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Growth response of weed and crop seedlings to deleterious rhizobacteria

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Abstract

Selected bacterial isolates previously demonstrated to be suppressive toward weed species in the laboratory were tested for effectiveness under greenhouse conditions. Rhizobacteria varied in ability to inhibit growth of host or nonhost weed species. Some bacterial isolates caused $\leqslant 75\%$ growth inhibition, while some isolates did not express inhibitory effects under greenhouse conditions. Host specificity of rhizobacteria also varied, with some isolates significantly suppressing growth of host plants as well as nonhost weed species and occasionally crop plants. For example, green foxtail [*Setaria viridis* (L.) Beauv.] growth was suppressed by 57% of rhizobacteria isolated from several weed hosts but morningglory (*Ipomoea hederacea* L.) and barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] were suppressed by only 32 and 37% of the rhizobacterial isolates, respectively. Isolates that only inhibited growth of weed plants without negatively affecting crop plants can be considered candidates for further tests as potential biological control agents. Because potential biological control agents would encounter more complex interactions with indigenous microorganisms and environmental factors when applied in the field, the greenhouse test is an important step in documenting the effectiveness and host specificity of deleterious bacteria.

Keywords: Biological control; Integrated weed management; Host range tests

1. Introduction

Naturally occurring deleterious rhizobacteria (DRB) have been implicated in the growth inhibition and yield depression of a variety of crop plants. Adverse effects of DRB are enhanced under certain management practices including direct drilling into crop residues (Fredrickson et al., 1987), unbalanced crop rotations, and continuous monoculture (Schippers et al., 1987; Turco et al., 1990). Further, it is important to determine the effects of crop management practices on DRB suppressive toward weeds in order to devise more sustainable systems that favor naturally occurring biological control microorganisms. For example, selected management tactics might be easily incorporated into cropping systems for manipulating the field environment to enhance survival, physiological behavior, and performance of natural antagonistic microorganisms of weeds. More research is needed to understand how this strategy for natural weed suppression, also known as "conservation biological control" (Newman et al., 1998), can be applied effectively to integrated weed management systems. Management practices including tillage, crop rotation, residue manipulation, and organic amendments enhance or induce favorable factors in the habitat for sustaining effective populations of natural biological control agents. Recent studies of crop management practices that involved reduced tillage, maintenance of high soil organic matter, and limited inputs of agrichemicals found increased levels of DRB associated with weed seedlings and high activities of specific soil enzymes that apparently contributed to natural weed suppression (Li and Kremer, 2000; Kremer and Li, 2003).

Specific effects of DRB include reduced seed germination, growth inhibition, reduced root elongation, root

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deformation and/or discoloration, as well as increased infection by root-colonizing fungi. Association of DRB with weed seedling roots either through management practices that encourage proliferation of indigenous populations or introduced as biological control agents can contribute to reduced herbicide use and minimal environmental contamination. Certain crop production systems shown to influence occurrence of DRB naturally associated with weed seedlings might be modified further for improved biologically based weed management (Li and Kremer, 2000). However, it is necessary to test selected DRB isolates on host plants to assess adequately the potential for natural growth suppression of weeds in particular agroecosystems.

DRB with potential as biocontrol agents for weeds have been described on downy brome (*Bromus tectorum* L.), green foxtail, and wild oat (*Avena fatua* L.) occurring in spring and winter wheat (Kennedy et al., 1991; Boyetchko, 1997) and on several broadleaf weed seedlings (Kremer et al., 1990). Rhizobacteria capable of suppressing plant growth in a species-specific manner have potential as bioherbicides (Mazzola et al., 1995; Kremer, 2002). Inhibition of plant growth by DRB can be species- or cultivar-specific (Kennedy et al., 2001). Only those phytotoxic isolates that specifically colonize and inhibit growth of weeds but not that of crop plants can be considered in the development of biological control technologies (Boyetchko, 1997; Kremer, 2002).

In previous seedling bioassays, rhizobacteria from rhizospheres of weeds originating from different crop management systems were tested for in vitro phytotoxicity toward their host plants (Li and Kremer, 2000). For both adequate assessment of the potential for natural growth suppression of weeds and for selection of promising DRB as weed biological control agents, tests for effectiveness must be conducted under more natural conditions than those in the laboratory. Based on the phytotoxicity screening carried out in the laboratory on host plants, the most virulent bacterial isolates were tested in the greenhouse on host plants and representative crop plants and weed species for growth suppression of intact plants under nonsterile conditions. This was to verify competitiveness and suppressive activity of DRB in the presence of other soil microorganisms and also determine host range for selecting isolates with potential as biological weed control agents.

