The Nature and Application of Biocontrol Microbes: *Bacillus* spp.The Role of *Bacillus*-Based Biological Control Agents in Integrated Pest Management Systems: Plant Diseases

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ABSTRACT

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Bacillus-based biological control agents (BCAs) have great potential in integrated pest management (IPM) systems; however, relatively little work has been published on integration with other IPM management tools. Unfortunately, most research has focused on BCAs as alternatives to synthetic chemical fungicides or bactericides and not as part of an integrated management system. IPM has had many definitions and this review will use the national coalition for IPM definition: "A sustainable approach to managing pests by combining biological, cultural, physical and chemical tools in a way that minimizes economic, health and environ-

mental risks." This review will examine the integrated use of *Bacillus*-based BCAs with disease management tools, including resistant cultivars, fungicides or bactericides, or other BCAs. This integration is important because the consistency and degree of disease control by *Bacillus*-based BCAs is rarely equal to the control afforded by the best fungicides or bactericides. In theory, integration of several tools brings stability to disease management programs. Integration of BCAs with other disease management tools often provides broader crop adaptation and both more efficacious and consistent levels of disease control. This review will also discuss the use of *Bacillus*-based BCAs in fungicide resistance management. Work with *Bacillus thuringiensis* and insect pest management is the exception to the relative paucity of reports but will not be the focus of this review.

Integration with host resistance. Integration of *Bacillus*-based biological control agents (BCAs) with disease resistant hosts has proven to be useful in management of several disease problems, particularly where high levels of disease resistance is not available, or where high yielding, highly resistant cultivars are not available. Published examples include *Fusarium* wilt of chickpea (8), *Pythium torulosum* damping-off of tomato (27), and *Cercospora* leaf spot of sugarbeet (11,20). In each of these studies, disease control involving a *Bacillus*-based BCA was better on the more resistant cultivar. Data in Table 1 show that the application of *Bacillus mycoides* isolate Bm J was more effective in controlling *Cercospora* leaf spot on the more resistant cv. HM 7054 than on the susceptible cv. Beta 2185. The research by Hervas et al. (8) showed that suppression of *Fusarium* wilt caused by race 5 of *Fusarium oxysporum* f. sp. *ciceris* was greater and more consistent on partially resistant cv. PV 61 with *Bacillus subtilis* isolate GB03 seed treatment than on the more susceptible cv. ICCV 4. Of interest is the interaction between inoculum load, host resistance, and influence of *Bacillus subtilis* isolate GB03. The effect of GB03 was significantly different from the pathogen control at the highest inoculum load on the susceptible cultivar and at only the lowest inoculum load on the partially resistant cultivar as measured by incubation period, disease index, and standardized area under the disease progress curve (AUDPC). Smith et al. (28), in their study of tomato inbreds with varying susceptibility to *Pythium torulosum* damping-off and *Bacillus cereus* isolate UW 85, showed that low inoculum doses are best for demonstrating differences in inbred disease resistance but that

high inoculum doses are needed to show disease control by UW 85. Smith et al. (28) proposed a dose response modeling approach to integrate information on pathogen inoculum load, BCA dose, and host resistance. In their study, they showed that inbred tomato lines differed independently regarding pathogen resistance and their ability to support the BCA, UW 85. They suggest that when breeding to improve disease resistance, both pathogen resistance and the ability to support BCAs should be considered.

