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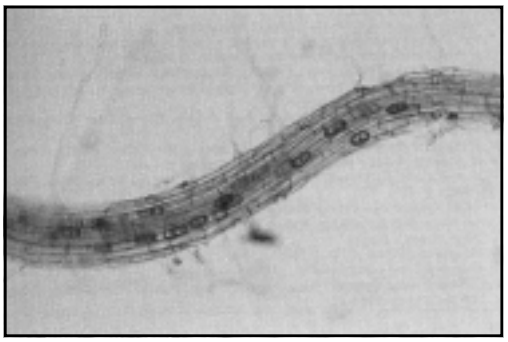
Creeping Bentgrass Cultivar Influences Biocontrol

A Healthy Ecosystem

In a recent study, our goal was to determine whether biological control activity of introduced inoculants was affected by the creeping bentgrass cultivar to which they were applied.

Inconsistent performance of biological control strategies for turfgrass disease control has limited the adoption of this technology into IPM programs. A major contributing factor to this variable performance in agricultural systems is the cultivar to which biological control organisms are applied. In a recent study, our goal was to determine whether biological control activity of introduced inoculants was affected by the creeping bentgrass cultivar to which they were applied. We reasoned that biological control performance should be enhanced on cultivars that are less susceptible to the disease in question as opposed to highly susceptible cultivars. The short-term applied goal of this research was to develop the understanding of how we might maximize the performance of microbial inoculants in turfgrass systems.



A normal appearing bentgrass root embedded with numerous Pythium *oospores. (Photo from* Creeping Bentgrass Management *by Peter Dernoeden.)*

The objectives of our research were to 1) determine the differential susceptibility of bentgrass cultivars to *Pythium aphanidermatum* and 2) determine the efficacy of known microbial inoculants in controlling Pythium damping-off on different bentgrass cultivars. The results of this study will ultimately be critical in making sound IPM recommendations for creeping bentgrass cultivars that will be most compatible with biological control strategies.

Methodologies

Bacterial inoculants used in this study (*Pseudomonas fluorescens* strain Pf-5 and *Enterobacter cloacae* strain EcCT-501) were chosen because of their known activities against Pythium diseases of turfgrasses and their consistent efficacy in controlling Pythium disease on a wide variety of plant species. For use in assays, bacteria were grown in 40 ml trypticase soy broth (TSB) shaken on a rotary shaker at 120 rpm for 16 hours at 27° C. Cells were rinsed in phosphate buffer and resuspended at the original concentration.

The *P. aphanidermatum* isolates used in this study (Pa-58 and PRR-147) were routinely grown in darkness on V8 agar at 27° C. The V8 medium was composed of the following: V8 juice, 100 ml, H_2O, 400 ml, $CaCO_3$, 1.5 g, Bacto Agar, 8.5 g. The medium was autoclaved for 25

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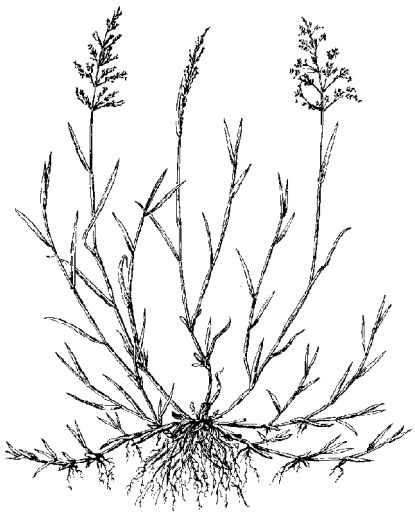
minutes on two consecutive days to avoid persistent contamination problems. After solidifying, plates were inoculated with a 24 hour culture of *P. aphanidermatum* and placed in a 27° C incubator.

To produce zoospores from *P. aphanidermatum* cultures, 18 mm diameter mycelial disks were excised from a 3-day old culture and placed in sterile plastic 60 x 15 mm petri dish (one disk/dish). Disks were flooded with 10 ml distilled deionized water and incubated at 24° C. After one hour, the water was removed and replaced with 10 ml fresh water, and returned to the 24° C incubator. Disks were then examined microscopically for zoospore activity every couple of hours. If sporangia and/or oogonia were not present, disks were then placed at 4° C for one hour, then returned to the 24° C incubator. On the other hand, if zoospores were present, they were enumerated by counting 200 µl aliquots with a haemacytometer and the number of zoospores/ml calculated. Zoospore suspensions were then diluted up to 1000X and used in seedling bioassays.



