

Application of Biologicals to Enhance Vegetable Seed Production and Quality

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Abstract

Beneficial bacteria and fungi provide promising alternatives or supplements to chemicals as seed treatments against soil borne pathogens. This review paper provides an assessment of biological control agents (BCA's) currently used with vegetable crop species, and key limitations to expanded use of BCA's as seed treatments. Research areas for improved biological efficacy and reliability in field and greenhouse settings are also discussed. The ability of BCA's to effectively colonize and grow with the seedling root system may be particularly useful when crops experience environmental stress. Improved application methods to optimize the density and uniformity of biologicals on seeds are increasing the reliability of BCA technology. Early results also suggest variable levels of BCA compatibility with chemical seed treatments and other microbial applications. Progress in storability, ease of application and economy of BCA production will be needed to move biologicals beyond their niche position in the seed production and treatment market.

Introduction

Research on the biological control of plant disease has a history of more than 70 years, and considerable attention has been directed to biological seed and transplant treatments (Cook, 1993; Nemeč et al., 1996; Lewis and Lumsden, 2001). Biological control agents (BCA's), especially beneficial bacteria and fungi, have shown promise in many seed enhancement studies, and the use of BCA's is consistent with the development of integrated crop management systems. Chemical treatments remain the dominant category of seed protectant, but chemical companies continue to be actively engaged in research and marketing of biological seed treatments. The potential for combined seed treatment technologies (chemical, biological, pelleting, coatings, etc.) provides opportunities and challenges for the vegetable seed industry.

Vegetable seed quality includes the physical, pathological, genetic and physiological traits ultimately culminating in successful crop establishment and yield (Basra, 1995). Use of BCA's as seed treatments can play an important role in most aspects of seed quality, and be of particular benefit for improved seedling growth under stressful conditions. An excellent historical and practical perspective of introduced microorganisms for general disease control was reviewed by Cook (1993) and provides a general context for the specific vegetable seed topics considered in this paper. Cook (1993) identified three stages of

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research and development necessary prior to the introduction of new BCA's for seed treatments or other uses: discovery, performance, and scale-up. In the discovery stage, naturally occurring or genetically improved biological antagonists are studied and evaluated in addition to improved fermentation and formulation procedures. Discovery stage research is mostly proprietary. When promising biological antagonists are discovered, there is generally a minimum of 15-20 site-years of performance testing. The most promising of these are then shifted to scale-up studies to identify specific biological strains performing best on given soils and environments. Many potential biological seed treatments fail to advance beyond performance testing (Cook, 1993). The erratic performance of BCA's in field/greenhouse trials is evidence that more information is required on mechanisms and factors governing the variable performance of beneficial microorganisms in the rhizosphere and spermosphere.

Biological control agents

Examples of vegetable seed BCA's. Beneficial fungi (*Trichoderma harzianum*, *Gliocladium vivens*) and bacteria (*Pseudomonas* spp., *Bacillus* spp., *Enterobacter cloacae*) have been studied and selected for disease-suppressive properties on a variety of vegetable species and other crops (Bennett, 1997). Results with some microorganisms have been quite surprising. For example, *Penicillium oxalicum*, an important seed pathogen of sweet corn (*Zea mays* L.), can protect pea (*Pisum sativum* L.) seedlings from Pythium seed rot (Windels and Kommedahl, 1978). Recent examples of commercial biocontrol products (including seed treatments) can be visited at a website maintained by Fravel (2002). Although biological seed treatments often display a narrower spectrum of control compared to some chemical treatments, because of soil chemistry and host specificity characteristics (Callan et al., 1997), the ability of many BCA's to colonize the crop rhizosphere can produce benefits beyond seedling emergence. Bjorkman et al. (1998) showed sweet corn root and shoot growth increased 66° for seedlings colonized with *Trichoderma* spp. in the absence of detectable disease organisms. The addition of bacteria to seed and seedlings provides the greatest benefit compared to untreated plants when crops encountered prolonged periods of stress (Lazarovits and Nowak, 1997). Low vigor *sh2* sweet corn plants responded the most to root colonization by *Trichoderma harzianum* strain 1295-22, while high-vigor seedling growth was not increased by this beneficial fungus (Bjorkman et al., 1998).

