Application of Antagonistic Microorganisms to Seeds to Control Fungal Plant Pathogens

Birgit Jensen, Inge M.B. Knudsen, Dan Funck Jensen, and John Hockenhull
Department of Plant Biology, Plant Pathology Section, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C

INTRODUCTION

During the next decade biological control may become an important component of plant disease management practices. The demand for alternatives to chemical control of plant pathogens has become stronger owing to concerns about the safety and environmental impacts of chemicals. Development of fungicide resistance, lack of effective chemical solutions for specific pathogens, and the fact that pesticides are not allowed in organic farming systems has increased the need for development of biological control agents (BCAs). Biological control can be practiced in three ways:

1) By deliberate application of beneficial microorganisms which suppress plant pathogens;

2) By deliberate application of organisms which induce resistance in the host plant;

3) By cultivation practices which enhance natural disease suppression (suppressive soils).

This paper deals with the first category and focuses primarily on biocontrol obtained by application of fungal antagonists to seeds.

CRITERIA FOR COMMERCIAL BIOLOGICAL SEED TREATMENT

The first requirement for successful biological control is the selection of a superior strain of an antagonistic microorganism. This is often done by screening for antagonism using either in vitro or in vivo systems or by genetic manipulation through mutation or protoplast fusion (Ahmad and Baker, 1987; Merriman and Russel, 1990; Harman 1991). However, there are numerous examples of potentially useful biocontrol agents which have been unable to secure stable and effective biological control under natural conditions (Renwick et al., 1991; Duczek, 1994). This has been attributed to a number of soil abiotic factors, including aeration, moisture, pH, temperature, and texture as well as biotic factors such as competition from, and predation by, the indigenous soil microbiota (Shah-Smith and Burns, 1996). However, difficulties related to the production, shelf-life and formulation of the antagonist are also important factors which strongly complicate the commercialization of products based upon living organisms. Therefore, in order to develop a biological product for seed treatment, the formulated antagonistic strain must fulfil a number of requirements: (1) Appropriate fungal structures must be produced rapidly at high and reproducible levels, (2) these structures must be able to withstand drying and storage, (3) they should be activated at sowing and be able to colonize the plant roots to suppress pathogens, (4) control efficiency should be high and stable under varying environmental conditions, and (5) the antagonist should be harmless to the germinating seed and the emerging roots (Baker, 1991; Harman

et al., 1991). Finally it is important to remember that registration of the BCA is a necessity for commercialization.

SHELF-LIFE

Survival and Shelf-life of the BCA . If BCAs are to become marketable products it is essential to ensure a good yield of efficient and viable propagules which also have a long and stable shelf-life. According to Powell (1993) the ideal objective is survival of the BCA at a temperature range from -5C to 30C for 2 years. Since any decrease in viability will increase production costs, one of the main problems in the production phase of a BCA is to hold viability of propagules as close as possible to 100% of the original production. Production of *Trichoderma harzianum* in liquid fermentation resulted in conidia of which only approximately 10% germinated after drying. However, desiccation tolerance could be enhanced to 50% to 70% germination by modifying the osmotic potential in the growth media (Harman et al.,1991; Jin et al., 1991). Production of conidia of *Gliocladium roseum* (IK726) on a solid substrate gave 50% survival after drying. A comparison of freshly harvested and dried conidia showed that germination began 2 to 4 h after inoculation and that all conidia capable of germinating had germinated 24 h after inoculation on water agar. However, the speed of germination was significantly affected by the drying process since freshly harvested conidia germinated faster than the dried conidia (Jensen et al., 1996). Rapid germination may be an important factor for control efficiency of pathogens like *Pythium ultimum* which germinate rapidly and begin to infect seeds within 4 to 6 h after planting (Stasz et al., 1980). Different techniques have been used to give the antagonist a competitive advantage over the indigenous microflora by ensuring favorable conditions around the seed at the time of planting. This will be discussed below. Once a high percentage of potentially germinable propagules has been ensured, the preparation should have a long shelf-life. Most fungal preparations can be stored at 4C for 6 to 12 months without significant loss of viability, while survival has generally proved to be poor at temperatures from 20 to 25C (Papavizas et al., 1984; Lewis and Papavizas, 1985; Dandurand and Knudsen, 1993; Jensen et al., 1996; Jensen, unpublished). However, Sivan et al. (1984) found a 91% decrease in viability of *T. harzianum* conidia after 1 year at 25C and recently we have shown that conidia of *G. roseum* can survive for 1 year at 20C with less than 70% decrease in viability. Finally, it must be stressed, that high viability and good shelf-life of a preparation do not necessarily mean that the antagonist has retained its activity and ability to control the pathogen effectively and consistently under a variety of environmental conditions, this has to be tested thoroughly.

