monitoring techniques to optimize the reliability of biological control. 4) We must improve the conservation and compatibility of biological control agents with other pest control methods. 5) We must effectively and rapidly train farmers to use all the available biological control tactics.

Those working to reestablish sustainable agriculture realize that biological control is not a panacea. However, it has not attained its rightful place in agriculture due, in part, to our unyielding dependence on chemical controls and our failure to develop the necessary strategies for its successful implementation. Expanding the use of biological control will require a multisystems approach, calling on pathologists, entomologists, horticulturists, ecologists, economists, commercial enterprises, and farmers. Integrating these numerous viewpoints and sources of research will help us progress in our theoretical and applied study of biological control.

Literature Cited

Agricultural Research Council. 1964. Report of the research committee on toxic chemicals. Agricultural Research Council, HMSO, London. p. 1-38.
Dreistadt, S.H. 1994. Pests of landscape trees and shrubs, an integrated pest management guide. Statewide IPM project, Univ. of Calif., Agr. & Natl. Res. Publ. 3359, Oakland.

Finch, S. 1994. Integrated crop protection in field vegetables- current advances and future possibilities. Med. Fac. Landbouww. Univ. Gent, 59/2a, 171-181.
Flint, M.L. 1990. Pest of the garden and small farm, a grower's guide to using less pesticide. Statewide IPM project, Univ. of Calif., Agr. & Natl. Res. Publ. 3332, Oakland.
Hoffman, M.P. and A.C. Frodsham. 1993. Natural enemies of vegetable insect pests. Cornell Univ., Ithaca, N.Y.
Hoitink, H.A.J., Y. Inbar, and M.J. Boehm. 1991. Status of compost-amended potting mixes naturally suppressive to soilborne diseases of floricultural crops. Plant Dis. 75:869-873.
Hoy, M.A. 1985. Almonds (California): Integrated mite management for California almond orchards, p. 299-310. In: W. Helle and M.W. Sabelis (eds.). Spider mites, their biology natural enemies and control. Elsevier Press, Amsterdam, Netherlands.
Hoy, M.A. 1991. Challenges for biological control: Enhancing its role in agriculture by the year 2000. Bul. Entom. Soc. New Zealand, 10:9-23.
Hunter, C.D. 1994. Suppliers of beneficial organisms in North America. Calif. E.P.A. Bul. PM 94-03, Sacramento.
Raupp, M.J., R.G. Van Driesche, and J.A. Davidson. 1993. Biological control of insect and mite pests of woody landscape plants: Concepts, agents, and methods. Univ. of Maryland, College Park.
Steiner, M.Y. and D.P. Elliott. 1987. Biological pest control for interior plantings. Second ed. Alberta Environ. Centre, Vegreville, Alberta, Canada.

Biological Seed Treatments: Factors Involved in Efficacy

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INTRODUCTION

Biological pest control is an integral part of a sustainable agriculture. Biological seed treatments for control of seed and seedling diseases offer the grower an alternative to chemical fungicides. While biological seed treatments can be highly effective, it must be recognized that they differ from chemical seed treatments by their utilization of living microorganisms. Storage and application conditions are more critical than with chemical seed protectants, and differential reaction to hosts and environmental conditions may cause biological seed treatments to have a narrower spectrum of use than some chemicals. Conversely, some biocontrol agents applied as seed treatments are capable of colonizing the rhizosphere, potentially providing benefits to the plant beyond the seedling emergence stage.

Effective biological control agents have been developed for control of seed and seedling pathogens such as *Pythium* spp., *Rhizoctonia solani* Kuhn, and *Fusarium* spp. Several reviews that include discussions of biological seed treatments have been published (Bennett et al., 1992; Chet and Inbar, 1994; Harman, 1991; Nelson and Maloney, 1992; Taylor and Harman, 1990; Weller, 1988; Whipps and McQuilken, 1993), and Cook (1993) has summarized factors hindering the development of biological plant disease control strategies.

A wide variety of microorganisms are currently in various stages of development for use as seed treatments. The most intensively studied include bacteria of the genera *Pseudomonas*, *Enterobacter*,

Erwinia, and *Bacillus*; the fungi *Trichoderma* and *Gliocladium*; and the actinomycete *Streptomyces* (Becker and Schwinn, 1993). However, once an effective biocontrol agent has been identified for use as a seed treatment, there is no guarantee that consistent disease control will be obtained from its use. The efficacy of biological seed treatments can be affected by soil pH and iron concentration (Weller, 1988), moisture, temperature, and pathogen inoculum density (Mathre et al., 1994). Efficacy can also be influenced by certain characteristics of the biocontrol agent and of the seed treatment itself. These include the inoculum density of the biocontrol agent on the seed, adjunct treatments such as priming, formulations and additives that enhance the activity and survival of the biocontrol agent in the formulated product, crop and pathogen specificity of the biocontrol agent, and compatibility with other microbial inoculants or chemical fungicides. A discussion of these characteristics will be illustrated, where possible, with data from our research with biological control of *Pythium* seed rot of *sh2* sweet corn (*Zea mays* L.) by *Pseudomonas aureofaciens* AB254, a naturally occurring bacterial isolate from western Montana soil (Callan et al., 1990, 1991).