Optimum inoculation densities for maximum beneficial response of *Pseudomonas* spp. on cucurbits, tomato (*Lycopersicon esculentum* Mill.), sweet corn (*Zea mays* L.) and sugar beet (*Beta vulgaris* L.) seeds are generally a minimum of log 7.0 to 7.5 colony-forming units (cfu's)/seed (Warren and Bennett, 1998; Callan et al., 1997; Osburn et al., 1989). Application methods influence the density and uniformity of BCA's on a seed. Biopriming, bio-osmopriming, drum priming, solid-matrix priming (SMP), and specialized pellets/coatings have been suggested as promising techniques for uniformly applying BCA's to crop seeds (Harman and Taylor, 1988; Lewis et al., 1987; Khan, 1992; Bennett et al., 1992; Kubik, 1995; Pill, 1995; Warren and Bennett, 1997). But, optimum drying protocols following seed hydration and BCA application still remain poorly understood. For example, levels of *P. aureofaciens* AB254 applied to *sh2* sweet corn declined slightly (-0.2 to -0.6 log cfu's/seed) following air drying at 24°C. However, the effects of drying BCA treated seed at higher or lower temperatures and controlled RH levels have not been reported (Callan et al., 1997). Pressure infusion of *sh2* sweet corn seed with *P. aureofaciens* was not a useful method for increasing the effectiveness or density of this beneficial bacterium (Reese et al., 1998). For any proposed inoculation procedure, it is important to ensure that seed contamination (pathogenic and saprophytic fungi) due to physiological seed treatments such as

osmopriming (Tylkowska and Biniek, 1994) or other hydration techniques is minimized. This precaution is further warranted based on similar seed hydration techniques used to uniformly contaminate seedlots for plant pathology research.

Limitations to BCA's as vegetable seed treatments. By placing biological control agents at the center of the classic host-environment-pathogen disease triangle (Fig. 1), factors that limit the effectiveness of most biological seed treatments can be identified. Failure of BCA's to adequately populate and protect the seedling root system due to unfavorable soil (temperature, moisture, pH, iron concentration), plant, and pathogen influences has been reported (Cook, 1993). Additional research on the desiccation and flooding tolerance of beneficial microbes, and the growth and population dynamics of microbes on vegetable seeds and roots will reduce the variability in BCA performance (Potts, 1994). Many bacteria produce protective polysaccharide shells (Costerton and Irvin, 1981), but such survival structures should permit a rapid protective response to pathogen attack (Bennett et al., 1992). Improved understandings of microbial communities and soil/plant habitat effects on BCA persistence also deserve further study. Bacteria and fungi in nature are often dormant or in stationary phases instead of actively growing. *Pseudomonas* spp. on sugar beet seed have been shown to compete for nutrients and produce secondary metabolites even in the absence of increasing BCA cell numbers (Fukui et al., 1994b). This so-called quiescent period may allow the accumulation of important antibiotic precursors or other physiological responses leading to improved cell fitness.

Variable levels of compatibility of BCA's with chemical seed treatments (e.g., insecticides, fungicides) have been noted, and may complicate the development of combination treatments (Bennett, 1997; Callan et al., 1997). Results from seed health testing in the Netherlands (Table 1) illustrate the benefit of 2- and 3-way fungicide treatments for carrot (*Daucus carota* L.) seed. Similar benefits can be expected by combining chemical and biological vegetable seed treatments assuming that compatibility exists. Variable levels of BCA compatibility with other applied microbes can also limit the development of effective seed treatments, but most results suggest the problem is manageable (Fukui et al., 1994a; Callan et al., 1997). Minimal competition was reported when two pseudomonad strains were inoculated at low density levels (10^4 cfu/seed) on sugar beet seed, but antagonism often occurred when seeds were coinoculated with one strain at a high density (10^7 cfu/seed) and the other at a low density (Fukui et al., 1994a). A beneficial bacterium (*Bacillum cereus*) placed on soybean (*Glycine max* L. Merr.) seed planted in Wisconsin fields was fully compatible with *Rhizobium* and *Bradyrhizobium* inoculants (Osburn et al., 1995). In fact, root nodulation increased on snap bean (*Phaseolus vulgaris* L.) plants treated at seeding with *Gliocladium virens* (Smith, 1996). Additionally, pseudomonads have been shown to be compatible with vesicular-arbuscular mycorrhizal fungi (Paulitz and Linderman, 1989). As genetically engineered lines for high production of antibiotics become more common, microbe compatibility issues may become more serious. It is also likely that more antagonistic (and compatible) microbe combinations will be developed as more species of bacteria, fungi, and viruses are discovered and tested for BCA potential. It has been estimated that the known species of microbes represent only 4-10% of the total number in the world vs. 81% known for vascular plant species (Hawksworth, 1991).

