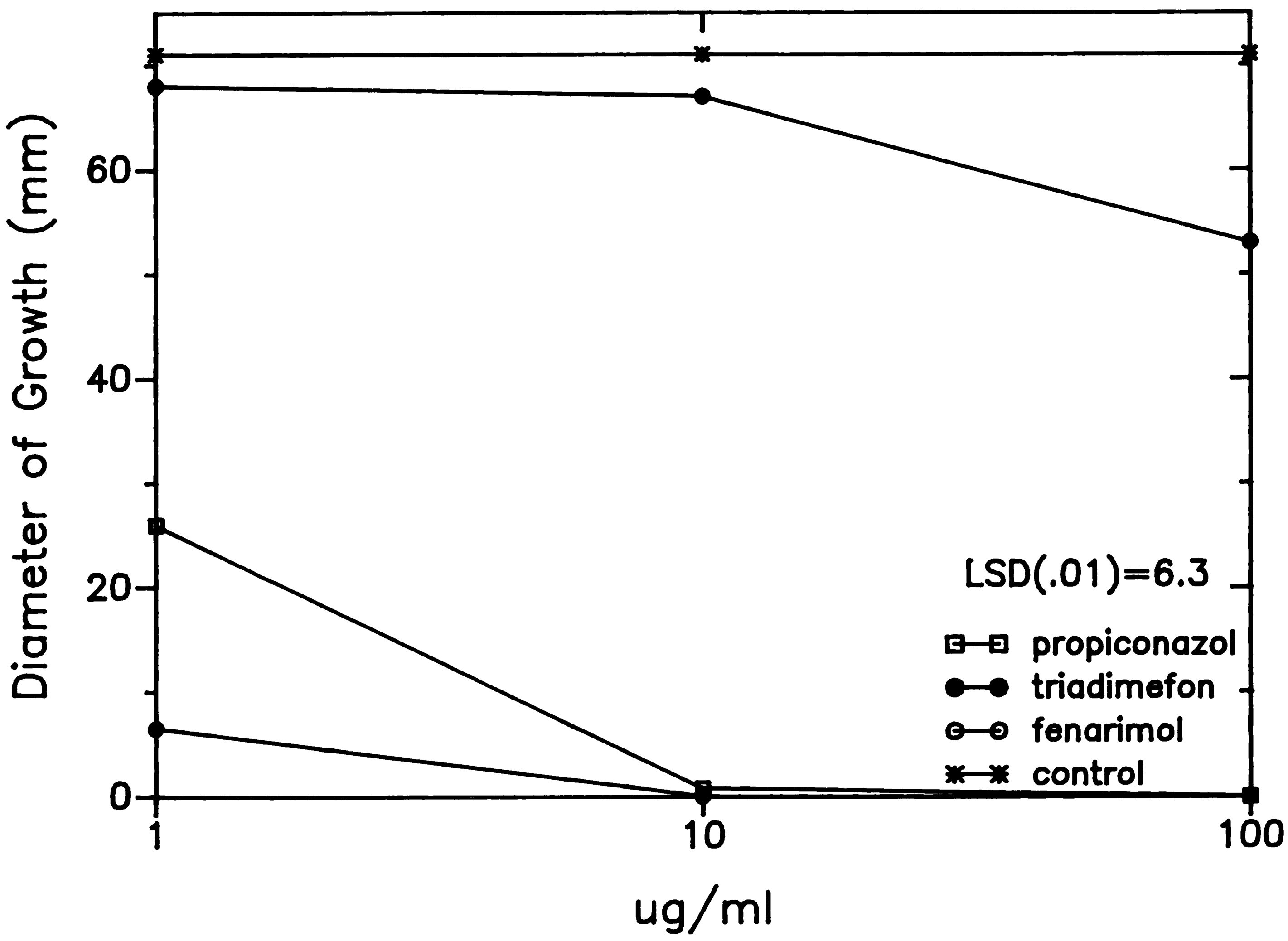

Diameter of Growth (mm)
60
40
20
0
1
10
100
ug/ml
LSD(.01)=6.3
propiconazol
triadimefon
fenarimol
control

Figure 5

In vivo Fungicide Bioassays

Three fungicide bioassays were performed in the greenhouse to test the efficacy of several fungicides as well as some biological control products in controlling necrotic ring spot on *P. pratensis* cv. Fylking.

MATERIALS AND METHODS

Preventive Greenhouse Fungicide Bioassay

The first of these studies was designed to test the protective effect of eight fungicides at three rates. Sterile, 76.2 mm diameter plastic pots were filled with sterile greenhouse mix. The surface was evenly seeded with approximately one ml of Fylking seed. Three weeks later, five wheat seeds infested with *L. korrae* isolate #1 (Novi) were placed ten mm beneath the soil surface in these pots. After two weeks no disease symptoms had occurred and three replicate pots were given drench treatments of the following fungicides, at the rate of 1.5, 3, and 6 Kg ai/ha.

1. Iprodione (Chipco 26019, Rhone Poulenc, Monmouth Junction, NJ)
2. Benomyl (Tersan 1991, E.I. DuPont de Nemours, Wilmington, De)
3. Vinclozalin (Vorlan, Mallinkrodt, St. Louis, Mo)
4. Thiophanate-methyl (Fungo-50, Mallinkrodt)
5. Thiophanate (Cleary's 3336, Cleary Chem. Corp., Somerset, NJ)
6. Triadimefon (Bayleton, Mobay, Indianapolis, In)
7. Fenarimol (Rubigan, Elanco, Indianapolis, In)
8. Propiconazol (Banner, Ciba-Geigy, Greensboro, NC)

Three weeks after treatment, control pots showed definite reddening of leaf blades which is considered a symptom of necrotic ring spot. At this time the severity of this symptom seemed to vary between treatments so the number of reddened leaf blades per pot was used as a measure of disease severity. In addition to this, the root systems of ten plants per pot were examined under a dissecting microscope for ectotrophic mycelium characteristic of L. korrae and the number of plants with such mycelium was recorded. Re-isolations were performed from such root segments. These data were subjected to 8x3 factorial analysis of variance and LSD analysis.

Curative Greenhouse Fungicide Bioassays (CGFB)

101.6 mm diameter plastic pots were seeded with P. pratensis cv. Fylking and were inoculated on 5/7/85 with five L. korrae isolate #1 (Novi) infested wheat seeds. These pots were used for all subsequent in vivo fungicide bioassays. These plants were fertilized with Hoagland's solution once monthly and maintained at approximately 25.4 mm height of cut in the greenhouse.

CGFB I

Two month old plants which had been showing the reddened leaf blade symptom for approximately one month were drenched with the following five fungicides:

1. Iprodione
2. Benomyl
3. Triadimefon
4. Fenarimol
5. Propiconazol

Five replications and two rates (1.5 and 6 kg ai/ha) of each fungicide were used. Five inoculated pots were not treated and five pots were left uninoculated as controls. Plants were maintained in the greenhouse and watered lightly at midday. Percent necrosis ratings of tops were taken one month and five months after treatment.

CGFB II

This bioassay was performed on six month old Fylking which had been repotted once and root pruned once, fertilized with Hoagland's solution monthly and maintained at about 25.4 mm in the greenhouse.

In this study the same five fungicides and two rates were used as in the previous study. In addition a 480 kg/ha rate of Lawn Restore (equivalent to 48 kg N/ha), and an equivalent rate of 10-4-4 urea fertilizer were applied to four replications of pots. Plants were maintained in the greenhouse as before. Percent necrosis of tops was again recorded.

RESULTS AND DISCUSSION

Preventive Bioassay I

8x3 factorial analysis of variance showed no significant differences for rates (P=.05). Significant differences were seen for treatments with LSD(.05), however.

The following rankings were obtained from averaging replications and rates for each fungicide.

Table 11. Effects of eight fungicides in preventing symptom development[a] in the greenhouse on Poa pratensis cv. Fylking inoculated with Leptosphaeria korrae[b]

Treatment	mean[c] no. red leaves per pot		mean[c] no. roots w/ectotrophic mycelium[d]	
Propiconazol	2.3	A[z]	6.8	AB
Iprodione	2.7	A	5.9	A
Benomyl	3.0	AB	5.6	A
Vinclozalin	3.9	AB	8.7	C
Fenarimol	5.0	AB	8.8	C
Thioph-methyl	7.1	BC	5.6	A
Thiophanate	10.0	CD	8.1	BC
Check (no fungic)	11.2	D	9.2	C
Triadimefon	12.2	D	7.9	BC
	LSD(.05)=4.17		LSD(.05)=1.82	

[z]Treatments followed by the same letter are not significantly different at the (.05) level by LSD.
[a]Counts taken 3 weeks after treatment.
[b]L. korrae isolate #1 (Novi).
[c]Average of ratings for 3 replications and 3 rates (1.5, 3, 6 kg a.i./ha).
[d]Based on 10 plants examined under a dissecting microscope.

Table 11 shows that when the number of reddened leaf blades per pot was used as an indicator of disease, propiconazol, iprodione, benomyl, vinclozalin, fenarimol and thiophanate-methyl were all significantly better at preventing disease development (P=.05) than no treatment. When the number of root systems out of ten plants, which contained ectotrophic mycelium was used as the disease measurement, thiophanate-methyl, benomyl, iprodione and propiconazol all showed significantly less disease than the check. Since the re-isolations performed yielded *L. korrae* in nearly all cases, the ectotrophic mycelium was concluded to be *L. korrae*.

