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Rhizobacteria associated with weed seedlings in different cropping systems

Jianmei Li
Department of Soil and Atmospheric Sciences, University of Missouri, Columbia, MO 65211

Robert J. Kremer
Corresponding author. U.S. Department of Agriculture, Agricultural Research Service, University of Missouri, Columbia, MO 65211; kremerr@missouri.edu

Rhizobacteria isolated from the rhizospheres of dominant weed species in six representative cropping systems and one native prairie ecosystem in mid-Missouri were screened for phytotoxicity on *Lactuca sativa* seedlings and their host plants in the laboratory. The proportions of deleterious rhizobacteria (DRB) were compared among different cropping systems to determine possible effects of crop management practices on the occurrence of DRB. Phytotoxicity screening on *L. sativa* seedlings revealed that an integrated crop management system with a *Zea mays–Glycine max–Triticum aestivum* cover crop rotation under no-tillage had the highest proportion of DRB at 25.3%, followed by an organic farming system with continuous *Fragaria virginiana* (strawberry) and organic amendments under minimum tillage at 22.9%. A continuous cool-season grass–legume meadow with no agrochemical inputs had the lowest proportion of DRB at 13%. Crop management practices that maintained high soil organic matter had higher proportions of DRB compared to cropping systems with lower organic matter. Phytotoxicity screening on host plants greatly reduced the proportion of rhizobacteria characterized as DRB, likely because of the high sensitivity of *L. sativa* seedlings to phytotoxins. Although screening on *L. sativa* is an effective method to detect phytotoxic rhizobacteria, our research indicates that it is essential to test selected cultures on their host weed species for accurate assessment of their occurrence in the field. Using this approach, we found that crop management practices influence the occurrence of DRB naturally associated with weed seedlings. Results suggest that crop production systems can be developed to favor soil microorganisms such as DRB that affect weed growth and thereby become important considerations in overall weed management.

Nomenclature: *Amaranthus retroflexus* L. AMARE, redroot pigweed; *Ipomoea hederacea* (L.) Jacq. IPOHE, ivyleaf morninglory; *Setaria faberi* Herrm. SETFA, giant foxtail; *Zea mays* L., corn; *Lactuca sativa* L. 'Blackseeded simpson', lettuce; *Glycine max* (L.) Merr., soybean; *Fragaria virginiana* L., strawberry; *Triticum aestivum* L., wheat.

Key words: Fertility management, integrated weed management, soil microorganisms, soil organic matter, crop rotation, tillage, *L. sativa* bioassay.

Various investigations of microorganisms associated with plant roots have provided evidence of a general occurrence of nonparasitic bacteria, termed deleterious rhizobacteria (DRB), with a potential to inhibit or reduce plant growth (Campbell et al. 1986; Cherrington and Elliott 1987). These bacteria may induce detrimental effects in plants through the production of substances that affect root cell permeability or in other ways interfere with root physiological processes (Schippers et al. 1987). Inoculation of seeds or roots with DRB results in growth inhibition, foliar abnormalities, and root deformation or necroses (Campbell et al. 1986).

Cropping systems affect the size and composition of the soil microbial communities. Management practices influence microbial activities in long-term agricultural fields (Bolton et al. 1985; Doran and Linn 1994; Ramsay et al. 1986; Rovira 1994). The ecology of root–microbe interactions in soils under minimum tillage differs greatly from that in soils subjected to extensive moldboard plowing. The changes in the physical and chemical properties of soil resulting from tillage greatly alter the matrix supporting growth of the microbial population (Höflich et al. 1999; Kennedy and Smith 1995). Recent reports indicate that DRB communities are encouraged by some crop management practices. Elliott and Stott (1997) found that the incidence of inhibitory microorganisms correlated with poor *Triticum aestivum* growth when no-till seeded into heavy residues from previous crops. It was postulated that some of the detrimental microorganisms might be closely associated with previous crop residues, especially when the residues remained on the soil surface. Populations on residues in the no-till plots were approximately 10-fold higher than those in the tilled plots (Stroo et al. 1988), suggesting that the crop may support and transmit DRB to subsequent crops. DRB associated with residues with no-till seeding may become a major cause of plant growth inhibition as crop rotation frequency decreases (Elliott and Stott 1997).

Crop rotation can influence the development and activity of DRB (Rovira et al. 1990; Schippers et al. 1987). Yield reductions of *T. aestivum* and, especially, of *Solanum tuberosum* L. (potato) result partly from growth inhibitory effects of DRB that extensively colonize crop root surfaces. In the Pacific Northwest, populations of DRB primarily composed of pseudomonads increased on the *T. aestivum* rhizoplane with increased *T. aestivum* cropping frequency (Rovira et al. 1990). Turco et al. (1990) found that the greatest frequency

TABLE 1. Characteristics of crop management systems selected for isolation of rhizobacteria and endorhizal bacteria.