2. Materials and methods

2.1. Rhizobacteria and culture conditions

Rhizobacterial isolates that exhibited the greatest growth suppressive activity on host plants in agar bioassays were tested for effectiveness under more rigorous greenhouse conditions (Table 1). The details of isolation of the rhizobacteria from various weed hosts, soils, and ecosystems and their characterization were reported previously (Table 1; Li and Kremer, 2000). Selected rhizobacterial isolates were cultured in half-strength King's B broth (King et al., 1954) on a rotary shaker at 27 °C at 100 rpm for 36 h. The number of cells present in the broth culture was determined by spread-plating appropriate dilutions of serially diluted bacterial suspensions on half-strength King's B agar. Rifampicin (Rif)-resistant mutants of each inoculant DRB were used in plant growth studies to differentiate between inoculated strains and similar bacteria indigenous to the soil used in the growth medium. Rif mutants were generated by growing the selected DRB strains on half-strength King's B agar amended with 100 μg/ml Rif (King's + Rif) and selecting colonies with similar growth rates and morphology as the wild-type strains. The growth-suppressive activity of Rif mutants was verified in in vitro bioassays using host seedlings (Li and Kremer, 2000) in which the wild-type DRB strains served as controls to assure that growth suppressiveness remained identical to the wild-type DRB strains.

2.2. Growth medium

The growth medium for greenhouse studies consisted of one part soil, one part potting mix (Pro-Mix "G," Hummert International, Earth City, MO), and 0.5 part of vermiculite. Each growth medium component was passed through a coarse screen (100-mm mesh) and thoroughly mixed. This provided a standard growth medium for all isolates and plants tested and allowed growth responses of all treatments under uniform conditions. Soil was collected from the upper 10 cm of the profile of a Kaintuck fine sandy loam (coarse-loamy, siliceous, superactive, nonacid, mesic Typic Udifluvent) located in a flood plain (0–2% slope) of a stream in Osage County, MO (91°45.0′W, 38°34.0′N) under unmanaged meadow vegetation and had not received pesticides for >20 years. Properties of the growth medium were: pH 6.9; organic matter, 6.0%; P, 40 mg/kg; Ca, 1450 mg/kg; Mg, 360 mg/kg; K, 130 mg/kg.

2.3. Plant species and inoculation

For each rhizobacterial isolate, the host weed species, two crop species [soybean (*Glycine max* L. Merr. "Williams 82") and wheat (*Triticum aestivum* L. "Cardinal")], and at least one representative monocotyledoneous and dicotyledoneous nonhost weed species were tested in the greenhouse to determine the growth response of these plants to bacterial inoculation. Although rhizobacteria were isolated from giant foxtail (*Setaria faberi* Herrm.) seedlings collected in the field, green foxtail was used as the test species in greenhouse assays because germination was more consistent than giant foxtail and effects of DRB were similar on both species (Li and Kremer, 2000). A total of 43 isolates were screened; however, the number of isolates screened in each host weed and crop trial varied based on the number of growth-suppressive isolates identified from each host. For example, 13 growth-suppressive isolates from giant foxtail were screened on green foxtail while only seven isolates from *Amaranthus* spp. were screened on redroot pigweed (*Amaranthus retroflexus* L.). Intact seeds of uniform

Table 1
Characterization of deleterious rhizobacterial strains collected from various ecosystems in central Missouri, USA and screened for host specificity in the greenhouse

