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# Kodiak®—a successful biological-control product for suppression of soil-borne plant pathogens of cotton

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**Cotton (*Gossypium* spp) has been the first large-scale, agronomic crop in the United States treated with a biological-control agent (BCA) 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 *Bacillus subtilis* strain GB03, registered as Kodiak® (Gustafson, Inc, Plano, TX, USA). Responses are typically a mixture of growth promotion (increased root mass) and disease suppression (*Rhizoctonia* and *Fusarium* spp). Strain GB03 shows exceptional rhizosphere competence, colonizing the rhizosphere of monocots and dicots. Though the initial success of strain GB03 has been observed in the production of cotton, other crops have shown positive yield responses following bacterization. Since *B. subtilis* is a spore-forming organism, it is extremely tolerant of environmental stresses, including seed-treatment pesticides, soil and seed pH, cultivar effects, edaphic factors and long-term storage. More importantly, it is readily produced with current fermentation technology. Other BCAs, such as *Pseudomonas* spp, do not readily adapt to large-scale production methods, and stability is a limiting factor. In order to be successful, scale-up production must provide a product with efficacy equivalent to the laboratory model. A better understanding of how fermentation and other production processes affect the efficacy and rhizosphere competence of biocontrol organisms is now required by the industry. Processes have to be carefully optimized for both maximum production and maximum efficacy. A strong collaboration and understanding between the agricultural industry and industrial microbiologists are required to continue the advance of new biologicals such as Kodiak®.**

**Keywords:** biological control; *Bacillus subtilis*; rhizosphere; *Rhizoctonia* spp; *Fusarium* spp; *Gossypium* spp

## Introduction

Spermosphere- and rhizosphere-active biological-control agents (BCAs) are living organisms that control or suppress soil-borne pathogens. Biological control or suppression of soil-borne pathogens has been addressed in academic research for more than 40 years. However, technology transfer has been largely limited to a few products with limited markets. This has largely been due to problems with industrial production, stabilization of biological products, and lack of consistent efficacy. However, in recent years, the promise of biologicals in large-scale agricultural markets has at least been realized in part. Cotton (*Gossypium* spp) has been the first large-scale, agronomic crop treated with a BCA 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 Kodiak® (Gustafson, Inc, Plano, TX, USA). Kodiak® is a formulated concentrate of *Bacillus subtilis* spores applied to seed for the control of soil-borne pathogens.

## Strain development

Historically, development of Kodiak® started in Australia. Isolate A-13 [7,19,20] has been well documented as a biocontrol and growth-promoting *B. subtilis* strain. Broadbent *et al* [7] reported that strain A-13 increased plant stands for several plant genera in a commercial greenhouse nursery. In addition, growth of wheat plants under nutrient-deficient conditions was improved following treatment with A-13. In a peanut system, Turner and Backman [19,20] reported increased root growth, increased plant vigor, and reduced levels of root cankers caused by *Rhizoctonia solani*. They utilized multiple host passages to select for a peanut-adapted variant of A-13, and field results were improved [20]. Based on results obtained with the A-13 strain, Kenney [13] utilized multiple host passages of A-13 through cotton to select for a cotton-adapted strain designated as GB03. This strain is currently registered and sold in the United States as Kodiak®. It is applied in combination with classical fungicides as a seed treatment for stand improvement, but it is also targeted for suppression of chronic diseases of the cottoon plant (US Patent 5-215-747).

## Modes of action

Generally accepted modes of action for BCAs are antagonism (antibiosis), competition (niche exclusion), parasitism or predation, and induced systemic resistance [11]. Though the mode-of-action for strain GB03 has not been fully determined, antibiosis is likely. Iturin antibiotics, generated by numerous *B. subtilis* strains, are compounds that exhibit strong antifungal activity against most pathogenic fungi and several bacterial species [15]. The iturin antibiotics increase cell membrane permeability, forming ion-conducting pores. Strain GB03 produces an iturin-class antibiotic, and it exhibits *in vitro* antibiosis against numerous cotton pathogens. Though difficult to prove, indirect evidence does indicate a key role of these antibiotics in antagonism against patho-

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gens such as *R. solani*. Antibiotic-negative mutants of *B. subtilis* strains are not efficacious [9].

## Activity spectrum

Strain GB03 has shown clear suppression of pathogenic *Fusarium* spp [5,22] and *R. solani* [6]. Recent results from *R. solani*-inoculated plots indicate the improvement in stand observed when using Kodiak® in combination with standard chemical fungicides (Table 1). Both *R. solani* and *Fusarium* spp are associated with the cotton seedling-disease complex [12]. However, strain GB03 colonizes the cotton rhizosphere season-long [4]. Kodiak® may therefore affect the suppression of chronic, long-term, and subacute diseases of the cotton plant, in addition to any effects it may have on seedling-stage pathogens. Kodiak® has also been implicated in promotion of a more vigorous cotton root system [14].

