Combined Biological and Chemical Seed Treatments for Control of Two Seedling Diseases of *Sh2* Sweet Corn

D. E. Mathre and **R. H. Johnston,** Department of Plant Pathology, Montana State University, Bozeman 59717; **N. W. Callan,** Western Montana Ag Research Center, Corvallis 59828; **S. K. Mohan,** Idaho Research and Extension Center, Parma 83660; **J. M. Martin,** Department of Plant, Soil, and Environmental Science, Montana State University, Bozeman 59717; and **J. B. Miller,** Western Montana Ag Research Center, Corvallis 59828

ABSTRACT

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Experiments were conducted in both the glasshouse and in the field to determine if biological and chemical control agents could be used together on sweet corn *(Zea mays* L.) seed to control *Pythium ultimum* damping-off or *Penicillium oxalicum* seedling disease. *Pseudomonas aureofaciens* AB254 and *Pseudomonas* sp. AB842 were used for control of *P. ultimum* and *P. oxalicum*, respectively. Metalaxyl seed treatment for control of *P. ultimum* was used at rates from 100 to 0.01% of the recommended rate either alone or in combination with *P. aureofaciens* AB254. Imazalil seed treatment for control of *P. oxalicum* was used at rates from 100 to 1% of the recommended rate either alone or in combination with *Pseudomonas* sp. AB842. In field tests, combining a chemical treatment with the biological agent did not affect the efficacy of disease control. Nor did combining low rates of chemical with the biocontrol agent increase the efficacy or reliability of disease control.

Interest in microbial agents for control of seedling diseases is increasing as concerns about the use of agricultural pesticides increase. Chemical seed treatments for control of seed and seedborne pathogens have been highly successful because very small amounts of pesticide per unit area are required and environmental impacts are quite low. Biocontrol strategies are of interest for those producers who wish to either avoid pesticides or reduce their pesticide use.

To date, biocontrol agents for control of seed and seedling diseases have not been widely adopted due to their limited availability and concerns about their reliability. Several strategies have been developed to improve reliability, including the integration of pesticides with biocontrol agents (9–12,18,21,22). Coordinated application of biocontrol agents with pesticides can reduce the deleterious actions of competitive microbes on beneficial biocontrol agents, and may enhance crop performance due to possible growth-promoting effects of the biocontrol agent (20). For example, planting canola (*Brassica napus* L.) seed treated with fungicide-tolerant bacteria combined with fungicides enhanced seedling emergence in the presence of *Rhizoctonia solani* Kühn (22). This effect may be due to the additive effects of growth promotion and disease control. On pea (*Pisum sativum* L.), a combination of *Bacillus subtilis* plus carboxin and thiram provided superior control of *R. solani* compared with either chemical or biological seed treatment alone (14). For control of *R. solani* in radish (*Raphanus sativus* L.) *Trichoderma harzianum* Rifai as a seed treatment combined with benodanil soil application produced levels of control that were additive but not synergistic (15). On cotton *(Gossypium hirsutum* L.), rates of metalaxyl necessary for control of *Pythium ultimum* Trow were reduced 100-fold if the metalaxyl seed treatment was combined with fluorescent pseudomonads (5).

Corn cultivars with the sh2 gene for shrunken endosperm have a high ratio of sugar to starch and are susceptible to a number of seed and soilborne plant pathogens (6). If corn is like pea, leakage of sugars from the seed during imbibition and emergence is favored by cool, moist conditions (19), which also favor development of seed decay organisms. *Pythium ultimum*, a soilborne oomycete, causes pre-emergence seed rot in sweet corn (2). *Penicillium oxalicum* Currie & Thom causes pre-emergence and post-emergence damping-off as well as seedling blight and stunting of young seedlings (13). *Penicillium oxalicum* occurs in soil, but greatest damage occurs from seed infested during maturation or harvest. These diseases are currently controlled by application of the fungicides metaxalyl for *P. ultimum* or imazalil for *P. oxalicum* (17). In addition, we have shown that *Pseudomonas aureofaciens* AB254 has activity against *P. ultimum* while *Pseudomonas* sp. AB842 has