Strain code	Host plant	Ecosystem origin[a]	Identification[b]
I2-12	*Amaranthus retroflexus*	Corn–soybean; MT	*Agrobacterium radiobacter*
K1-15	*Amaranthus retroflexus*	Corn–soybean–wheat; NT	*Pseudomonas aureofaciens*
K1-30	*Amaranthus retroflexus*	Corn–soybean–wheat; NT	*Vibrio* sp.
TPH10	*Amaranthus retroflexus*	Native tallgrass prairie	*Aeromonas hydrophila*
TPH2	*Amaranthus retroflexus*	Native tallgrass prairie	*Chryseomonas luteola*
TPH4	*Amaranthus retroflexus*	Native tallgrass prairie	*Burkholderia cepacia*
TPR15	*Amaranthus retroflexus*	Native tallgrass prairie	*Burkholderia cepacia*
TPR16	*Amaranthus retroflexus*	Native tallgrass prairie	*Pseudomonas aureofaciens*
TPR40	*Amaranthus retroflexus*	Native tallgrass prairie	*Pseudomonas fluorescens*
A1-1	*Convolvulus arvensis*	Continuous corn; CT	*Pseudomonas aureofaciens*
A1-10	*Convolvulus arvensis*	Continuous corn; CT	*Flavobacterium indologenes*
A1-15	*Convolvulus arvensis*	Continuous corn; CT	*Pseudomonas fluorescens*
D2-10	*Convolvulus arvensis*	Corn–wheat–red clover; CT	*Aeromonas hydrophila*
D2-11	*Convolvulus arvensis*	Corn–wheat–red clover; CT	*Aeromonas hydrophila*
D2-26	*Convolvulus arvensis*	Corn–wheat–red clover; CT	*Chryseomonas luteola*
CMH2	*Ipomoea* sp.	Cool season pasture	*Aeromonas hydrophila*
CMH3	*Ipomoea* sp.	Cool season pasture	*Aeromonas hydrophila*
CMR2	*Ipomoea* sp.	Cool season pasture	*Aeromonas hydrophila*
M1-10	*Ipomoea* sp.	Organic vegetable farm; NT	*Pseudomonas fluorescens*
M2-3	*Ipomoea* sp.	Organic vegetable farm; NT	*Chryseomonas luteola*
TMH16	*Ipomoea* sp.	Native tallgrass prairie	*Pseudomonas aureofaciens*
TMR12	*Ipomoea* sp.	Native tallgrass prairie	*Pseudomonas fluorescens*
TMR13	*Ipomoea* sp.	Native tallgrass prairie	*Aeromonas hydrophila*
B1-7	*Setaria faberi*	Continuous corn; CT	*Pseudomonas putida*
CFH15a	*Setaria faberi*	Cool season pasture	*Chryseomonas luteola*
CFH33	*Setaria faberi*	Cool season pasture	*Aeromonas hydrophila*
G1-1	*Setaria faberi*	Corn–soybean; MT	*Pseudomonas fluorescens*
G2-10	*Setaria faberi*	Corn–soybean; MT	*Agrobacterium radiobacter*
G2-11	*Setaria faberi*	Corn–soybean; MT	*Pseudomonas fluorescens*
G1-16	*Setaria faberi*	Corn–soybean; MT	*Chromobacterium violaceum*
J1-44	*Setaria faberi*	Corn–soybean–wheat; NT	*Pseudomonas fluorescens*
J1-45	*Setaria faberi*	Corn–soybean–wheat; NT	*Pseudomonas fluorescens*
J2-4	*Setaria faberi*	Corn–soybean–wheat; NT	*Aeromonas caviae*
L1-12	*Setaria faberi*	Organic vegetable farm; NT	*Agrobacterium radiobacter*
L1-41	*Setaria faberi*	Organic vegetable farm; NT	*Pseudomonas aureofaciens*
L2-12	*Setaria faberi*	Organic vegetable farm; NT	*Pseudomonas fluorescens*
L2-19	*Setaria faberi*	Organic vegetable farm; NT	*Pseudomonas fluorescens*
TFR1	*Setaria faberi*	Native tallgrass prairie	*Xanthomonas maltophilia*
TFR4	*Setaria faberi*	Native tallgrass prairie	*Aeromonas hydrophila*
CCH27	*Xanthium strumarium*	Cool season pasture	*Agrobacterium radiobacter*
TCH9	*Xanthium strumarium*	Native tallgrass prairie	*Aeromonas hydrophila*
TCR34	*Xanthium strumarium*	Native tallgrass prairie	*Aeromonas hydrophila*
TCR44	*Xanthium strumarium*	Native tallgrass prairie	Not identified

[a] Tillage: CT, conventional tillage (moldboard plowing followed by disking and harrowing); MT, minimum tillage (fall or spring chisel plowing followed by field cultivator for seedbed preparation); NT, no tillage.
[b] Details of bacterial identification procedures are provided in Li and Kremer (2000).