Seedling bioassays were conducted in 12-well tissue culture plates. Each plate was set up to include one or two sets of 4 non-inoculated wells and one or two sets of 4 inoculated wells to which either no bacteria were added or to which each of the bacterial treatments were added. In pathogenicity experiments where mycelial inoculum was used, one set of four wells each were inoculated with a 2 mm diameter disk removed from a 24 hour V8 culture of the appropriate *P. aphanidermatum* isolate. To each of four wells, 23 mg of seed of the particular bentgrass cultivar was added. Tissue culture plates were placed in clear plastic boxes to reduce evaporation and incubated in a 28° C incubator (14 hour day, 10 hour night). Seed germination was assessed daily, beginning three days after inoculation (time at which shoots were first visible) by estimating the percentage of seeds in the well that had germinated relative to the non-inoculated control.

For studies with zoospore inoculum, 450 µl of water was added to each set of 4 replicate wells. To inoculate wells with zoospores, 50 µl of the appropriate zoospore dilution was added to each set of 4 replicate wells providing a range of zoospore densities in a total well volume of 500 µl. After the addition of zoospores, 23 mg of seed of the appropriate bentgrass cultivar was added to each well. Tissue culture plates were then placed in clear plastic boxes to reduce evaporation and incubated in a 28° C incubator (14 hour day, 10 hour night). Seed germination was assessed daily, beginning three days after inoculation, by estimating the percentage of seeds in the well that had germinated relative to the non-inoculated control.

Results

Tolerance of Bentgrass Cultivars to Pythium aphanidermatum

Mycelial Inoculum. Bentgrass cultivars varied in their response to the two different *Pythium aphanidermatum* isolates used in this study (see Figure 1). Pa58 was again more virulent than isolate PRR-147 against all cultivars tested. Those cultivars most tolerant of isolate Pa58 were Penn G-6, Seaside II, Providence, L-93, Southshore, and SR7100. Those cultivars most sensitive were Penncross, Regent, Sefton, and Tiger. Those cultivars most tolerant of PRR-147 were Penn G-6, Seaside II, Sefton, Providence, Southshore, Trust, and L-93. Those cultivars most sensitive to mycelial inoculum of PRR-147 were Lopez, SR7100, Putter, Cato, 18th Green, Backspin, Penncross, and Tiger.

Zoospore Inoculum. As with mycelial inoculum, bentgrass cultivars also varied in their response to zoospore inoculum of each of the *Pythium aphanidermatum* isolates. In general, PRR-147 was less virulent than Pa58 at zoospore dosages that ranged from 250 to

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100,000 zoospores/well. Among the more sensitive cultivars to isolated PRR-147 were Lopez, LCB-703, Penncross, Procup, Putter, Regent, Trust, Seaside II, Sefton, Southshore, SR1020, SR1119, and Tiger. Most cultivars were highly sensitive to isolate Pa58. Those cultivars that were the least sensitive were Exeter, L-93, Penn G-6, Providence, SR7100, and Viper. In general, zoospore concentrations as little as 250/well were sufficient to result in 100% seedling mortality 7 days after inoculation. Since isolate Pa58 was uniformly more virulent, our subsequent bacterial inoculations were all conducted using this isolate.

Efficacy of Bacterial Inoculants on Different Bentgrass Cultivars

Based on reactions of the different bentgrass cultivars to infection by the two different *Pythium aphanidermatum* isolates, we predicted that inoculants would be more effective on cultivars such as Exeter, L-93, Penn G-6, Providence, or SR7100 that were less susceptible to *P. aphanidermatum* isolate Pa58 than the other more susceptible cultivars such as Lopez, LCB-703, Penncross, Procup, Putter, Regent, Trust, Seaside II, Sefton, Southshore, SR1020, SR1119, and Tiger. We reasoned that lower disease pressure alone would be a dominant host plant factor favoring the activity of introduced bacterial inoculants. Our results indicate that this reasoning appears to be invalid. For example, even though *E. cloacae* was more effective on Penn G-6, Providence, and SR7100 than on cultivars such as Lopez, LCB-703, Penncross, and SR1020 and thus fitting our predicted model, it was also more effective on cultivars such as Southshore, Regent, Putter, and Tiger than on cultivars such as Exeter or L-93. This latter scenario did not fit our predicted model.

Each of the bacterial strains evaluated in this study behave differently from each other on the different cultivars. Of all the cultivars tested, *E. cloacae* was effective in suppressing Pythium damping-off on all cultivars except Cobra, LCB-103, 18th Green, Cato, SR1020, and Penncross. *P. fluorescens*, on the other hand, behaved quite differently from *E. cloacae*. For example, *P. fluorescens* produced compounds that were phytotoxic to seeds of many of the bentgrass cultivars tested. Of the cultivars tested that were not sensitive to the *P. fluorescens* phytotoxins, *P. fluorescens* was highly effective only on L-93, LCB-703, Lopez, Exeter, Crenshaw, Cobra, LCB-103, 18th Green, and Cato. *P. fluorescens* was less effective on Backspin.