Curative Greenhouse Fungicide Bioassay I

CGFBI

Table 12. Effects of five fungicides, applied curatively, on percent leaf necrosis of Poa pratensis cv. Fylking inoculated with Leptosphaeria korrae[a] in the greenhouse

Treatment	Rate	mean % leaf necrosis[b] 1 mo.	5 mo.
no inoculum		14 A[z]	10 A
Benomyl	6.0 kg/ha	26 A	86 D
Benomyl	1.5 kg/ha	42 B	88 D
Propiconazol	1.5 kg/ha	48 B	28 B
Iprodione	6.0 kg/ha	50 B	90 D
Iprodione	1.5 kg/ha	52 BC	88 D
no fungicide		64 CD	86 D
Fenarimol	6.0 kg/ha	64 CD	48 C
Fenarimol	1.5 kg/ha	76 DE	50 C
Triadimefon	1.5 kg/ha	78 E	90 D
Triadimefon	6.0 kg/ha	82 E	90 D
Propiconazol	6.0 kg/ha	86 E	30 B
		LSD(.05)=1.261	LSD(.05)=0.571

[z]Treatments followed by the same letter are not significantly different at the (.05) level by LSD.
[a]L. korrae isolate #1 (Novi).
[b]Based on an estimate of proportion of necrotic leaf tissue/pot.

Table 12 summarizes the results of two-way analysis of variance of percent leaf necrosis ratings taken one month after and five months after treatment. In both ratings the check treatment which received no inoculum and no fungicide rated significantly better than the rest. One month after treatment, both rates of benomyl, 1.5 kg propiconazol, and 6 kg iprodione all were significantly better than the no fungicide treatment. After four additional months in the greenhouse, however, both rates of propiconazol ranked significantly better than any of the other treatments followed by the two rates of fenarimol; in fact these were the only treatments that still appeared green. All other treatments were not significantly better than the check. These results, although never reproduced to this degree, indicate that the initial favorable effect produced by benomyl may be transitory, while fenarimol and propiconazol, especially at the higher rates may have an initial phytotoxic effect but also a greater long-term fungicidal effect. Some phytotoxicity has also been noted with fenarimol in field studies on P. pratensis at high rates, 24 and 48 kg/ha, (R. Detweiler, personal communication).

Curative Greenhouse Fungicide Bioassay II

CGFB II

Table 13. Effects of five fungicides and two fertility supplements, applied curatively, on percent leaf necrosis of *Poa pratensis* cv. Fylking inoculated with *Leptosphaeria korrae*[a] in the greenhouse

Treatment	Rate	mean % leaf necrosis[b]
Propiconazol	1.5 kg/ha	4.0 A[z]
10-4-4	480 kg/ha	5.0 AB
Lawn Restore	480 kg/ha	6.5 B
CM-A1	6.2 kg/ha	6.5 BC
Benomyl	6 kg/ha	6.75 CD
Propiconazol	6 kg/ha	7.25 CDE
Fenarimol	1.5 kg/ha	7.25 CDE
Benomyl	1.5 kg/ha	7.25 CDE
Iprodione	6 kg/ha	7.5 CDEF
Iprodione	1.5 kg/ha	8.0 CDEFG
CM-A2	6.2 kg/ha	8.25 DEFG
CM-R1	6.2 kg/ha	8.5 EFG
YMG broth control	25 ml/pot	8.75 EFG
Triadimefon	1.5 kg/ha	9.0 FG
Triadimefon	6 kg/ha	9.0 FG
No treatment		9.0 FG
Fenarimol	6 kg/ha	9.5 G

LSD(.05)=1.679

[z]Treatments followed by the same letter are not significantly different at the (.05) level by LSD.
[a]*L. korrae* isolate #1 (Novi).
[b]Based on an estimate of proportion of necrotic leaf tissue/pot.

Table 13 summarizes the results of a two way analysis of variance of the mean percent necrosis from four replications of 16 different treatments. Ratings were taken one month after treatments. In this study several treatments ranked significantly better than the check. Included among these were Lawn Restore and 10-4-4, both fertilizers, which were not significantly different from each other. CM-A1, one of the Ringer actinomycetes grown in .4% yeast-1% malt-.4% glucose broth, was as effective as Lawn Restore. Of the fungicides, propiconazol, benomyl and the low rate of fenarimol all ranked significantly better than the check, while the high rate of fenarimol showed a phytotoxic response. Once again, triadimefon rated poorly.

General trends among these studies indicate that propiconazol, fenarimol(barring phytotoxicity), benomyl and iprodione may give some degree of control _in vivo_ relative to the check. The fertilizers (10-4-4, and Lawn Restore), ranked very highly in the study in which they were included, indicating a tendency for disease escape with this pathogen (Garrett 1956), and a potential for biological or cultural management for this disease.

In Vitro Antagonism of Four Bacteria and Two Actinomycetes to *L. korrae*

INTRODUCTION

Ringer Corporation of Eden Prairie, MN. produces a biological fertility product known as Lawn Restore for use on home lawns as well as golf courses. This product has a fertilizer analysis of 10-4-4 and is known to contain several species of fungi, bacteria and actinomycetes. The nitrogen source consists mainly of feather meal, soybean meal and bone meal which may provide a food source for the bacteria and actinomycetes, allowing them to become established under field conditions. It has been postulated that the organisms, which are contained in this product in their dormant state, may provide some sort of biological control for necrotic ring spot of *P. pratensis*. For this reason, studies were established to investigate the effects of these "Ringer organisms" against *L. korrae*, as well as several other disease causing organisms, on agar.

MATERIALS AND METHODS

Ringer Corp. provided two actinomycete cultures and four bacterial cultures for use in these studies. Based on cultural characteristics the actinomycetes were tentatively identified as *Streptomyces* spp. The bacteria had the following characteristics:

<u>R-1</u> (Gm-) <u>Bacillus</u> sp.- rod, long, in pairs. Cream colored on Nutrient agar. Bacteria cream to blue on PDA.

<u>R-5</u> (Gm-) <u>Bacillus</u> sp.- rod, short, singly. Cream colored on Nutrient agar.

<u>R-6</u> (Gm+) <u>Bacillus</u> sp.- rod, long, in chains and pairs. Cream-tan on Nutrient agar.

R-7 (Gm-) <u>Bacillus</u> sp.- rod, long, in pairs and chains. Pinkish-white on Nutrient agar.

The Ringer actinomycete cultures were grown on agar medium (YMG) containing .4% yeast extract, 1% malt extract, .4% glucose and 2 % agar. Cultures were transferred to two replicate plates of this medium using an inoculation loop to form a streak of the organism down the center of the plate. Four isolates of the test organism, <u>L. korrae</u> in this case, were grown on potato dextrose agar (Gibco labs, Madison, WI 53713) plates, and six mm plugs of the fungus were cut using a cork borer. One such plug was aseptically transferred to either side of the actinomycete streak on the same day, after 3 days, 7 days, 14 days, and 21 days. The plates were then observed for at least two weeks for any signs of abnormal growth and zones of inhibition as the fungal growth approached that of the actinomycete.

The Ringer bacteria were grown on nutrient agar (BBL Microbiology Systems, Becton Dickinson and Co, Cockeysville, MD 21030). Using an inoculation loop, bacteria were transferred in a streak to fresh plates of either nutrient

agar or PDA. Plugs of the test fungus were transferred to either side of this streak on the same day.
The plates were then observed for approximately two weeks for signs of fungal growth inhibition.

These same bacterial cultures were also tried in combination against the test organisms. The four bacterial cultures were grown on plates of nutrient agar and each individual bacterial culture was transferred as before to two replicate plates of nutrient agar and YMG. Every possible combination of the four bacteria was tried. Some plates received streaks of two different bacteria, some three different bacteria. Plugs of the test fungi, *L. korrae* and *P. graminicola*, were transferred as before, on the same day as the bacteria, and the plates were then observed for two weeks.

RESULTS AND DISCUSSION

Actinomycete studies:

Tables 14 - 16 show that more definite and reproducible inhibition was seen when the actinomycetes were left on agar longer than one week. At 7-9 days the combination of CM-A1 & CM-A2 showed less inhibition than either of the organisms alone. When left two or more weeks before transferring the test fungus to the plates, definite thin zones of inhibition of fungal growth developed around the actinomycete streak on most plates.

Increasing time from one week to two weeks, between plating the actinomycetes and the test fungi, caused fungal growth inhibition in a higher percentage of cases.

TABLE 14. Antagonism[a] of Ringer actinomycetes[b] to Leptosphaeria korrae on agar[c] when both were plated the same day[d]

	L. korrae isolate #1	#2	#3	#4
CM-A1(1)	-		-	-
(2)	+?	+	-	+?
CM-A2(1)	+	+	+	+
(2)	+?	-	-	-

[a](+) indicates less fungal growth in presence of producer than on the control plate, (-) = no inhibition.
[b]CM-A1 and CM-A2 = probable Streptomyces spp.
[c]YMG agar used = .4% yeast, 1% malt, .4% glucose, 2% agar.
[d]Ratings made at 14 days.

TABLE 15. Antagonism[a] of Ringer Actinomycetes[b] to Leptosphaeria korrae on agar[c] when L. korrae was plated 7-9 days after Actinomycete[d]

	L. korrae isolate #1	#2	#3	#4
CM-A1(1)	+	+	+	
(2)	+	+	+	+
CM-A2	+	+	+	+
CM-A1 & CM-A2 (1)	+		+	
(2)	+?	+?	+?	+?

[a](+) indicates less fungal growth in presence of producer than on the control plate, (-) = no inhibition.
[b]CM-A1 and CM-A2 = probable Streptomyces spp.
[c]YMG agar used = .4% yeast, 1% malt, .4% glucose, 2% agar.
[d]Ratings made at 14 days.

TABLE 16. Antagonism[a] of Ringer Actinomycetes[b] to *Leptosphaeria korrae* on agar[c] when *L. korrae* was plated 14 days after Actinomycete[d]

	L. korrae isolate			
	#1	#2	#3	#4
CM-A1	++	++	++	++
CM-A2	++	++	++	++
CM-A1& CM-A2	++	++	++	++

[a](++) indicates zone of very thin growth between the 2 organisms, (-) = no inhibition.
[b]CM-A1 and CM-A2 = probable *Streptomyces* spp.
[c]YMG agar used = .4% yeast, 1% malt, .4% glucose, 2% agar.
[d]Ratings made at 14 days.

