Iowa State University

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

INVESTIGATORS:

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EXECUTIVE SUMMARY

IOWA STATE UNIVERSITY

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

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Principal Investigator

Endogenous ethylene is generated in the leaves of Poa pratensis in response to infection by Bipolaris sorokiniana and the ethylene contributes substantially to the loss of chlorophyll from the infected leaves. This research project was initiated to determine if the endogenous ethylene, or its mode action, can be manipulated to prevent the loss of chlorophyll in infected leaves and prevent yellowing. Prevention of ethylene induced yellowing could result in the control of symptom expression, specifically yellowing of infected turf, independent of the infection. This could reduce use of fungicides and provide a new approach to disease management.

Research conducted in the last year has concentrated on decreasing ethylene in infected leaves by applying ethylene inhibiting substances to roots of inoculated plants. The following materials have been evaluated for their effectiveness when applied to the soil.

1. Aminooxyacetic Acid (AOA)
2. Aminoisobutyric Acid (AIBA)
3. Benzoic Acid (BNZ)
4. Canaline (CAN)
5. Carbonyl Cyanide m-Chlorophyenylhydrazone (CCCP)
6. Cobalt Chloride (COCL)
7. Propyl Gallate (PGA)

Endogenous ethylene in healthy leaves ranged from 276 to 321 $\mu l\ l^{-1}$. Endogenous ethylene of inoculated leaves increases by 24h, peaked at 48h (1476 $\mu l\ l^{-1}$), and then declined at 72h and 96h. CAN, AOA, CCCP, and PGA applied to roots reduced leaf ethylene in response to infection. Of the materials that decreased ethylene, only CAN and AOA prevented substantial loss of chlorophyll. Inoculated leaves of plants treated with CAN and AOA retained 74% and 80% of their Chlorophyll, respectively.

Preliminary results from leaf treatments with CAN and AOA show a greater decrease in the surge of endogenous ethylene associated with infection than that achieved with soil treatment. Ethylene levels have averaged 30% of that in inoculated controls with as much as a 91% retention of chlorophyll.

These observations suggest that manipulation of symptom expression in this host-pathogen interaction (and perhaps others) is feasible. Our 1993, studies will concentrate on the function and control of senescence processes during pathogenesis.

A. OBJECTIVES

The primary objective of this project for 1992 was to evaluate substances known to prevent the biosynthesis and/or mode of action of the endogenous ethylene generated during the infection of Poa pratensis by Bipolaris sorokiniana. The endogenous ethylene generated is a major physiological factor in the yellowing of infected leaf tissue. Control of this process could feasibly reduce or eliminate the use of fungicides for the control of this disease (and possibly others) by preventing the yellowing associated with disease development.

B. OBSERVATIONS

The primary source of endogenous ethylene during infection is from the biosynthetic pathway in the host plant. Some ethylene also is produced by the pathogen from a second pathway, but the amount is believed to be negligible in most cases. Therefore, prevention of biosynthesis and/or mode of action of endogenous ethylene produced by the host was the target of the 1992 studies. The following substances have been evaluated for their ability to prevent the biosynthesis of ethylene and to prevent or reduce yellowing:

1. Aminooxyacetic Acid (AOA)
2. Aminoisobutyric Acid (AIBA)
3. Benzoic Acid (BNZ)
4. Canaline (CAN)
5. Carbonyl Cyanide m-Chlorophyenylhydrazone (CCCP)
6. Cobalt Chlorophylloride (COCL)
7. Propyl Gallate (PGA)

1. Soil Treatment Studies.

Ten milliliters of each substance at concentrations of 10^{-3}M were applied to the soil in pots containing P. pratensis each of 3 days preceding inoculation, and then each of 4 days during pathogenesis which included the assay days for endogenous ethylene evolution by the infected leaves. The four youngest visible leaves were inoculated with four agar plugs (4mm) containing the mycelium of the pathogen.

a. Ethylene Production:

Inoculated plants were assayed for endogenous ethylene generation at 24, 48, 72, and 96 hours after inoculation. The normal levels of endogenous ethylene in healthy leaves ranged from 276 to 321 $\mu l\ l^{-1}$. All ethylene inhibiting substances, except AIBA, decreased endogenous ethylene at one or more of the four 24h sampling periods. AOA and PGA were among the more consistent substances at decreasing endogenous ethylene in healthy control leaves.