Conclusions

Our results clearly indicate that the performance of introduced microbial inoculants for the biological control of *Pythium aphanidermatum*-incited damping-off of bentgrasses is strongly influenced by the cultivar. Biocontrol efficacy on some cultivars exceeds that observed on other cultivars. With some cultivars, no biocontrol activity was supported.

Our results further indicate that the reaction of inoculants to the various cultivars vary with inoculant. *Enterobacter cloacae* strain EcCT-501 behaved differently from *Pseudomonas fluorescens* strain Pf-5 on most cultivars. We originally hypothesized that efficacy of inoculants would be enhanced on those cultivars that are least susceptible to *Pythium aphanidermatum*. However, this was not the case and there was no apparent relationship between susceptibility and biocontrol performance. We were able to demonstrate that the different bentgrass cultivars differed in their sensitivity to different isolates of *Pythium aphanidermatum*. It is likely that introduced inoculants would also be affected by the isolates of the pathogen tested.

The reasons for the phytotoxicity of *P. fluorescens* to some cultivars of bentgrass are unknown but are likely related to the produc-

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Injury from a Pythium root infection. (Photo from Creeping Bentgrass Management by Peter Dernoeden.)

Figure 1. Virulence of Mycelial Inoculum of Pa58 and PRR-147 on Creeping Bentgrass Cultivars

	Seed Germination (% of Control)[a]			Seed Germination (% of Control)[a]	
Cultivar	Pa58	PRR-147	Cultivar	Pa58	PRR-147
Penn G-6	42.5	50.0	Penn A-1	1.0	15.0
Seaside 2	12.5	40.0	18th Green	1.0	10.0
Providence	10.1	30.0	SR 1119	0.8	20.0
L-93	8.8	27.5	Princeville	0.8	20.0
Southshore	7.8	30.0	Crenshaw	0.8	17.5
SR 7100	7.8	10.0	SR 7200	0.5	15.0
Penn G-1	6.5	20.0	Cobra	0.3	12.5
Exeter	6.5	17.5	LCB-103	0.3	12.5
Viper	5.5	20.0	LCB-703	0.3	12.5
Putter	5.3	10.0	SR 1020	0.3	12.5
Trust	3.3	30.0	Backspin	0.3	10.0
Pro Cup	2.8	12.5	Lopez	0.3	7.5
Cato	1.8	10.0	Sefton	0.0	40.0
Mariner	1.5	17.5	Regent	0.0	15.0
National	1.5	15.0	Tiger	0.0	10.0
Penn A-2	1.5	12.5	Penncross	0.0	10.0
Penn A-4	1.0	20.0			

[a] Seed germination assessed 7 days after sowing and inoculation

tion of antibiotics such as pyoluteorin and 2,4-diacetylphloroglucinol. These antibiotics are known to be produced by strain Pf-5 and they are known to be phytotoxic in sufficient concentrations. It is likely that seedling turfgrasses are much more susceptible than mature turf to these antibiotics so that reactions on mature turfgrasses are likely to be somewhat different.

Additional Research Required

Much additional research is required and it will be important to have answers to many other questions regarding the performance of introduced inoculants on different bentgrass cultivars. For example, important research priorities will be to understand 1) how different grass species (not just cultivars) affect performance, 2) how cultivars react in different soil types, 3) how inoculants react to different cultivars infected by different strains of Pythium and different genera of pathogens, 4) how reactions of seedling turf compare with those of mature turf, 5) how different microbial inoculants behave on the different cultivars, and 6) how these reactions hold up under field conditions. These studies will comprise the focus of this work in coming years.

Contributions to the Current Knowledge Base

The utilization of different cultivars of bentgrasses to improve disease control has been largely ignored over the years. There are now over 40 different bentgrass varieties that can be used in golf course turf. With disease control being the major challenge of golf turf managers, it is surprising that these cultivars have not been exploited more fully. Our findings that cultivars vary in their susceptibility to *Pythium aphanidermatum* is significant in and of itself, since it may be possible to utilize more disease resistant cultivars for integration with other disease control practices. However, even more significant is the finding that inoculants behave differently on different cultivars. This may provide a new means of understanding why inoculants might fail in the field and also to allow us to design appropriate inoculant-cultivar combinations for enhanced biological disease control. We feel that these new findings are just beginning to reveal some of the factors that will ultimately allow us to successfully implement microbial inoculants into turfgrass systems for effective and consistent disease control.

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