TABLE 17. Antagonism[a] of Ringer Bacteria[b] to *Leptosphaeria korrae* on agar[c] when both were plated the same day[d]

	L. korrae isolate			
	#1	#2	#3	#4
N agar				
R-1	-		-	
PDA				
R-1(1)	-	-	-	-
(2)	+	+	+	+
N agar				
R-5(1)	+?	+?		
(2)	+?	+,-	+,-	-
PDA				
R-5(1)	+	+	+	+
(2)	+	+	+	+
N agar				
R-6(1)	+?	+?		
(2)	-	-	-	-
PDA				
R-6(1)	-	-	-	-
(2)	-	-	-	-
N agar				
R-7	-		-	
PDA				
R-7(1)	-	+	-	-
(2)	-	-	-	-

[a] (+) indicates less fungal growth in presence of producer than on the control plate, (-) = no inhibition.
[b] R-1, R-5, R-6, R-7 probable *Bacillus* spp.
[c] Nutrient agar and Potato Dextrose agar.
[d] Ratings made at 14 days.

TABLE 18. Antagonism[a] of Ringer Bacterial Combinations[b] to Leptosphaeria korrae & Phialophora graminicola on agar[c] when both were plated on the same day[d]

	Bacterial Combinations[e]									
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
YMG agar										
L.k. #1	-	-	-	-	-	-	+	-	+	-
P.g. #1	-	-	+?	+?	+?	+	-	+	+	+
N agar										
L.k. #1	+	+	+	+	+	+	+	+	+	+
P.g. #2	+	+	+	+	-	+	+	+	+	-

[a] (+) indicates less fungal growth in presence of producer than on the control plate, (-) = no inhibition.
[b] R-1, R-5, R-6, R-7 probable *Bacillus* spp.
[c] Nutrient agar and YMG agar (.4% yeast, 1% malt, .4% glucose, 2% agar).
[d] Ratings made at 14 days.

[e] Key to Bacterial Combinations
#1: R-1, R-5
#2: R-1, R-6
#3: R-1, R-7
#4: R-5, R-6
#5: R-5, R-7
#6: R-6, R-7
#7: R-1, R-5, R-6
#8: R-1, R-5, R-7
#9: R-1, R-6, R-7
#10: R-5, R-6, R-7

Bacterial studies:

Table 17 shows that of the four bacteria, R-5 showed the most inhibitory potential. This was especially evident in the trials done on PDA. Both R-1 & R-5 seemed to be more inhibitory on PDA than on nutrient agar, while R-6 & R-7 failed to show much inhibition on either.

In contrast, most of the bacterial combinations, as seen in Table 18, showed inhibition on nutrient agar of both *L. korrae* and *P. graminicola*, while much less inhibition was shown on YMG agar. Combinations of bacteria seem to be more effective inhibitors than any of the bacteria alone, except perhaps R-5.

No consistent correlation was noted between the type of medium and the type or amount of inhibition noted. Nutrient agar, a standard medium for growing bacteria, did not seem to be a better medium than YMG or PDA for producing inhibition with the bacterial cultures.

The mode of inhibition is yet to be discovered but has been postulated to be due to the production of inhibitory substances formed by the producer organisms which inhibit or retard growth of the test organisms in culture. Another possibility is that the faster growing organism, or the organism that was placed on the plate first might compete with the test organism for nutrients that are contained in the growth medium. Whether this inhibition occurs *in vivo* is unknown.

"The outcome of competition between a pathogen and associated microbiota in the rhizosphere or other regions where nutrients are available is determined largely by effects of the physical environment on the relative competitive advantage of the pathogen vs that of the other organisms. This can partially explain the frequent observation that temperature, pH, water potential and other factors optimal for growth of a pathogen in pure culture are often different from those most favorable to infection of plants in the field. All factors of the environment combined determine the ecological niche available to the pathogen and antagonist" (Cook and Baker 1983 p. 202).

Suppressant Effects of Nature's Touch Green Magic and Synthetic Nutrient Solution on Growth of *L. korrae in vitro*.

INTRODUCTION

Nature's Touch Green Magic is an inorganic, liquid, lawn fertilizer with an analysis of 20-0-2. It consists mainly of urea nitrogen (18.8 %), potash (2%), and micronutrients derived from and formulated with Hemlock bark and other plant extracts. This product has been shown to help in rejuvenation of lawns infected with necrotic ring spot (Ross & Vargas, 1984). The mode of action of Green Magic in causing such an effect has not previously been investigated. One theory is that the nutrition, especially the nitrogen supplied by Green Magic, helps the plants to grow back, overcoming disease symptoms while not actually eliminating the disease. Another theory is that something in the product itself is antagonistic or inhibitory to the pathogen(s) causing the disease. This study was conceived to test the second hypothesis.

MATERIALS AND METHODS

This study was performed once as a preliminary study with three rates, 1, 10, and 100 ug/ml (ppm) of Green Magic and nutrient solution and then repeated twice using six different rates, 1, 10, 25, 50, 75, and 100 ug/ml.

Nutrient solution was prepared by mixing the following amounts of macro and micronutrients together in one liter of distilled water to simulate the composition of Green Magic.

Nutrient Solution		Green Magic analysis (%)	
$CO(NH_4)_2$ (Urea)	502.53g	N	20
KNO_3	51.86g	K_2O	2
$CuSO_4.5H_2O$	2.37g	Cu	.05
$FeSO_4.7H_2O$	37.31g	Fe	.62
$MnSo_4.H_2O$	10.03g	Mn	.27
$ZnSo_4.7H_2O$	5.33g	Zn	.10
S(52% flowable)	10.54g	S	1.04

In all three trials potato dextrose agar was prepared from mix, autoclaved and partially cooled before the appropriate amounts of the two test solutions were added. In addition to this, in the preliminary trial Green Magic and nutrient solution were added to separate flasks of PDA before being autoclaved. Approximately 20 ml of agar was poured into each 15 x 100 mm plastic petri dish. Two isolates of L. korrae were used in this experiment, #1 (Novi), and #2, (Okemos).

Four replicate plates of each Green Magic and nutrient solution treatment plus four PDA controls were inoculated with a six mm plug of fungus taken from the edge of an actively growing colony of each of the isolates. These plates were then incubated on the laboratory bench in a plastic bag for two weeks. Diameters of growth were recorded at four, seven, and ten days. Factorial analysis of variance was performed and LSD's calculated on seven day diameters of growth because of good differentiation between treatments. Autoclaved and non-autoclaved Green Magic-amended agar were analysed separately.

RESULTS AND DISCUSSION

Table 19. Mean growth[a] of *Leptosphaeria korrae*[b] on agar amended[c] with several concentrations of nutrient solution[d] and Green Magic[e] fertilizer

	mean growth (mm) *L. korrae*	
rate (ug/ml)	Isolate #1	Isolate #2
0 - control	29.0	30.2
Nutrient Sol		
1	28.3	29.5
10	27.0	28.2
25	24.8	21.6
50	19.4	16.2
75	5.8*[f]	1.2*
100	0.0*	0.0*
Green Magic		
1	29.0	31.2
10	13.5*	12.5*
25	12.0*	13.0*
50	11.0*	9.5*
75	11.0	8.0
100	13.0	11.8
	LSD(.05)=2.2	LSD(.05)=3.2

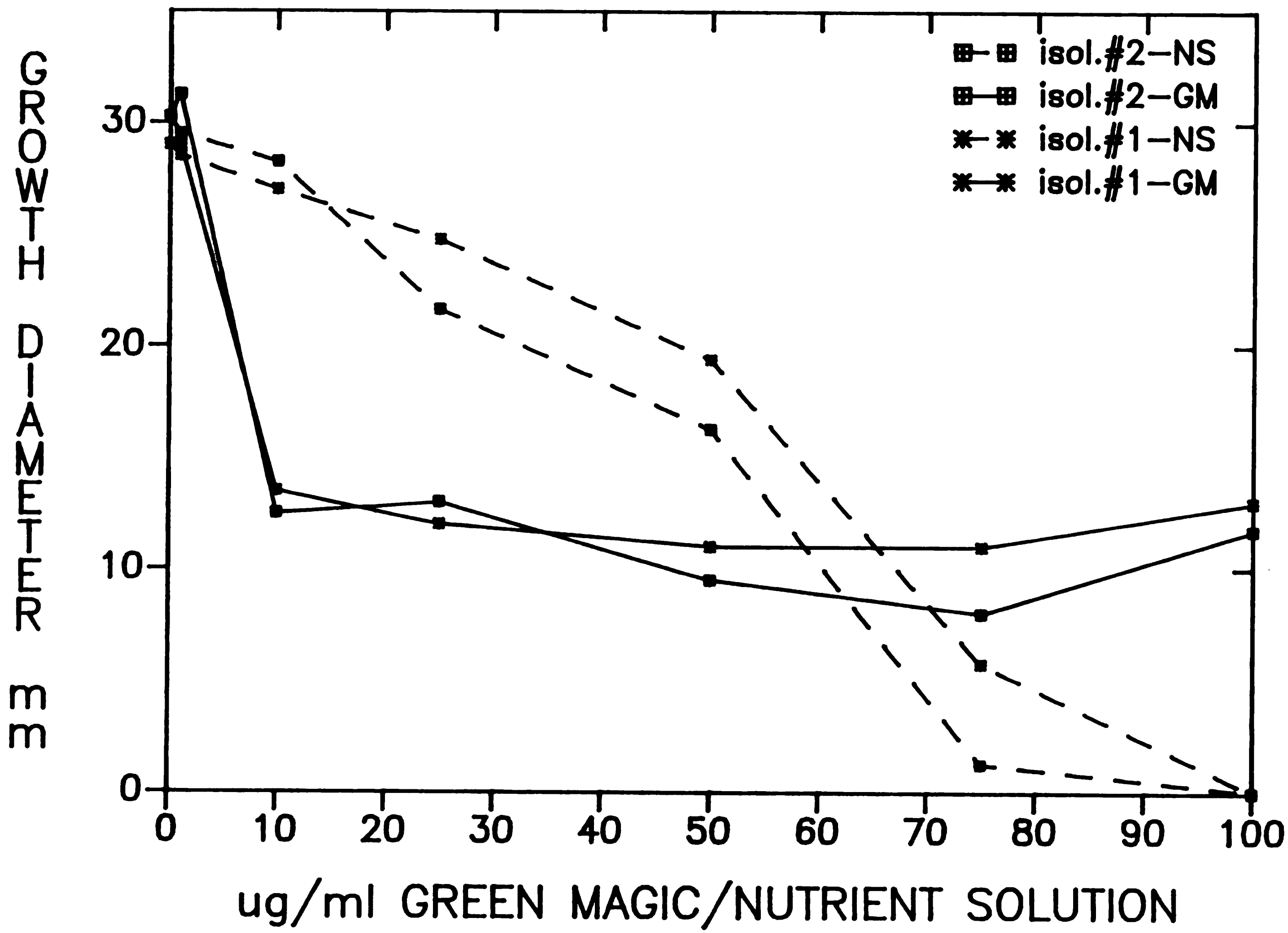
[a] Growth measured at 7 days.
[b] *L. korrae* isolate #1=Novi, MI; isolate #2=Okemos, MI.
[c] Nutrient solution and Green Magic added to partially cooled PDA.
[d] Nutrient solution simulates macro and micro nutrient composition of Green Magic.
[e] Green Magic (Agro-Chem Inc.), 20-0-2 liquid fertilizer.
[f] (*) indicates significantly less growth at a single rate in a column between Green Magic and nutrient solution.