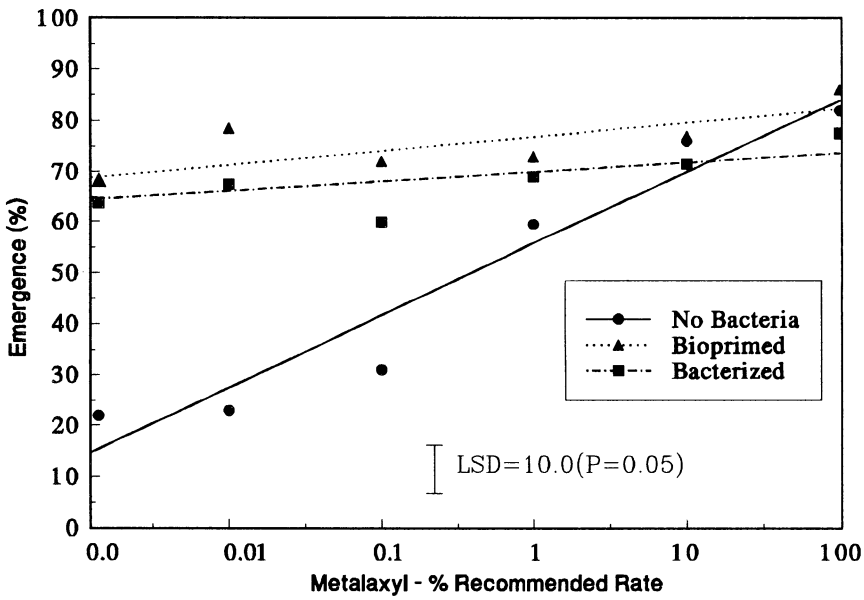

Fig. 1. Effect of low rates of metalaxyl seed treatment on *sh2* sweet corn in combination with *Pseudomonas aureofaciens* AB254 for control of *Pythium ultimum* damping-off in the glasshouse. The recommended (100%) rate of metalaxyl was 0.3 g ai/kg. Plots of means were fitted with best-fit curves.

Corresponding author: D. E. Mathre
E-mail: UPLDM@msu.oscs.montana.edu

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activity against *P. oxalicum* (7,8; N. Callan, *unpublished data).*

The purpose of this study was to determine if these biological antagonists combined with low rates of fungicide would provide enhanced control of these corn seedling diseases.

MATERIALS AND METHODS

Production of biocontrol agents. Both *Pseudomonas aureofaciens* AB254 and *Pseudomonas* sp. AB842 were grown for 48 h on potato dextrose agar (PDA) and the bacterial matrix from four plates of each culture was scraped into 4 ml of 1.5% methyl cellulose solution (4,000 centipoises, Sigma Chemical Co., St. Louis, Mo.) prepared in distilled water. Sixteen milliliters of this suspension was added to 350 seeds (Crisp 'n' Sweet 710 corn seed-Crookham Seeds, Caldwell, Idaho) and the mixture was agitated to insure uniform coating of the seed surface. Concentrations of viable bacteria on each seed were estimated at 10^8 cfu from counts produced by washing three replications of five seeds each in 0.01 M phosphate buffer (pH 6.8), followed by serial dilutions and plating on PDA. Seeds coated with bacteria were air-dried to original moisture (9%) at room temperature before planting as bacterized seeds. *Pseudomonas aureofaciens* AB254 and *Pseudomonas* sp. AB842 were used for control of *P. ultimum* and *P. oxalicum*, respectively.

Seeds were bio-primed by placing 50 g of bacteria-coated seed between double layers of germination blotter paper in a sealable 1-gallon plastic bag to which was added 30 ml of sterile water. The seed was incubated at 23°C for 20 h and either removed and planted immediately in glasshouse tests or dried to original moisture for field tests.