INOCULUM DENSITY OF THE BIOCONTROL AGENT

A minimum number of cells or propagules of a biocontrol agent are often required to provide an adequate level of seed protection (Fukui et al., 1994b; Osburn et al., 1989; Paulitz et al., 1992). Protection of 'Crisp 'N Sweet 710' (Crookham Co., Caldwell, Idaho) *sh2* sweet corn from *Pythium* seed rot increased with *P. aureofaciens* AB254 inoculum density in both the greenhouse and field at the Western Agricultural Research Center (WARC), Corvallis, Mont. In the field, log 7.1 colony-forming units (cfu) of AB254 provided 90% seedling emergence, equivalent to that from treatment with metalaxyl [N-(2,6-dimethylphenyl)-N-(methoxyacetyl) alanine methyl ester; Ciba-Geigy, Greensboro, N.C.), a fungicide that is selective for oomycetes, including *Pythium* spp. (Fig. 1). Under severe disease conditions in the greenhouse, log 7.5 cfu provided maximum seedling emergence. These experiments indicate that a minimum of log 7 to 7.5 cfu of *P.*

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aureofaciens AB254/seed is required to protect sweet corn from *Pythium* seed rot. These results are similar to those of Osburn et al. (1989), who determined that log 7.5 cfu/seed of *Pseudomonas putida* R20 provided maximum protection of sugar beet (*Beta vulgaris* L.) pericarps from colonization by *P. ultimum*.

BIOLOGICAL CONTROL AGENTS AND SEED PRIMING

Seed priming encompasses a variety of physiological treatments that enhance seed germination and vigor through the addition of moisture (Bennett and Waters, 1987; Heydecker and Coolbear, 1977; Khan, 1992; Taylor et al., 1988). Membrane repair processes are postulated to occur during priming, and seed germination and seedling vigor are often enhanced (Khan, 1992; Sung and Chang, 1993).

The addition of microbial biocontrol agents during priming (bio-priming) allows for colonization of the seed prior to planting and adds a new dimension to the seed priming treatment. Precolonization provides the biocontrol agent with a competitive advantage over seed-attacking pathogens, such as *P. ultimum*, and often provides superior seed protection when compared to simple seed coating (Callan et al., 1990, 1991; Harman, 1991; Harman and Taylor, 1988; Harman et al., 1989; Kubik, 1995).

The bio-priming seed treatment developed for control of *Pythium* seed rot of *sh2* sweet corn combines microbial inoculation with preplant seed hydration (Callan et al., 1990, 1991). Bio-priming involves coating seed with a bacterial biocontrol agent such as *P. aureofaciens* AB254 and hydrating for 20 h under warm (23 °C) conditions in moist vermiculite (Callan et al., 1990) or on moist germination blotters in a self-sealing plastic bag (Callan et al., 1991). The seeds are removed before radicle emergence. The bacterial biocontrol agent may multiply substantially on seed during bio-priming. In three experiments conducted at WARC, 'Crisp 'N Sweet 710' *sh2* sweet corn seed was coated with various inoculum densities of *P. aureofaciens* AB254 before bio-priming. Bacterial enumeration was performed on seed after coating and again after bio-priming. The final population of *P. aureofaciens* AB254 on seed after 20 h of bio-priming depended on the initial inoculum density (Fig. 2). *Sh2* sweet corn seed appears to be able to sustain a maximum of log 9 cfu/seed, as bacteria applied to seed at this level did not show a net increase during bio-priming. The maximum inoculum carried by seed will vary with seed size and seed surface characteristics.