Shelf-Life on Seeds. Commercialization of a biological seed treatment product will be a more attractive alternative to chemicals if seeds coated with antagonists can be stored for several months. Survival of conidia following their application to seeds is not well documented. Cliquet and Scheffer (1996) showed that survival of conidia applied onto radish seeds through an industrial film-coating process varied according to the strain of *T. harzianum*. For two strains, a better conidial viability was observed, with a decrease of one order of magnitude after storage for 3 months at 15C and 5 months at 4C. Another strain, which had also controlled damping off (caused by *P. ultimum)* in growth chamber assays, failed to survive at both temperatures (Cliquet and Scheffer, 1996). The viability of conidia of an isolate of *G. roseum*

(IK726) applied to barley seeds and stored for more than 6 month at 4C was stable (Jensen, unpublished). The activity of germinable conidia also seemed to be intact, because sowing of kernels infected with *Bipolaris sorokiniana* and coated with conidia of the antagonist gave more than 90% control after 8 months of storage at low temperature. Conidial viability on seeds at 20C declined after 1 to 3 months depending on specific storage conditions (Jensen, unpublished). These results show the feasibility of biocontrol of seed-borne and seedling diseases by application of specific strains of antagonists to seeds. However, care should be taken since strains which have proven to be superior in disease control tests are not necessarily those with the best capacity for survival on seeds stored under commercially acceptable conditions.

SEED TREATMENT

Various strategies have been used for applying BCAs. Seed application has several advantages compared to spraying or incorporation of the BCA into soil or soilless growing mixture. Only a small amount of active ingredient (antagonist) is used especially compared to soil application and, besides this, the antagonist is placed close to the pathogen, both in time and space. In addition to protecting the plant from seed-borne infections, biological seed treatment also has the potential of protecting against attack from soil-borne pathogens as well.

Seed-borne Pathogens. In many countries seed lots of cereals are routinely treated with chemicals (Rennie and Cockerell, 1994). A Nordic project was initiated in order to screen for microorganisms antagonistic to a variety of important seed-borne diseases on cereals and adapted to the North European soil habitats and microenvironments. In Denmark, an antagonistic isolate of *G. roseum* (IK726) was isolated from the field and tested in field trails. Results showed that seed treatment with freshly harvested conidia of *G. roseum* controlled *Fusarium culmorum* as effectively as seed treatment with the fungicide Sibutol LS 280 (Knudsen et al., 1995). In another field experiment, with barley naturally infected with *Bipolaris sorokiniana,* it was demonstrated that *G. roseum* also was effective against this disease as both plant dry weight 1 month after sowing, and the thousand-grain weight at harvest, were significantly increased. In addition, control was as good as that of the fungicide Fungazil TBZ (Knudsen et al., 1995). Also a mixed natural infection of *Fusarium* spp. (including *Gerlachia nivale*) was controlled by *G. roseum* in a sand test, and a 70% reduction in the disease index was obtained (Knudsen, unpublished). In field trials, seed treatment with a dried and stored formulation of *G. roseum* conidia gave good protection against the seedborne pathogen, *Fusarium culmorum,* on winter wheat. At harvest the grain yield was as high as the yield harvested in plots which had received a chemical seed treatment with Sibutol (Jensen, unpublished). These promising results show that seed treatment with suitable formulated antagonistic preparations can become a realistic alternative to chemical seed treatment.

Soil-borne Pathogens. Most of the work on biological seed treatment has been directed towards protecting seeds and seedlings against soil-borne diseases. However, in many cases highly effective strains selected in preliminary experiments have been unable to ensure effective biological control under natural conditions. It is not sufficient to have viable propagules, the propagules also have to be properly

formulated to give effective and consistent results. In this connection the composition of coating material (binder, solid carrier) plays an important role. Harman and Taylor (1988) showed that using a carrier with pH 4.1, seed treatment with *T. harzianum* gave better protection against soil-borne pathogens than a formulation with a more alkaline carrier. This was mainly because the acidic environment favored growth of *Trichodema* compared to most other microorganisms (Harman and Taylor, 1988). A conducive environment for the bioprotectant can also be created through a physical barrier. Taylor et al. (1991) used a liquid-coating system consisting of *T. harzianum*, a solid carrier, and a binder. The application technique resulted in the formation of a continuous, uninterrupted, <0.1-mm-thick coating around the seed, which was sufficient to slow down the infection of the seed by *P.ultimum* by 5 to 6 h (Harman, 1991). The creation of such a physical barrier between the antagonist and pathogen also favored the utilisation of exudates from the seed by the antagonist rather than the pathogen during initial seed germination. McQuilken et al. (1990), showed that modern seed technologies like pelleting and film-coating are compatible with the use of BCAs. Cliquet and Scheffer (1996) have demonstrated the same with film-coating.