size (not surface-sterilized) of each species were planted in 250-cm^3 pots and placed in flats holding 25 pots (NuPots, Hummert International, Earth City, MO). Seeds of each plant host were placed on the growth medium in five replicate pots each of which was inoculated with 5-ml of the selected bacterial suspension at the time of planting. Control plants received the same volume of sterile King's B nutrient broth. The seedlings were grown at 28 °C during a 16-h light period and at 21 °C during an 8-h dark period. Light was supplemented by incandescent lamps at a photon flux density of 230–250 μmol/m^2/s. Emergence rates were recorded and plants were harvested 14 days after emergence. During the growth period, observations of abnormal development of treated plants were recorded. At harvest, growth medium components were gently removed from the roots to reduce the loss of root biomass. After washing with water and blotting with paper towels, root injury was evaluated relative to the control, according to a visual rating scale (0 = best, healthy, and very extensive tap and lateral root development; 4 = necrotic, inhibited tap, and lateral root development). Roots were severed from shoots, and fresh shoot and root weights were determined. Dry shoot and root weights of each treatment were determined by oven-drying at 70 °C for 3 days. Segments of roots were randomly collected and placed on King's + Rif to verify that the inoculant DRB strain was present in the soil and

colonized the roots of inoculated plants. Plants inoculated with the wild-type DRB strains were included as comparative checks with the antibiotic-resistant mutant strains. Root segments of these plants were placed on King's agar to assure that the colonizing rhizobacteria were similar to those on plants inoculated with the mutant strains based on morphological characteristics.

2.4. Statistical analysis

The greenhouse test was arranged as a completely randomized block design. Fresh and dry weights of root and shoot on a single plant basis were subjected to analysis of variance (ANOVA). Treatment means were compared by Tukey's Studentized range test at the 5% level of probability to determine if bacterial inoculation had a significant effect on the growth of either weed species and/or the crop species tested. The greenhouse tests were conducted three times.

3. Results

Roots of inoculated seedlings plated on King's + Rif were consistently colonized by the inoculant DRB, which suggested that observed plant growth effects were caused by the applied DRB strains. Roots of plants inoculated with wild-type strains were similarly colonized based on morphological features of their antibiotic mutant types. This strategy strengthened confidence that the majority of Rif-resistant colonies developing on plates originated from the applied inocula rather than from the small segment of the soil bacterial community that may exhibit low-level Rif resistance. About 45% of the isolates significantly inhibited green foxtail growth when measured as shoot dry weight (Table 2). Shorter tap roots, reduced lateral root and root hair development and root biomass, discoloration of roots, lesion development, and the fragility of the root system were the most common symptoms of root injuries caused by bacterial inocula. Shoot growth inhibition was characterized by reduced shoot biomass and heights, and smaller leaves. Isolate *Aeromonas hydrophila* strain CFH33 from the giant foxtail rhizosphere significantly suppressed root or shoot growth of green foxtail. Fewer isolates suppressed root growth; however, it is noteworthy that the phytotoxic activity of *Pseudomonas fluorescens* strain G2-11 was extremely strong resulting in more than 75% inhibition of both root and shoot growth of green foxtail. None of the three isolates from foxtail rhizospheres, *Chryseomonas luteola* strain CFH15a and *A. hydrophila* strains CFH33 and TFR4, significantly inhibited the growth of barnyardgrass (Table 2). Interestingly, only two DRBs originating from giant foxtail, *P. fluorescens* strains G2-11 and L2-19, suppressed the growth of the nonhost monocotyledonous weed, barnyardgrass.

The shoot biomass of Morningglory (*Ipomoea* sp.) and field bindweed (*Convolvulus arvensis* L.) was reduced by four DRB strains from giant foxtail (Table 2), indicating that although originally from a monocotyledonous weed, these isolates were aggressive rhizosphere colonizers able to adapt to and thrive in rhizosphere microenvironments of dicotyledonous plants. For example, giant foxtail isolates *P. fluorescens* strains L2-19 and G2-11 caused more than 75 and 64% reduction in root biomass, respectively.

Pseudomonas fluorescens strain M1-10, *A. hydrophila* strains CMR2 and TMR13, and *P. aureofaciens* strain TMH16 all isolated from morningglory rhizospheres, significantly reduced shoot growth of morningglory (Table 3). No significant differences in root growth were detected

Table 2
Growth response of weed hosts to inoculation of rhizobacteria isolated from giant foxtail (*Setaria faberi*) collected in various cropping systems