Inoculation of seed at a low density before bio-priming would allow a substantial increase in bacterial population, implying the potential for significant metabolic activity on the seed. To determine the effect of initial inoculum density of *P. aureofaciens* AB254 on seed before bio-priming, we treated 'Crisp 'N Sweet 710' *sh2* sweet corn seed with either a relatively low density (log 6.0) or a density near the inoculum capacity of the seed (log 8.6), and bio-primed. For comparison, seed was coated with log 8.6 cfu/seed and planted without bio-priming in WARC soil naturally infested with *P. ultimum*. Biological control of *Pythium* seed rot was not affected by initial AB254 inoculum density as seed protection equivalent to metalaxyl treatment was obtained with either log 6.0 or log 8.6 cfu/seed of AB254 before bio-priming (Table 1). Coating seed with AB254 without priming increased emergence over the methylcellulose (MC)-coated control, but emergence was less than from bio-primed seed. Seed hydration alone also increased emergence, presumably by allowing the seed to pass through a vulnerable early stage before exposure to the pathogen (Stasz et al., 1980). Fukui et al. (1994b) found that a high level of metabolic activity was maintained by bacterial cells on inoculated sugar beet (*Beta vulgaris* L.) seed, even though no increase in population was detected. This result is in agreement with our observation that *P. aureofaciens* AB254 protected *sh2* sweet corn seed from *Pythium* seed rot at low temperatures at which no net bacterial increase occurred (Mathre et al., 1994).

Bio-primed seeds may be planted directly in a moist state or air-dried at room temperature to ~10% moisture for planting or storage (Callan et al., 1991). Planting hydrated seed, where practical, may increase emergence speed (Bennett and Waters, 1987). Protection of seeds by bio-priming was similar to metalaxyl treatment regardless of seed hydration level in six field plantings of *sh2* sweet corn in *P.*

Fig. 1. Effect of seed inoculum density of *Pseudomonas aureofaciens* AB254 and metalaxyl on biological control of *Pythium* seed rot of *sh2* sweet corn ('Crisp 'N Sweet 710') in the field and greenhouse. Metalaxyl was applied at 0.3 g a.i. kg/seed. LSD_{0.05} for seedling emergence was 8.3% in the field and 9.0% in the greenhouse. Seedling emergence with metalaxyl seed treatment was 88% in the field and 87% in the greenhouse.

Fig. 2. Increase of *Pseudomonas aureofaciens* AB254 on *sh2* sweet corn ('Crisp 'N Sweet 710') seed during bio-priming. The symbol types represent data from three experiments.

Table 1. Effect of inoculum density of *Pseudomonas aureofaciens* AB254 on *sh2* sweet corn ('Crisp 'N Sweet 710') seed before bio-priming on biological control of *Pythium* seed rot in the greenhouse. Western Agricultural Research Center, Corvallis, Mont.^z

Seed treatment	<i>P. aureofaciens</i> AB254 (log cfu/seed)		Seedling emergence (%)	Seedling height (cm)
	At treatment	At planting		
Bio-prime with AB254	6.0	8.4	91 d ^y	16.7 c
Bio-prime with AB254	8.6	9.0	92 d	17.2 c
Hydrate with MC ^x	---	0	74 b	15.9 bc
Coat with AB254	---	8.6	85 c	15.0 ab
Coat with MC	---	0	43 a	14.3 a
Metalaxyl ^w	---	0	91 d	15.9 bc

^z*Pythium ultimum* 333 propagules/gram soil; AB254, cfu = colony-forming unit.

^yMean separation within columns by protected LSD_{0.05}.

^xMC = methylcellulose.

^wMetalaxyl applied at 0.3 g a.i./kg seed.

ultimum-infested soil (Mathre et al., 1994). In only one experiment did the planting of hydrated seed increase seedling emergence over that from seed that had been air-dried after bio-priming.

Drying conditions after priming may affect seed performance. Parera and Cantliffe (1994) found that rapid drying of solid matrix primed *sh2* sweet corn seed resulted in better field emergence and

seedling vigor than when seeds were dried slowly at lower temperatures. The effects of drying conditions on seed-inoculated biocontrol agents must also be considered. *Pseudomonas aureofaciens* AB254 appears to withstand air-drying on seed at room temperature, decreasing from log 8.9 cfu/seed after bio-priming to log 8.7 after air-drying in one experiment and from log 8.6 to log 8.0 in another. However, the effect of elevated drying temperature on survival of AB254 on seed is not known.

FORMULATION AND SURVIVAL OF FORMULATED PRODUCT

Biological seed treatments are more vulnerable to adverse conditions during transport and storage than are chemical seed treatments. Most microorganisms cannot tolerate desiccation or extreme heat, and viability loss will render the formulation ineffective. A successful biocontrol formulation must retain viability until used by the consumer, and ideally the unused portion could be stored for use the following year. Furthermore, the longevity of formulated biocontrol products may be limited even under ideal storage conditions. Microbial inoculants composed of endospore-forming bacteria such as *Bacillus* or of fungal spores would be expected to have a longer shelf life than would inoculum of non-spore-forming bacteria (i.e., *Pseudomonas*) or fungal mycelial preparations.

