BIOLOGICAL SEED TREATMENT AS A COMPONENT IN MAINTAINING SEEDLING HEALTH

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Abstract

Cotton (Gossypium spp.) has been the first large-scale, agronomic crop treated with biological-control agents for suppression of seedling diseases and long-term chronic diseases of the rhizosphere. The vast majority of cotton seed planted in the United States is now treated with one or more biological-control agents (organisms). Standard chemical fungicides have two major weaknesses as seed treatments; they actively select for increased pathogen populations of organisms that are not controlled by their activity spectrum, and efficacy rapidly diminishes after planting. Biological-control agents are living organisms that control or suppress cotton rhizosphere pathogens. Generally accepted modes of action for biocontrol agents are antagonism (antibiosis), competition (niche exclusion), parasitism or predation, and induced systemic resistance (Deacon and Berry, 1993). Biological-control agents colonize the rhizosphere, and rhizosphere-competent strains maintain colonization until harvest. Biologicals supplement standard chemical fungicides through early synergy with chemicals. Biologicals also expand the activity spectrum, and they provide long-term activity. Use of biologicals in combination with chemical fungicides provides a classic example of integrated pest management, using the advantages of each component to provide optimum disease control. In order to be successful, biocontrol organisms must be dependable and efficacious, provide long-term storability under standard warehouse conditions, be compatible with chemical fungicides and insecticides applied to cotton seed, be compatible with current production practices, and provide demonstrated dollar returns to producers.

Seedling-Disease Pathogens

Rhizoctonia solani, Pythium spp., Fusarium spp., and Thielaviopsis basicola are associated with the cotton seedling-disease complex (Johnson et al., 1978). The relative importance of the organisms causing this disease complex has been debated, largely due to regional and environmental variation. Currently, R. solani and Pythium spp. are generally considered the most important seedling disease pathogens (Kucharek, 1991).

Though R. solani and Pythium spp. are mainly associated with seedling diseases, they have also been reported as potential contributors to yield loss in cotton throughout the growing season. Damage from R. solani can occur through the flowering stage in cool, wet springs (Neal, 1942), but the cotton plant can recover from severe, prolonged damage if subsequent growing conditions are optimal. However, under continued stress, reduced shoot and root growth have been reported (Brown and McCarter, 1976). Brown and McCarter (1976) hypothesized that chronic R. solani infections, though initiated during the seedling stage, delay flowering and boll maturity, resulting in yield losses. Though yield reductions have not been correlated with long-term Pythium spp. infections, there is evidence that Pythium spp. act as chronic pathogens by attacking tap and secondary roots of older cotton, causing soft, light-brown lesions (Watkins, 1981).

Nearly all cotton planted today is treated with a combination of fungicides for control of seedling diseases. However, chemicals applied on seed or at planting do not provide adequate protection against chronic pathogens and deleterious organisms throughout the growing season (Suslow, 1982). In addition, a pathogen such as Fusarium oxysporum f.sp. vasinfectum, both a seedling disease organism (Johnson, 1978; Colyer, 1988) and causal agent of Fusarium wilt (Atkinson, 1892; Starr et al., 1989), is not readily controlled by registered fungicides (Watkins, 1981). Some chemical treatments actually enhance Fusarium spp. populations, presumably through elimination of fungal competition in the rhizosphere (Bush and Bird, 1977; Batson, 1982; Colyer, 1988). Biocontrol organisms, through colonizing the rhizosphere of the cotton plant for an extended period, have potential to fill a void by improving season-long plant health and defending against pathogens or other deleterious organisms not addressed by conventional fungicidal treatments. Potential biocontrol organisms for cotton seedling diseases have been reported from three broad categories: bacteria, fungi, and insects/nematodes.

Bacterial Biological Control Organisms

Numerous bacterial species have been screened for biocontrol activity against seedling diseases of cotton, and specific screening methods have been reported (Hagedorn, 1989; Kloepper, 1991; Hagedorn, 1993). Among those reported in the literature are Bacillus subtilis (Podile and Dube, 1988; Kenney et al., 1992; Brannen and Backman, 1993), Bacillus megaterium (Vargas and Ramirez, 1983), Pseudomonas flourescens (Howell and Stipanovic, 1979; Howell and Stipanovic, 1980; Suslow, 1985; Loper, 1988; Howie and Suslow, 1991), Aeromonas caviae (Inbar and Chet, 1991), Streptomyces spp. (Turhan, 1981), Enterobacter cloacae, and Erwinia herbicola (Nelson, 1988).

Though a Pseudomonas flourescens strain was briefly produced as a biological in-furrow product (Dagger G®; Ecogen, Inc., Langhorne, PA), the only successful

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biological products developed thus far for direct seed treatment have used Bacillus subtilis strains. Bacilli have advantages over other bacterial biocontrol agents; bacilli produce stable spores that can be applied to seed in slurry or planter-box treatments. Bacillus spp. spores maintain viability for years under standard conditions observed in cotton seed storage. Non-spore formers generally have viability and storage problems. Products with non-spore formers are generally limited by short-term storability, and slurry treatment is often not possible, limiting application to planter-box and in-furrow applications.