DRB strain	Growth reduction (%)[a]							
	Green foxtail (*Setaria viridis*)		Barnyardgrass (*Echinochloa crus-galli*)		Field bindweed (*Convolvulus arvensis*)		Ivyleaf morningglory (*Ipomoea hederacea*)	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
G1-1	61.4*[b]	33.6*	20.0	0.0	(14.3)[c]	47.0*	53.1*	26.2*
G1-16	66.0*	56.4*	ND[d]	ND	7.5	25.0*	3.8	27.8*
G2-11	77.2*	79.1*	62.0*	70.2*	27.6*	59.6*	66.2*	31.2*
J1-45	0.0	36.4	(55.4)	(36.0)	ND	ND	(2.6)	(9.1)
J2-4	(13.6)[d]	18.2	10.0	(40.0)	ND	ND	21.5	19.2
L1-12	25.0	24.5	20.0	30.4	ND	ND	(24.5)	3.2
L1-41	18.2	46.0*	(7.0)	7.2	ND	ND	(13.5)	10.5
L2-6	31.8	36.4*	16.8	5.0	ND	ND	(28.5)	10.1
L2-19	31.8	45.4*	67.3*	73.5*	(6.3)	64.3*	78.9*	22.7*
CFH15a	10.3	3.8	(32.0)	0.0	ND	ND	(12.3)	18.0
CFH33	37.8*	7.6	(6.0)	(14.0)	ND	ND	(51.2)	1.0
TFR1	17.2	2.5	(17.0)	10.9	ND	ND	(7.6)	12.2
TFR4	(47.6)	(25.7)	ND	ND	(4.0)	4.6	ND	ND

[a] % Growth reduction = [(Control root or shoot length − DRB strain root or shoot length)/Control root or shoot length] × 100; % Growth increase = [(DRB strain root or shoot length − Control root or shoot length)/Control root length] × 100.
[b] Values followed by an asterisk indicate significant growth reduction from noninoculated control seedlings according to Tukey's range test ($P = 0.05$).
[c] Values in parentheses indicate growth promotion of host plant compared to noninoculated control seedlings.
[d] Treatment effect was not determined in this trial.

Table 3
Growth response of weed hosts to inoculation of rhizobacteria isolated from morningglory (*Ipomoea* spp.) and field bindweed (*Convolvulus arvensis*) seedlings collected in various cropping systems

DRB strain	Original weed host	Growth reduction (%)[a]							
		Green foxtail (*Setaria viridis*)		Barnyardgrass (*Echinochloa crus-galli*)		Field bindweed (*Convolvulus arvensis*)		Ivyleaf morningglory (*Ipomoea hederacea*)	
		Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
M1-10	*Ipomoea* sp.	22.7	50.0*[b]	ND[c]	ND	1.5	(38.8)	(18.5)	24.4*
CMH2	*Ipomoea* sp.	13.8	(15.3)	ND	ND	13.7	14.0	(20.0)	1.0
CMH3	*Ipomoea* sp.	34.5*	34.6*	ND	ND	15.6	13.5	11.2	14.4
CMR2	*Ipomoea* sp.	24.1	1.2	ND	ND	(15.6)	2.7	3.6	21.6
TMH16	*Ipomoea* sp.	34.5*	34.6*	ND	ND	24.5*	28.3*	20.5	27.0
TMR12	*Ipomoea* sp.	(47.6)[d]	(25.7)	ND	ND	ND	ND	(1.3)	8.1
TMR13	*Ipomoea* sp.	41.4*	21.0*	ND	ND	11.8	28.8*	(17.4)	25.2
A1-10	*Convolvulus arvensis*	29.5	19.0	ND	ND	(20.0)	40.0*	42.5*	17.0
A1-15	*Convolvulus arvensis*	56.8*	37.2*	ND	ND	(5.0)	(20.0)	(38.5)	12.6
D2-10	*Convolvulus arvensis*	25.0	47.2*	ND	ND	13.8	37.2*	27.6*	15.2
D2-11	*Convolvulus arvensis*	70.4*	50.9*	40.0*	38.8*	(4.5)	30.7*	3.0	5.2
D2-26	*Convolvulus arvensis*	34.1*	47.2*	45.5*	33.8*	19.5*	55.0*	43.4*	21.5*

[a] % Growth reduction = [(Control root or shoot length − DRB strain root or shoot length)/Control root or shoot length] × 100; % Growth increase = [(DRB strain root or shoot length − Control root or shoot length)/Control root length] × 100.
[b] Values followed by an asterisk indicate significant growth reduction from noninoculated control seedlings according to Tukey's range test ($P = 0.05$).
[c] Treatment effect was not determined in this trial.
[d] Values in parentheses indicate growth promotion of host plant compared to noninoculated control seedlings.

among different treatments, which indicated low inhibitory activity by the isolates or high tolerance of morningglory to inhibitory substances produced by the isolates colonizing the roots. *Aeromonas hydrophila* strain TMR13 and *P. aureofaciens* strain TMH16 suppressed field bindweed shoot biomass (Table 3), but not root biomass, and strains CMH3 and TMH16 from the morningglory rhizosphere significantly suppressed root or shoot growth of green foxtail.