Formulation technology has the potential to enhance the effectiveness of biological seed treatments (Lewis and Papavizas, 1991). Most formulation research is directed toward the production of an inoculum with high densities of viable propagules, long shelf life, and stability under adverse conditions. The product should also be easily applied to seed and economically produced.

Several investigators have noted improved biocontrol activity or shelf life through modifications of microbial formulations. Biological control activity of two *Trichoderma* species against *Pythium* seed rot was enhanced by the addition of polysaccharides or carboxylic acids to the seed treatment (Nelson et al., 1988). Caesar and Burr (1991) found that the addition of sucrose to talc-MC preparations of several bacteria improved survival in storage. Harman and Taylor (1988) improved the performance of *Trichoderma*-treated seeds by modifying the pH to favor the biocontrol agent.

Biological seed treatments may be formulated as a product to be applied to the seed at planting or seed may be pretreated and ready to plant. We conducted storage tests to examine survival of *P. aureofaciens* AB254 on sweet corn seed. *Pseudomonas aureofaciens* AB254 was applied to seed in MC and air-dried overnight to 9% to 10% moisture before being stored at 8 °C or at room temperature (24 °C) for up to 7.5 months. Serial dilutions from four replications were plated to enumerate bacterial populations. Seeds were either planted directly from storage or were removed from storage and bio-primed for 20 h at 23 °C before planting. Metalaxyl-treated and freshly bio-primed seed were planted for comparison. Seeds were planted in naturally *P. ultimum*-infested soil in the MSU Plant Growth Center after 1, 3.5, and 7.5 months. Disease severity in the greenhouse plantings varied, so comparisons should be made only within planting dates.

Storage temperature was a major factor in the longevity of *P. aureofaciens* AB254 on sweet corn seed (Fig. 3). Inoculum viability on seed stored at both temperatures decreased most rapidly during the first month. After 7.5 months of storage, seed had lost 1.4 log units when stored at 8 °C and 3 log units at 24 °C. Results of an 11.5-month test (not shown) conducted in a similar manner concurred with these data.

Storage conditions affected the survival of AB254 on seed, but bio-priming seed after storage resulted in a recovery of the AB254 population and, consequently, its ability to protect seed. Seedling emergence in *P. ultimum*-infested soil declined more rapidly when the seed was stored at 24 °C than at 8 °C (Table 2). Emergence from seed coated with AB254 and stored at 24 °C declined to the level of nontreated seed after 3.5 months in storage, while seed stored at 8 °C retained some protection for 7.5 months. Emergence from seed stored for up to 3.5 months at 8 or 24 °C was restored to the level of metalaxyl treatment when seed was bio-primed before planting. After 7.5 months of storage, only seed held at 8 °C and then bio-primed provided emergence equal to metalaxyl treatment.

Fig. 3. Effect of storage temperature on survival of *Pseudomonas aureofaciens* AB254 on sh2 sweet corn ('Crisp 'N Sweet 710') seed. Means within a storage duration denoted by different letters are different ($P < 0.05$).

Table 2. Effect of storage temperature on viability of *Pseudomonas aureofaciens* AB254 on sh2 sweet corn ('Crisp 'N Sweet 710') seed and on biological control of *Pythium* seed rot, MSU Plant Growth Center, Bozeman, Mont.

Seed treatment	Seedling emergence (%)			
	Storage time (months)			
	0	1	3.5	7.5
None	25 a ^z	43 a	18 a	38 a
Metalaxyl ^y	46 a	91 d	72 b	82 d
Coated with AB254 (unstored)	43 a	---	---	---
Bio-primed with AB254 (unstored)	56 a	87 cd	75 b	88 d
Coated with AB254				
Stored at 8 °C	---	78 bc	64 b	70 c
Stored at 24 °C	---	76 b	34 a	48 b
Bio-primed with AB254				
After storage at 8 °C	---	89 d	74 b	85 d
After storage at 24 °C	---	82 b-d	67 b	64 c

^zMean separation within columns by protected LSD_{0.05}.

^yMetalaxyl applied at 0.3 g a.i./kg seed.

Table 3. Seedling emergence of sh2 sweet corn ('Crisp 'N Sweet 710') after bio-priming or coating seed with *Pseudomonas aureofaciens* AB254 and storing at ≈15 °C for 6 months. Western Agricultural Research Center, Corvallis, Mont.