Fungicide seed treatment. The fungicides metalaxyl (Gustafson's Apron-FL 28.35% a.i.) and imazalil (Gustafson's Flo-Pro IMZ 31% a.i.) were applied to seed at rates ranging from none to the highest rate currently recommended by the manufacturer (0.3 g a.i./kg seed for metalaxyl and 0.1 g a.i./kg for imazalil). Varying concentrations of each formulation were prepared in distilled water, and 4.0 ml of each concentration was added to 100 g of seed contained in a 1-liter glass flask to achieve the appropriate application rate. The seed was agitated to distribute the fungicide uniformly over the seed surface and allowed to air dry until used.

When seeds received both biocontrol agent and fungicide, the fungicide treatment was applied first and bacteria were applied after the seeds had dried. For chemical control, metalaxyl was used for experiments with *P. ultimum* and imazalil was used against *P. oxalicum.*

Experimental sites. Glasshouse experiments for *P. ultimum* control were conducted at the Plant Growth Center at Montana State University (MSU) using soil collected from the Western Agricultural Research Center (Burnt Fork Sandy Loam, pH 7.4) near Corvallis, Mont. This soil has a naturally occurring population of *P. ultimum,* determined by the method of Ali-Shtayeh et al. (1). Field experiments with *P. ultimum* were located at the Western Agricultural Research Center. Both glasshouse and field experiments were planted with 50 seeds per treatment and treatments were replicated six times in randomized complete block designs. Glasshouse experiments were repeated once. In glasshouse experiments, individual treatments were seeded in metal flats (30 cm length × 20 cm width × 5 cm depth). Field experiments were seeded in single rows 2.5 m long with 0.7 m between rows.

Glasshouse experiments for *P. oxalicum* control were conducted in the MSU Plant Growth Center with pasteurized Plant Growth Center mix (1:1:1; Bozeman silt loam, sand, peat moss; vol/vol/vol). Conidia of *P. oxalicum* produced on PDA were suspended in sterile distilled water with 1 drop of Tween 20 per 100 ml and added to seed in a flask to obtain 5 × 10^3 con-

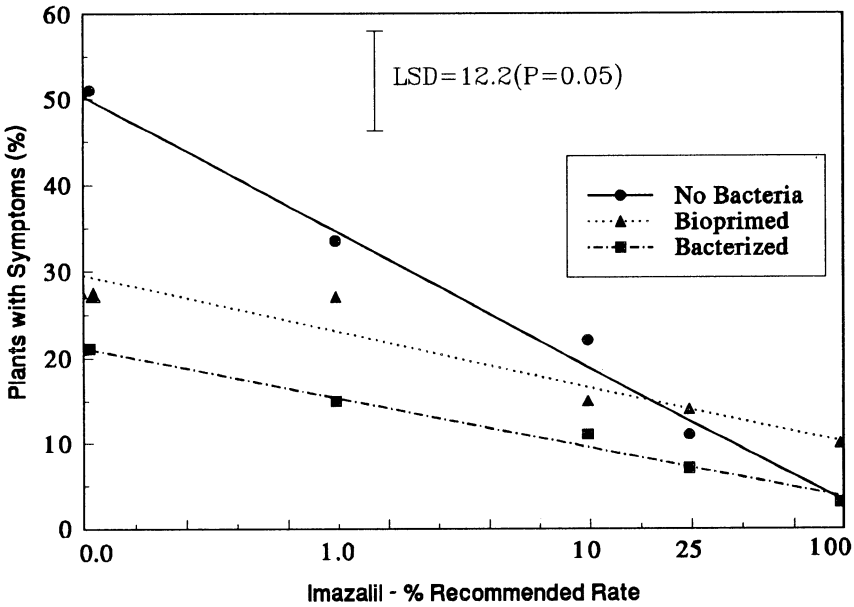

Fig. 2. Effect of low rates of imazalil seed treatment on *sh2* sweet corn in combination with *Pseudomonas* sp. AB842 seed treatment for control of *Penicillium oxalicum* seedling blight in the glasshouse. The recommended (100%) rate of imazalil was 0.1 g ai/kg. Seed was infested with 5,000 conidia/seed of *Penicillium oxalicum.*