Rhizobacteria from redroot pigweed (*A. retroflexus* L.), *P. aureofaciens* strain K1-15, and *Agrobacterium radiobacter* strain I2-12 reduced both root and shoot growth of redroot pigweed and green foxtail (Table 4). *Chryseomonas luteola* strain TPH2, *P. aureofaciens* strain TPR16, and *Burkholderia cepacia* strains TPR15 and TPH4 from the redroot pigweed rhizosphere reduced shoot growth of redroot pigweed (Table 4). With the exception of *B. cepacia* TPH2, these strains also suppressed shoot and/or root growth of green foxtail. Differences in root biomass caused by bacterial inoculation were not significant. Due to the inconsistent germination of common cocklebur (*Xanthium strumarium* L.), only two treatments provided sufficient replication to be analyzed statistically (Table 4). DRB strains from common

Table 4
Growth response of weed hosts to inoculation of rhizobacteria isolated from *Amaranthus* spp. and common cocklebur (*Xanthium strumarium*) seedlings collected in various cropping systems

DRB strain	Original weed host	Growth reduction (%)[a]					
		Green foxtail (*Setaria viridis*)		Redroot pigweed (*Amaranthus retroflexus*)		Common cocklebur (*Xanthium strumarium*)	
		Root	Shoot	Root	Shoot	Root	Shoot
I2-12	*Amaranthus* sp.	29.5*[b]	30.9*	74.2*	30.5*	ND[c]	ND
K1-15	*Amaranthus* sp.	54.5*	66.4*	61.3*	58.9*	ND	ND
TPH2	*Amaranthus* sp.	9.5	6.8	(72.4)[d]	33.3*	ND	ND
TPH4	*Amaranthus* sp.	23.8*	33.2*	17.1	25.7*	ND	ND
TPR15	*Amaranthus* sp.	23.5*	41.6*	(80.9)	40.2*	ND	ND
TPR16	*Amaranthus* sp.	23.0*	17.4	(1.0)	26.1*	ND	ND
TPR40	*Amaranthus* sp.	7.1	6.8	(47.6)	(17.6)	ND	ND
CCH27	*Xanthium strumarium*	14.2	25.2*	ND	ND	49.5*	40.0*
TCH9	*Xanthium strumarium*	14.2	15.2	ND	ND	ND	ND
TCR34	*Xanthium strumarium*	(40.4)	(18.4)	ND	ND	39.0*	33.3*
TCR44	*Xanthium strumarium*	(30.9)	(20.5)	ND	ND	ND	ND

[a] % Growth reduction = [(Control root or shoot length − DRB strain root or shoot length)/Control root or shoot length] × 100; % Growth increase = [(DRB strain root or shoot length − Control root or shoot length)/Control root length] × 100.
[b] Values followed by an asterisk indicate significant growth reduction from noninoculated control seedlings according to Tukey's range test ($P = 0.05$).
[c] Treatment effect was not determined in this trial.
[d] Values in parentheses indicate growth promotion of host plant compared to noninoculated control seedlings.

cocklebur were host-specific, both significantly suppressing root and shoot growth of common cocklebur seedlings. *Agrobacterium radiobacter* strain CCH27 and *A. hydrophila* strain TCR34 were host-specific, both significantly suppressing shoot and root growth of common cocklebur seedlings (Table 4).

Of 27 DRB strains tested, five had potential negative effects on root growth of soybean and wheat seedlings. No DRB strain reduced shoot growth of the crop seedlings. *Aeromonas hydrophila* strain D2-10 reduced root growth of both crop seedlings; *P. fluorescens* strain G1-1 and *A. radiobacter* strain I2-12 suppressed root growth of wheat; and *P. aureofaciens* strain TMH16 and *A. hydrophila* strain TMH13 reduced root growth of soybean (Table 5). All strains reducing growth of crop seedlings also suppressed growth of at least one weed species. Any consideration of developing these isolates as bioherbicides would be restricted to use in crops other than soybean or wheat. Because the activity of introduced microorganisms may be confounded by soil management effects on the microbial ecology of the soil (Hartel et al., 1994), subsequent screening in field trials is necessary so that efficacy of such DRB strains is predictable.