Code	Management system	Crops	Herbicides[a]	Predominant weeds[b]
SF-6	Conventional tillage, monoculture	*Zea mays*	1× atrazine, PPI	CONAR, IPOHE, SETFA
SF-26	Conventional tillage, crop rotation	*Z. mays, Triticum aestivum, Trifolium pratense*	*Z. mays*: 1× atrazine, PPI *T. aestivum, T. pratense*: None	CONAR, SIDSP
CS-1	High agrichemical input, minimum tillage, crop rotation	*Z. mays, Glycine max*	*Z. mays*: 1× atrazine + 1× metolachlor, PPI *G. max*: 1× metolachlor + 1× imazaquin	AMARE, IPOHE, SETFA, XANST
CS-5	Integrated management, no-tillage, crop rotation	*Z. mays, G. max, T. aestivum,* cover crop	*Z. mays*: 0.5× atrazine + 1× dicamba, burndown; 1× nicosulfuron + 1× bromoxynil, POST *G. max*: 1× glyphosate, burndown; 1× clethodim + 1× acifluorfen, POST *T. aestivum,* None	AMARE, SETFA
CS-6	Cool-season pasture[c]	Cool-season grasses and legumes	None	AMARE, IPOHE, SETFA, XANST
OF	Organic farming system	*Fragaria virginiana*	None	AMARE, IPOHE, POLPY, SETFA
TP	Uncultivated native prairie[c]	Warm-season grasses and forbs	None	AMARE, IPOHE, SETFA, XANST

[a] Application rates for atrazine: 1× = 2.24 kg ai ha^{-1} and 0.5× = 1.12 kg ai ha^{-1}; for metolachlor: 1× = kg ai ha^{-1}; for imazaquin: 1× = 0.17 kg ai ha^{-1}; for dicamba: 1× = 0.56 kg ai ha^{-1}; for nicosulfuron: 1× = 0.035 kg ai ha^{-1}; for bromoxynil: 1× = 0.28 kg ai ha^{-1}; for glyphosate: 1× = 0.42 kg ai ha^{-1}; for clethodim: 1× = 0.10 kg ai ha^{-1}; for acifluorfen: 1× = 0.15 kg ai ha^{-1}. PPI, preplant incorporated; POST, postemergence.

[b] Weed seedling growth stages at sampling (number of true leaves): AMARE, *Amaranthus retroflexus* L., redroot pigweed (3 to 4); CONAR, *Convolvulus arvensis* L., field bindweed (2 to 3); IPOHE, *Ipomoea hederacea* (L.) Jacq., ivyleaf morningglory (2 to 3); POLYP, *Polygonum pensylvanicum* L., Pennsylvania smartweed (3 to 4); SETFA, *Setaria faberi* Herrm., giant foxtail (3 to 4); SIDSP, *Sida spinosa* L., prickly sida (2 to 3); XANST, *Xanthium strumarium* L., common cocklebur (2 to 3).

[c] Weed seeds planted in soils of these sites served as "bait plants" for isolating rhizobacteria and endorhizal bacteria. Weed seedling growth stages at sampling (number of true leaves): AMARE (3 to 4); IPOHE (2 to 3); SETFA (3 to 4); XANST (2).

of phytotoxic rhizobacteria was associated with continuous *Zea mays,* indicating that crop monoculture may favor a build-up of microorganisms inhibitory to plant growth compared to crop rotation.

The amount and availability of carbon and nitrogen from soil organic matter can greatly influence general soil microbial growth and activity (Bolton et al. 1985; Wardle 1992). However, soil organic matter effects on the occurrence and activity of DRB has received less attention. Knowledge of factors that stimulate or repress DRB is necessary for understanding the ecology of these bacteria and their effects on plant and, especially, weed growth. Understanding the influence of existing cropping systems and the soil properties developed under each specific cropping system on the incidence of inhibitory microorganisms might aid in predicting the level of naturally occurring biological control one might expect (Duffy et al. 1997). Also, once we understand factors that limit or stimulate the incidence of DRB or the survival of applied DRB (Skipper et al. 1996), field management practices may be devised that will increase the effect of these microorganisms on weed growth.

An awareness of the effect of rhizosphere microbial communities on weed growth is critical, but this is often not realized. Cropping systems incorporating certain soil management practices can adversely affect weed dynamics and provide opportunities to design alternative methods for suppressing weeds (Gallandt et al. 1999). Because DRB will likely play a major role in the suppression of weeds under these management conditions, it is important to understand the influence of existing production systems on the occurrence and activity of DRB. The objective of our study was to determine the relationship of growth-inhibitory (phytotoxic) rhizobacteria associated with weed seedlings under selected crop management systems on mid-Missouri claypan soils.

