

# Biological control of *Helminthosporium* foot rot of Kentucky bluegrass

Robert M. Endo  
D. Egel

The fungal pathogen *Helminthosporium sorokinianum* overwinters on infected Kentucky bluegrass litter. In the spring, the pathogen grows as a saprophyte (feeds on dead or decaying organic matter) on the litter and produces spores that cause primary infection of living bluegrass plants. Therefore, if the saprophytic activity leading to the production of the pathogen spores can be suppressed biologically on the litter, the disease can be controlled.

The activity of the pathogen on the litter is determined by its ability to compete saprophytically against the myriad of microorganisms naturally colonizing and decomposing the litter. S. D. Garrett and others have shown that, on wheat, the pathogen has a very poor innate competitive saprophytic ability and an extreme susceptibility to competing saprophytes. We reasoned that if we could show that suppressive microorganisms also occur on Kentucky bluegrass and its litter, we might be able to manipulate this suppressiveness to control spore production and therefore biologically control the *Helminthosporium* disease.

## Suppressive microorganisms in seeds

Untreated and surface-disinfested seeds of Merion Kentucky bluegrass were each placed in separate petri dishes containing moist, sterile sand for 4, 8, 16, and 32 hours. They were then spray-inoculated with spores of *H. sorokinianum*.

The sterile seeds developed abundant infection, and the pathogen produced abundant spores on the seed. In contrast, the fungus was able to infect only those untreated seeds that were pre-incubated for 4 to 8 hours before inoculation; seeds that were pre-incubated for 16 to 32 hours were protected from infection by the surface microorganisms originally present on the seed. The time required for the suppressive effect to appear is thought to represent the multiplication time for the suppressive microorganisms. This and other types of experiments with seeds of two other cultivars of Kentucky bluegrass confirmed that seeds of Kentucky bluegrass harbor a surface microflora that is suppressive to *H. sorokinianum*.

## Suppressive microorganisms in plants and litter

Experiments with stem and leaf pieces of Merion and Newport Kentucky bluegrass taken from the field showed that, on those moistened and incubated at least 16 hours before inoculation with spores of *H. sorokinianum*, the germination, infection, and sporulation of *H. sorokinianum* was inhibited, whereas on stem pieces that were pre-incubated dry, the patho-

gen caused many infections and sporulated abundantly. Presumably the prior period of moist incubation favored the development of a suppressive surface microflora that already existed on the stem and leaf surfaces. This and other types of experiments with field-grown Kentucky bluegrass plants confirmed that these plants harbor a surface microflora that is suppressive to *H. sorokinianum*. The source of the suppressive microorganisms is unknown, but some may have come from bluegrass seed as well as from the soil and from air-borne contaminants.

There is evidence that Kentucky bluegrass litter harbors a microflora that is suppressive to *H. sorokinianum*. Kentucky bluegrass litter was collected from field-grown plants and dried. The dried litter was placed in petri plates containing sterile, dry sand. The litter in half of the plates was left dry, whereas the litter in the remaining half was sprayed with sterile water and incubated for 4, 8, 16, and 32 hours. At the end of the incubation periods, the litter was sprayed with spores of *H. sorokinianum*.

Colonization and sporulation by the pathogen occurred on all the litter samples that had been pre-incubated for only 4 or 8 hours, but the fungus was completely suppressed on litter that had been pre-incubated for 16 and 32 hours. These results indicated that the field litter of Kentucky bluegrass also harbors a microflora that is suppressive to *H. sorokinianum*.

We also noted that dry litter, immediately upon rewetting, greatly stimulated spore germination of the pathogen, its mycelial growth, and its ability to colonize the litter and to sporulate. We found that this stimulation of the pathogen was caused by the enhanced release of nutrients from the dry litter following its rewetting. This was an important finding, because it suggested how the pathogen is able to develop and sporulate on the litter under field conditions. When the litter is moist, the saprophytic microorganisms present in and on the litter suppress the development of the pathogen, but when the litter dries out, the suppressive microorganisms cease their activity. When the dry litter is remoistened, the litter releases nutrients that greatly stimulate the activity of *H. sorokinianum*, leading to its sporulation before the saprophytic microorganisms can build back up to suppressive levels. Controlling sporulation of the pathogen thus apparently depends upon keeping the litter continually moist and therefore biologically active.

These laboratory findings suggest that we might be able to control the disease in the field by the simple expedient of irrigating lawns in such a way that the litter is kept moist. We have noted that, if bluegrass turf in Riverside and Santa Ana is sufficiently dense, once-a-week watering from April to May and twice-a-week watering from June to September is sufficient to keep the litter moist.

## Irrigation

To determine if an irrigation program of keeping the litter moist will suppress spore production by the pathogen on field

litter, we selected two one-year-old plots of common Kentucky bluegrass, one at the South Coast Field Station, Irvine, and the other at UC Riverside. Each plot was divided into two subplots measuring 10x20 feet. One subplot was irrigated twice a week and the other every 10 days. The litter in the well-watered subplot was maintained in a moist condition, whereas the litter in the second subplot dried out on the seventh day following irrigation. At this time, both subplots received supplemental irrigation of 12 hours to encourage the *Helminthosporium* fungus present on the litter to develop and sporulate.

Examination 72 hours later revealed that the litter that was allowed to dry out and remoistened supported sporulation of the *Helminthosporium* fungus at both Riverside and at Santa Ana, as predicted. Although the continuously moist litter at Riverside did not support sporulation of the *Helminthosporium* fungus as predicted, the moist litter at Santa Ana did, a result not predicted. This suggests that the necessary suppressive microflora was present on the litter at Riverside but not at Santa Ana. Although these results are encouraging, they suggest that the responsible suppressive microorganisms are not always present on moist field litter. This complicates the problem, because it suggests that in some cases litter will have to be artificially inoculated with the suppressive microorganisms. The saprophytic suppressive microorganisms will therefore have to be isolated and identified, and their degree of suppressiveness determined.

### **Isolating suppressive microorganisms**

Progress in isolating and identifying *Helminthosporium*-suppressive microorganisms from suppressive Kentucky bluegrass litter can be reported. Of 46 isolates of actinomycetes bacteria and fungi that were isolated from suppressive litter, 24 were antibiotic to *H. sorokinianum* when the pathogen was paired with each of the isolates on sterile potato dextrose agar. The most inhibitory isolates were: *Bacillus subtilis*, *Trichoderma viride*, *Trichothecium roseum*, several *Penicillium* species and several *Aspergillus* species. Within each of the species, considerable clonal variation in suppressive ability occurred. When all 24 isolates were used to inoculate steam-sterilized litter and the moist litter was allowed to incubate for 36 hours, the litter became suppressive to *H. sorokinianum* spores.

Although we have identified many of the suppressive microflora, further research is needed to demonstrate the range of organisms responsible for suppression, the mechanisms necessary, and whether artificial inoculations of field litter with suppressive microorganisms will result in the successful biological control of the disease.

*Robert M. Endo is Professor, and D. Egel is Graduate Research Assistant, Department of Plant Pathology, University of California, Riverside.*