4. Discussion

In a preliminary greenhouse trial in which only fresh shoot biomass was measured, effects of DRB on plant growth was inconsistent; however, root ratings showed that root growth was suppressed due to bacterial inoculation (results not shown). We concluded that growth-inhibitory effects were not consistently reflected as reduced shoot biomass despite damage to roots by introduced rhizobacteria. Therefore, root biomass was considered important in assessing the phytotoxicity of bacterial isolates. This agrees with Horwath et al. (1998), who suggested both root and shoot components need to be determined to adequately measure the performance of DRB.

Various deleterious effects of rhizobacteria on weed seedling growth were observed under greenhouse conditions. The deleterious effects of rhizobacteria on shoot and root morphogenesis may be related to the production of phytotoxins (Nehl et al., 1997). Several of the strains produced toxic metabolites including hydrogen cyanide and excessive concentrations of indole compounds detected via in vitro bioassays (Kremer and Souissi, 2001; Kim and Kremer, 2005). Many strains that were phytotoxic based on agar bioassays failed to severely inhibit growth of weed

Table 5
Growth response of soybean and wheat to inoculation of rhizobacteria isolated from weed seedlings collected in various cropping systems

DRB strain	Original weed host	Growth reduction (%)[a]			
		Soybean (*Glycine max*)		Wheat (*Triticum aestivum*)	
		Root	Shoot	Root	Shoot
G1-1	*Setaria faberi*	(48.6)[b]	(5.0)	30.8*[c]	1.6
G2-11	*Setaria faberi*	(6.5)	(1.2)	(3.5)	(16.0)
L2-19	*Setaria faberi*	23.9	1.6	20.0	10.6
CFH15a	*Setaria faberi*	3.8	6.8	18.6	(15.6)
CFH33	*Setaria faberi*	(27.8)	3.4	18.4	(14.0)
TFR1	*Setaria faberi*	(60.0)	13.6	(60.0)	(14.6)
TFR4	*Setaria faberi*	(45.9)	(1.6)	20.0	3.7
A1-10	*Convolvulus arvensis*	1.0	14.1	(4.0)	(29.0)
D2-10	*Convolvulus arvensis*	30.1*	12.5	32.3*	9.8
D2-26	*Convolvulus arvensis*	11.4	14.0	24.6	5.6
CMH2	*Ipomoea* sp.	25.3*	(8.5)	(53.5)	(14.0)
CMH3	*Ipomoea* sp.	19.6	(10.9)	(25.1)	(2.6)
CMR2	*Ipomoea* sp.	20.6	(6.2)	(28.4)	(6.6)
TMH16	*Ipomoea* sp.	29.9*	(2.7)	(33.3)	9.2
TMR12	*Ipomoea* sp.	(28.6)	4.5	(28.6)	(13.0)
TMR13	*Ipomoea* sp.	28.5*	(16.5)	(18.0)	3.8
I2-12	*Amaranthus* sp.	15.0	8.7	31.2*	11.0
K1-15	*Amaranthus* sp.	(26.6)	(3.0)	21.0	(5.4)
TPH2	*Amaranthus* sp.	(30.8)	10.6	(30.8)	(11.6)
TPH4	*Amaranthus* sp.	(6.0)	12.1	(10.1)	0.9
TPR15	*Amaranthus* sp.	(4.4)	22.7	(4.4)	16.1
TPR16	*Amaranthus* sp.	(9.6)	25.8*	(9.6)	3.0
TPR40	*Amaranthus* sp.	(50.6)	9.1	(50.0)	(28.6)
CCH27	*Xanthium strumarium*	(50.0)	16.6	(56.0)	(4.0)
TCH9	*Xanthium strumarium*	(14.5)	10.6	(14.5)	0.0
TCR34	*Xanthium strumarium*	(18.5)	10.0	(18.9)	6.8
TCR44	*Xanthium strumarium*	(25.6)	(18.2)	(34.4)	0.0

[a] % Growth reduction = [(Control root or shoot length − DRB strain root or shoot length)/Control root or shoot length] × 100; % Growth increase = [(DRB strain root or shoot length − Control root or shoot length)/Control root length] × 100.

[b] Values in parentheses indicate growth promotion of host plant compared to noninoculated control seedlings.