None of the ethylene inhibiting substances applied to the

roots of P. pratensis prevented infection by B. sorokiniana and lesion development was typical of that on inoculated control plants. All ethylene inhibiting substances, except for BNZ, significantly decreased the surge of endogenous ethylene at 24h and 48h during the early stages of pathogenesis (Table 1). BNZ enhanced endogenous ethylene production in inoculated leaves at 24h , 48h, and 72h. At 72h, as pathogenesis progressed, AIBA and COCL ceased to decrease ethylene evolution. CAN, AOA, CCCP, and PGA continued to decrease endogenous ethylene production at 72h and 96h.

b. Chlorophyll Loss:

Inoculation of leaves of untreated control plants with B. sorokiniana decreased the chlorophyll content of the leaves to 43% of that in healthy control leaves at 96h after inoculation. Ethylene inhibiting materials applied to the roots of healthy plants did not significantly change the chlorophyll content of the leaves. Plants treated with AIBA, BNZ, CCCP, COCL, and PGA and leaf inoculated with B. sorokiniana failed to prevent the loss of chlorophyll during pathogenesis. Inoculated plants treated with CAN and AOA substantially decreased the loss of chlorophyll compared to that of inoculated control plants. Inoculated leaves of plants treated with CAN and AOA retained 74% and 80% of their chlorophyll, respectively.

2. Leaf Treatment Studies.

The soil treatment studies recently completed did not prove to be as effective as we had hoped. Although CAN and AOA contained the rise in ethylene and maintained a substantial amount of the chlorophyll during infection, the response was not as strong as we would have like to have seen. Therefore, studies are in progress in which leaves are being treated directly with the various ethylene inhibiting substances. Leaves of plants are treated two days before inoculation and at the time of inoculation. Environmental parameters include a 12h photoperiod and a temperature of 22°C.

a. Ethylene Production and Chlorophyll Content:

The leaf treatment studies are still in progress, but the general trends emerging from them suggests that the plants are more responsive to leaf treatment. CAN is especially promising in that it has held the ethylene surge during infection to about 30% of that occurring in infected control plants while maintaining 91% of the chlorophyll. AOA and CCCP also seem more effective at decreasing ethylene when applied to leaves; however, their ability to maintain the chlorophyll of the leaf does not seem to be as effective as

that of CAN. Some materials like PGA that were effective controllers of ethylene in the soil studies have proven to be ineffective in the leaf studies. All leaf treatment observations are preliminary and may change with completion of the studies.

C. CONCLUSIONS AND OUTLOOK

1. CAN, AOA, CCCP, and PGA proved effective in decreasing the surge of endogenous ethylene during infection when applied to the roots of inoculated plants. However, only CAN and AOA maintained a substantial portion of the chlorophyll during pathogenesis. The treatment of roots did not prove as effective as we had hoped and we are now in the process of conducting foliar application tests that we believe will be more effective with a broader spectrum of materials. The primary drawback of foliar application is the possibility of increased phytotoxicity. Preliminary observations with foliar applications of CAN and AOA look more promising than the results obtained with soil treatments.

2. The soil treatment studies conducted in this project utilized very high levels of inoculum in an effort to see how effective the materials tested could be at containing ethylene production and subsequent chlorophyll loss. In retrospect, it is probable that the inoculum pressure exerted by this approach simply overwhelmed the plant tissue and any good that the substances being tested might have done. The inoculum levels originally used would be several hundred times greater than any level encountered in the field. Foliar treatments are currently being reexamined with substantially lower levels of inoculum and somewhat different environmental parameters. This could make a major difference in the results.

3. It is unlikely that the ethylene inhibiting substance alone will prevent loss of chlorophyll from infected leaves. Some chlorophyll loss may be related to other hormones and to senescence induced by the pathogen. Work on the potential manipulation of these factors will be a goal of our 1993 research. Control of senescence physiology is a primary goal. If senescence retardation can be achieved and combined with a decrease in ethylene (which induces senescence) production, the potential for symptom expression control is very good.

D. PUBLICATIONS

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

Principle Investigator: _____ Date: 10/7/92

Table 1. Endogenous ethylene in Bipolaris sorokiniana infected leaves of Poa pratensis root treated with substance antagonistic to ethylene biosynthesis.

Chemical Tested	Endogenous Ethylene ($\mu l\ l^{-1}$)			
	24 hr	48 hr	72 hr	96 hr
Healthy Control Leaves	298	321	276	302
Inoculated Control Leaves	1115	1474	1090	578
Inoculated Plants Soil-Treated with Test Chemicals				
Aminooxyacetic Acid (AOA)	732 (66%)[1]	642 (44%)	622 (57%)	427 (74%)
Aminoisobutyric Acid (AIBA)	821 (74%)	1052 (71%)	1061 (97%)	620 (107%)
Benzoic Acid (BNZ)	1238 (111%)	1578 (107%)	1330 (122%)	499 (86%)
Canaline (CAN)	684 (61%)	959 (65%)	672 (62%)	415 (72%)
Carbonyl Cyanide m-Chlorophenyl-hydrazone (CCCP)	723 (65%)	624 (42%)	859 (62%)	358 (62%)
Cobalt Chloride (COCL)	535 (48%)	1556 (106%)	1094 (101%)	495 (86%)
Propyl Gallate (PGA)	567 (51%)	1118 (76%)	574 (53%)	347 (60%)

[1]Percentage of ethylene compared with inoculated control leaves.