Physiological Management of *Bipolaris sorokiniana* Leaf Spot Symptom Expression by Kentucky Bluegrass

A unique approach to disease management that involves suppression of visual symptoms.

by DR. CLINTON F. HODGES

BIPOLARIS SOROKINIANA infects grasses throughout the temperate regions of the world. Among the grass species used for turf, *B. sorokiniana* is best known for causing a leaf spot of Kentucky bluegrass (7, 24). It also can damage creeping bentgrass, perennial rye, and red fescue. In addition to causing leaf spot, *B. sorokiniana* can infect crowns, rhizomes, stolons, roots, and inflorescences of turfgrass plants.

The leaf spot disease caused by *B. sorokiniana* is often referred to as *spring leaf spot* in turf disease textbooks (24). Leaf spot is commonly observed in the spring, but damage to leaves can be more extensive in the fall, especially with wet, moderately cool, overcast conditions (19, 20, 21, 23). The spots produced on the leaves of Kentucky bluegrass are initially dark brown, to deep purple, to almost black in coloration. Spots occur individually or are scattered over the surface of infected leaf blades. As the individual spots enlarge, their centers typically become tan-colored and they often are surrounded by a chlorotic halo. If large numbers of spots occur on a single leaf, they often coalesce and cause rapid blighting of the leaf. Fewer spots on a leaf results in a progressive yellowing of the entire leaf blade. Yellowing of infected leaves occurs in the early spring, but it is the dominant symptom in fall and early winter.

Control of leaf spot (and the other diseases caused by this pathogen) can be difficult. The resistance shown by Kentucky bluegrass cultivars is not very reliable and generally breaks down under cultural and environmental stresses (12, 23, 24). Several fungicides are effective against *B. sorokiniana* (24), but to be most effective they must be on or in the plants before infections are initiated. Once leaf spot has been initiated (and other organs of the plant also are infected), chemical control often is ineffective due to the inaccessibility of the fungicides to infected crowns, rhizomes, and roots (23). Hence, when Kentucky bluegrass is severely leaf-spotted, very large areas of turf become yellowed and the quality of the grass becomes aesthetically unacceptable. If the condition persists throughout the growing season, or from season to season, the turf will progressively thin and large areas can be completely lost.

Developmental Physiology of Leaf Spot

Control of leaf spot by means of cultural practices, host plant resistance, and fungicides is difficult and expensive under the best of circumstances. Because of the difficulty of controlling leaf spot and the prevalence of the disease in the north central states, studies on the developmental physiology of leaf spot of Kentucky bluegrass have been an ongoing part of the turf disease program at Iowa State University for a number of years. The ultimate purpose of this research has been to determine the physiology of disease development and to develop new approaches to the control and/or management of the disease.

The most damaging developmental characteristic of leaf spot is the yellowing of infected leaves that decreases the aesthetic value of the turf. Research conducted in our laboratory has shown that the yellowing of infected leaves is related to light, senescence processes, hormone action, and a phytotoxin produced by the pathogen. The yellowing of infected leaves can also be enhanced by postemergence herbicides.

Light and Senescence: The severity of yellowing by leaf-spot infected leaves typically increases with the aging of the individual leaves. This developmental characteristic of leaf spot is substantially influenced by photoperiod. As daylength increases from 10 to 14 hours, the size of the leaf spots and the yellowing of infected leaves decreases (18, 21). Infected leaves subjected to continuous dark for 96 hours will have up to 70% of the leaf tissue turn yellow; infected leaves subjected to continuous light show yellowing of only 9% of the leaf tissue. These observations explain in part why leaf spot becomes less severe from spring to summer as daylength increases. It also

provides part of the evidence for why this disease is potentially more important in the fall (with progressively shorter daylengths) than in the spring.