In a 3-year study, Larson (20) showed that a BCA, *Bacillus mycoides* isolate Bm J, provided control of *Cercospora* leaf spot equal to synthetic fungicides as measured by AUDPC on a sugarbeet hybrid that had a moderate level of resistance, but isolate Bm J provided only 41% of the control afforded by four fungicide sprays on the susceptible cultivar (Table 1). In a multi-year study with Bm J, this BCA provided 38 to 91% of the control of labeled fungicides on susceptible cultivars (1). The effect of Bm J application was to flatten the disease progress curve such that the economic threshold was exceeded too late in crop maturity to significantly affect yield. The moderately resistant cultivar, HM 7054, had a flatter disease progress curve than susceptible cv. Beta 2185 such that the economic threshold was exceeded later in crop maturity. This is characteristic of the rate limiting resistance found in these cultivars (27). By utilizing a moderately resistant cultivar and Bm J, the level of disease control was not statistically different from that of four sprays of fungicide on the susceptible cultivar. In this same study, Larson (20) compared AUDPC and yields of other moderately resistant and susceptible cultivars produced under a standard fungicide spray regime with a spray program utilizing Bm J. The data were similar to that described previously.

Osburn et al. (24) reported a similar set of experiments with *Bacillus cereus* isolate UW 85 seed treatments and soybean cultivars resistant, tolerant, or susceptible to *Phytophthora* root rot. In

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this research, UW 85 increased yields only on the susceptible cultivar where *Phytophthora* root rot was a factor. At another site where *Phytophthora* root rot was not a factor, both metalaxyl (in furrow and seed treatment) and UW 85 increased stands significantly over untreated seeds regardless of *Phytophthora* root rot resistance. A similar nonadditive response was shown by McGovern (23) for *Fusarium* wilt of basil and by Everts and Armentrout (6) for powdery mildew of pumpkin.

Integration with fungicides or bactericides. Since the review paper by Jacobsen and Backman (10) dealing with biological and cultural controls as alternatives and supplements to chemicals in integrated pest management (IPM) systems (10) there have been additional reports of using *Bacillus*-based BCAs with fungicides to achieve more stable control. Perhaps the most common combination of BCAs with fungicides is with seed treatments. Today nearly all cotton planted is treated with Kodiak (*Bacillus subtilis* isolate GB03) and fungicides (4). Brannen and Kenney (4) indicated that combining Kodiak with fungicides provides control of pathogens that are not controlled by available fungicides, and the BCA provides control via colonization of the rhizosphere long after the fungicides have degraded. Brannen and Kenney (4) suggest that success of Kodiak in the cotton market is due to integration with fungicides. Although not addressed by these authors, the fungicide will provide control under environmental conditions unfavorable for the BCA, or before the BCA has sufficiently colonized the rhizosphere. In addition, this *Bacillus* isolate also increases growth and plant vigor (14). In results reported by Multistate Research Project S 302 on cotton for 2001 (2), the combination of Kodiak and the standard fungicide treatment carboxin-PCNB-metalaxyl provided better stands than the standard fungicide treatment in one of five locations and was not significantly different from the standard fungicide in the other four locations, whereas Kodiak alone provided stands lower than the fungicide standard in four of five locations and was not statistically different from the standard fungicide in the other location. In 2002, cottonseed treatment tests of Kodiak plus carboxin-PCNB-metalaxyl provided stands not statistically different from the standard carboxin-PCNB-metalaxyl seed treatment alone (3). Batson et al. (2,3) showed similar data. Other *Bacillus* spp. were examined in these same trials, but none performed better than Kodiak.

Cook et al. (5) reported on the use of *Bacillus* sp. isolate L324-92, used alone and in combination, commonly used seed treatments, including difenoconazole plus metalaxyl, difenoconazole plus mefexam, tebuconazole plus thiram, and tebuconazole plus metalaxyl for control of the wheat root rot complex that includes *Gaeumannomyces graminis* var. *tritici*, *Rhizoctonia solani* AG8, *R. oryzae*, and several *Pythium* spp. Yield increases were significantly higher for the difenoconazole plus mefexam plus L324-92 treatment than for either the fungicide or the BCA alone on winter wheat. This effect was not significant for the other fungicide treatments on winter wheat, and there were no significant differences on spring wheat. There were no significant differences for control of take-all, but at one of four locations there were treatment differences for *Rhizoctonia* root rot on winter wheat. However, the BCA alone was as effective as the fungicides alone and the combination was not statistically different from these treatments, except at one location where the combination of tebuconazole plus thiram plus L324-92 provided a lower disease index than the fungicide alone, but disease control was not statistically different from the BCA alone. At another location, similar effects were seen with difenoconazole plus mefexam.