Seed treatment	Seedling emergence (%)	
	In greenhouse immediately after seed treatment ^z	In field after 6 months ^y
AB254 bio-primed, stored	79 c ^x	68 b
AB254 coated, stored	66 b	48 a
AB254 coated at time of planting	---	66 b
Metalaxyl ^w	84 c	69 b
None	24 a	50 a

^x*Pythium ultimum* 910 propagules/g soil.

^y*Pythium ultimum* 650 propagules/g soil.

^zMean separation within columns by protected LSD_{0.05}.

^wMetalaxyl applied at 0.3 g a.i./kg seed.

Bio-priming may improve the shelf life of treated seed. Many bacteria are capable of producing protective exopolysaccharides in situ (Costerton and Irvin, 1981) and may do so during bio-priming. A 6-month storage test conducted at WARC showed that bio-priming 'Crisp 'N Sweet 710' sh2 sweet corn with *P. aureofaciens* AB254 provided better protection from *Pythium* seed rot after 6 months of storage than did coating seeds with the bacteria (Table 3). This result agrees with those of Kubik (1995), who found that survival of *Enterobacter cloacae* EC102 on seed was improved when the bacterium was allowed to increase on the seed during solid matrix priming.

CROP AND PATHOGEN SPECIFICITY

Several modes of action may be operating simultaneously in biological seed protection, including competition with the pathogen

for seed-derived compounds (Elad and Chet, 1987; Paulitz et al., 1992), production of antibiotics and siderophores (Weller, 1988), direct inhibition of pathogen hyphal development (Nelson et al., 1986), and mycoparasitism (Al-Hamdani et al., 1983). Nutritional dissimilarities or predominance of certain mechanisms over others may result in better protection of one crop than another by a biocontrol agent or greater effectiveness against one pathogen than another.

A complex nutritional relationship may exist between the plant, the pathogen, and the spermosphere or rhizosphere microorganism, as shown in studies by Nelson et al. (1986, 1988), in which adhesion of *Enterobacter cloacae* to hyphae of *P. ultimum* was regulated by the presence of specific carbohydrates. Seeds that exuded high levels of carbohydrates during germination were not protected by *E. cloacae*.

Bacteria also use components of seed exudates to produce inhibitory compounds such as antibiotics and siderophores that may be involved in biocontrol. Maurhofer et al. (1994) found that while *Pseudomonas fluorescens* CHA0 controlled *Pythium* damping-off of cress (*Lepidium sativum* L.) and cucumber (*Cucumis sativus* L.), its antibiotic pyoluteorin was biologically involved only on cress. Seed surface characteristics undoubtedly contribute to crop specificity in some biological seed treatments. The acidic nature of tomato (*Lycopersicon esculentum* Mill.) seeds may have been responsible for relatively better protection of tomato by the more acid-tolerant *Trichoderma* than by *E. cloacae* (Harman and Taylor, 1988). An interesting example of crop specificity is the ability of *Penicillium oxalicum*, a common seedborne pathogen of sweet corn (Callan et al., 1996; Halfon-Meir and Solel, 1990), to protect pea (*Pisum sativum* L.) seeds from *Pythium* seed rot (Callan, unpublished; Windels and Kommedahl, 1978).

Pseudomonas aureofaciens AB254 has routinely protected sweet corn seeds from *P. ultimum* seed rot at a level equivalent to that provided by metalaxyl. In contrast, seedling emergence of beets (*Beta vulgaris* L.) and sugar snap peas, while often increased by AB254, was generally less than with metalaxyl. Recently, AB254 has been found to be an effective seed protectant for cucurbitaceous crops in the greenhouse and field. In 1995, three field experiments with 'Senator' zucchini squash (*Cucurbita pepo* L.) were conducted at the Southwest Florida Research and Education Center (SWFREC) at Immokalee. Six replications of three seeds/hill and 16 hills/plot were planted in double-cropped white plastic mulched beds at a 46-cm spacing on 1.8-m centers. *Pythium* spp. were detected on dead and dying plants by R.J. McGovern of SWFREC. In the first two plantings, both bio-priming and coating seed with *P. aureofaciens* AB254 provided protection from *Pythium* seed rot equal to metalaxyl treatment. No disease was detected in the third planting as soil temperatures at planting depth increased (Table 4).

Some biocontrol agents may be more effective against one pathogen than another, in part due to production of pathogen-specific antibiotics (Howell and Stipanovic, 1979, 1980). *Pseudomonas aureofaciens* AB254 is highly effective in controlling *Pythium* seed rot of sweet corn, but exhibits less in vitro activity against *P. oxalicum*. However, *Pseudomonas* sp. AB842, also isolated from soils near Corvallis, Mont., is highly effective in controlling *P. oxalicum* seed decay and seedling blight but has little antagonism toward *P. ultimum*. Bio-priming *sh2* sweet corn seed with *Pseudomonas* AB842, AB254, or a combination of the two isolates reduced seedborne fungi when compared with the level on nontreated seed. In some field experiments, this combination of isolates increased seedling stand and vigor over that resulting from individual application (Callan, unpublished).

