EXECUTIVE SUMMARY

IOWA STATE UNIVERSITY

Potential for Physiological Management of Symptom Expression by Turfgrasses Infected by *Bipolaris sorokiniana*

Final report: 1993

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Dr. Clinton F. Hodges
Principal Investigator

The endogenous ethylene synthesized in leaves of *Poa pratensis* in response to infection by *Bipolaris sorokiniana* is responsible for a substantial portion of the chlorophyll (Chl) lost from the leaves during pathogenesis. Previous studies have examined the potential for preventing chlorosis of infected leaves by inhibiting the biosynthesis and/or mode of action of ethylene during pathogenesis. Studies conducted during 1992-93, established that control of the mode of action of ethylene during pathogenesis is ineffective at decreasing the loss of Chl from infected leaves. Conversely, inhibition of ethylene biosynthesis during pathogenesis by means of pyridoxal enzyme inhibitors substantially decreases Chl loss from infected leaves. Several pyridoxal enzyme inhibitors have proven effective, but L-canaline has been most effective with minimal side effects. L-canaline is hydrolytically cleaved from L-canavanine, a naturally occurring amino acid in jack beans and other legumes.

L-canaline has been evaluated over a concentration range of 10^{-4} to 10^{-12}M. Ethylene biosynthesis in infected leaves treated with 10^{-4} or 10^{-6}M L-canaline was 40-50% and 50-60% less than that in nontreated infected leaves for each concentration, respectively. At these concentrations (10^{-4} and 10^{-6}M), L-canaline increased the retention of Chl in infected leaves to 91 and 82%, respectively, of that in healthy control leaves. The Chl content of nontreated infected leaves was 67% of the healthy control leaves. Dilutions of L-canaline beyond 10^{-6}M were less effective at decreasing ethylene biosynthesis and Chl retention failed to occur during pathogenesis. No phytotoxic responses have been observed with L-canaline except for a slight inhibition of hyphal growth at 10^{-4}M.

There is evidence that leaf spot may enhance senescence processes in infected leaves. Preliminary studies with cytokinins, gibberellins, and some antibiotics known for their ability to slow Chl loss during senescence have been initiated to determine if these substances can decrease Chl loss during pathogenesis. Kinetin riboside, zeatin riboside, and the antibiotic cordycepin decreased Chl loss and ethylene biosynthesis during pathogenesis at a concentration of 10^{-4}M. These compounds also showed some inhibition of hyphal growth and phytotoxicity to leaves; both problems seem concentration related and are believed to be correctable. Gibberellic acid decreased ethylene biosynthesis in infected leaves but failed to prevent Chl loss.

The physiological evidence to date suggests that a symptom expression management system for leaf spot may be feasible. Research will continue on senescence inhibition, and new studies will be initiated on the function of the fungal toxin helminthosporal in symptom expression.

FINAL PROGRESS REPORT

November, 1993

PROJECT TITLE

Potential for Physiological Management of Symptom Expression by Turfgrasses Infected by *Bipolaris sorokiniana*

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DATE OF PROGRESS REPORT

September 15, 1993

SUBMITTED TO

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A. OBJECTIVES

The primary objectives of this project for 1993, were to determine the effective range of activity for L-canaline as an inhibitor of ethylene biosynthesis in *Poa pratensis* infected by *Bipolaris sorokiniana*, and to initiate studies to evaluate the ability of cytokinins, gibberellins, and antibiotics to slow the loss of chlorophyll during pathogenesis. Of the various substances found to prevent biosynthesis and/or mode of action of ethylene during infection, L-canaline has proven most effective. L-canaline is a pyridoxal enzyme inhibitor that interrupts the biosynthesis of ethylene; materials that interfere with the mode of action of ethylene during pathogenesis have not proven effective. Preliminary studies with cytokinins, gibberellins, and antibiotics were initiated to evaluated their ability to slow the loss of chlorophyll during pathogenesis by slowing senescence processes believed to be accelerated by the disease.

B. OBSERVATIONS

1. Canaline Studies:

L-canaline [L-α-amino-γ-(aminooxy)-n-butyric acid] (dipicrate salt) is a natural product derived from the amino acid L-canavanine. L-canavanine occurs naturally in jack bean (*Canavalia ensiformis)* and at least 1,200 legumes. In our studies, it has proven to be an effective pyridoxal enzyme inhibitor that substantially decreases the biosynthesis of ethylene during leaf spot development with minimal side effects. This decrease in ethylene results in a decrease in the loss of chlorophyll during disease development. Other pyridoxal enzyme inhibitors are also effective and may be further evaluated in the future.