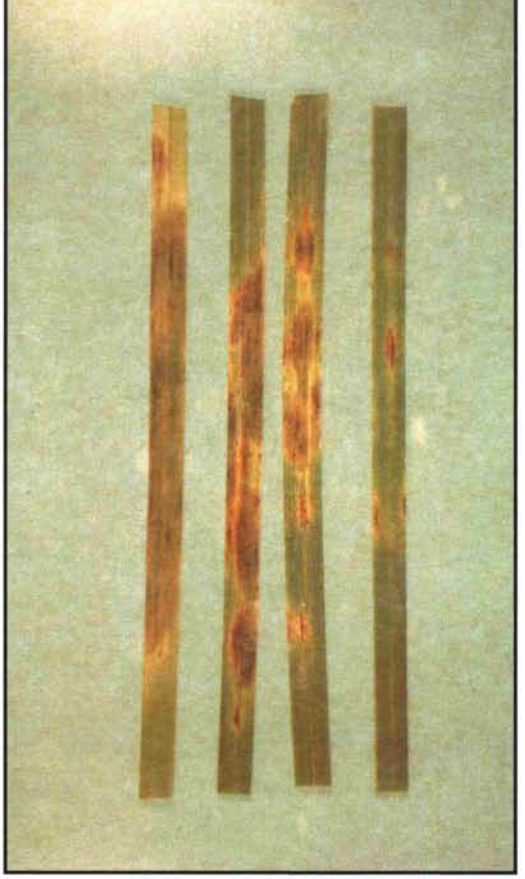
Daylength also interacts with the natural senescence processes of leaves to further enhance yellowing of infected leaves. When progressively older infected leaves are subjected to a natural light spectrum with a daylength of 14 hours, there is little difference in the yellowing of leaves of different ages. When the infected leaves of different ages are subjected to 10- or 14-hour daylengths, yellowing of the two youngest leaves does not differ, but the two oldest infected leaves subjected to the 10-hour daylength show substantial yellowing (20). These responses imply an interaction between photoperiod and leaf senescence that influences severity of leaf spot development and subsequent yellowing of infected leaves. Long daylengths (14 hours or more) delay senescence and the yellowing of infected leaves. Short daylengths (10 hours or less) promote leaf senescence, especially among older leaves, and this condition is exploited by the pathogen to increase the severity of yellowing.

This light phenomenon is further illustrated by increasing the amount of far-red light in the light spectrum. Far-red light is known to promote senescence processes, and it will increase the severity of yellowing by infected leaves irrespective of daylength (20). The older leaves of the shoot that are beginning to senesce are especially sensitive to far-red light. As light passes through the upper, younger leaves of the shoot, the proportion of far-red light striking older undercover leaves increases and further enhances the yellowing associated with infected leaves. The effect of far-red light combined with the senescence-promoting effect of short daylength in the fall further predisposes infected leaves to accelerated yellowing.

Hormones: The interaction between photoperiod, light quality, and senescence implies that hormones are involved in the yellowing of infected leaves. The first evidence of this came from studies on the effect of postemergence herbicides on leaf spot development. Applications of 2,4-D, MCPP, or dicamba to Kentucky bluegrass were found to substantially increase the yellowing of leaf-spot-infected leaves (8). It was further observed that the herbicides increased the severity of yellowing on each older infected leaf (9, 10). These postemergence herbicides are auxin-type growth regulators. One of the side effects of auxin-type herbicides in perennial grasses and other plant species is that they elicit the production of the hormone ethylene by the plant, and the increase in ethylene can enhance the rate of senescence in mature tissues (1). This enhanced senescence caused by the herbicide-generated ethylene is exploited by the pathogen, and the yellowing of older infected leaves is further enhanced.

The potential involvement of ethylene in the yellowing of leaf-spot-infected leaves of Kentucky bluegrass provided the first critical information on the physiology of the disease that could be tested experimentally. During the infection of Kentucky bluegrass leaves by *B. sorokiniana*, studies revealed that substantial quantities of ethylene were produced in the leaves and that the increase in ethylene was correlated with the yellowing of infected leaves (15, 18). Natural levels of ethylene in leaves of Kentucky bluegrass range from about 250 to 350 ppb. The ethylene content of leaves infected by *B. sorokiniana* increases to 2,400 ppb or higher within 48 to 72 hours after infection and then progressively declines as the disease progresses. Visible yellowing of infected leaves can be seen within 24 hours of peak ethylene production. The severity of the yellowing increases on each older infected leaf, illustrating the link between the ethylene surge and leaf senescence (11).

Although a good cause-and-effect correlation was developed between the rise in ethylene and the subsequent yellowing of infected leaves, proof that the ethylene was responsible for the yellowing of infected leaves was still needed. Ethylene, as a plant hormone, is unique because it is a gaseous substance. This characteristic permits the ethylene to be evacuated from the leaf tissue during the infection process by placing the leaves of intact plants under a vacuum. When ethylene is evacuated from infected leaves, the leaf spots develop normally, but about 80% of the chlorophyll is retained by the infected leaves and they remain visibly green (15). In other studies in which plants were treated with norbornadiene (NBD), a substance known to block the mode of action of ethylene, infected leaves retained over 90% of their chlorophyll while the leaf spots developed normally (11). These studies clearly established ethylene as the primary cause of yellowing of leaf-spot-infected leaves.