Results of trial II are summarized in Table 19 and Figure 6 with significance shown at (P=.05).

At the 1 ug/ml rate significant differences were only shown in the preliminary trial and in trial I, in both cases nutrient solution inhibited growth of L. korrae isolate # 1 more than did the Green Magic. In most instances Green Magic proved to be more toxic at the low to intermediate rates, 10, 25, 50 ug/ml, and nutrient solution to be more toxic at high rates, 75, and 100 ug/ml. From these studies, Green Magic appears to have some added inhibitory potential relative to the nutrient solution at lower rates, (10, 25, and 50 ug/ml).

Results from the preliminary study, testing autoclaved vs. non-autoclaved Green Magic, showed that autoclaved and non-autoclaved product yielded nearly identical results. Part of Green Magic's action has been attributed to the presence in this product of poly-organic complexes obtained from plant extracts. Such molecules would presumably lose their integrity when autoclaved, causing the loss of their inhibitory potential. The reason for Green Magic's superiority at lower rates is unknown.

Figure 6. Inhibition of *Leptosphaeria korrae* growth on agar amended with several concentrations of Green Magic fertilizer and a similar nutrient solution.



isol.#2—NS
isol.#2—GM
isol.#1—NS
isol.#1—GM
GROWTH DIAMETER mm
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
ug/ml GREEN MAGIC/NUTRIENT SOLUTION

Figure 6

IN VIVO NECROTIC RING SPOT MANAGEMENT - FIELD STUDIES

Field studies have been conducted at three different *Poa pratensis* lawn sites in Michigan over the past three years to study the preventive and curative effectiveness of several fertilizer products and anti-fungal compounds on necrotic ring spot (NRS).

MATERIALS AND METHODS

In May 1984 a curative study was established on a heavily diseased *P. pratensis* sodded area in Novi, MI. The study was laid out with three replications of 1.83 x 2.44 m^2 plots in a randomized block design. The condominium site at Novi was irrigated with an automatic irrigation system and in 1984 received, in addition to our treatments, the same four fertility and herbicide treatments as the rest of the complex, including a total of 180 kg N/ha. In 1985 and 1986 this area did not receive any additional fertilizer treatment other than those we applied.

In May 1985 a curative study was established on the sodded *P. pratensis* lawn of the Prince Corp. in Holland, MI where disease had developed the previous summer. Rather diffuse symptoms were present at the start of this study. Percent area infected of each plot was determined approximately twice monthly throughout the season. Reduction in percent area infected was calculated at the end of the season by subtracting the rating of 11/19/85 from the 5/28/85 rating and dividing this by the 5/28/85 rating.

Therefore a positive value equals reduction in disease symptoms, and negative, an increase.

In May 1986 a curative study was established at Dearborn, MI on the P. pratensis sodded lawn of Oakwood Hospital. This site contained many distinct diseased patches so monthly ratings included number of diseased patches per plot. The percent reduction in number of patches from the first rating (5/16) was calculated for several dates.

Both the Holland and Dearborn sites were irrigated with an automatic system and were also core cultivated once in those respective years, (in the spring at Dearborn, and in the fall at Holland). The same treatments were applied to 1.83 x 2.44 m plots in a randomized block design at all three sites.

Chemicals were used at rates recommended by the manufacturers and applied at several different timing intervals as indicated in Tables 20 and 21. Treatments that were sprayed on were applied using a CO_2 small plot sprayer at a volume of 454.2 l/ha, equipped with a .914 m boom. Drenches were applied by mixing the treatment in 7.57 l of water in a sprinkling can and drenching over each plot individually. Dry materials were applied by hand to individual plots.

Soil samples taken at these sites consisted of composite samples of 21 plugs (seven plugs taken from each of the three replications for a particular treatment.) A

25.4 mm soil probe was used to take 50 mm depth samples. Where indicated, the thatch was separated from the subsoil and the two samples analyzed separately.

RESULTS AND DISCUSSION

In 1984, no new symptom development was observed in the Novi plot area (Table 22). Disease reduction was most likely due to the effects of fertility in causing recuperation of diseased areas. Two plots in this study, (one replication of treatment 10 and of the check), showed an increase in severity of disease symptoms in one replication each, but this was thought to be due to localized droughty areas caused by a poor irrigation system.

In 1985 the number of patches per plot were again monitored over the course of the season (Table 23). After the initial rating (5/30), disease reduction was noted in all treatments up until the 10/1 rating. Percent disease reduction from the initial (5/23/84) counts to the 10/1/85 counts and analysis of variance indicated several treatments including the high rates of Green Magic (treatments 8 and 9), and the sulfur treatment (#16) to be significantly better at reducing and suppressing disease symptoms than the check (Table 24). Treatments 16 and 17 (sulfur and check) had receved 24 kg N/ha in August and September because of red thread (*Laetisaria fuciformis*) and general chlorosis.

At the start of the 1986 season at Novi, only one plot, (#15), showed symptoms of necrotic ring spot (Table 24). Scattered patches developed as the season progressed with the most severe symptoms apparent by late July, presumably because of the high temperatures and drought stress during this period. An increase in disease symptoms was noted in some plots on 8/14/86. Analysis of variance of percent disease reduction at that date indicated that all fertilizer treatments except treatment 2 were significantly better than the check at reducing or suppressing disease (Table 25). Although no other differences were noted between treatments, and some of the symptoms appeared to be localized, these results indicate the importance of nutrition in suppressing disease symptoms. Symptoms began to decrease into September and October and become obscured by other diseases such as dollar-spot, (*Lanzia* and *Moellerodiscus* spp.), and red thread.

The Holland study in 1985 yielded an analysis of variance which showed no treatments significantly better than the untreated check (Table 26). The site did undergo core cultivation in September of 1985 which may have helped to obscure differences between treatments.

In the Dearborn study in 1986 some statistical differences were noted in counts of 9/11 and 11/22 (Table 27). Neither date showed any treatments significantly better than the untreated check. A trend was noted, however, in that nearly all of the fertility treatments showed disease reduction at the time of the 9/11 rating while the untreated check had slightly more disease than at the start of the season.

The total number of patches increased in most treatments at Dearborn from 6/13/86 to 7/14/86 (Table 28). The total number of patches sharply declined after the 7/14 treatments, leveled off to a plateau from 7/31 to 9/11, dropped sharply again between 9/11 and 10/7, (a period of heavy rainfall), and remained fairly constant thereafter. Since L. korrae was isolated from plant roots infected by ectotrophic mycelium it was assumed that these areas were affected by necrotic ring spot. No P. graminicola was ever obtained from the numerous isolations performed from any of these plot areas.

Several general conclusions may be drawn from these studies. All three field sites experienced a general decline in symptoms throughout the period we monitored them. At Novi, symptom decline (recuperation of turf) was dramatic in 1984, probably due to the unfortunate addition of 180 kg N/ha over the whole plot area. The heavy dose of nitrogen in 1984 seemed to play a large part in allowing the grass to recover from previous damage as well as obscurring differences between treatments. Disease counts taken in 1985 and 1986 also indicated that nutrition is important in disease prevention.

The disease has been present at this Novi site since at least 1981, exhibited severe symptoms in the fall of 1983, and only scattered symptoms have developed since then. This pattern resembles the phenomenon of "Take-all decline" reported on wheat and bentgrasses due to build-up of biological antagonism to the fungal pathogen, Gaeumannomyces graminis, an ascomycete with a similar growth and infection habit to L. korrae (Asher & Shipton, 1981; Smiley & Craven-Fowler, 1984a; Worf et al., 1982,1985).

The sulfur treatment (#16), appeared to be fairly effective in both the Novi study (Tables 22, 23, 24, 25) and the Dearborn study (Table 27). Although statistical significance was not shown in all cases, largely because of the large variability between replications caused by uneven distribution of this disease, this treatment showed consistent reduction in disease. When combined with balanced (10-4-4) fertility in 1986 these plots showed good density and color. Both control of patch disease (Take-all patch of Agrostis spp.) and improvement of turf color and density have been reported through the use of sulfur treatments (Davidson & Goss, 1972). No lowering of soil or thatch pH was noted with this treatment after two years at the Novi site (Table 29).