Combining Seed Priming and Biological Control. Various physiological seed conditioning treatments have been reported to enhance and stabilize the efficacy of biological control agents. One of the most promising is seed priming, in which controlled hydration initiates the physiological processes of germination, without radicle emergence. Treatment of cucumber seeds with *T. harzianum* was not sufficient to control *P. ultimum*. When seeds were coated and primed, however, control efficacy was strongly increased (Taylor et al., 1991). Priming favored the antagonist as it could colonize and take possession of substrates including exudates from the germinating seed before either the competing microflora or plant-pathogenic fungi (Taylor et al., 1991).Treating seeds of sweet corn with an antagonistic isolate of*Pseudomonas aureofaciens* effectively controlled seed decay induced by*P. ultimum* in soils with low to medium water content. However, when the water content of the soils was high coating was unable to control the disease but if seed coating was combined with priming, control was obtained (Mathre et al., 1994). They concluded that a combination of preplant seed hydration and the use of a BCA provided the most consistent protection against seed decay. Although priming is a promising technology for enhancement of biological control, technical problems concerning storage of primed seeds without loss of seed germinability and variability of the antagonist have to be solved.

RHIZOSPHERE COMPETENCE

Rhizosphere competence of antagonistic strains is a very important trait since not only the seed but also the root need to be protected from attack by soil-borne plant pathogens. Rhizosphere competence may be defined as the ability of a microorganism, applied by seed treatment, to colonize the rhizosphere of developing roots (Baker, 1991). In general, bacteria apparently have greater root colonization capabilities than fungal antagonists (Rovira and Campbell, 1974). One of the fungal genera — *Trichoderma* — which has been extensively used for biocontrol of soil-borne diseases has seldom been reported to be rhizosphere competent (Ahmed and Baker, 1987). However, Sivan and Chet (1989) did demonstrate rhizosphere competence in a wild strain of *T. harzianum*. An antagonistic isolate of *G. roseum*

(IK726) was able to colonize young barley roots in sand (Knudsen et al., 1996) and in field soil *G. roseum* was reisolated 4 months after sowing in a significantly higher number from roots of plants derived from seeds coated with the antagonist than from roots derived from noncoated seeds (Knudsen et al., 1996). In preliminary greenhouse tests, seed treatment with this isolate has shown good ability to control *P. ultimum* on sugar beet (Jensen, unpublished). Rhizosphere competence has been demonstrated in *T. harzianum* following protoplast fusion (Sivan and Harman, 1991) and by mutation (Ahmed and Baker, 1987) and these strains were shown to exhibit a significant improvement in the control of *P. ultimum* compared to the original strains (Harman et al., 1989; Baker, 1991). The trait of rhizosphere competence cannot be generalized from one plant-antagonist combination to another. Sivan and Harman (1991) showed that the rhizosphere competent isolate of *T. harzianum* colonized roots of maize and roots of cotton differently. The responses of two plant species to root colonization by *Streptomyces griseoviridis* were also different (Kortemaa et al., 1994). Therefore, rhizosphere competence has to be investigated for each combination of host plant and antagonist.

CONCLUDING REMARKS

The prospects for successful biological seed treatments to control seed-borne diseases are very promising. A storable formulation of *G. roseum* has proven highly efficient under field conditions when applied as a seed treatment. Furthermore, seeds coated with this antagonistic isolate can be stored for several months. Biocontrol of soil-borne diseases have also been achieved by coating seeds with antagonistic fungi. In this context rhizosphere competence of the BCA seems to be a very important trait in order to obtain or improve control efficacy. On the basis of the good results achieved with biological seed treatment in small-scale experiments the commercialization of biological control agents for seed treatment is now becoming a realistic possibility. However, the resources of commercial companies and foundations are required for large-scale production, wide-scale field testing, and registration including risk assessment and marketing.

LITERATURE CITED

Ahmad, J.S. and **R. Baker.** 1987. Rhizosphere competence of *Trichoderma harzianum*. Phytopathology 77, 182-189.

Baker, R. 1991. Induction of rhizosphere competence in the biocontrol fungus Trichoderma, pp. 221-228. In: D.L. Keister and P.B. Cregan (eds.). The rhizosphere and plant growth. Klüwer Academic Publishers, Netherlands.

Cliqurt, S. and **J. Scheffer**. (1996). Biological control of damping-off caused by *Pythium ultimum* and *Rhizoctonia solani* using *Trichoderma* spp. applied as industrial film coatings on seeds. Europ. J. Plant Pathol. 102:247-255.

Dandurand, L.M. and **G.R. Knudsen**. 1993. Influence of *Pseudomonas fluorescens* on hyphal growth and biocontrol activity of *Trichoderma harzianum* in the spermosphere and rhizosphere of pea. Phytopathology 83:265-270.