## Integrated pest management

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 [18]. In addition, a pathogen such as *Fusarium oxysporum* f sp *vasinfectum*, both a seedling disease organism [10,12] and causal agent of Fusarium wilt [1,17], is not readily controlled by registered fungicides [21]. Some chemical treatments actually enhance *Fusarium* spp populations, presumably through elimination of fungal competition in the rhizosphere [2,8,10]. Biocontrol agents, 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.

The success of Kodiak® in the cotton market has largely been due to the integration of a BCA with standard chemical fungicides. Kodiak® supplements standard chemical fungicides through an early synergy, expands the activity spectrum, and provides long-term activity. Use of Kodiak® in combination with chemical fungicides provides a classic example of integrated pest management (IPM), using the advantages of each component to provide optimum disease control.

**Table 1** Suppression of *Rhizoctonia solani*, a cotton seedling pathogen, through use of Kodiak® (*Bacillus subtilis*) biocontrol agent as a seed treatment

| Seed treatments | Stand counts (plants per 9.1 m)[a,b] | | |
|---|---|---|---|
| | Cultivar DP50 | Cultivar SV453 | Seed treatment means[c,d] |
| 1. Untreated control | 1.4 | 0.2 | 0.8 a |
| 2. Chemical standard | 15.2 | 12.2 | 13.7 b |
| 3. Chemical standard + Kodiak® | 34.4 | 52.5 | 43.5 c |
| Cultivar means[d] | 17.0 | 21.6 | |

[a]Randomized complete block design with a 2 × 3 factorial (cultivar × seed treatment).
[b]21 days after planting. Each value is the mean of six replications.
[c]Means in a column followed by the same letter are not significantly different ($P$ = 0.05) when using Fisher's protected LSD.
[d]LSD (0.05) among seed treatment means = 12.1 LSD (0.05) among cultivar means = 7.0. Cultivar × seed treatment interaction = 0.24.

## Lessons for technology transfer

Though the cotton industry can be proud of the fact that it has promoted Kodiak® as the first large-scale, fungicidal BCA, 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 provide excellent results under laboratory conditions, there may currently be no practical way to produce an economical product from the organism. Researchers should be aware of industrial production and agricultural restraints. Secondly, good laboratory results do not necessarily correlate to good field results. Lab tests are often conducted with biologicals in a form that does not closely resemble that produced through fermentation technology. Further development of fermentation technology must also be achieved in order to optimize activity of BCAs [16]. A third reason for biological failure is observed when biologicals are touted as replacements for chemical fungicides. Though this may be a possibility in the future, the near-term use of biologicals requires integration with chemical fungicides. Compatibility with currently applied fungicides is a requirement, and failure to recognize this can lead to futile research and development efforts.

The above pitfalls were avoided with development of Kodiak®. Kodiak® has been relatively easy to produce with current industrial fermentation technology, and the fermentation product has performed well in the field. Bacilli have advantages over other potential bacterial and fungal BCAs; bacilli produce stable spores. Spores can be applied to seed in slurry or planter-box treatments—the current seed-treatment technology standards. *Bacillus* spp spores maintain viability for years under standard conditions observed in storage of product or treated seed lots. Non-spore formers generally have viability and storage problems. Products formed from non-spore formers, such as *Pseudomonas* spp, are therefore limited by short-term storability, and slurry treatment is often not possible, limiting application to planter-box and in-furrow applications.

In order to be successful, BCAs must be dependable and efficacious, provide long-term storability under warehouse conditions, be compatible with chemical fungicides and insecticides applied to 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 plant health, resulting in higher yields for the producer.

## The path forward

Though a fungicidal BCA is now widely used in the cotton market, the potential of BCAs has not yet been fully realized. New, more efficacious strains of bacteria and fungi will certainly be introduced into this 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. 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.

Becker and Schwinn [3] give a review of the market potential of BCAs for control of soil-borne pathogens. The potential for BCAs is excellent! The use of BCAs may open many untapped markets worldwide. Registration of BCAs requires less testing than required for conventional chemical fungicides. Therefore, registration costs are reduced, and the time required for registration is also reduced substantially. Numerous crops do not provide the acreage requirements or high values that justify product development of chemical fungicides. However, reduced registration costs for BCAs allow for product development in smaller markets. Some large markets, such as seed treatment of wheat and soybean, are still largely unpenetrated by chemical seed-treatment fungicides, due to difficult cost-return ratios or the desire to use carryover seed for consumption. In these examples, the introduction of inexpensive biological products, even if less efficacious than chemical fungicides, may provide better market penetration. In addition, many biologicals have an EPA exemption from tolerance, allowing consumption of treated seed. Environmental concerns may also dictate the utilization of biologicals, though these concerns have not driven markets thus far.

As industrial microbiologists become more aware of the needs of the marketplace, additional alliances and research at the industrial level will help to make biological control of plant pathogens a greater reality. The success of Kodiak® has provided a clear example of the potential of BCAs as part of an integrated system of disease control.

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