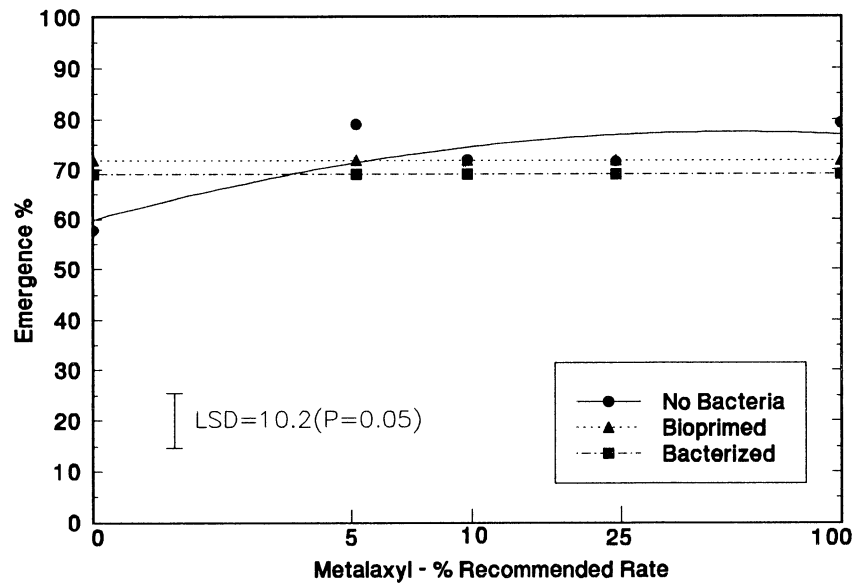

Fig. 3. Percent emergence from *sh2* sweet corn seed as affected by the integrated control of *Pythium ultimum* under field conditions at the Western Ag Research Center, Corvallis, Mont., in 1992 using combinations of metalaxyl and *Pseudomonas aureofaciens* AB254. The recommended (100%) rate of metalaxyl was 0.3 g ai/kg.

idia/seed. Each treatment of 50 seeds, planted in metal flats, was replicated four times in a randomized complete block design. Tests were repeated a minimum of two times.

Field tests of seed infested with *P. oxalicum* were located at the Northwestern Agricultural Research Center near Kalispell, Mont., in 1992, and the Research and Extension Center near Parma, Idaho, in 1992 and 1993. Soil from these sites contained no detectable propagules of *P. ultimum*. Field tests were planted with 50 seeds per treatment in single rows 2.5 m long spaced 0.7 m apart. Treatments were arranged in a randomized complete block design with six replications.

Test evaluations. All experiments were comparisons among three seed treatments (no treatment, bio-primed, and bacterized) in combination with a fungicide (metalaxyl or imazalil) applied at different rates. In glasshouse tests with *P. ultimum*, emergence of healthy plants was determined, while in the tests with *P. oxalicum*, incidence of diseased plants was determined 6 weeks post-seeding. In field tests, stand was determined 30 days post-seeding. Field and glasshouse data were evaluated by regression analysis using the general linear model (GLMODEL) in MSUSTAT (16). Means from treatments with significant (P = 0.05) regression values were separated by least significant difference tests. In glasshouse tests, the magnitude of disease that developed in the repetitions of the experiments was different since the tests were done at different times of the year. However, the relative performance of the treatments was similar. Therefore, data selected from single representative glasshouse experiments are displayed in Figures 1 and 2. Field data from individual years were analyzed separately (Figs. 3, 4). In all cases, the fungicide rates were converted to logarithms (base 10) for analysis.