COMPATIBILITY WITH OTHER MICROBIAL INOCULANTS

According to Weller (1988), a combination of compatible bacterial biocontrol agents may provide better disease control than the same isolates applied alone. This feature may be due to a wider range of adaptation of the bacteria to physical, biological, and chemical conditions. Combining strains may not yield the desired results if competition occurs among biocontrol agents. Competition among bacterial strains inoculated onto seed may depend on their initial inoculum density. Fukui et al. (1994a) found that competition between two

Table 4. Zucchini squash ('Senator') seedling emergence following seed treatment with *Pseudomonas aureofaciens* AB254 at SWFREC, Immokalee, Fla.

Seed treatment	Seedling emergence (%)		
	Planting date		
	26 Jan. 1995	21 Feb. 1995	22 Mar. 1995
Bio-prime AB254	86 b ²	92 b	90 a
Coat AB254	81 b	92 b	93 a
Primed only	74 ab	82 a	89 a
Metalaxyl ³	84 b	91 b	94 a
None	64 a	78 a	88 a

²Mean separation within columns by protected LSD_{0.05}.

³Metalaxyl applied at 0.3 g a.i./kg seed.

pseudomonad strains on sugar beet seed was minimal when both were inoculated at a low level. Apparently, the two bacterial strains were initially distributed among the limited number of favorable sites and competition was nonsignificant. In comparison, after one strain was inoculated at high density and the other at low density, antagonism between the strains was more evident.

Ideally, a biological seed protectant should be compatible with other microbial inoculants, particularly strains of nitrogen-fixing bacteria routinely used with leguminous crops. For example, some strains of *Pseudomonas* (Nishijima et al., 1988) and *Bacillus cereus* (Halverson and Handelsman, 1991; Osburn et al., 1995) increase nodulation by *Rhizobium* and *Bradyrhizobium* inoculants.

COMPATIBILITY WITH CHEMICAL SEED TREATMENTS

The combination of biological and chemical controls is viewed as a means of increasing the reliability of crop protection under conditions that are unfavorable for the performance of the biocontrol agent alone. Biological and chemical controls may be targeted for the same or different pathogens. Sweet corn seed is frequently infested or infected with *P. oxalicum* and *Fusarium* spp., although the effects of these fungi may be masked by *Pythium* seed rot. The fungicide imazalil [1-(2-(2,4-dichlorophenyl)-2-(2-propenyl)ethyl)-1H-imidazole; Gustafson, Plano, Texas] is effective in controlling these seedborne fungi (Callan et al., 1996). Mathre et al. (1995) found that metalaxyl was compatible with *P. aureofaciens* AB254 for control of *P. ultimum*, as was imazalil with *Pseudomonas* sp. AB842 for control of *P. oxalicum*. In these experiments, the biological and chemical treatments were highly effective alone, so additive effects were not always evident. Complementary effects were seen when *P. aureofaciens* AB254 was combined with imazalil in a seed treatment, resulting in control of *Pythium* seed rot and an increase in seedling vigor. Similarly, *Rhizoctonia solani* was controlled on pea by a combination of *Bacillus subtilis* and thiram/carboxin (tetramethyl-thiuram disulfide/5,6-dihydro-2-methyl-N-phenyl-1,4-oxathiin-3-carboxamide) (Hwang and Chakravarty, 1992) and on canola (*Brassica napus* L.) with Captan (cis-N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide) and *Pseudomonas fluorescens* and *Serratia* sp. (Zablotowicz et al., 1992).

CONCLUSIONS

Biological pest control is certain to play an important role in agriculture in the future. To be competitive with conventional chemical controls, biological seed treatments must be effective, economical, and dependable. The incorporation of biocontrol agents into seed priming processes offers a means to enhance efficacy, as does the combination of biological and chemical treatments and the application of multiple biocontrol agents. Advances in formulation technology are resulting in biocontrol products with extended shelf life and an adequate inoculum density of the biocontrol agent on the seed at planting. An appreciation of the uniqueness of biocontrol strategies will aid in ensuring their proper handling and use on the appropriate crops and against the intended pathogens, thus enabling the full potential of biological control to be realized.