[c] Values followed by an asterisk indicate significant growth reduction from noninoculated control seedlings according to Tukey's range test ($P = 0.05$).

and/or crop seedlings in soil. Seedling growth suppression, readily detected in agar bioassays, may not be expressed as greatly, if at all, when DRBs are confronted with competition from other root colonizers and exposed to complex soil biological and environmental factors (Kennedy et al., 2001; Rovira, 1965). Survival of DRB in nonsterile conditions may determine the potential success of the microorganisms to suppress weed growth in the field. Erratic performance of many DRB strains in soil can be attributed to several factors including the loss of ecological competence, inability of the DRB to produce growth-inhibitory compounds at the appropriate time and place, and inconsistent root colonization (O'Sullivan and O'Gara, 1992; Weller and Thomashow, 1993). Bacterial metabolites and culture medium breakdown products transferred during inoculation may affect early seedling growth. However, these effects are likely transient because sustained inhibition is associated with activity of bacteria established on the root surface within 48–72 h of inoculation of the seedling (Li et al., 2002; Kim and Kremer, 2005).

Plant species, as well as genotypes within a plant species, vary in response to inoculation with rhizobacteria (Åström and Gerhardson, 1988; Cherrington and Elliott, 1987). DRB isolates tested in this study differentially affected growth of plant species, each having a specific host spectrum, which agrees with previous host-range determinations for other DRB (Åström and Gerhardson, 1988, 1989; Elliott and Lynch, 1984; Kennedy et al., 2001). Different plants may also influence introduced DRB, either directly by affecting root colonization, growth, and physiology, or indirectly by affecting the indigenous rhizosphere microflora that interact with the introduced isolates (Åström and Gerhardson, 1989). Differential response of plants was demonstrated in our study in which green foxtail was suppressed by nearly twice as many isolates (57%) as were morningglory (32%) or barnyardgrass (30%). Our study was not designed to follow growth-suppressive effects beyond 14 days. Growth suppression by DRBs later in the growing season may diminish and allow the weed to recover vigorous growth. However, limited field testing has shown that within this period, crop plant growth advances to out-compete weed interference (Kremer, 2000).

A majority of the rhizobacteria screened in the bioassays enhanced root and/or shoot growth of the representative crop species, soybean, and wheat. An earlier study showed that *P. fluorescens* D7, a DRB that aggressively reduces growth of downy brome seedlings in soil, stimulated growth of oilseed rape (*Brassica napus* L.) (Kennedy et al., 2001). Boyetchko (1997) reported that rhizobacteria reduced growth of green foxtail with simultaneous growth increase in spring wheat and conferred an advantage to the crop by allowing it to out-compete this weed. Similarly, we demonstrated that *P. fluorescens* strain G2-11, which suppressed growth of several weed species, enhanced growth of soybean and wheat. Based on these results, strain G2-11 has potential to serve a dual function as a weed biological control agent and a crop growth promoter. Subsequent studies that examined performance of strain G2-11 in different formulations confirmed its ability to suppress weed seedling growth and enhance wheat growth in soil environments (Zdor et al., 2005). Because rhizobacteria with dual abilities to suppress weed growth and enhance crop growth were identified, other programs that screen bacteria for single traits of plant growth promotion or pathogen suppression might include similar screening for growth suppression of specific weeds associated with their cropping systems.

The present study verifies potential activity in the soil environment of native DRBs from various cropping systems and also aids in initially determining the adaptability of a selected isolate for development as a bioherbicide for weeds (Kremer, 2002). Kennedy et al. (1991) identified several rhizosphere-inhabiting bacteria that selectively inhibited root elongation or seed germination of downy brome with no apparent deleterious effects on growth and development of winter wheat. Their results were consistent with our study in that some DRBs selectively suppress weed growth without significant deleterious effects on crop plants. The host specificity of DRBs shown in these studies indicates that biological control agents can be developed by surveying specific crop or soil management systems to select rhizobacteria that are suppressive toward weeds but not crop plants. Further, these management systems provide insight toward development of certain cultural practices that might promote the development of weed-suppressive microorganisms in the field rather than reliance on introduction of biological control agents.

Despite the number of trials showing successful control of some weeds in the greenhouse (Horwath et al., 1998), commercial use of deleterious rhizobacteria as biological control agents, however, must await further improvements in the efficacy of strains and a more profound understanding of environmental factors influencing growth, survival, and colonization of rhizobacteria. For rhizobacteria to have a significant role in sustainable agriculture, especially for weed management, consistent and effective efficacy must be achieved under field conditions (Skipper et al., 1996; Boyetchko, 1997). The effectiveness of weed-suppressive rhizobacteria in a biological control approach might best be demonstrated as a component of weed management where it can be integrated with other weed control methods (Boyetchko, 1996). Therefore, further field studies are necessary to determine the adaptability and persistence of applied rhizobacteria under a wide range of soils, production and management regimes, crops, weed species, and environmental conditions.

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