In previous studies, L-canaline has proven effective when applied to the soil or to the leaves of infected plants at a molar concentration of 10^{-4}. This years studies have concentrated on determining the effective concentration range of L-canaline when applied to the leaf surface, and to determine its potential toxicity to the pathogen.

A dilution series of L-canaline (10^{-4}, 10^{-6}, 10^{-8}, 10^{-10}, and 10^{-12}M) was evaluated for potential inhibition of *B. sorokiniana* hyphal growth. Hyphal growth was slightly inhibited by L-canaline at 10^{-4}M (Fig. 1). This response provides evidence that the L-canaline is not preventing or significantly slowing infection by the pathogen and that its primary function is that of decreasing ethylene biosynthesis during pathogenesis.

L-canaline at 10^{-4}M applied to leaves and subsequently inoculated with *B. sorokiniana* decreased the biosynthesis of ethylene by about 50% at each of the 24h observation periods through 96h (Fig. 2). As the L-canaline was diluted through 10^{-8}M, the ethylene increased; dilutions of 10^{-10} and 10^{-12}M were about the same as

those observed for 10^{-8}M. At the 24h and 96h sampling periods the ethylene content of inoculated leaves treated with L-canaline dilutions of 10^{-8}M and greater did not differ from that of the inoculated controls. The dilution series had little effect on the ethylene content of healthy control leaves (Fig. 2).

The chlorophyll content of infected control leaves 96h after inoculation was 67% of that in the healthy control. L-canaline at 10^{-4} and 10^{-6}M increased the chlorophyll content of diseased leaves to 91 and 82% of healthy control leaves, respectively (Fig. 3). The chlorophyll content of infected leaves treated with L-canaline at 10^{-8}, 10^{-10}, and 10^{-12}M did not differ from that of the inoculated control leaves.

The overall results of the L-canaline studies show that it is an effective inhibitor of ethylene biosynthesis in the concentration range of 10^{-4} and 10^{-6}M. At these concentrations, chlorophyll loss during infection is minimal. This substance also seems effective applied to leaves or roots.

2. Cytokinin and Antibiotic Studies:

Preliminary studies have been initiated with cytokinins to determine their ability to slow the loss of chlorophyll during pathogenesis. Cytokinins are known to slow the loss of chlorophyll associated with senescence processes. In that there is some evidence that leaf spot enhances the ageing of infected leaves, the cytokinins may help slow the loss of chlorophyll during pathogenesis. In addition to the cytokinins, some antibiotics known for their ability to slow chlorophyll loss during senescence are also being evaluated.

Ten cytokinins and one antibiotic have been examined in our initial screening studies. All were applied to leaves of *P. pratensis* at a concentration of 10^{-4}M prior to inoculation. Of the materials evaluated, kinetin riboside, zeatin riboside, and the antibiotic cordycepin decreased chlorophyll loss during pathogenesis. The chlorophyll content of the treated, infected leaves ranged from 93 to 102% of the healthy control leaves; the chlorophyll content of inoculated control leaves was 69%. All three substances had the unexpected affect of decreasing ethylene biosynthesis during pathogenesis. However, some problems exist with these compounds that must be resolved. All showed some inhibition of the hyphal growth of *B. sorokiniana*. From the standpoint of disease control this is not a negative effect; however, for the purposes of testing our hypothesis of symptom manipulation, it may be a problem if it prevents normal infection and disease development. A second problem involves some mild phytotoxic effects on healthy control leaves. It is believed that both problems are concentration and application oriented. The preliminary studies have been conducted with relatively high concentrations of the compounds (10^{-4}M) and it is believed that

the problems can be solved with dilution and some changes in application.

3. **Gibberellin Studies:**

Gibberellins (GA) are known to slow senescence in some plant species and they are active growth regulating compounds in grasses. GA_3 at concentrations of 10^{-4}, 10^{-6}, and 10^{-8}M applied to leaves before inoculation decreased ethylene biosynthesis in a narrow time frame during pathogenesis and failed to prevent chlorophyll loss as the disease developed. GA_3 decreased ethylene biosynthesis to at least 50% of the inoculated control at 48 and 72h after inoculation, however, it had no effect at 24 and 96h. No phytoxicity was observed and leaves with active intercalary meristems showed growth stimulation in some instances in excess of one-inch within the 96h assay period. The GA_3 response is more complex than that observed with the cytokinins and will, therefore, be placed on hold in favor of continuing the cytokinin studies.