Literature Cited

- Al-Hamdani, A.M., R.S. Lutchmeah, and R.C. Cooke. 1983. Biological control of *Pythium ultimum*-induced damping-off by treating cress seed with the mycoparasite *Pythium oligandrum*. Plant Pathol. 32:449-454.
- Becker, J.O. and F.J. Schwinn. 1993. Control of soil-borne pathogens with living bacteria and fungi: status and outlook. Pestic. Sci. 37:355-363.
- Bennett, M.A., V.A. Fritz, and N.W. Callan. 1992. Impact of seed treatments on crop stand establishment. HortTechnology 2:345-349.
- Bennett, M.A. and J.L. Waters. 1987. Seed hydration treatments for improved sweet corn germination and stand establishment. J. Amer. Soc. Hort. Sci. 112:45-49.
- Callan, N.W., D.E. Mathre, and J.B. Miller. 1990. Bio-priming seed treatment for biological control of *Pythium ultimum* preemergence damping-off in *sh2* sweet corn. Plant Dis. 74:368-372.
- Callan, N.W., D.E. Mathre, and J.B. Miller. 1991. Field performance of sweet corn seed bio-primed and coated with *Pseudomonas fluorescens* AB254. HortScience 26:1163-1165.
- Callan, N.W., D.E. Mathre, S.K. Mohan, J.B. Miller. 1996. Soil moisture and temperature effects on *shrunk2* sweet corn seed decay and seedling blight caused by *Penicillium oxalicum*. J. Amer. Soc. Hort. Sci. 83-90.
- Caesar, A.J. and T.J. Burr. 1991. Effect of conditioning, betaine, and sucrose on survival of rhizobacteria in powder formulations. Appl. and Environ. Micro. 57:168-172.
- Chet, I. and J. Inbar. 1994. Biological control of fungal pathogens. Appl. Biochem. and Biotechnol. 48:37-43.
- Cook, R.J. 1993. Making greater use of introduced microorganisms for biological control of plant pathogens. Annu. Rev. Phytopathol. 31:53-80.
- Costerton, J.W. and T. Irvin. 1981. The bacterial glycocalyx in nature and disease. Annu. Rev. Microbiol. 35:229-324.
- Elad, Y. and I. Chet. 1987. Possible role of competition for nutrients in biocontrol of *Pythium* damping-off by bacteria. Phytopathology 77:190-195.
- Fukui, R., M.N. Schroth, M. Henderson, and J.G. Hancock. 1994a. Interaction between strains of pseudomonads in sugar beet spermospheres and their relationship to pericarp colonization by *Pythium ultimum* in soil. Phytopathology 84:1322-1330.
- Fukui, R., M.N. Schroth, M. Henderson, J.G. Hancock, and M.K. Firestone. 1994b. Growth patterns and metabolic activity of pseudomonads in sugar beet spermospheres: relationship to pericarp colonization by *Pythium ultimum* in soil. Phytopathology 84:1331-1338.
- Halfon-Meir, A. and Z. Solel. 1990. Factors affecting seedling blight of sweet corn caused by seedborne *Penicillium oxalicum*. Plant Dis. 74:36-39.
- Halverson, L.J. and J. Handelsman. 1991. Enhancement of soybean nodulation by *Bacillus cereus* UW85 in the field and in a growth chamber. Appl. Environ. Microbiol. 57:2767-2770.
- Harman, G.E. 1991. Seed treatments for biological control of plant disease. Crop Protection 10:166-171.
- Harman, G.E. and A.G. Taylor. 1988. Improved seedling performance by integration of biological control agents at favorable pH levels with solid matrix priming. Phytopathology 78:520-525.
- Harman, G.E., A.G. Taylor, and T.E. Stasz. 1989. Combining effective strains of *Trichoderma harzianum* and solid matrix priming to improve biological seed treatments. Plant Dis. 73:631-637.
- Heydecker, W. and P. Coolbear. 1977. Seed treatments for improved performance - survey and attempted prognosis. Seed Sci. Technol. 5:353-425.
- Howell, C.R. and R.D. Stipanovic. 1979. Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. Phytopathology 69:480-482.
- Howell, C.R. and R.D. Stipanovic. 1980. Suppression of *Pythium ultimum*-induced damping-off of cotton seedlings by *Pseudomonas fluorescens* and its antibiotic, pyoluteorin. Phytopathology 70:712-715.
- Hwang, S.F. and P. Chakravarty. 1992. Potential for the integrated control of *Rhizoctonia* root-rot of *Pisum sativum* using *Bacillus subtilis* and a fungicide. J. Plant Dis. and Protection 99:626-636.