The potential to use lower rates of fungicides when combined with a *Bacillus*-based biological control has not been well explored. Our lab group has studied the use of *Bacillus pumilis* isolate 341-16-5 as a seed treatment for the control of sugarbeet damping-off and root rot caused by *Aphanomyces cochlioides*, *Pythium ultimum*, and *R. solani* in combination with varying rates

of hymexazol. In 14 location-years of testing, this isolate has increased both stand at harvest and yield when combined with 20, 30, or 45 g of hymexazol per 100,000 seed compared with that of hymexazol treatments alone. In addition, the 20-g rate was as effective as the 45-g rate when combined with the BCA. *Bacillus pumilis* isolate 341-16-5 was not as effective as hymexazol when used alone, and the 20-g rate was not as effective as the 45-g rate when used alone.

Reports on the use of *Bacillus*-based BCAs with fungicides for control of foliar diseases are relatively scarce. In a study of control of avocado black spot caused by *Pseudocercospora purpurea*, Korsten et al. (19) showed that, in eight location-years of research, *Bacillus subtilis* isolate B246 applied with copper oxychloride or with benomyl and copper oxychloride provided more consistent control than either the BCA or the fungicides alone. The integrated program allowed for fewer fungicide sprays, and Korsten et al. (19) suggested that the integration of fungicides acted as a safeguard in those years unfavorable to BCA activity. In other work on sugarbeet, Kiwnick et al. (16) reported that the combination of *Bacillus* sp. isolate MSU 127 and 76 g (active ingredient [ai]) of azoxystrobin per ha had the lowest *Rhizoctonia* crown and root rot index (1.89) and was not statistically different from the 304 g ai/ha rate of azoxystrobin fungicide applied alone (1.99), while the BCA alone had a disease index of 2.63 and the 76 g ai/ha rate of azoxystrobin had a disease index of 2.44. In other work on this topic, Jacobsen et al. (12) showed similar results with another strobilurin fungicide, pyraclostrobin. The use of MSU 127 in combination with these fungicides allows the rate of fungicide to be reduced by 50% without loss of efficacy.

In studies on bacterial spot of pepper, Wright and Miller (30) showed that Gustafson Biologicals LS 213, LS 254, and LS 257 (all preparations containing *Bacillus*, *Brevibacillus*, and *Paenibacillus* spp.) or *Paenibacillus macerans* isolate OSU 142 incorporated into the transplant media (Gustafson LS preparations) or used as a transplant dip (OSU 142) provided better control when used with foliar applications of Kocide 101 or Kocide 101 plus Manex than the bacterial treatments or the Kocide plus Manex treatments alone. Kiwnick and Jacobsen (15) showed that *Bacillus* isolates MSU 129, MSU 131, and MSU 133 combined with

TABLE 1. Integration of varietal resistance to *Cercospora* leaf spot, fungicides, fungicide application number, and the biological control Bm J for management of *Cercospora* leaf spot of sugarbeet data for 2001 and 2002 combined

Treatment	Beta 2185 KWS ^a = 6.3		HM 7054 KWS ^a = 4.3	
	AUDPC ^b	Sugar (kg/ha)	AUDPC ^b	Sugar (kg/ha)
Unsprayed	55.7	8,989	34.7	9,581
4 sprays ^c	19.3	9,940	11.9	10,192
3 sprays	22.8	9,485	11.4	9,796
2 sprays	22.3	9,495	15.7	9,706
Bm J preventive + 14, 28, and 42 days	46.7	10,148	21.2	9,830
Tetraconazole 0.075 liter/ha + Bm J onset + Bm J 14, 28, and 42 days	25.3	10,056	18.2	9,521
FLSD 0.05 ^d	4.15	659	4.15	659

^a Score for *Cercospora* leaf spot severity based on the 1-to-9 Kleinwanzleber Saatzeit (KWS) scale. Cultivars with lower KWS scores are more resistant to *Cercospora* leaf spot.