Duczek, L.J. 1994. Relationship between a greenhouse and field assay for control of common root of spring wheat and barley. Can. Plant Disease Survey 74:135-140.

Harman, G.E. 1991. Seed treatment 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 Disease 73:631-637.

Harman, G.E., X. Jin, T.E. Stasz, A.C. Peruzzotti, A.C. Leopold, and **A.G. Taylor.** 1991. Production of conidial biomass of *Trichoderma harzianum* for biological control. Biological Control 1:23-28.

Jensen, B., I.M.B. Knudsen, D.F. Jensen, and **J. Hockenhull.** 1996. Development of a formulation of *Gliocladium roseum* for biological seed treatment. In: C. Alabouvette (ed.), Biological and integrated control of root diseases in soilless cultures IOBC/wprs Bulletin 19(6):164-169.

Jin, X., G.E. Harman, and **A.G. Taylor.** 1991. Conidial biomass and desiccation tolerance of *Trichoderma harzianum* produced at different medium water potentials. Biological Control 1:237-243.

Kortemaa, H., H. Riata, K. Haahtela, and **A. Smolander.** 1994. Root-colonization ability of antagonistic *Streptomyces griseoviridis*. Plant and Soil 163, 77-83.

Knudsen, I.M.B., J. Hockenhull, and **D.F. Jensen.** 1995. Biocontrol of seedling diseases of barley and wheat caused by *Fusarium culmorum* and *Bipolaris sorokiniana*: effects of selected fungal antagonists on growth and yield components. Plant Pathol. 44:467-477.

Knudsen, I.M.B., B. Jensen, D.F. Jensen, and **J. Hockenhull.** 1996. Colonization of *Gliocladium roseum* on barley roots from sand and field soil, pp. 33-37. In: D. Funck Jensen, H-B. Jansson, and A. Tronsmo (eds.). Monitoring antagonistic fungi deliberately released into the environment. Kluwer Academic Publishers, Netherlands.

Lewis, J.A. and **C. Papavizas.** 1985. Characteristics of alginate pellets formulated with *Trichoderma* and *Gliocladium* and their effect on the proliferation of the fungi in soil. Plant Pathol. 34:571-577.

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 aurefaciens* AB254. Crop Protection 13:301-307.

McQuilken, M.P., J.M. Whipps, and **R.C. Cooke.** 1990. Control of damping-off in cress and sugar beet by commercial seed-coating with *Pythium oligandrum*. Plant Pathol. 39:452-462.

Merriman, P. and **K. Russell.** 1990. Screening strategies for biological control, pp. 171-190. In: D. Hornby (ed.). Biological control of soilborne plant pathogens. CAB International, Wallingford.

Papavizas, G.C., M.T. Dunn, J.A. Lewis, and **J. Beagle-Ristaino.** 1984. Liquid fermentation technology for experimental production of biocontrol fungi. Phytopathology 74:1171-1175.

Powell, K.A. 1993. The commercial exploitation of microorganisms in agriculture, pp. 441-459. In: D.G. Jones (ed.). Exploitation of microorganisms. Chapman and Hall, London.

Rennie, W.J. and **V. Cockerell.** 1994. Strategies for cereal seed treatment, pp. 17-26. In: Martin, T. (ed.). Seed treatment: Progress and prospects, BCPC monograph no 57.

Renwick, A, R. Campbell, and **S. Coe.** 1991. Assessment of *in vivo* screening systems for potential biocontrol agents of *Gaeumannomyces graminis*. Plant Pathol. 40:524-532.

Rovira, A.D. and **R. Campbell.** 1974. Scanning electron microscopy of microorganisms on the roots of wheat. Microbial Ecol. 1:15-23.

Shah-Smith, D.A. and **R.G. Burns.** 1996. Biological control of damping-off of sugar beet by *Pseudomonas putida* applied to seed pellets. Plant Pathol. 45:572-582.

Sivan, A. and **I. Chet.** 1989. The possible role of competition between *Trichoderma harzianum* and *Fusarium oxysporum* in rhizosphere colonization. Phytopathology 79:198-203.

Sivan, A and **G.E. Harman.** 1991. Improved rhizosphere competence in a protoplast fusion progeny of *Trichoderma harzianum*. J. Gen. Microbiol. 137:23-29.

Sivan, A., Y. Elad, and **I. Chet.** 1984. Biological control effect of a new isolate of *Trichoderma harzianum* on *Pythium aphanidermatum*. Phytopathology 74:498-501.

Stasz, T.E., G.E. Harman, and **G.A. Marx.** 1980. Time and site of infection of resistant and germinating pea seeds by *Pythium ultimum*. Phytopathology 70:730-733.

Taylor, A.G., T.-G. Min, G.E. Harman, and **X. Jin.** 1991. Liquid coating formulation for the application of biological seed treatment of *Trichoderma harzianum*. Biological Control 1:16-22.