C. CONCLUSIONS AND OUTLOOK

1. **Ethylene Biosynthesis and Pathogenesis:**

Our studies to date establish that endogenous ethylene biosynthesis in leaves of *P. pratensis* infected by *B. sorokiniana* can be substantially decreased with pyridoxal enzyme inhibitors, and that the decrease in endogenous ethylene results in an increase in the chlorophyll retained by the infected leaves. Therefore, the outlook for the eventual use of pyridoxal enzyme inhibitors as part of a disease symptom management program seems to be increasingly promising.

The most effective pyridoxal enzyme inhibitor evaluated to date is L-canaline. Other effective pyridoxal enzyme inhibitors that remain to be evaluated in detail are 1-aminoethoxyvinylglycin (AVG) and aminooxyacetic acid (AOA). In earlier studies, AOA and AVG seemed more effective than L-canaline at decreasing ethylene biosynthesis. However, some unexpected toxicity problems occurred with both compounds that must be resolved before they can be evaluated in more detail. Studies with L-canaline are complete at this point. Resumption of studies with L-canaline will occur pending the outcome of the cytokinin and antibiotic studies. Should effective substances be found, they will be combined with L-canaline for further evaluation.

In order to maximize the inhibition of ethylene biosynthesis during pathogenesis by pyridoxal enzyme inhibitors, additional information is needed relative the specifics of ethylene production in infected leaves. We know from previous research that the level of ethylene generated during infection decreases in progressively older leaves. However, damage to older leaves is

more severe than in young leaves because the threshold of sensitivity to the ethylene decreases in older leaves. We do not know, however, if ethylene biosynthesis in response to infection is localized or systemic. For example, if the youngest or oldest leaf of a shoot is infected, does ethylene rise only in the infected leaf or does it rise in all leaves of the shoot. This information is of importance relative the effectiveness of the pyridoxal enzyme inhibitors across the various age leaves of the shoot. Studies to evaluate this problem will be initiated this year.

2. **Cytokinins and Antibiotics:**

Preliminary studies with kinetin riboside and zeatin riboside, and with the antibiotic cordycepin, suggest that these substances can decrease the biosynthesis of ethylene and the loss of chlorophyll during disease development. However, these substances also inhibit hyphal growth and show some evidence of phytotoxicity. The outlook for incorporating these materials into a disease symptom management system remains uncertain at this time and will require additional study.

These substances will be further evaluated over a dilution range and hopefully we will find a concentration range that is effective at preventing chlorophyll loss without other detrimental effects. To fully assess the effectiveness and mode of action of the cytokinins and antibiotic in a symptom management system, it will be necessary to characterize the process of senescence in healthy and infected leaves. The leaves of the shoot will be evaluated for protein, amino nitrogen, and chlorophyll content as a measure of their degree of aging. The ability of the cytokinins and antibiotic to alter these factors in healthy and infected leaves will provide evidence for their mode of action.

D. PUBLICATIONS

1. Hodges, C. F. and Campbell, D. A. 1993. Regulation of endogenous ethylene in leaves of *Poa pratensis* infected by *Bipolaris sorokiniana* by means of root applied substances inhibitory to ethylene biosynthesis. J. Plant Physiol. (accepted)

2. Hodges, C. F. and Campbell, D. A. 1994. Inhibition of endogenous ethylene biosynthesis by L-canaline in leaves of *Poa pratensis* infected by *Bipolaris sorokiniana*. Plant Physiol. (in preparation)

