Turfgrass Seedling Treatments For the Control of Pythium Diseases And the Improvement of Stand Establishment

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Seedling establishment is the most critical stage in new turfgrass installations of renovations. Establishment efficiency can be affected by many different factors, including speed of germination, inherent competitiveness with other turfgrass varieties and weed species, and susceptibility to seed rotting and damping-off pathogens. The latter is perhaps the most troublesome, yet one of the most infrequently recognized causes of establishment failures. To understand ways of improving stand establishment, it is important to understand the nature and control of seed rotting and seedling pathogens of turfgrasses.

Seed and Seedling Pathogens

Seed rotting and damping-off fungi are often the major limiting factors to stand establishment. The more common seed and seedling rotting fungal pathogens include species of *Pythium, Fusarium,* and *Rhizoctonia.* More than 15 fungal genera are represented in this category. Few studies have focused on the ecology, epidemiology, and control of *Fusarium-* and *Rhizoctonia-* incited damping-off diseases of turfgrasses. Diseases caused by *Pythium* species have been studied most widely because they appear to be the most important in limiting stand establishment.

Not only are *Pythium* species major pathogens of seed and seedling rotting, but they also become major root rooting pathogens once established in a turfgrass planting. In a survey of pathogenic *Pythium* species recovered from mature bentgrass turf, the more aggressive creeping bentgrass damping-off pathogens included *P. myriotylum, P. tardicrescens,* and *P. volutum.* All of the highly aggressive isolates were more virulent to creeping bentgrass seedlings at warm temperatures (28-32 degrees C) than cooler ones (16 degrees C).

In a survey of *Pythium* species on creeping bentgrass and perennial ryegrass, Nelson and Craft found that Pythium graminicola was isolated most frequently from mature stands of turfgrasses and nearly all isolates tested were highly virulent as seed rotting pathogens of creeping bentgrass and perennial ryegrass. Among other pathogenic species recovered were isolates of *P. aphanidermatum, P. aristosporum, P. torulosum,* and *P. vanterpoolii.* At least one isolate within each species was highly virulent to creeping bentgrass seeds and seedlings. *Pythium torulosum* was the species most frequently recovered from turfgrass roots and crowns. However, nearly all isolates were nonpathogenic. Five pathogenic isolates of *P. torulosum* were recovered. With the exception of one isolate, all were only weakly virulent to creeping bentgrass seedlings at cool (13 degree C) or warm (28 degrees C) temperatures.

Conditions Favoring Pythium Damping Off That Affect Stand Establishment

Pythium damping off of turfgrasses is known to be affected by a number of factors that directly impact establishment efficiency. These include a number of important environmental and cultural factors. They are:

1.) **Germination Time.** The longer seeds spend in moist soil, the greater the potential for seed and seedling rots. Kentucky bluegrass can take two to three weeks after planting before emergence, whereas fescues and bentgrasses take only a week to ten days for emergence. Perennial ryegrass generally requires less than one week.

2.) **Soil Moisture and Oxygen Levels.** All *Pythium* species require abundant moisture for germination and dispersal. Prolonged wet soils favor seed and seedling rots. Unfortunately, these prolonged wet periods are necessary for adequate seed germination and seedling establishment. Oxygen levels are generally lower in soils with high soil moisture. Increased soil compaction also decreases soil oxygen levels by decreasing the amount of air-filled pore space.

3.) **Soil Temperatures**. *Pythium* species are capable of inciting severe seed and seedling rots at both cool and warm temperatures. We have found in laboratory tests that the majority of *P. graminicola* isolates and all *P. aristosporum* isolates recovered from mature stands of turf are highly virulent as seed and seedling rot pathogens at both 13 and 28 degrees C.

In these studies, damping-off severity of specific isolates of *P. graminicola* and *P. vanterpoolii* on creeping bentgrass was favored by either cool or warm temperatures, depending upon the isolate. Although isolates of *P. aphanidermatum* were virulent at both temperatures, in general, they were more virulent at 28 degrees C than at 13 degrees C. At 28 degrees, some isolates of *P. graminicola*, *P. aphanidermatum* and *P. aristosporum* were pathogenic to perennial ryegrass in growth chamber experiments, whereas none of the isolates of *P. torulosum* and *P. vanterpoolii* were pathogenic. On perennial ryegrass, isolates of *P. graminicola* ranged from nonpathogenic to highly virulent. Because of the wide temperature optima of individual strains, as well as the presence of both "cool temperature" and "warm temperature" strains in many turfgrass soils, it is difficult to find temperature conditions that are not favorable for seed and seedling diseases caused by *Pythium* species.