Soil tests from Novi and Holland indicated a phosphorus deficiency (Table 29), so a 144 kg P/ha treatment was included in this study in 1985. Turf quality in 1985 indicated that P alone did not give satisfactory results, so in 1986 10-4-4 applications totalling 144 kg N/ha and 57.6 kg K_2O and P_2O_5 per ha were substituted. This treatment simulated the nutrient constitution of the Lawn Restore treatment and gave similar results at Dearborn in 1986.

Table 20. Treatments, rates and dates of application at Novi, Holland and Dearborn, MI 1984-1986

Treatment	Rate	application dates[a]
1 [b]Lawn Restore	480 kg/ha	M, S
2. Lawn Restore	"	M
3. Lawn Restore	"	S
4. Lawn Restore	"	M,J,S
5. Lawn Restore	"	J
6. Lawn Restore	"	S,O
7. Lawn Restore	"	O
8 [c]Soil Aid,	40.6 l/ha	M
[c]Green Magic	203.2 l/ha	M,J,S
9. Soil Aid,	40.6 l/ha	M
[c]Catzyme	12.7 l/ha	M
Green Magic	203.2 l/ha	M
[c]Strengthen & Restore	203.2 l/ha	J,S
[d]Spring Equalizer	203.2 l/ha	Ap
10. Gr. Magic	203.2 l/ha	O
[d]Spr. Eq.	203.2 l/ha	Ap
11. Gr. Magic	203.2 l/ha	S
[b](Holland 0-46-0)	144 kg/ha	
12.[b] Fungus Rx	180 kg/ha	M,J,S
13. Gr. Magic	203.2 l/ha	M
14. Gr. Magic	203.2 l/ha	M,J, O
Spr. Eq.(Novi)	203.2 l/ha	Ap
15. Gr. Magic	203.2 l/ha	J
16.[e]Cleary Sulfur	48 kg/ha	M,J,S
[f]10-4-4	48 kg N/ha	M,J,S
17. Check (No Tmt.)		
(Novi-1985 46-0-0)	24 kg N/ha	A,S
18.[g]20-0-2	48 kg N/ha	M,J,S,O
19.[b]10-4-4 (Dearborn only)	48 kg N/ha	M,J,S
20.Sp. Equalizer (Dearborn only)	203.2 l/ha	Ap
20. 0-46-0(Novi 1985)	144 kg P_2O_5/ha	
20.[b]10-4-4(Novi 1986)	48 kg N/ha	M,J,S
21. Sp.Eq. (Novi 1986 only)	203.2 l/ha	Ap

[a]Ap=April, M=May, J=July, A=Aug., S=September, O=October.
[b]Dry treatments applied by hand.
[c]Liquid formulations applied with CO_2 small plot sprayer.
[d]Spring equalizer applied foliarly (8-13-0).
[e]Flowable (52%) sulfur applied as drench. 1985 received a 24 kg N/ha application of 46-0-0 in August and September.
[f]All 10-4-4 treatments 1986 only.
[g]20-0-2, 4 applications in 1984-1985 reduced to 3 applications in 1986.

Table 21. Dearborn- 1986 fungicide field treatments[a]

Treatment	Rate(kg ai/ha)
21. Iprodione (Chipco 26019)	6
22. Iprodione (Chipco 26019)	12
23. Benomyl (Tersan 1991)	9
24. Benomyl (Tersan 1991)	18
25. Triadimefon (Bayleton)	6
26. Triadimefon (Bayleton)	12
27. Fenarimol (Rubigan)	3
28. Fenarimol (Rubigan)	6
29. Banner (Propiconazol)	6
30. Banner (Propiconazol)	12

[a]All fungicides applied once monthly from June through October 1986 as drenches.

Table 22. Novi 1984 - percent reduction[a] in necrotic ring spot patches from 5/23/84 to 10/25/84

Tmt	Rep I % red.[b]	Rep II % red.	Rep III % red.	mean[c]	Mean separation[d]
1	83	100	100	94	a
2	100	100	100	100	a
3	100	100	67	89	ab
4	100	100	100	100	a
5	89	100	100	96	a
6	100	100	100	100	a
7	100	100	100	100	a
8	100	80	100	93	a
9	100	100	80	93	a
10	100	-400	100	-67	bc
11	83	100	100	94	a
13	100	100	86	95	a
14	100	100	86	95	a
15	0	91	100	63	abc
16	50	79	100	76	ab
17	33	-400	100	-88	c
18	100	100	88	95	a .
LSD(.05)				159	

[a]Calculated by subtracting number of patches per plot on 10/25/84 from the number on 5/23/84, dividing this by the number present on 5/23/84, and multiplying by 100.
[b]Percent disease reduction.
[c]Mean of 3 replications.
[d]Means followed by the same letter are not significantly different by LSD(.05).

Table 23. Necrotic ring spot patches per plot - 1984 and 1985 Novi field trials

	1984						1985																				
Tmt	5/23			10/26			5/30			6/19			7/25			8/9			9/13			10/1			11/7		
	replication																										
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	6	6	7	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
2	7	1	11	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	6	1	0	0	0
3	9	4	9	0	0	3	2	0	2	0	0	0	1	0	0	2	0	0	0	0	1	0	3	0	0	0	0
4	6	2	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0
5	9	3	3	1	0	0	1	0	1	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0
6	8	7	8	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	3	0	0	0
7	14	7	5	0	0	0	3	3	1	0	0	0	0	0	1	0	0	2	0	1	4	1	2	7	0	0	1
8	5	10	6	0	2	0	0	4	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0
9	5	2	5	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	9	1	9	0	5	0	2	3	0	0	0	0	0	0	1	0	0	1	0	1	0	1	3	1	0	0	0
11	6	4	4	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12							1	5	0	0	0	0	0	0	0	0	0	0	1	0	0	2	1	0	0	0	0
13	2	4	7	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
14	4	4	7	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
15	4	11	6	4	1	0	0	4	1	0	0	0	0	0	6	0	1	8	0	0	8	2	2	9	0	0	0
16	4	14	4	2	3	0	1	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	6	1	5	4	5	0	3	3	1	0	0	0	2	0	1	0	0	3	1	0	1	6	5	2	0	0	1
18	4	4	8	0	0	1	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	1	3	0	0	0	0
20							0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	0	0	0

Table 24. Necrotic ring spot patches per plot - 1986 Novi field trials

Tmt	4/18			7/14			7/31			8/14			9/5		
	replication														
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
2	0	0	0	0	3	0	0	3	0	2	6	3	3	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	2	0	0	0	0	1	0	0
5	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	1	3	1	3	0	0	3
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
12	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	7	1	0	5	5	1	4	3	2	3	5	0	1
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	1	0	0	2	8	2	3	11	3	2	4	0
18	0	0	0	0	0	0	1	0	0	1	2	0	0	0	0
20	0	0	0	0	1	0	0	3	0	1	3	0	0	0	0
21	0	0	0	1	4	0	3	3	0	3	5	0	7	2	0

Table 25. Mean percent reduction[a] in necrotic ring spot patches from 5/23/84 to 10/1/85 and 8/14/86 at Novi, MI

Treatment	10/1/85 mean[b] % red.	mean[c] sep.	8/14/86 mean % red.	mean sep.
9	100	a	100	a
11	100	a	94	a
5	100	a	89	a
16	100	a	100	a
8	97	a	100	a
1	95	a	94	a
14	92	a	100	a
6	88	a	100	a
13	87	a	100	a
3	75	ab	100	a
18	67	ab	75	a
7	41	ab	68	a
15	27	ab	52	a
10	-7	ab	96	a
4	-33	ab	100	a
2	-108	b	-119	ab
17	-113	b	-303	b
LSD(.05)	192		271	

[a]Calculated by subtracting number of patches per plot on 10/1/85 or 8/14/86 from the number on 5/23/84, dividing this by the number present on 5/23/84, and multiplying by 100.

[b]Percent disease reduction. Mean of 3 replications.

[c]Means followed by the same letter are not significantly different by LSD(.05).

Table 26. Holland - 1985 mean percent reduction[a] in percent area infected[b] from 5/18/85 to 10/18/85

Trt	Mean % red.	Mean Separation[c]
4	68	a
7	61	a
8	58	a
17	56	a
11	53	a
13	51	a
5	51	a
14	51	a
15	49	a
2	41	a
12	41	a
16	32	a
18	28	ab
6	24	ab
1	23	ab
10	17	ab
3	16	ab
9	- 25	b
LSD(.05)	54	

[a]Mean of 3 replications. % Reduction = 5/18/85 rating - 10/18/85 rating divided by 5/18/85 rating, times 100.
[b]Rating of % of plot area affected by necrotic ring spot symptoms.
[c]Treatments followed by the same letter are not significantly different by LSD(.05).

Table 27. Dearborn 1986 mean percent reduction[a] in number of patches from 5/16/86 to 9/11/86 and from 5/16/86 to 11/22/86

Trt	9/11/86 Mean % red.	Mean Separation[b]	11/22/86 Mean % red.	Mean Separation[b]
9	81	a	82	a
12	53	ab	83	a
4	48	ab	48	ab
5	42	ab	36	abc
16	37	abc	78	a
6	27	abc	60	a
24	26	abc	35	abc
8	24	abc	51	ab
30	22	abc	61	a
13	19	abc	34	abc
19	19	abc	40	abc
18	16	abc	28	abc
15	3	abc	49	ab
20	2	abc	19	abc
1	2	abc	70	a
22	0	abc	-50	bcd
14	-3	abc	27	abc
3	-3	abc	47	ab
17check	-8	abc	38	abc
2	-11	abc	3	abcd
11	-26	abc	12	abc
27	-33	abc	78	a
21	-39	abc	-22	abc
10	-43	abc	19	abc
7	-67	abc	-13	abcd
25	-83	bc	-100	de
23	-83	bc	-167	e
26	-88	bc	-8	abcd
29	-113	c	9	abc
28	-333	d	-60	cd
LSD(.05)	154		106	

[a]Mean of 3 replications. Percent reduction=# patches on 5/16/86 - # patches on 9/11 or 11/22 divided by # patches on 5/16 times 100.