Research and technology for improved BCA activity. Biocontrols are available and effective for many seed treatment niches, but for the biological seed protectant market to move beyond 1--2% of total chemical sales (Tryon, 1994), progress in BCA formulation and reliability must occur. Key goals of product quality research include (1) 'long' shelf-life (1-2 years), (2) high density of viable propagules,

(3) stability under adverse conditions, (4) ease of application to seed and (5) economy of production (Lewis and Papavizas, 1991). Studies have been conducted to address these goals. For example, storage temperature (8° vs. 24°C) affected the longevity of *P. aureofaciens* AB254 applied to *sh2* sweet corn seed (Callan et al., 1997). After 7.5 months of storage, AB254 levels decreased 1.4 log units at 8°C and 3.0 log units at 24°C. Bioprimering after storage resulted in a recovery of AB254 populations, but after 7.5 months of storage, only seed maintained at 8°C and then bioprimered provided seedling emergence equal to metalaxyl [N-(2,6-Dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester] treated controls (Callan et al., 1997). Similar losses of AB254 cfu's were observed on tomato seed treated with BCA applied in two ways (bio-osmoprimed and methyl cellulose coated) and stored at 5°C for 4 and 8 months (Warren and Bennett, 1999). Bio-osmoprimed seeds had less BCA loss compared to methyl cellulose coated seed after 4 months (-0.1 vs. -0.6 log units) and 8 months of storage (-1.5 vs. -1.8 log units), but these differences were unlikely to reduce infection by *Pythium* spp. (Table 2). Kubik (1995) noted that survival of *Enterobacter cloacae* EC102 on vegetable crop seeds was improved for SMP vs. methyl cellulose coated seeds.

Other strategies for improving BCA activity or shelf life involve the addition of various polysaccharides, carboxylic acids and other nutrient sources during formulation (Table 3). Modified pH levels during seed hydration using such techniques as SMP resulted in improved biological seed treatment performance (Harman and Taylor, 1988). Tailoring biological seed treatments to inoculum levels and mixtures on seedlots may also improve control reliability (van den Bulk and Langerak, 1995). Paine (1993) found detectable levels of *Bacillus pasteurii*, *Pseudomonas putida*, *Aspergillus* spp., and *Fusarium* spp. on two commercial *sh2* sweet corn cultivars ('Camelot', 'Crisp'n' Sweet 710') seed lots, while other microbes were cultivar or production-location specific. 'Camelot' seeds also tested positive for the presence of *Paecilomyces* spp. and *Verticillium* spp., while *Penicillium* spp. and *Trichoderma* spp. were found only on the 'Crisp'n' Sweet' seedlot. Dramatic differences in pathogen species and inoculum levels were also observed by Mohan and Bijman (1997) on six *sh2* sweet corn seedlots (Table 4). More detailed seed health testing could also indicate whether a BCA treatment is necessary against superficial pathogenic inocula (less persistence and disease risk) or embryo-borne (internal) inocula with greater persistence (van den Bulk and Langerak, 1995, Anfinrud, 1998).

The levels of storage and field pathogens on specific seedlots should also be identified. Seeds of cabbage (*rassica oleracea* L.), cucumber (*ucumis sativus* L.), pepper (*Capsicum annuum* L.), radish (*Raphanum sativus* L.), and turnip (*rassica rapa* L.) were inoculated with two *Aspergillus* spp. to examine susceptibility to pathogen invasion in storage (Kulik, 1973). After 30 days storage at 85% RH and 22-25°C, little fungal invasion was observed on the vegetable seeds compared to serious pathogen growth on the cereal (corn, wheat) seeds stored in a similar fashion. These results suggest that coat characteristics and seed composition of many vegetable species may provide protection against storage fungi. It is known that most seed storage fungi cannot invade seeds in equilibrium with a relative humidity of 65% or less, although xerophytic strains of fungi can be pathogenic at relative humidity levels just above this value (Kulik, 1995). However, seedlot moisture contents represent an average of each individual seed comprising the lot, and even seed tissues vary in seed moisture content within a seed, emphasizing the need for attention to satisfactory control of storage pathogens while simultaneously ensuring a favorable storage environment for BCA's. Further differences in seed coat

composition, pathogen tolerance, and BCA colonization are likely to vary among cultivars within species, just as cultivars vary in storability (Priestly, 1986; Coolbear, 1995). Levels of seed leachate from various seedlots will also impact the success of BCA treatments, especially if seed leachates increase the colonization of beneficial microbes rather than pathogens in the spermosphere/rhizosphere.