5.) **Planting Depth**. Planting depth affects susceptibility of seedlings to damping off by increasing the amount of time susceptible tissue spend in soil exposed to pathogens. The more quickly the seedling emerges, generally the more quickly the plant can escape infection from seed rotting pathogens.

6.) **Sowing Density**. Seeds of turfgrasses are sown into a variety of habitats. Typically, seeds are sown into a well-prepared, plant-free seed bed or they are overseeded into an established turfgrass stand. It is common practice to continually overseed thinning areas of turf or to overseed a warm-season turfgrass with a cool-season one during the fall. In both cases, seeding rates are generally excessive.

The notion among most turfgrass managers is the more seed you sow, the better the stand. However, it has been shown in studies with other plant species that increased seedling densities can enhance Pythium damping-off severity. The increased seedling densities are comparable to increasing the soil inoculum of *Pythium*. Increasing the seedling density increase the germi-

Table 1. Fungicide Seed Treatments for the Control of Seedling Pathogens

Fungicide	Target Pathogen	Grass Species	Rates
Benomyl	*Fusarium culmorum*	Perennial Ryegrass	1.5g a.i./kg.
Captan	*Fusarium culmorum*	Perennial Ryegrass	1.5g a.i./kg.
	F. oxysporum	Perennial Ryegrass	3.0g a.i./kg
Chloroneb	*Pythium aphanidermatum*	Annual Ryegrass	4 oz./100 lb.
Etridiazole	*Pythium aphanidermatum*	Annual Ryegrass	4 oz./100 lb.
Iprodione	*Fusarium culmorum*	Perennial Ryegrass	1.5g a.i./kg
Metalaxyl	*Pythium spp.*	Perennial Ryegrass	1.5g a.i./kg
	P. graminicola	Creeping Bentgrass	3.2g a.i./kg
	Cladochytrium caespitis	Kentucky bluegrass	7.0g/kg
Thiram	*F. oxysporum*	Perennial Ryegrass	3.0g a.i./kg

nation frequency of *Pythium* propagules and also enhances the plant-to-seed spread of the pathogen.

7.) **Cultivar Selection.** In a dated and limited survey of turfgrass species, bermudagrasses and all of the cool-season were susceptible to *Pythium aphanidermatum*. Warm-season grasses, with the exception of bermudagrass, were resistant. No complete and contemporary studies on susceptibility of bentgrass varieties to Pythium damping off have been carried out and no known resistance to Pythium blight or Pythium root rot exists among cultivars currently in commercial use.

Why Are Seeds and Seedlings So Susceptible To Damping-Off Pathogens?

Of all stages of plant development, the germinating seed and seedling stages are perhaps the most vulnerable to a variety of stress-related factors that can be fatal. Not only are plants at these stages more vulnerable than mature plants to water deficits, temperature extremes, and pesticide toxicity, they are also much more susceptible to infection by soil-borne pathogens.

One of the principal reasons for the increased susceptibility of germinating seeds and seedlings to infection, particularly by *Pythium* species, is the exudation of cellular compounds into the soil surrounding the seed (or spermosphere) during the germination process. During initial stages of seed germination, the uptake of water into the seed results in the physical damage to cell membranes. Even though the plant eventually repairs this damage, many cell constituents leak out of the seed into the surrounding soil during the first few hours of germination before these repair processes are complete. Under high moisture conditions or suboptimal conditions for seed germination, seeds release more exudates.

Nearly all seed- and seedling-rotting pathogens utilize these exudates as a food source and to sense the presence of a susceptible host plant. Many pathogens, such as *Pythium* species, are ecologically adapted to respond very rapidly to the presence of these exudates since they do not persist for long periods of time in the soil.

The presence of seed and seedling exudates are critically important in regulating responses of pathogens to plants and in supporting microbial interactions and processes in the spermosphere. If there are insufficient concentrations of exudates in the spermosphere, *Pythium* species do not respond to the presence of the plant and do not infect the seed or seedling.

Seed Treatments To Improve Stand Establishment

Pregerminated Seed. Presoaking seed treatments that result in the emergence of the radicle are referred to as pregermination treatments. These treatments generally involve the soaking of seed in water until radicle emergence. The germinated seed is then planted as a slurry. This is done to enhance the germination rate of seed once planted in the soil. Even though this method greatly decreases the establishment time, seeds planted in this manner are difficult to handle and must be planted immediately. Specialized equipment is also required. Germinated seeds are much more susceptible to physical damage than are ungerminated seeds.