One B. subtilis strain (GB03) is now applied to the vast majority of cotton planted in the United States. Isolate A-13 (Broadbent et al., 1971) was previously documented as a biocontrol and growth-promoting B. subtilis strain. Based on results obtained with the A-13 strain, Kenney (Kenney et al., 1992) utilized multiple host passages of A-13 through cotton to select for a cotton-adapted strain designated as GB03. This strain has better cotton inoculation capabilities, and it is currently registered and sold in the United States as a fungal biocontrol agent, Kodiak® (Gustafson, Inc., Plano, TX). It is applied in combination with classical fungicides as a seed treatment, but it is targeted for control of primarily chronic, long-term diseases of the cotton plant. Strain GB03 has shown clear suppression of pathogenic Fusarium spp. (Brannen and Backman, 1994; Zhang and Howell, 1995) and R. solani (Brannen, 1995).

A second strain of B. subtilis, GB07 (MBI 600), has biocontrol activity against R. solani and P. ultimum in cotton (Rossall and McKnight, 1991; McKnight, 1993). This strain is also available for seed treatment as Epic® (Gustafson, Inc., Plano, TX). Both B. subtilis strains GB03 and GB07 colonize the rhizosphere of cotton plants season-long (Brannen and Backman, 1993).

Fungal Biological Control Organisms

Among the potential fungal biocontrol organisms reported are Trichoderma harzianum (Harman, 1989; Sivan and Chet, 1989), Gliocladium virens (Howell and Stipanovic, 1983; Howell, 1987; Lumsden and Locke, 1989; Howell, 1991; Howell, 1995), Laetisaria arvalis (Lewis and Papavizas, 1992), Stilbella aciculosa (Lewis and Papavizas, 1993), Chaetomium globosum (Walther and Gindrat, 1988), and hypovirulent Rhizoctonia solani (Sneh et al.,1989a; Sneh et al., 1989b).

Though Gliocladium virens has been produced as an in-furrow product (GlioGuard®, W. R. Grace and Company, Columbia, MD), no fungal seed treatment has been previously manufactured for use in cotton. However, Wilbur Ellis will soon be marketing a planter box Trichoderma harzianum, T22 Planter Box®. The product is compatible with most common chemicals used in seed treatment, and it has activity against Pythium spp., Fusarium spp., and R. solani (D. Schulteis, *personal communication*). Though this product does not allow a slurry treatment, it does provide the producer with an additional on-farm, biological seed-treatment option.

Insect and Nematode Biological Control Organisms

At least two insect and one nematode species are reported as potential biocontrol agents of cotton seedling diseases: Proisotoma minuta (Curl et al., 1985; Lartey et al., 1994), Orychiurus encarpatus (Curl et al., 1987), and Aphelenchus avenae (Caubel et al., 1981).

No products have been developed from insect or nematode biocontrol agents. Though the research is sound, the practical potential for such organisms is not good. The difficulty of providing a cost-effective, stable, product will likely be too great for the near term. However, researchers and producers should look for cultural practices that maximize the natural production of such ecologically important organisms in the field.

Requirements for a Successful Biological Control Agent

Though the cotton industry can be proud of the fact that it has promoted the first large-scale use of a fungicidal, biological product (Kodiak® and Epic®), cotton has had its biological failures as well. There are several reasons why specific organisms will probably not be developed, and there are reasons for past failures of marketed products. Though many organisms listed in this review may provide excellent results under laboratory conditions, there is currently no practical way to produce an economical product from the organism. Secondly, good laboratory results do not necessarily correlate to good field results. In order to be successful, biocontrol organisms must be dependable and efficacious, provide long-term storability under warehouse conditions, be compatible with chemical fungicides and insecticides applied to cotton seed, be compatible with current production practices, and provide demonstrated dollar returns to producers. Any deviation from these guidelines will result in failure. However, if these guidelines are endorsed, we can expect to see numerous biological products entering the market within the next few years. Through the use of integrated chemical and biological combinations, we can expect an increase in seedling and long-term cotton plant health, resulting in higher yields for the producer.

Path Forward

Though biologicals are now widely used in the cotton market, the potential of biologicals has not yet been fully realized. New, more efficacious strains of bacteria and fungi will certainly be introduced into the market within the next few years. As better methods of stabilizing Pseudomonas spp. and fungi become available, these organisms will also be developed into marketable products. The use of genetically-engineered microorganisms (GEMS) as biocontrol agents will also provide better strains for product development. However, the public stigma attached to GEMS will have to be counteracted through education. The potential may also exist to genetically engineer plants that promote stronger symbiosis with particular biocontrol organisms, similar in concept to the multiple-adversity resistance (MAR) breeding program (Bird, 1982). The advent of induced-systemic resistance biocontrol agents may help to alleviate seedling diseases as well as foliar diseases through seed treatment. Other ideas will also certainly surface as this realm is explored more thoroughly.

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