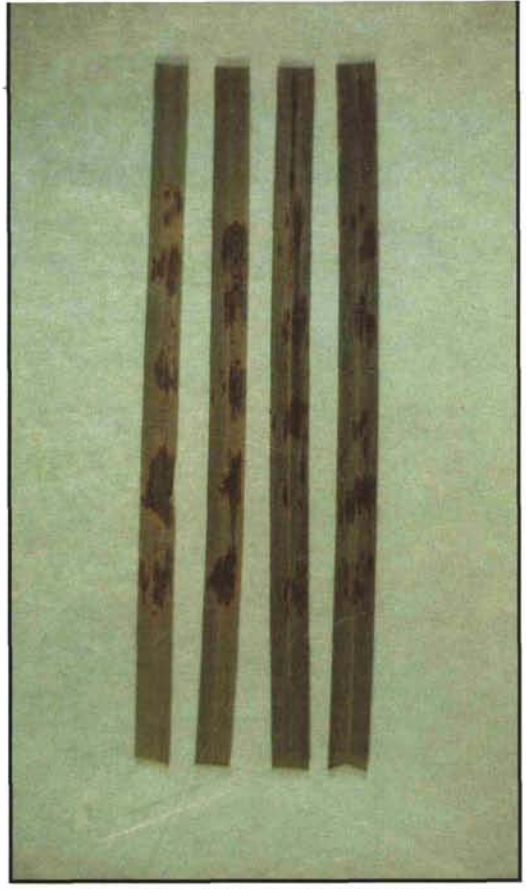


Development of Bipolaris sorokiniana *on Kentucky bluegrass leaves results in yellowing 96 hours after inoculation.*

Phytotoxins: When ethylene is evacuated from infected leaves, or its mode of action is interrupted by NBD, there is still a 10% to 20% loss of chlorophyll from the leaves. This loss seems to be associated with the chlorotic halo surrounding individual leaf spots on the leaves. Development of chlorotic halos is not prevented by the control of ethylene action. It is believed that the halos are the result of direct injury by a nonspecific phytotoxin (helminthosporal or prehelminthosporol) produced by *B. sorokiniana* during the infection process (4, 25, 26). The phytotoxin functions by disruption of cell membranes.

The Potential for Management of Symptom Expression

The establishment of ethylene as a primary cause of yellowing of leaf-spot-infected leaves provided an opportunity to develop a new approach to the management of this disease. The traditional approaches to disease control are development of plant resistance and/or prevention of infection by means of fungicides. Both approaches have substantial limitations for the control of



Leaves treated with canaline (CAN) to prevent the biosynthesis of ethylene by the plant during infection affects the development of Bipolaris sorokiniana.

diseases induced by *B. sorokiniana*. However, with increasing knowledge of the physiology of leaf spot development, it may be possible to prevent the yellowing of infected leaves without preventing infection. If the yellowing of leaf-spot-infected leaves could be prevented, the presence of small leaf spots would be of little consequence to the aesthetic value of the turf, and the diseased leaves would be periodically mowed off. Development of a system for management of symptom expression (specifically the yellowing of infected leaves) could provide a new approach to leaf spot management that could compliment, or replace, present systems that rely solely on prevention of infection.

There are two possible approaches to how management of symptom expression by leaf-spot-infected leaves might be accomplished. One approach involves the chemical inhibition of ethylene biosynthesis, or its mode of action, during the infection of the leaves. The second approach involves the regulation of ethylene biosynthesis during infection by means of genetic modification of the plant. To understand the work we have conducted to date on these approaches, it is necessary to return to ethylene and its biosynthesis during the infection process.

Potential for Chemical Management of Symptom Expression

Studies were initiated in 1991 to determine if ethylene biosynthesis could be reduced or prevented in leaves infected by *B. sorokiniana*, and thereby prevent a substantial portion of the yellowing of infected leaves. The ethylene generated during infection is produced primarily by the host plant in response to the infection (22), with relatively small amounts coming from the pathogen (5, 6). The biosynthesis of ethylene in infected leaves is as follows: methionine (Met) → *S*-adenosyl-L-methionine (AdoMet) → 1-aminocyclopropane-1-carboxylic acid (ACC) → ethylene (27). The conversion of AdoMet to ACC is mediated by the enzyme ACC synthase (2). This enzyme can be inhibited by a number of compounds that prevent the biosynthesis of ethylene (3, 17), but prior to our studies, none of the substances had been evaluated for their ability to prevent ethylene biosynthesis in a host-pathogen interaction.

Our initial studies evaluated two known enzyme inhibitors (aminooxy)-acetic acid (AOA) and canaline (CAN) by applying them to the soil in which Kentucky bluegrass plants were growing (13). Infection of leaves on treated and nontreated plants did not differ, but infected leaves of nontreated plants produced 1,476 ppb ethylene within 48 hours. The leaves of plants treated with AOA or CAN produced 700 and 950 ppb of ethylene in response to infection, respectively. The infected leaves of AOA-treated plants retained 80% of their chlorophyll and the CAN-treated plants 74%. These values represent substantial increases in chlorophyll retention (and prevention of yellowing) compared to the 43% chlorophyll retention in the nontreated, infected leaves.