RESULTS

Control of *Pythium ultimum*. In glasshouse experiments with *P. ultimum*, emergence was positively influenced by increasing metalaxyl concentration (Fig. 1). At rates lower than 10% of the recommended rate, emergence declined significantly. Bio-primed and bacterized seeds showed a slightly positive but nonsignificant ($P < 0.05$) response to metalaxyl rate. The bio-primed treatment (slope = 0.13) was not significantly different from the bacterized treatment (slope = 0.11) across metalaxyl rates ($P < 0.05$).

In the 1992 study planted near Corvallis, Mont., (*P. ultimum* > 500 propagules per gram), emergence of plants with metalaxyl rates of 5% or more did not differ significantly ($P < 0.05$) from emergence of plants with the full rate. Both bio-priming and bacterization were effective against *Pythium* independent of chemical applica-

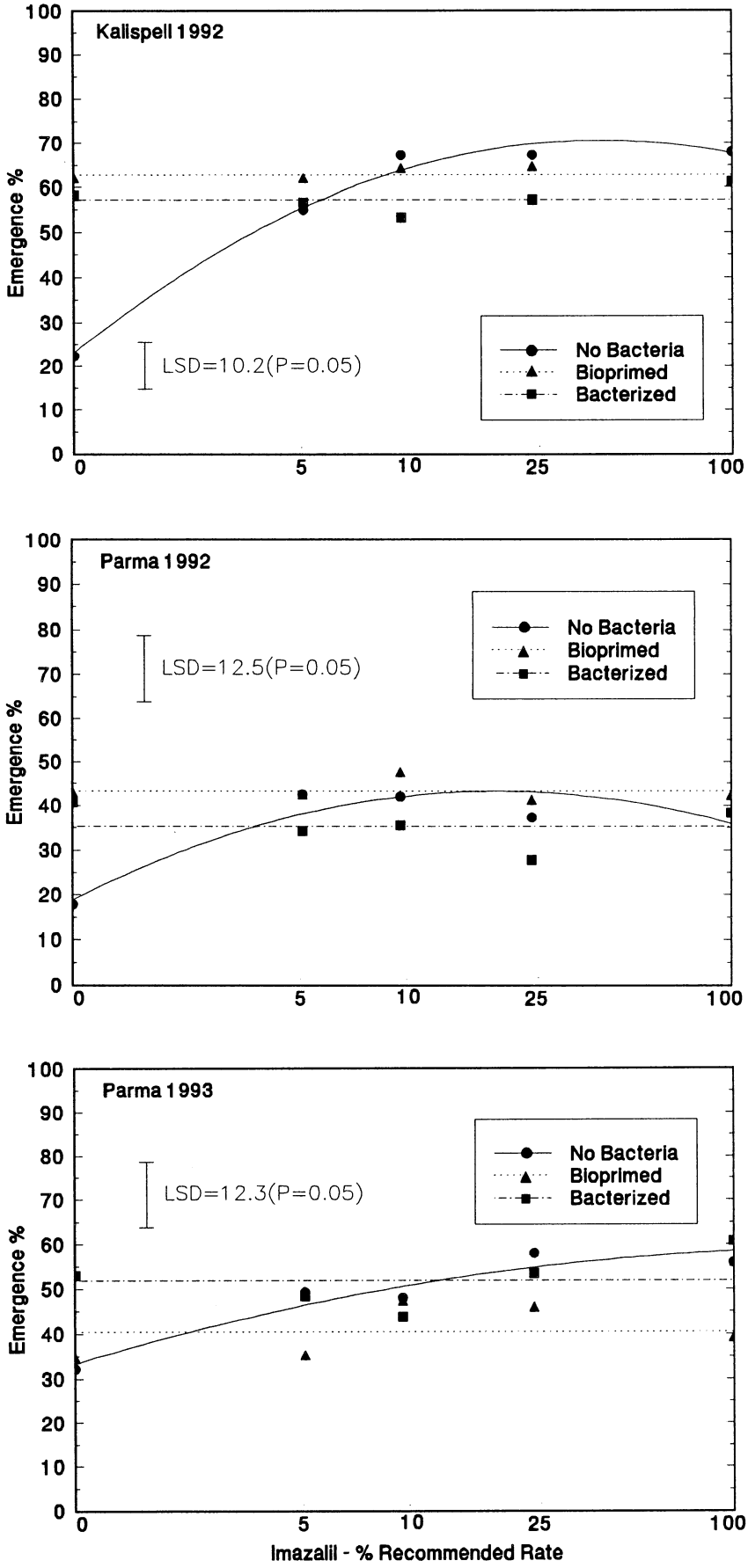

Fig. 4. Percent *sh2* sweet corn stand as affected by the integrated control of *Penicillium oxalicum* seedling blight under field conditions at the Northwestern Ag Research Center, Kalispell, Mont., in 1992 and at the Irrigation Research and Extension Center, Parma, Idaho, in 1992 and 1993 using combinations of imazalil and *Pseudomonas* sp. AB842. The recommended (100%) rate of imazalil was 0.1 g ai/kg.

tion rates. The mean emergence from the bio-primed seed was not significantly different ($P < 0.05$) from that from the bacterized seed. (Fig. 3).

Control of *Penicillium oxalicum*. In glasshouse tests of seed infested with *P. oxalicum,* disease control was positively influenced by fungicide rate (Fig. 2). Incidence of disease in plants from seed treated with imazalil at 25 and 100% of the recommended rate was not significantly different from each other. Disease incidence in plants from bio-primed seed was consistently, but not significantly more, than that in plants from bacterized seed across imazalil rates. Overall, the bio-primed and bacterized treatments were significantly improved by fungicide treatment and emergence of seed treated with AB842 was improved significantly by chemical application rates of 10% or higher.