[b]Treatments followed by the same letter are not significantly different by LSD(.05).

Table 28. Dearborn 1986 - Total number of diseased patches per treatment[a]

Tmt [b]	5/16	6/13	7/14	7/31	8/19	9/11	10/7	10/18	11/22
1	24	26	26	26	23	23	13	11	10
2	23	31	25	20	15	24	16	17	15
3	29	25	30	28	24	27	15	17	12
4	29	21	35	18	13	11	7	7	9
5	28	24	20	18	14	15	9	14	15
6	36	25	31	25	28	28	11	13	12
7	15	15	20	18	22	25	19	18	17
8	29	25	27	24	23	23	8	12	15
9	24	17	19	17	5	4	0	0	3
10	28	38	43	36	38	38	29	32	22
11	24	26	38	28	30	33	14	18	22
12	31	36	33	21	19	13	5	3	5
13	28	19	23	21	20	20	18	18	16
14	34	28	42	27	31	31	14	20	18
15	34	31	42	24	25	25	21	21	17
16	20	20	27	18	16	14	5	5	6
17	15	10	13	10	13	16	11	10	10
18	21	21	28	17	12	16	10	7	13
19	24	26	26	15	18	16	10	9	9
20	24	16	26	23	22	22	18	16	19
21	10	9	18	12	10	14	10	12	12
22	4	5	6	5	4	4	5	6	6
23	5	7	11	10	9	8	5	13	12
24	13	11	15	8	8	7	2	9	8
25	4	5	6	4	7	7	4	6	8
26	8	31	24	23	15	24	12	17	19
27	7	8	14	11	7	10	3	3	2
28	7	8	16	11	14	17	11	7	8
29	10	6	7	10	9	14	8	6	7
30	12	15	14	13	9	8	3	9	3
total	600	585	705	541	503	537	316	356	350

[a] Total of 3 replications
[b] Treatment dates in bold type

Table 29. Soil tests[a] from Novi, Holland and Dearborn field sites

Site	date	soil or thatch[b]	pH	P205 (kg/ha)	K20 (kg/ha)	Ca (kg/ha)	Mg (kg/ha)	meq/ 100g CEC
Novi 1	5/20/85	S	7.6	5.5	204	9199	436	
Novi 2	"	S	7.8	13.2	610	9590	452	
Novi #16	7/29/85	Th	7.6	16.5	313	8984	528	
" "	"	S	8.1	1.1	176	9512	432	
Novi #17	7/29/85	Th	7.6	29.7	361	8123	528	
" "	"	S	8.0	1.1	176	9336	467	
Novi #4	11/85	Th	8.1	31.9	264	8709	648	51.2
"	"	S	7.9	3.3	151	9101	504	17.3
Novi #16	"	Th	7.6	9.9	231	8318	593	41.0
" "	"	S	8.2	3.3	100	7829	313	8.2
Novi #17	"	Th	7.6	13.2	210	7438	556	41.4
" "	"	S	7.7	3.3	167	9101	504	13.8
Novi #4	4/86	Th	7.2	37.4	299	7046	520	
" "	"	S	7.8	4.4	149	8611	394	
Novi #9	"	Th	-	22	308	7242	512	
" "	"	S	8.0	5.5	139	8415	436	
Novi #16	"	Th	7.3	19.8	352	8024	555	
" "	"	S	7.9	3.3	149	8220	470	
Novi #17	"	Th	7.5	20.9	326	8709	625	
" "	"	S	7.8	4.4	157	8807	503	
Novi #4	9/86	Th	7.5	44	384	7801	568	
" "	"	S	7.8	1.1	151	8472	360	
Novi #9	"	Th	7.4	17.6	287	6942	521	
"	"	S	8.1	1.1	126	7968	433	
Novi #16	"	Th	7.4	23.1	260	7801	548	
"	"	S	8.1	1.1	143	8136	433	
Novi #17	"	Th	7.6	17.6	214	7633	558	
"	"	S	8.1	1.1	167	8640	489	
Holl. #16	11/85	S+T	7.9	39.6	160	6253	537	11
" #4	"	S+T	7.9	62.8	275	6253	640	16.4
" #20	"	S+T	7.7	74	220	5440	575	11.1
" #17	"	S+T	7.9	72.7	264	6870	602	12
Dearborn	5/86	Th	-	70.4	432	6119	788	17
"	"	S	-	47.3	194	5469	667	.15
" #4	9/86	Th	7.3	56.1	250	3843	537	
" "	"	S	8.0	11	185	6582	537	
" #9	"	Th	7.3	38.5	287	3843	556	
" "	"	S	8.0	8.8	241	5748	602	
" #16	"	Th	7.2	74.9	204	3430	521	
" "	"	S	8.1	11	185	6490	667	
" #17	"	Th	7.4	39.6	185	3338	505	
" "	"	S	8.0	18.7	214	6119	621	

[a]Michigan State University Soil Testing Lab
[b]Twenty composite samples from each treatment to a 5 cm depth. Soil and thatch analyzed separately where indicated.

Summary and Conclusions

Patch symptoms on Poa pratensis were first reported in the late 1950's in many eastern states. This coincided with the time that "improved" P. pratensis cultivars began to be used. During the 60's the disease was epiphytotic in these bluegrass stands, and intensive study led to the incitants being named as Fusarium roseum and F. tricinctum, a diagnosis based primarily on the abundant presence of these organisms on dying grass tillers, as well as their induction of a foliar blighting in controlled experiments. Although Koch's postulates were never completed since the characteristic patch symptom was never reproduced on mature turf, the original hypothesis was generally accepted, and the name Fusarium blight has since been associated with the patch or "frogeye" symptom on Kentucky bluegrass. Though L. korrae has been proven to be a cause of patch symptoms of P. pratensis in Michigan and several other states, the interactions of this pathogen with other biotic and abiotic factors in both the cause and the disappearance of this disease should not be ignored.

Leptosphaeria korrae was identified as an incitant of patch disease symptoms of Poa pratensis in Michigan. This fungal pathogen has previously been reported in other eastern, northwestern, and mid-western states as the cause of necrotic ring spot of Poa pratensis L. (Smiley & Craven-Fowler, 1984a; Chastagner, 1984a,1984b; Jackson, 1984; Worf et al., 1986). It has also been cited recently as an incitant of spring dead spot of Cynodon dactylon Pers. in the south western U.S. (Endo et al., 1984). This is the first confirmed report of L. korrae in Michigan.

The Michigan isolates resembled L. korrae in cultural characteristics and temperature-growth relations. Pseudothecia were induced on stems and roots of P. pratensis infected with three Michigan isolates. Ascospore measurements compared favorably with values cited by other researchers (Chastagner et al., 1984a; Smiley & Craven-Fowler, 1984a; Worf et al., 1986; Walker & Smith, 1972).

Pathogenicity of the Michigan isolates on Poa pratensis was proven in the growth chamber, greenhouse and in the field. Inoculations incited root necrosis and wilting and death of the leaf tissue. Patch symptoms were induced under field conditions. Reddening of leaf tissue was also occasionally observed in growth chamber, greenhouse, and field inoculations.

Identification of three Michigan isolates as L. korrae was accomplished through cultural characteristics, temperature - growth relations, ascospore measurements, and pathogenicity studies.

Host range studies did not vary greatly from those of other researchers. P. pratensis cv. Fylking proved highly susceptible followed closely by P. pratensis cv. Adelphi. Adelphi has previously been reported as a fairly resistant bluegrass cultivar (Smiley & Craven-Fowler, 1985c; Worf et al., 1986). Festuca rubra cv. Pennlawn , reportedly susceptible (Smiley 1985c), reacted variably - from moderate to highly susceptible at both 20 and 28 C. Lolium perenne cv. Manhattan appeared to be of intermediate susceptibility. Festuca arundenacea cv. K-31, reportedly resistant, reacted variably, and Agrostis palustris cv. Penncross, reportedly susceptible, appeared moderate to susceptible at both temperatures. The cultivar of Cynodon used was resistant although pseudothecia did develop on root tissue of these plants at 28 C.

From growth chamber studies and field observations it is clear that L. korrae can infect and cause symptoms over a wide range of temperatures. In addition, it was shown by field inoculations that this disease can be spread by turf/soil cores, a factor that should be considered when cultivation practices are used.

In 1986 a direct relationship was noted between soil moisture and necrotic ring spot symptoms on a site that had been artificially inoculated in 1985. During periods of prolonged high soil moisture, symptoms decreased dramatically, and increased again when soil dried. This coincides with the majority of reports in the literature which list drought stress as an important factor in predisposing turf to Fusarium blight (see literature review). Chastagner (1984b) reported necrotic ring spot to be a disease of overwatered turf, however, and Smiley has reported Fusarium blight to follow heavy rains, or alternating wetness and drought in New York state (1980a, 1980b). In light of this, detailed studies at more locations should be performed to further determine the role of soil moisture in the epidemiology of this disease. More controlled studies are also needed to delineate the water potential extremes and optima under which L. korrae pathogenesis and growth occur.

The results of in vitro screening of fungicides for inhibition of L. korrae compares well with the reports in the literature. Fenarimol, propiconazol, and the benzimidazols all were highly inhibitory to growth at all rates. Iprodione and vinclozalin were less effective, and triadimefon was ineffective.

In greenhouse bioassays fenarimol, propiconazol, benomyl and iprodione all gave some degree of control. In one study, however, pots that received fertilizer either as 10-4-4 solution or dry Lawn Restore (10-4-4 analysis) ranked significantly better than all fungicides other than the low rate of propiconazol, indicating a potential for recuperation when plants are supplied with adequate balanced fertilizer.

Field studies over the past three years have not been successful in demonstrating chemical control of this disease. Studies conducted by Vargas and Detweiler in 1984, 1985, and 1986 (personal communication), also were unsuccessful due to lack of symptom development. At Dearborn in 1986 no significant differences were obtained among the fungicide treatments from factorial analysis of variance. The fact that some fungicides were significantly less effective than no treatment in a two-way analysis of variance, may be an indication that non-target effects such as inhibition of favorable micro-organisms or more direct effects on the plant are occurring.