Conclusions

Private and public sector research scientists are making valuable improvements in the efficacy, reliability and utility of biological seed treatments for vegetable crops and other species. A partnership between Gustafson, Inc. (Dallas, TX) and Speedling, Inc. (Sun City, FL) also reflects the need for BCA application to vegetable transplants as well as seeds for optimal results with biological protectants (Anon., 1998). High quality vegetable seed production typically relies on the combination of chemical foliar sprays to disease free stock grown in isolated, relatively disease free areas with suitable climates (McDonald and Copeland, 1997). Adding biological agents by colonizing seed with beneficial microbes may be an important advancement in seed production (Gonzalez and Trevathan, 2001). Long term objectives in BCA research must still focus on establishing resident antagonists (leading to 'suppressive soils'), using proper rotations, cover crops, etc., but BCA's as seed treatments appear to be an excellent short term production strategy (Cook, 1993). GPS/GIS technology employed in precision farming systems may also prove useful in future seed production. Just as soil fertility, seeding depth and plant population decisions are improved by treating a field as a grid rather than a uniform whole of subunits, seed quality and seed health could be improved by recognizing this variability in a production field (Barr, 1998; Weiland et al., 2001). Viewing the work and progress with BCA's applied to seed as a subset of microbial ecology illustrates the many areas yet to be explored.

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Table 1. Sensitivity of eight fungi commonly found on carrot seeds to three fungicides.

Fungi	Fungicides [†]		
	Thiram	Iprodione	Carbendazim
<i>Alternaria dauci</i>	x [‡]	xxx	-
<i>A. radicina</i>	x	xx	-
<i>A. alternata</i>	x	xx	-
<i>Stemphylium botryosum</i>	x	xx	-
<i>Botrytis</i> spp.	x	xx	-
<i>Fusarium</i> spp.	x	-	x
<i>Penicillium</i> spp.	-	x	-
<i>Phoma</i> spp.	x	x	xx

[†] Thiram - Bis(dimethylthio-carbamoyl)disulfide; or tetramethylthiuram disulfide Iprodione - 3-(3,5-dichlorophenyl)-N-(1-methylethyl)2,4-dioxo-1-imidazolidinecarboxamide
Carbendazim - Methyl benzimidazol-2-ylcarbamate

[‡] xxx = highly sensitive; xx = moderately sensitive; x = weakly sensitive; - = insensitive

(Adapted from van den Bulk and Langerak, 1995)

Table 2. Bacterial (*Pseudomonas aureofaciens* AB 254) populations on tomato seeds after 4 and 8 months of storage at 5°C (Warren, 1997).

<u>Bio-osmoprimered lots</u>	<u>Colony forming units (CFU)/tomato seed</u>		
	<u>Initial</u>	<u>4 mo.</u>	<u>8 mo.</u>
1	7.7 x 10 ⁵	6.6 x 10 ⁵	3.3 x 10 ⁴
2	6.1 x 10 ⁵	4.7 x 10 ⁵	1.4 x 10 ⁴
<u>Methyl cellulose coated lots</u>	<u>Initial</u>	<u>4 mo.</u>	<u>8 mo.</u>
1	7.5 x 10 ⁸	1.9 x 10 ⁸	8.7 x 10 ⁶
2	5.6 x 10 ⁸	1.2 x 10 ⁸	8.4 x 10 ⁶

Table 3. Examples of proposed techniques for improving BCA activity or shelf-life.

<u>Citation</u>	<u>Technique</u>	<u>BCA</u>
Caesar & Burr, 1991	add sucrose to talc and methylcellulose preps	<i>Pseudomonas</i> spp. and <i>Enterobacteriaceae</i> spp.
Harman & Taylor, 1988	pH modifications	<i>Trichoderma</i> treated seeds
Lewis et al, 1991	vermiculite, bran and dilute acid added to fungal biomass	<i>Gliocladium virens</i>
Nelson et al., 1988	add polysaccharides or carboxylic acids	Two <i>Trichoderma</i> species
Warren and Bennett, 1999	nutrient broth	<i>Pseudomonas aureofaciens</i> AB254 bio-osmoprimered seeds

(Adapted from Callan et al., 1997)

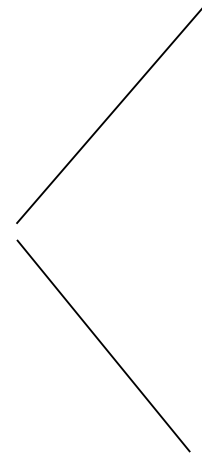
Table 4. Occurrence of three major seed-borne pathogens on six seedlots of *shrunk-2* sweet corn seed produced in Parma, ID.

Seedlot	<i>Penicillium oxalicum</i>	<i>Fusarium</i> spp.	<i>Rhizopus</i> spp.
	-----%-----		
1	3 [†]	0	74
2	42	60	50
3	0	0	40
4	0	0	85
5	85	62	49
6	38	33	75

[†] Average percentage of seed with fungi

(Adapted from Mohan and Bijman, 1997)

The Disease Triangle



host (seed/seedling)
·seed leachates,
root exudates

environment
·soil temps, pH, moisture

BCA's