In field tests with *P. oxalicum*–infested seed planted into *P. ultimum*–free soil, emergence was significantly improved by application of imazalil (Fig. 4). In these tests, imazalil rates of 10% or higher were as effective as the full rate. Unlike the glasshouse data, field data revealed that AB842 alone was as efficient as AB842 in combination with imazalil. Emergence of bio-primed seeds was usually higher than emergence of seeds that were bacterized but, as in the glasshouse tests, differences were not significant.

DISCUSSION

For those interested in reducing or eliminating use of fungicides, the combination of biological control organisms with chemical controls may be of interest and value. This concept, however, raises several practical questions. Could a grower decrease the rate of a fungicide seed treatment if the treatment was combined with a biocontrol bacterium? Could low fungicide doses increase reliability of control provided by a biological seed treatment? The work of Baker and Scher (3) and the work reported here support the first premise, but not the second.

Our results with *Pythium* and *Penicillium* showed that either organism will respond to biocontrol with the appropriate *Pseudomonas* isolate and control is equal to that obtained with a fungicide treatment. Our field tests did not reveal an advantage to combining low fungicide rates with biocontrol agents to increase the reliability of the agent. This may be due to a limited number of trials or perhaps these particular biocontrol agents are efficacious enough that they do not exhibit the reliability problems noticed by others. The control using *P. aureofaciens* AB254 in the field suggests that the antagonism is reliable over locations and years (8).

Combining biocontrol treatments with low levels of fungicide has been valuable in other systems (5,14,22), but exposing an organism to low levels of toxic materials could select for resistance. One way to prevent this would be to combine a biocontrol organism effective against one or more disease organisms with a fungicide, applied at the recommended rate, effective against a different spectrum of organisms. The fungicides that have been developed over the last 10 years tend to be effective against specific groups of organisms (4).

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