More recent studies with CAN applied directly to the leaves of Kentucky bluegrass held the ethylene surge to about 850 ppb without interfering with infection, and the infected leaves retained 90% of their chlorophyll (14). A 90% retention of chlorophyll during disease development keeps the infected leaves green with only the brown lesions present. These studies demonstrated that the yellowing of leaf-spotted leaves could be physiologically managed without preventing infection and leaf-spot development on the leaves.

Several questions still remain unanswered relative to management of the yellowing of leaf-spotted leaves. It is still unclear as to how effective chemical treatments will be on senescing leaves. Studies are in progress to determine the ability of CAN (and other substances) to slow or prevent yellowing on the oldest infected leaves of the shoot. Also, the compounds worked with to date (CAN, AOA) would not be effective for field use because of toxicological problems, but other substances and treatment techniques are still under study. Development of a practical field control technology is not out of the realm of possibility. Overall, the control of yellowing of leaf-spot-infected leaves with CAN and AOA has been effective and suggests that physiological management of the yellowing symptom is possible.

Management of ethylene biosynthesis might also have some unforeseen benefits in addition to controlling the yellowing of leaf-spotted leaves. Since ethylene is a known promoter of senescence, reducing the levels of ethylene in the plant might prolong the life of aging leaves. By slowing the aging of leaves, the yellowing of infected leaves might be further reduced and infection by secondary pathogens and saprophytes also decreased. Controlling symptom expression, as opposed to infection, might also slow the natural selection processes of the pathogen. If the pathogen is permitted to infect and develop normally, the need for it to change genetically in order to overcome a fungicide or plant resistance would be greatly diminished.

Potential for Genetic Management of Symptom Expression

Blockage of ethylene biosynthesis by means of exogenous application of chemical inhibitors to leaf-spot-infected leaves of Kentucky bluegrass can substantially decrease the yellowing of infected leaves. Continued research on this approach could result in a practical chemical treatment for control of symptom expression; however, development of a safe chemical treatment system could be a long process. A nonchemical solution to the control of symptom expression would be environmentally more acceptable and is within the realm of possibility.

Discovery of the gene for the enzyme ACC deaminase (16), which regulates the availability of ACC for ethylene biosynthesis, may provide the most

expedient approach to genetic control of ethylene biosynthesis by infected leaves. The enzyme degrades ACC and prevents it from forming ethylene. The by-products of ACC degradation are metabolized to amino acids commonly found in higher plants. The ACC deaminase gene has been introduced into tomatoes, where it effectively decreases ethylene biosynthesis, but the gene has not been established in a perennial grass.

Research is in progress to determine whether the deaminase gene can be established in Kentucky bluegrass. The process of establishing the gene in Kentucky bluegrass first necessitates establishing cells of the plant in callus culture. This process has been achieved and whole plants have been successfully regenerated from the callus. It now remains to be determined if the ACC deaminase gene, which originates from a *Pseudomonas* bacterium, can be established in the Kentucky bluegrass callus, and if the gene will be incorporated into the cells of plants regenerated from the callus cells. Lastly, if the gene is incorporated into the regenerated plants, will it control the ethylene surge during infection and decrease the yellowing of the leaves?

The outlook for sucessfully establishing the ACC deaminase gene in Kentucky bluegrass is guardedly optimistic. It has been determined that like infected leaves, Kentucky bluegrass callus cultures inoculated with *B. sorokiniana* generate ethylene. If the gene can be incorporated into the callus cells, a callus bioassay system could be developed for determining the effectiveness of the gene for controlling ethylene prior to regenerating whole plants. Work is presently in progress for introducing the ACC deaminase gene into Kentucky bluegrass callus. A DNA vector is being constructed that will include the ACC deaminase gene, and the vector will be attached to another gene that is specific for expression in grasses. The vector will then be introduced to the callus cells by means of a particle gun.

The research conducted over many years on the physiology of leaf spot symptom expression by Kentucky bluegrass has provided information that will be effectively used in development of new disease management strategies. The knowledge gained relative to photoperiod, light quality, hormones, phytotoxins, and postemergence herbicides has provided the foundation for exploration of radically different approaches to the management of *B. sorokiniana* leaf spot (and perhaps some other leaf-infecting pathogens). Prevention of ethylene biosynthesis and yellowing of Kentucky bluegrass leaves in response to infection by *B. sorokiniana* by means of chemical treatment or genetic alteration is feasible. Whether this approach succeeds or fails, the knowledge gained on how the symptoms are produced has opened the door to an unlimited number of possibilities that will ultimately alter how we approach the management of many turfgrass diseases.

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