- Khan, A.A. 1992. Preplant physiological seed conditioning. Hort. Rev. 13:131-181.
- Kubik, K.K. 1995. Seed priming and survival of desiccated *Enterobacter cloacae* inoculated during seed priming. PhD Diss., Univ. of Nebraska, Lincoln.
- Lewis, J.A. and G.C. Papavizas. 1991. Biocontrol of plant diseases: The approach for tomorrow. Crop Protection 10:95-105.
- Mathre, D.E., N.W. Callan, R.H. Johnston, J.B. Miller, and A. Schwend. 1994. Factors influencing the control of *Pythium ultimum*-induced seed decay by seed treatment with *Pseudomonas aureofaciens* AB254. Crop Protection 13:301-307.
- Mathre, D.E., R.H. Johnston, N.W. Callan, S.K. Mohan, J.M. Martin, and J.B. Miller. 1995. Combined biological and chemical seed treatments for control of two seedling diseases of *sh2* sweet corn. Plant Dis. 79:1145-1148.
- Maurhofer, M., C. Keel, D. Haas, and G. Defago. 1994. Pyoluteorin production by *Pseudomonas fluorescens* strain CHA0 is involved in the suppression of *Pythium* damping-off of cress but not of cucumber. Eur. J. Plant Pathology 100:221-232.
- Nelson, E.B., W.-L. Chao, J.M. Norton, G.T. Nash, and G.E. Harman. 1986. Attachment of *Enterobacter cloacae* to hyphae of *Pythium ultimum*: possible role in the biological control of *Pythium* preemergence damping-off. Phytopathology 76:327-335.
- Nelson, E.B., G.E. Harman, and G.T. Nash. 1988. Enhancement of *Trichoderma*-induced biological control of *Pythium* seed rot and pre-emergence damping-off of peas. Soil Biol. Biochem. 20:145-150.
- Nelson, E.B. and A.P. Maloney. 1992. Molecular approaches for understanding biological control mechanisms in bacteria: Studies of the interaction of *Enterobacter cloacae* with *Pythium ultimum*. Can. J. Plant Pathol. 14:106-114.
- Nishijima, F., W.R. Evans, and S.J. Vesper. 1988. Enhanced nodulation of soybean by Bradyrhizobium in the presence of *Pseudomonas fluorescens*. Plant Soil 111:149-150.
- Osburn, R.M., M.N. Schroth, J.G. Hancock, and M. Henderson. 1989. Dynamics of sugar beet seed colonization by *Pythium ultimum* and *Pseudomonas* species: effects on seed rot and damping-off. Phytopathology 79:709-716.
- Osburn, R.M., J.L. Milner, E.S. Oplinger, R.S. Smith, and J. Handelsman. 1995. Effect of *Bacillus cereus* UW85 on the yield of soybean at two field sites in Wisconsin. Plant Dis. 79:551-556.
- Parera, C.A. and D.J. Cantliffe. 1994. Dehydration rate after solid matrix priming alters seed performance of *shrunk-2* corn. J. Amer. Soc. Hort. Sci. 119:629-635.
- Paulitz, T.C., O. Anas, and D.G. Fernando. 1992. Biological control of *Pythium* damping-off by seed-treatment with *Pseudomonas putida*: relationship with ethanol production by pea and soybean seeds. Biocontrol Sci. and Technol. 2:193-201.
- Stasz, T.E., G.E. Harman, and G.A. Marx. 1980. Time and site of infection of resistant and susceptible germinating pea seeds by *Pythium ultimum*. Phytopathology 70:730-733.
- Sung, F.J.M. and Y.H. Chang. 1993. Biochemical activities associated with priming of sweet corn seeds to improve vigor. Seed Sci. and Technol. 21:97-105.
- Taylor, A.G., D.E. Klein, and T.H. Whitlow. 1988. SMP: solid matrix priming of seeds. Scientia Hort. 37:1-11.
- Taylor, A.G. and G.E. Harman. 1990. Concepts and technologies of selected seed treatments. Annu. Rev. Phytopathol. 28:321-339.
- Weller, D.M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annu. Rev. Phytopathol. 26:379-407.
- Whipps, J.M. and M.P. McQuilken. 1993. Aspects of biocontrol of fungal plant pathogens, p. 45-79. In: D.G. Jones (ed.). Exploitation of microorganisms. Chapman and Hall, London.
- Windels, C.E. and T. Kommedahl. 1978. Factors affecting *Penicillium oxalicum* as a seed protectant against seedling blight of pea. Phytopathology 68:1656-1661.
- Zablottowicz, R.M., C.M. Press, N. Lyng, G.L. Brown, and J.W. Kloepper. 1992. Compatibility of plant growth promoting rhizobacterial strains with agrichemicals applied to seed. Can. J. Microbiol. 38:45-50.