^b AUDPC = area under the disease progress curve.

^c Mean of spray at disease onset plus 14, 28, and 42 days and Minnesota predictive model since they were the same. First spray at disease onset for 2001 = tetraconazole at 0.15 liter/ha, second spray = benomyl at 92 g/ha, and third and fourth spray = triphenyltin hydroxide at 57 g/ha; and 2002 first spray = tetraconazole at 0.15 liter/ha, second spray = trifloxystrobin at 110 ml/ha, third spray = triphenyltin hydroxide at 57 g/ha, and fourth spray = trifloxystrobin at 110 ml/ha.

^d FLSD = Fisher's least significant difference.

thiabendazole provided better control of *Fusarium* dry rot of potato than thiabendazole or the BCAs alone regardless of whether the *F. sambucinum* isolate used was sensitive or insensitive to thiabendazole.

Combinations of BCAs. Control of plant diseases by BCAs depends on interaction with the host, most commonly by colonization or establishment on the phyllosphere or rhizosphere, or by induction of induced resistance. This is clearly affected by the environment, and combinations of BCAs may allow for greater consistency of control when they are selected to be adapted over a range of environmental conditions. In addition, mixtures may confront the pathogen or pathogens with different modes of action. Unfortunately, most reports on *Bacillus*-based BCAs are for single isolates or in comparison to other BCAs used alone. The following examples illustrate the benefits of combinations of BCAs.

Research by Guetsky et al. (7) showed that control of *Botrytis cinerea* on strawberry leaves was more consistent over a range of environmental conditions when a yeast, *Pichia guilhermondii*, was applied with *Bacillus mycoides* isolate B16 than that of either organism applied alone. Under specific environmental conditions, each of the organisms was superior to the other, but for control over the range of environments (1 to 30°C and 78 to 100% relative humidity) the coefficient of variability was reduced from 9.7 to 75% when these BCAs were applied alone to 0.4 to 9.0% when both BCAs were applied together. Another example of the effect of environment is the work of Raupach and Kloepper (26), who showed that, when *Bacillus subtilis* isolate INA7 was used with *Bacillus subtilis* isolate GBO3 and *Curtobacterium flaccumfaciens* isolate ME1 as seed treatments, greater growth promotion and numerically better angular leaf spot control of cucumber was achieved where methyl bromide fumigation was used compared with that of nonfumigation. This latter mixture of BCAs is the basis for the BioYield product marketed by Gustafson Inc., Plano, TX.

Besides variability in environments, pathologists and others are confronted with control of multiple diseases on the same crop or cropping system. The importance in selecting *Bacillus*-based BCAs adapted to specific environments is shown in the research reported by Leibinger et al. (21), where *Bacillus subtilis* isolates AG704 and HG77 showed good colonization of apples in the field but poor colonization and apple fruit rot control in storage compared with that of two yeast species. Leibinger et al. (21) also demonstrated that compatibility between BCAs is important in that the yeast *Aureobasidium pullans* was reduced by the *Bacillus* BCAs with a resultant decrease in efficacy when these BCAs were mixed. Jetiyanon et al. (13) showed similar benefits for mixtures of *Bacillus* spp. applied as seed treatments. They studied *Bacillus amyloliquefaciens* isolate IN937A applied alone and in combination with *Bacillus pumilis* strains SE34, SE49, T4, and INR7 and demonstrated greater growth promotion and anthracnose disease control in the winter season on long cayenne pepper with the combinations than with IN937A alone. Control of southern blight on tomato, growth promotion on tomato, and control of *Cucumber mosaic virus* and growth promotion on cucumber were not improved by using combinations. Thus, mixtures may not work with all crops or pathosystems.