Principle Investigator

9/16/93
Date

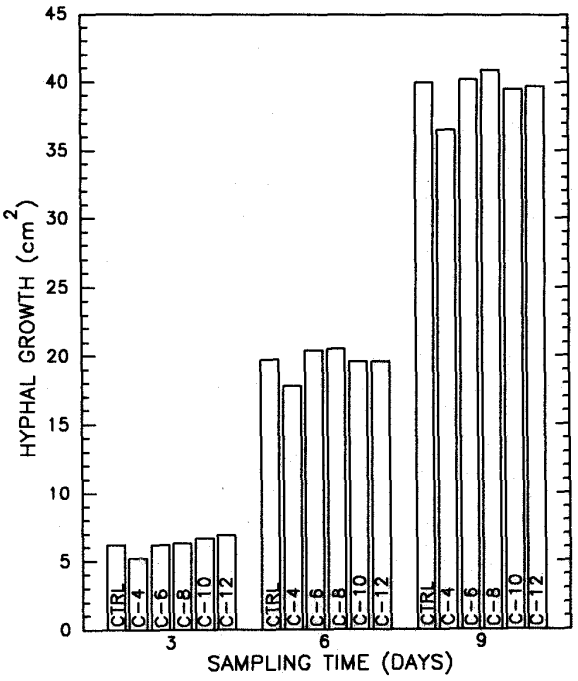

Fig. 1. Hyphal growth of *Bipolaris sorokiniana* on different concentrations of L-canaline ranging from 10^{-4} to 10^{-12}M. Note: These are preliminary values and have not been analyzed statistically.

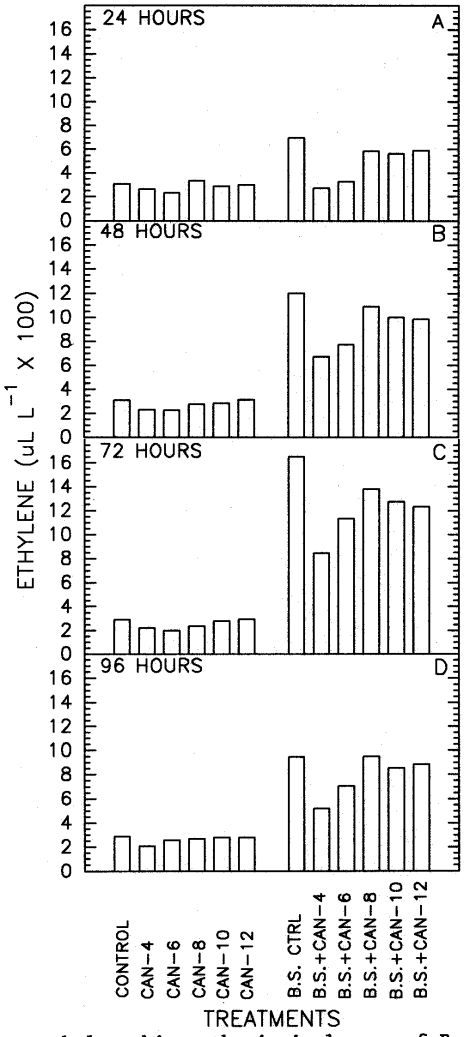

Fig. 2. Endogenous ethylene biosynthesis in leaves of *Poa pratensis* treated with different concentrations of L-canaline ranging in concentration from 10^{-4} to 10^{-12}M and infected by *Bipolaris sorokiniana*. Note: These are preliminary values and have not been analyzed statistically.

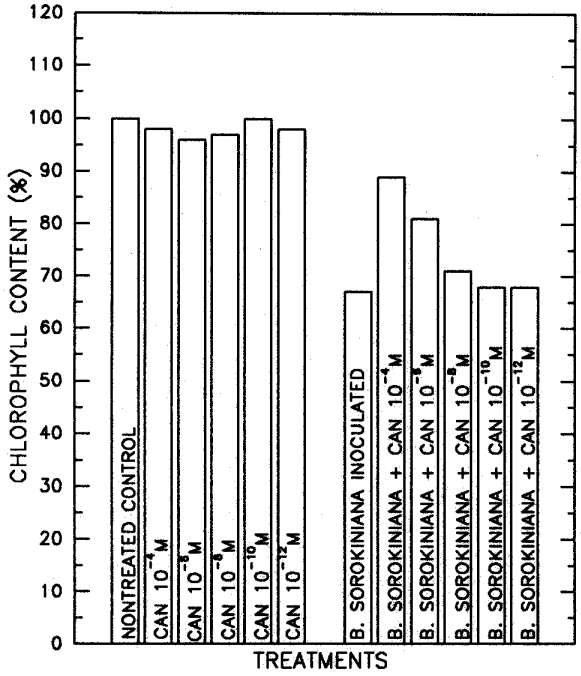

Fig. 3. Chlorophyll content of leaves of *Poa pratensis* exposed to L-canaline ranging in concentration from 10^{-4} to 10^{-12}M and infected by *Bipolaris sorokiniana*. Note: These are preliminary values and have not been analyzed statistically.