Field studies have also indicated the importance of fertility in recuperation of necrotic ring spot diseased areas, and it appears that proper balanced nutrition is especially important in encouraging new root growth which allows the plants to escape serious damage even in the presence of the pathogen. This is a concept used by Garrett (1956) to describe Take-all of wheat caused by Gaeumannomyces graminis. In essence, because of the ectotrophic growth habit, the rate of root infection is relatively slow in comparison to new root growth in well fertilized areas. Increased root volume may permit the plant to overcome the effects of the pathogen in reducing water uptake and nutrient absorption. Although nitrogen has in some cases been shown to increase susceptibility to disease, it can still increase disease escape (Garrett 1956).

Preliminary studies have shown two commercial fertilizers to be especially effective in causing turf recovery in diseased areas (Ross & Vargas). One of these products, Lawn Restore (Ringer Corp., Eden Prairie, MN), contains several species of dormant microorganisms said to be isolated from soils suppressive to plant disease (Dr. D.

Lovness, personal communication). Cultures of four species of bacteria and two species of actinomycetes were tested separately and in mixed culture for *in vitro* antagonism to *L. korrae*. When the bacteria were plated in mixed culture against *L. korrae*, zones of inhibition were formed in most cases. Although the relevance of *in vitro* inhibition to what occurs in the natural ecosystem has been challenged (Sivasithamparam, 1978; Bowen & Theodorou, 1979; Asher et al., 1981), these results warrant further investigation into the potential for biological control of this disease.

The other fertilizer tested, Green Magic (Agro-Chem Inc), is composed of plant by-products and soluble nitrogen and micronutrient sources. Part of the activity of this product is said to be due to the antibiotic nature of some of its organic constituents. In *in vitro* tests, significantly less *L. korrae* growth was seen on agar amended with Green Magic at low to intermediate rates than with a nutrient solution prepared to simulate it. The activity of this product in field studies may be of a nutritional nature.

Significant progress has been made towards development of a management program for necrotic ring spot. *In vitro* studies indicate that Lawn Restore and Green Magic may have activity in addition to nutritional to aid in the management of this disease. In addition, greenhouse and field studies have indicated the importance of nutrition in recovery of diseased areas. Soil moisture has also been shown to be an important factor in necrotic ring spot development. Thus, the biological and cultural control of necrotic ring spot deserves further investigation. Laboratory and greenhouse studies have indicated the potential of several anti-fungal chemicals in managing this disease, but adequate data from field tests are still needed.

Bibliography

Abernethy, T, Larsen, P.O. 1984. Yellow Patch Update. ALA Technology. June 1981. p.26.

Anonymous. 1983. Wisconsin's Experience With Yellow Patch. Patch of Green. Sept. 1983.

Arx, J.A. von, Oliver, D.I. 1952. The Taxonomy of Ophiobolus graminis Sacc. Trans. Brit. Mycol. Soc. 35:29-33.

Asher, M.J.C., Shipton, P.J. (eds). 1981. Biology and Control of Take-all. Academic Press, New York. 538 p.

Bean, G.A. 1966. Observations on Fusarium Blight of Turfgrasses. Plant Disease Reporter. 50:942-945.

---, Cook, N., Rabbitt, A.E. 1967. Chemical controls of Fusarium Blight of Turfgrass. Plant Disease Reporter. 51:839-841.

--- 1969. The Role of Moisture and Crop Debris in the Development of Fusarium Blight of Kentucky Bluegrass. Phytopathology. 59:479-481.

Beard, J.B. 1973. Turfgrass Science and Culture. Prentice Hall, Englewood Hills, N.J. 658 p.

Bowen, G.D., Theodorou, C. 1979. Interactions Between Bacteria and Ectomycorrhizal Fungi. Soil Biol. and Biochem. 11:119-126.

Brown, C.L. 1983. The Isolation, Characterization and Control of Rhizoctonia cerealis Causing Yellow Patch on Poa pratensis L. in Michigan. MS Thesis. Michigan State University. 61 p.

---, Vargas, J.M. Jr., Roberts, D.L. 1983. The Occurrence of Yellow Patch of Turf in Michigan. (abstr.) Phytopathology. 73:839.

Brown, Kristen J., Kachadoorian, Roseann V., Worf, Gayle, L. 1983. Survey of Necrotic Ring Spot Disease in Wisconsin Lawns. (abstr). Phytopathology. 73:839.

Burpee, Lee. 1980. Rhizoctonia cerealis Causes Yellow Patch of Turfgrasses. Plant Disease. 64:1114-1116.

Chastagner, G.A., Goss, R.L., Staley, J.M., Hammer, W. 1984. A New Disease of Bluegrass Turf and its Control in the Pacific Northwest. (abstr.) Phytopathology. 74:812.

--- 1984. NRS : Research on a New Disease of Bluegrass Turf and its Control in the Pacific Northwest. Presented at 38th NW Turfgrass Conference, Spokane, WA. Sept 18-20 1984.

--- 1985. Symptoms of Necrotic Ring Spot. Grounds Maintenance. May 1985. p.52.

Cole, H. Jr. 1976. Factors Affecting Fusarium Blight Development. Weeds, Trees & Turf. July 1976. p. 35-37.

---, Baker, K.F. 1974. Biological Control of Plant Pathogens. W.H. Freeman and Co., San Francisco.

---, ---. 1983. The Nature and Practice of Biological Control of Plant Pathogens. APS, Minneapolis, MN.

Couch, H.B., Bedford, E.R. 1966. Fusarium Blight of Turfgrasses. Phytopathology 56:781-786.

Cutright, N.J., Harrison, M.B. 1970. Chemical Control of Fusarium Blight of Merion Kentucky Bluegrass Turf. Plant Disease Reporter. 54:771-773.

---,---. 1970. Some Environmental Factors Affecting Fusarium Blight of Merion Kentucky Bluegrass. Plant Disease Reporter. 54:1018-1020.

Dale, J.L. 1978. Atypical Symptoms of Rhizoctonia Infection on Zoysia. Plant Disease Reporter. 62:645-647.

Davidson, R.M., Goss, R.L. 1972. Effects of P,S,N, Lime, Chlordane, and Fungicides on Ophiobolus Patch Disease of Turf. Plant Disease Reporter. 56:565-567.

Deacon, J.W. 1974. Further Studies on Phialophora radicicola and Gaeumannomyces graminis on Roots and Stem Bases of Grasses and Cereals. Trans. Brit. Mycol. Soc. 63:307-327.

Dernoeden, P.H., O'Neill, N.R. 1983. Occurrence of Gaeumannomyces Patch Disease in Maryland and Growth and Pathogenicity of the Causal Agent. Plant Disease. 67:528-532.

Endo, R.M., and Colbaugh, P.F. 1974. Fusarium Blight of Kentucky Bluegrass in California. In: E.C. Roberts (ed.) Proceedings of the 2nd International Turfgrass Research Conference. Am. Soc. Agron., Blacksburg, VA p. 325-326.

---, Ohr, H.D., Krausman, E.M. 1984. The Cause of the Spring Dead Spot Disease of Cynodon dactylon (L.) Pers. in California. (abstr.) Phytopathology. 74:812.

---,---,---. 1985. L. korrae, a Cause of the Spring Dead Spot Disease of Bermudagrass in California. Plant Disease. 69:235-237.

Fermanian, T.W., Ahring, R.M., Huffine, W. 1981. The Isolation of A Toxin from SDS Areas in Bermdagrass. In: R.W. Sheard (ed.) Proceedings 4th International Turfgrass Research Conference. (Guelph) Am. Soc. Agron., Madison, WI. pp. 433-441.

Fulton, D.E., Cole, H., Nelson, P.E. 1973. Fusarium Blight Symptoms on Seedling and Mature Merion Kentucky Bluegrass Plants Inoculated With Fusarium roseum and Fusarium tricinctum. Phytopathology. 64:354-357.

Funk, C.R. 1976. Developing Genetic Resistance to Fusarium Blight. Weeds, Trees, and Turf. July 1976. p. 41-44.

Garrett, S.D. 1956. Biology of Root Infecting Fungi. Cambridge University Press. 292 p.

Goldberg, C.E., Cole, H., Burpee, L. 1974. Absence of Interaction Between Systemic Fungicides and Nematicides for the Control of Fusarium Blight in Kentucky Bluegrass. (abstr.) Phytopathology. 64:581.

Hornby, D., Slope, D.B., Gutteridge, R.J., and Sivanesan, A. 1977. Gaeumannomyces cylindrosporus, a New Ascomycete from Cereal Roots. Trans. Brit. Mycol. Soc. 69:21-25.

Huisman, O.C. 1982. Interrelations of Root Growth Dynamics to Epidemiology of Root Invading Fungi. Annual Review of Phytopathology. 20:303-327.

Jackson, N. 1981. Take-all Patch of Turfgrasses in the Northeast U.S. In: R. W. Sheard (ed.) Proceedings of the 4th International Turf Research Conference. (Guelph) Am Soc. Agron. Madison, WI. pp. 421-424.

---. 1984. A New Cool Season Patch Disease of Kentucky Bluegrass Turf in the Northeastern U.S. (abstr.) Phytopathology. 74:812.

Kane, R.T., Smiley, R.W. 1983. Plant Growth Regulating Effects of Systemic Fungicides Applied to Kentucky Bluegrass. Agronomy Journal. 75:469-473.

Kozelnicky, G.M. 1974. Updating 20 Years of Research : SDS on Bermudagrass. USGA - Greens Section Record. 12(3) 12-15.

Lucas, L.T. 1980. Control of Spring Dead Spot of Bermudagrass With Fungicides in North Carolina. Plant Disease. 64:868-870.

---. 1980. Spring Dead Spot of Bermudagrass. In: B.G. Joyner and P.O. Larsen (eds.) Advances in Turfgrass Pathology. Harcourt Brace Jovanovich, Duluth, MN. pp. 183-187.