Studies with pregerminated seeds of other plant species have shown that the soaking process causes the releases of pathogen stimulating exudates within the first 24 hours. When these seeds are then planted after soaking, in soil infested with pathogenic *Pythium* species, seed and seedlings are much less susceptible to disease.

Seed Priming. Seed priming has also been referred to as osmoconditioning. This process differs from presoaking treatments because the radicle never emerges from the seed coat. During the priming process, seeds are soaked in a solution of various salts or polyethylene glycol to limit water availability to the seed. Concentrations of these solutes are adjusted to allow the seed to imbibe just enough water to initiate the biochemical processes required for seed germination without radicle emergence. Unlike pregermination treatments, primed seed can be rinsed and dried after the priming process. Therefore, it can be planted just like untreated seed.

Table 2. Effective microbial seed treatments for the control of Pythium damping off incited by *Pythium graminicola*

Species	Strain #	Original target pathogen
Azospirillum brasiliense	Cd-1	None
Enterobacter cloacae	MN9	*Pythium ultimum*
	EcCT-501	*Pythium aphanidermatum*
		Sclerotinia homoecarpa
Enterbacter spp.	Bf-14	*Magnaporthe poae*
Paenibacillus macerans	144/88.4	Various
	91/15.3B	Various
Pseudomonas aureofaciens	Tx-1	*Sclerotinia homoecarpa*
Pseudomonas fluorescens	AN4	*Pythium ultimum*
	Pf-5	*Pythium ultimum*
	FN3	*Pythium ultimum*
Pseudomonas putida	11/91.72B	Various
Seratia marcescens	9M5	*Magnaporthe poae*

Both types of presowing treatments are most effective on slow-to-germinate species, such as Kentucky bluegrass or bermudagrass. They can reduce the emergence time by up to ten days over nontreated seed. These effects are particularly pronounced in cool soils where Pythium seed rots and damping off can be more serious problems.

The time these processes take to complete varies according to species, varieties, and even seed lots. Without knowing the exact priming times required, it is possible to end up with seed that has been insufficiently primed or deteriorating because it has been soaked too long.

In both pregermination and seed priming techniques, solutions must be aerated to supply sufficient concentrations of oxygen to the seed during water imbibition. This allows the seed to imbibe water normally. If done properly, pathogen stimulatory exudates are removed in both techniques, making seeds much less susceptible to *Pythium* seed and seedling diseases when planted in the soil.

Fungicide Seed Treatments. In nearly all of the studies with fungicide seed treatments on turfgrasses, positive improvements in seedling stands have been obtained. Nearly all of the published studies have been conducted with annual or perennial ryegrasses. To my knowledge, only one study has been conducted on creeping bentgrass and this has not been published in widely accessible sources. Table 1 summarizes what is known about effective rates for selected seedling pathogens. In some cases, improvements in seedling stands of more than 70 percent have been observed. In other cases, protection can last up to ten weeks after sowing.

Seed treatment fungicides are formulated either as a slurry or as a dust treatment and are applied at relatively low rates. Fungicide seed treatments provide control equivalent to spray applications at a fraction of the fungicide use.

Biological Seed Treatments For Control of Seed and Seedling Pathogens

Biological seed treatments and seedbed amendments have proven to be quite effective in suppressing damping-off diseases of turfgrasses incited by the *Pythium* species. In a study, nearly 45 percent of all bacteria recovered from mature stands of creeping bentgrass and perennial ryegrass were suppressive to damping off incited by *P. aphanidermatum*. A higher frequency of antagonistic strains was found among the general heterotrophic bacteria than within the selected groups of enteric bacteria and *Pseudomonas* spp., groups known to be antagonistic to many turfgrass pathogens.

Although isolations of general heterotrophic bacteria yielded higher frequencies of effective antagonists, strains of enteric bacteria, particularly strains of *Enterobacter cloacae*, were more highly suppressive to *P. aphanidermatum* on perennial ryegrass than general heterotrophic bacteria or *Pseudomonas* spp. The level of control was as good as that provided by the fungicide metalaxyl.

Other studies have shown that a wide variety of bacterial strains, in addition to *E. cloacae*, are suppressive to damping off of creeping bentgrass incited by *P. graminicola* when applied as seed treatments. These strains are effective at both cool (20 degrees C) and warm (28 degrees C) temperatures and suppression lasted for at least 11 days.