Combining cultural controls with *Bacillus*-based BCAs. Cultural controls are the basic means of disease management in traditional farming systems (29) and are the resilient skeleton on which all other pest management practices depend in most cropping systems (10). Research papers that address the incorporation of *Bacillus*-based BCAs with crop rotation, tillage, plant nutrition, sanitation, selection of favorable environments for planting, and improved drainage, etc., have not been found for this review, except for work by Ploper et al. (25). They showed that, when mixtures of two *Bacillus cereus* isolates were used with plastic row mulches, control of tomato early blight was equal to the control achieved with weekly applications of chlorothalonil on toma-

atoes grown on bare soils. Clearly, there is research needed that tests the hypothesis that BCAs perform better under conditions that are less favorable to disease development.

Use of *Bacillus*-based BCAs in fungicide resistance management. Integration of BCAs into pesticide resistance management strategies has not been well explored. However, use of different modes of action in sequential fungicide applications is considered a keystone of resistance management programs (9,18). Because *Bacillus*-based BCAs have modes of action different from that of synthetic chemical fungicides, it is logical that they can be used in fungicide resistance management programs. *Bacillus*-based BCAs have modes of action that include antibiosis, parasitism, and induced systemic resistance (1,10). Larson (20) studied the prevalence of benomyl, azoxystrobin, and tetraconazole resistance of *Cercospora beticola* for 2 years in replicated plots that received no treatments, a rotation of different classes of labeled fungicides, *Bacillus mycoides* isolate Bm J, or a program that integrated Bm J with these fungicides. In the first year of the study, use of Bm J alone did not reduce the percentage of conidia insensitive to 1 ppm of benomyl, azoxystrobin, or tetraconazole. However, the Bm J plus fungicide treatments had a statistically smaller percentage of conidia insensitive to 1 ppm of benomyl and tetraconazole than the plots treated with the rotation of fungicides and was equal to the fungicide rotation plots for azoxystrobin insensitivity. In 2002, no reduction of fungicide resistance was noted. Because these plots were only 3 by 10 m, it is likely that spores blown or splashed from adjacent plots masked treatment effects. For mycelial growth tests for insensitivity there were no statistical differences between treatments in either year.

Matheron and Porchas (22) reported on the use of Serenade (*Bacillus subtilis* isolate QST 713) alternated with sulfur (Microthiol 80DF), myclobutanil (Rally 40W), or trifloxystrobin (Flint 50WG) for control of powdery mildew of lettuce. Treatments in which four sprays of sulfur (8.96 kg/ha) or Serenade (2.24 kg/ha) plus sulfur (4.48 to 8.96 kg/ha) were applied were not statistically different, whereas Serenade alone (2.24 to 4.48 kg/ha) provided statistically more mildew damage. Alternate sprays of myclobutanil (70.6 g ai/ha) or trifloxystrobin (69 g ai/ha) with Serenade provided control not statistically different from that achieved with four applications of trifloxystrobin or myclobutanil. Thus, alternate sprays with the BCA Serenade saved 8.96 to 20.16 kg of sulfur per ha, 139 g ai of trifloxystrobin per ha, or 141 g ai/ha and achieved the same results. In addition, by using different modes of action, Matheron and Porchas (22) proposed that the BCA contributed to a fungicide resistance management program.

Concluding comments. This review points out the relative paucity of research involving integration of *Bacillus*-based BCAs with other disease management tools. We strongly encourage biological control researchers to not only compare the level of control achieved with BCAs to that of synthetic chemical standards but to make comparisons that integrate resistant cultivars, cultural controls, reduced rates of pesticides, and other BCAs. It is only with these types of comparisons that the full potential for integrating BCAs into modern production agriculture will be achieved. In addition, the use of BCAs as replacements for fungicides or bactericides with high potential for development of resistance under low disease pressure may provide for less fungicide selection pressure on pathogen populations. It is of interest that most *Bacillus*-based BCA product labels contain language that integration of these products within an overall IPM strategy is desirable. It is critical that the research base be built to make science-based recommendations for integration of BCAs into IPM systems.

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