Muse, R.R. 1971. Chemical Control of Fusarium Blight of Merion Kentucky Bluegrass. Plant Disease Reporter. 55:333-335.

Otto, M.E., Vargas, J.M. Jr. 1985. Isolation, Pathogenicity, and Inhibition of Growth with Fungicides of an L. korrae - like Fungus from Kentucky Bluegrass in Michigan. (abstr.) Phytopathology. 75:1286.

Pair, J.C., Crowe, F.J., Willis, W.G. 1986. Transmission of Spring Dead Spot Disease of Bermudagrass by Turf/Soil Cores. Plant Disease. 70:877-878.

Partyka, R.E. 1976. Factors Affecting Fusarium Blight in Kentucky Bluegrass. Weeds, Trees, and Turf. July 1976. pp. 37-38.

Ross, J.L., Vargas, J.M. Jr. 1984. Yellow Patch/ Cool Season Brown Patch. In: 54th Annual Michigan Turfgrass Conference Proceedings. 13:36-41.

Sanders, P.L., Burpee, L.L., Cole, H. Jr. 1978. Preliminary Studies on Binucleate Turfgrass Pathogens that Resemble Rhizoctonia solani. Phytopathology. 68:145-148.

---,---,---. 1978. The Effect of Soil Composition on Initial and Residual Efficacy of Four Systemic Fungicides on Pot Grown Creeping Bentgrass. Plant Disease Reporter. 62:545-549.

---. Pennypacker, B.W., Cole, H. Jr. 1980. Histological Study of Merion Kentucky Bluegrass Showing Symptoms of Fusarium Blight. (abstr.) Phytopathology. 70:468.

---, Cole, H. Jr. 1981. The Fusarium Diseases of Turfgrass. In: P.E. Nelson, T.A. Toussoun, and R.J. Cook,(eds.) Fusarium : Diseases, Biology and Taxonomy. Pennsylvania State Univ. Press., University Park, PA. pp. 195-209.

Sivasithamparam, K., Parker, C. A. 1978. Effects of Certain Isolates of Bacteria and Actinomycetes on Gaeumannomyces graminis var tritici and Take-all of Wheat. Australian Journal of Botany. 26:773-782.

Smiley, R.W., Craven M.M. 1977a. Control of Benzimidazole Tolerant Fusarium roseum on Kentucky Bluegrass. Plant Disease Reporter. 61:484-488.

---. 1977b. Reflections on Fusarium Blight. 1977. Weeds, Trees, and Turf. May 1977. pp. 22-28.

---, Craven, M.M. 1978. Fungicides in Kentucky Bluegrass Turf : Effects on Thatch and pH. Agronomy Journal. 70:1013-1019.

---,---. 1979a. Fusarium spp. in Soil, Thatch and Crowns of Poa pratensis Turfgrass Treated with Fungicides. Soil Biology and Biochemistry. 11:355-363.

---,---. 1979b. Microflora of Turfgrass Treated With Fungicides. Soil Biology and Biochemistry. 11:349-353.

---. 1980a. Fusarium Blight of Kentucky Bluegrass : New Perspectives. In : P.O. Larsen , B.G. Joyner (eds.) Advances in Turf Pathology. Harcourt, Brace, Jovanovich and Co., Duluth, MN. pp. 155-177.

---, Craven, M.M., and Bruin, J.A. 1980b. Fusarium Blight and Physical, Chemical, and Microbial Properties of Kentucky Bluegrass Sod. Plant Disease. 64:60-63.

---, Gallenberg, D.J., Palmer, J.R., Paysour, R.E. 1981. Poa pratensis L. Cultivar Origin in Relation to its Relative Susceptibility to Fusarium Blight. In: R.W. Sheard (ed.) Proc. 4th Int. Turfgrass Res. Conf. (Guelph). Am. Soc. Agron., Madison, WI. pp. 433-441.

---. 1983a. Compendium of Turfgrass Diseases. APS. St. Paul, MN. 102 p.

---, Kane R.T. 1983b. Plant Growth Regulating Effects of Systemic Fungicides Applied to Kentucky Bluegrass. Agronomy Journal. 75:469-473.

---, Craven Fowler, M. 1984a. L. korrae and P. graminicola Associated With Fusarium Blight Syndrome of Poa pratensis in New York. Plant Disease. 68:440-442.

---, ---, Kane, R.T. 1984b. Characteristics of Pathogens Causing Patch Diseases of Poa pratensis in New York. (abstr.) Phytopathology. 74:811.

---. 1984c. Fusarium Blight Syndrome Re-described as a Group of Patch Diseases Caused by Phialophora graminicola, Leptosphaeria korrae, or Related Species. (abstr.) Phytopathology. 74:811.

---, Craven-Fowler, M. 1984d. New Insights into Fusarium Blight Syndrome. Grounds Maintenance. Nov. 1984.

---. 1985a. Arsenate Herbicide Stress and Incidence of Summer Patch on Kentucky Bluegrass Turfs. Plant Disease. 69:44-48.

---, Craven-Fowler, M.,Kane, R.T., Petrovic, A.M., White, R.A. 1985b. Fungicide Effects on Thatch Depth, Thatch Decomposition Rate and Growth of Kentucky Bluegrass. Agromomy Journal. 77:597-602.

---,---. 1985c. New Insights into the Fusarium Blight Syndrome. ALA Technology. March 1985. p. 60.

---,---. 1985d. Techniques for Inducing Summer Patch Symptoms on Poa pratensis. Plant Disease. 69:482-484.

---, Kane, R.T., Craven-Fowler, M. 1985e. Identification of Gaeumannomyces - like Fungi Associated with Patch Diseases of Turfgrasses in North America. In: F. Lemaire (ed.). International Turfgrass Research Conference. (Avignon, France) 1985. pp. 609-618.

---, Craven-Fowler, M., Buchanan, L. 1985f. Bioassay of Phytotoxins Associated With Healthy and Patch Disease Affected Poa pratensis Turfgrass. In: F. Lemaire (ed.) International Turfgrass Research Conference. (Avignon, France) 1985. pp. 619-628.

Smith, A.M. 1965. Ophiobolus herpotrichus a Cause of Spring Dead Spot in Couch Turf. Agricultural Gazette of New South Wales. 76:753-758.

---. 1971. Spring Dead Spot of Couchgrass Turf in New South Wales. Journal of the Sports Turf Research Institute. no. 47 pp. 54-59.

Subriats, F.J., Self, R.L. 1972. Fusarium Blight of Centipede Grass. Plant Disease. 56:42-44.

Thompson, D.C., Smiley, R.W., Craven-Fowler, M. 1983. Oxidation Status and Gas Composition of Wet Turfgrass Thatch and Soil. Agronomy Journal. 75:603-609.

Troll, J. 1969. Fusarium Blight and Merion Bluegrass. U.S. USGA Green Section Record. Jan. pp. 7-9.

Turgeon, A.J., Meyer, W.R. 1974. Effects of Mowing Height and Fertilization Level on Disease Incidence in Five Kentucky Bluegrasses. Plant Disease Reporter. 58:514-516.

---. 1976. Effects of Cultural Practices on Fusarium Blight Incidence in Kentucky Bluegrass. Weeds, Trees, and Turf. July 1976.

---. 1980. Turfgrass Management. Reston Publishing Co., Reston, VA. 391 p.

Vargas, J.M. Jr., Laughlin, C.W. 1971. Benomyl for the Control of Fusarium Blight of Merion Kentucky Bluegrass. Plant Disease Reporter. 55:167-170.

---,---. 1972. The Role of Tylenchorhynchus dubius in the Development of Fusarium Blight of Merion Kentucky Bluegrass. Phytopathology. 62:1311-1314.

---, Turgeon, A.J. 1980. The Principles of Blending Kentucky Bluegrass Cultivars For Disease Resistance. In: J.B. Beard (ed.), Proceedings of the 3rd International Turfgrass Research Conference. Am. Soc. Agron., Munich, W. Germany. pp. 45-53.

---. 1981. Management of Turfgrass Diseases. 1981. Burgess Publ. Co., Minneapolis, MN. 204 p.

---. 1982. Fusarium Blight : Its Development and Management. ALA Technology. May/June 1982. pp. 16-18.

---, 1986. Cool Season Turf Diseases. Weeds, Trees, and Turf. April, 1986.

Wadsworth, D.F., Young, H.C. 1960. Spring Dead Spot of Bermudagrass. Plant Disease Reporter. 44:516-518.

Walker, J. 1972. Type Studies on Gaeumannomyces graminis and Related Fungi. Trans. Brit. Mycol. Soc. 58:427-457.

---, Smith, A.M. 1972. L. narmari and L. korrae, Two Long-spored Pathogens of Grasses in Australia. Trans. Brit. Mycol. Soc. 58:459-466.

Wong, P.T.W., Baker, R. Control of Wheat Take-all and Ophiobolus Patch of Turfgrass by Fluorescent Pseudomonads. In: Ecology and Management of Soilborne Plant Pathogens. 1985. APS Press, St. Paul, MN pp. 151-153.

Worf, G.L., Avenius, R.C., Stewart, J.S. 1982. A Gaeumannomyces - like Organism Associated With Diseased Bluegrass in Wisconsin. (abstr.) Phytopathology. 72:975.

---, Brown, K.J. et.al. 1983. Survey of NRS Disease in Wisconsin Lawns. (abstr.) Phytopathology. 73:839.

---, Stewart, J.S. 1985. Bluegrass Necrotic Ring Spot. ALA Technology. Feb. 1985. pp. 58.

---,---. 1986. Necrotic Ring Spot Disease of Turfgrass in Wisconsin. Plant Disease. 70:453-458.

Young, H.C. Jr., Sturgeon, R.V. Jr., Huffine, W.E. 1973. Soil Type Associated With Spring Dead Spot of Bermudagrass. (abstr.) Phytopathology. 63:450.