There are a number of products on the market now that consist of microbial preparations. These are marketed in a variety of ways, but are generally targeted at improving soil properties. Some of these products might provide some benefit in reducing problems with Pythium damping off, particularly when seed is sown in a high sand content environment where microbial activity is somewhat low. Unfortunately, there is no microbial-based product that is currently registered with the US Environmental Protection Agency for control of Pythium damping-off diseases of turf.

Some composted organic amendments are also suppressive to Pythium damping off and the subsequent symptoms from root rot damage. Amending sand with composts, prepared from a variety of feedstocks, suppressed seedling and root diseases of creeping bentgrass caused by *P. graminicola.*

Among the more suppressive materials in laboratory experiments are industrial sludge and composted municipal biosolids. Among those generally not suppressive are leaf, yard waste, food, spent mushroom composts, cow manure, chicken manure, and combinations of these two manures with leaf compost. Pythium-suppressive composts typically have higher microbial populations. Furthermore, a strong negative relationship between compost microbial activity and Pythium damping-off severity was observed, indicating that much of the suppressive activity was due to microbial activities present in the compost amendment. A number of microbes recovered from these suppressive composts are equally suppressive to damping off incited by *P. graminicola* when applied as seed treatments.

Conclusions

Pythium seed rot and damping off take a countless toll on newly developing turfgrass seedlings. In the past, seedling stand losses due to Pythium damping off have never been of particular concern because of the relatively low cost of turfgrass seed. To my knowledge, the magnitude of losses during seeding and overseeding programs has never been documented. However, there is much more interest currently in stand losses because of the ever-increasing cost of seed and the increasing amount of overseeding during transitioning of golf course turf. Seed treatments can provide a significant improvement in stand establishment as well as a significant savings in seed cost.

A variety of options are available for the treatment of turfgrass seed. Presowing germination and priming techniques appear to be of limited value to most turfgrass species, particularly those that germinate quickly, such as ryegrass. The greatest benefit of these techniques has been seen with slowly germinating varieties sown in cold soils in situations where rapid weed-free turf cover is essential.

Although presowing treatment methods have been described in publications, it is advisable to leave them to seed producers because light, temperature, oxygen levels, and solution concentrations are critical and must be monitored carefully. Any mistakes can result in the loss of the seed.

Fungicide seed treatments are currently the most effective approach for controlling Pythium damping off in newly sown areas. Several products are currently available as seed treatment formulations. Although microbial products are not presently labelled for seed and seed bed treatments, many of these types of products can be used successfully to improve stand establishment by reducing damage from seed rotting pathogens.

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References

1. Abad, Z.G.; Shew, H.D.; and Lucas, L.T.. 1994. Characterization and pathogenicity of *Pythium* species isolated from turfgrass with symptoms of root and crown rot in North Carolina. Phytopathology 84: 913-921
2. Baldwin, N.A.; and Margot, P. 1990. Seedling Disease of turfgrasses caused by *Fusarium culmorum* and *Cladochytrium caepitis* and their control by fungicide seed treatments. Brighton Crop Protection Conference 123-130.
3. Brede, J.; and Brede, A.D. 1989. Seed Priming. Grounds Maintenance, April, pp 42, 46,48.
4. Burdon, J.J.; and Chilvers, G.A. 1975. A comparison between host density and inoculum density effects on the frequency of primary infection foci in Pythium-induced damping-off disease. Aust. J. Bot. 23:899-904.
5. Burdon, J.J.; and Chilvers, G.A. 1975. Epidemiology of damping-off disease (*Pythium irregulare*) in relation to density of *Lepidium sativum* seedlings. Ann. Appl. Biol. 81:135-143.
6. Colbaugh, P.F. 1988. Apron controls *Pythium* in developing turf. Grounds Maintenance, Sept., pp 72, 74.
7. Craft, C.M. and Nelson, E.B.. 1996. Microbial properties of composts that suppress Pythium damping off and root rot of creeping bentgrass caused by Pythium graminicola. Appl. Environ. Microbiol. 62:1550-1557.
8. Danneberger, T.K.; McDonald, M.B., Jr., Geron, C.A. and Kumari, P. 1992. Rate of germination and seedling growth of perennial ryegrass following osmoconditioning. Hort. Science 27: 28-30.
9. Falloon, R.E.. 1987. Fungicide seed treatments increase growth of perennial ryegrass. Plant Soil 101: 197-203.
10. Freeman, T.E.1972. Seed treatment for control of Pythium blight of ryegrass. Plant Dis. Rptr. 56: 1043-1045.
11. Freeman, T.E. 1980. Seedling diseases of turfgrasses incited by *Pythium.* Advances in Turfgrass Pathology, Advanstar, Duluth, MN, pp 41-44.
12. Freeman, T.E.and Horn, G.C.. 1963. Reaction of turfgrasses to attack by *Pythium aphanidermaturm*, Plant Dis. Rptr. 47: 425-427.
13. Hummel, N.W. 1991. Coated seed. Grounds Maintenance, August, pp 20,22,26
14. Kobayashi, D.Y., and El-Barrad, N.E. 1996. Selection of bacterial antogonists for the control of summer patch disease on Kentucky bluegrass. Curr. Microbiol. 32: 106-110.
15. Kobayashi, D.Y.; Guglielmoni, M.; and Clarke B.B. 1995. Isolation of chitinolytic bacteria as biological control agents for summer patch. Soil Biol. Biochem. 27: 1479.
16. Kraus, J.: and Loper, J.E. 1995. Requirements for production of Pseudomonas fluorescens Pf5. Appl. Environ. Microbiol. 61:849-854.
17. Lewis, G.C. 1988. Fungicide seed treatments to improve seedling emergence of perennial ryegrass and the effect of different cultivars. Pestic. Sci. 31: 179-187
18. Lewis, G.C. 1988. Improvements to newly sown ryegrass by use of combined fungicide and insecticide treatment. Crop. Prot. 7:34-38
19. Lo, C.T.; Nelson, E.B.; and Harman, G.E.1996. Biological control of turfgrass diseases with a strain of *Trichoderma harzianum.* Plant Dis. 80: 736-741.
20. Lush, W.M. and Birkenhead, J.A. 1987. Establishment of turf using pregerminated seed. Aust. J. Exp. Agric. 27:323-327
21. Luz, W.C.D. 1990. Microbiological control of *Bipolaris sorokiniana.* Fitopath. Brasiliera 15:246
22. Luz, W.C.D. 1993. Microbiological control take-all of wheat by seed treatment. Fitopath. Brasiliera 18:82-85
23. Luz, W.C.D. 1994. Effect of seed microbialization on controlling root rot and seedborne pathogens of wheat. Fitopath. Brasiliera 19: 144-148
24. Nelson, E.B., Burpee, L.L., and Lawton, M.B. 1994. Biological control of turfgrass diseases. Integrated Pest Mgmt. for Turfgrass, Lewis Publishers, Chelsea, MI.
25. Nelson, E.B., and Craft, C.M. 1991. Identification of Pythium fro roots and crowns of turf. Phytopath 81:1529
26. Nelson, E.B., and Craft, C.M. 1991. Introduction of strains of Enterbacter cloacae for control of dollar spot. Plant Dis. 75:510-514.
27. Nelson, E.B., and Craft, C.M. 1992. Miniaturized bioassay for soil bacteria supportive to Pythium blight. Phytopath. 82: 206-210.
28. Nelson, E.B., and Maloney, A.P. 1992. Molecular approaches to understanding biological control mechanisms in bacteria. Can. J. Plant Pathol. 14:106-114
29. Perondi, N.L., Luz, W.C.D., and Thomas, R. 1996. Microbiological control of wheat scab. Fitopatol. Bras. 21:243-249.
30. Smith, J.D., Jackson, N., and Woolhouse, A.R. 1989. Fungal Diseases of Amenity Turf. E.&F.N. Spon, London.
31. Smith, M.A., and Baldwin, N.A. 1991. Fungicide treatments for control of damping off. Test Agrochem. Cultiv. Ann. Appl. Biol. Supp. 118:38-39.
32. Stockwell, C.T., Nelson, E.B., and Craft, C.M. 1994. Biological control of *Phytium graminicola.* Phytopath. 84
33. Thurn, M.C. 1993. Organic source effects on disease suppression of putting green root zone mixes. M.S. Thesis, Cornell University.
34. Umali-Garcia, M., Hubbell, D.H., Gaskins, M.H., and Dazzo, F.B. 1980. Association of Azospirillum with grass roots. Appl. Environ. Microbiol. 39: 219-226.
35. van Dijk, K. 1995. Seed exudate stimulant inactivation by *Enterbacter cloacae* and its involvement in the biological control of *Pythium ultimum.* M.S. Thesis, Cornell University.
36. Wilkinson, H.T., and Avenius, R. 1985. The selection of bacteria antagonistic to *Pythium* spp. pathogenic to turfgrass. Phytopaht. 75:812.
37. Williams, F., and Pulley, G. 1993. Seed Treatments. Grounds Maintenance, Sept. pp 66,68,70,72.