Materials and Methods

Cropping Systems

One native prairie ecosystem and six cropping systems that differed in fertilization, method and intensity of tillage, and crop rotation sequence were chosen as representative management systems in mid-Missouri (Table 1). Cropping systems of continuous *Z. mays* receiving full fertility under conventional tillage (SF-6) and *Z. mays–T. aestivum–Trifolium pratense* L. (red clover) rotation receiving full fertility under conventional tillage (SF-26) were established at Sanborn Field, a long-term experimental research site at the University of Missouri, Columbia. At Sanborn Field, full fertility consisted of application of fertilizers sufficient for a yield of 11,000 kg ha^{-1} *Z. mays* based on soil test recommendations and yield history of the plots, and conventional tillage comprised moldboard plowing followed by tandem disk secondary tillage for seedbed preparation. Three cropping systems at the Agricultural Systems for Environmental Quality (ASEQ) site near Centralia, MO, were selected. (1) CS-1, with high agrichemical input, minimum tillage, and *Z. mays–Glycine max* rotation, receiving 190 kg ha^{-1} N as well as lime, P, and K as needed based on soil test only for a *Z. mays* yield of 9,400 kg ha^{-1}. Minimum tillage consisted of field cultivation for fertilizer and herbicide incorporation, mid-season in-row cultivation, and fall or spring chisel plow after *Z. mays* harvest. (2) CS-5, an integrated crop manage-

TABLE 2. Selected chemical and physical characteristics of Mexico silt loam at each crop management site.

Site[a]	pH	O.M.	P	Ca	Mg	K	Clay	Silt	Sand
		%		kg ha^{-1}				%	
SF-6	5.8	3.2	90	1,210	75	140	24.5	67.7	7.8
SF-26	5.6	3.1	70	1,420	130	210	17.1	77.9	5.0
CS-1	6.4	3.1	60	1,690	180	190	17.0	75.4	7.6
CS-5	6.2	4.0	110	1,825	320	275	27.5	63.1	9.4
CS-6	6.2	2.6	30	3,260	435	265	19.0	76.6	5.4
OF	6.6	6.2	130	2,470	320	280	25.9	66.9	7.2
TP	4.9	5.3	20	1,800	315	250	21.0	75.0	4.1

[a] See Table 1 for site codes.

ment of no tillage and *Z. mays–G. max–T. aestivum* cover crop rotation receiving 150 kg ha^{-1} N and lime, P, and K based on soil test only for a *Z. mays* yield of 7,500 kg ha^{-1}. (3) CS-6, a 10-yr continuous cool-season grass and legume pasture system with no agrichemical input, representing previously cultivated land taken out of crop production. An organic farming system (OF) approximately 16 km east of Columbia, MO, was continuously cropped to *Fragaria virginiana* on raised beds with annual applications of 10 Mg ha^{-1} beef manure–sawdust compost and no agrichemical inputs. Tucker Prairie (TP), 32 km east of Columbia, MO, consisting of native warm-season grasses and forbs was selected to represent an uncultivated reference site. The soil at all sites was mapped as a Mexico silt loam (fine, montmorillonitic, Mesic, Aeric Vertic Epiaqualf). Soils from each site represented a diverse range in pH, soil organic matter content, and mineral nutrient contents (Table 2).

Sampling Procedures

The cropping systems differed in the composition of dominant weed species present (Table 1). Only *Setaria faberi, Amaranthus retroflexus,* and *Ipomoea hederacea* were present at each site. Weed seedlings were sampled during June 1997 and 1998. Six replicate seedlings of dominant weed species were randomly sampled following a "W" pattern in the field. Growth stages of the seedlings at sampling are shown in Table 1. Weed plants, including the roots, were carefully removed from the soil using a spatula sterilized with 70% ethanol. Special care was taken to keep enough soil on the seedling root system to avoid moisture loss and keep the root systems intact before further processing in the laboratory. Each individual sample was placed in a sterile plastic bag in the field. All bags with seedlings were transported in a cooler, to maintain the viability of the seedlings, then kept refrigerated at 4 C before processing within 24 h of collection.

Isolation of Rhizobacteria and Endorhizal Bacteria

Bacteria associated with the root surface (rhizoplane) and soil tightly adhering to roots were considered rhizobacteria and were obtained according to the following procedure. Roots were separated from weed seedlings, thoroughly shaken to dislodge loosely adhering soil, added to 95 ml sterile phosphate buffered saline (PBS; 10 mM K_2PO_4–KH_2PO_4, 0.14 M NaCl; pH 7.2), and shaken for 30 min at 200 rpm on a rotary shaker. Tenfold dilutions of root washings were made in PBS and plated on King's B agar medium, supplemented with 80 μg g^{-1} cycloheximide to avoid fungal contamination (Araujo et al. 1996). Although King's B medium was developed to selectively culture pseudomonads, species of other typical bacteria generally are readily cultured from rhizospheres using this medium (Kremer et al. 1990). Plates were incubated in an inverted position at 27 C for 3 d, after which bacterial colony forming units (CFUs) were enumerated. Representative colonies were selected and subcultured by streaking growth onto King's B and tryptic soy agars to obtain pure, single-colony isolates. Selection was based on distinct bacterial types observed according to morphological characterization, including pigment, colony form, elevation, margin, texture, and opacity (Smibert and Krieg 1994). Fluorescent pigment production was detected by exposing bacterial colonies to ultraviolet light (< 260 nm wavelength) for 1 to 2 s.

Bacteria within the root epidermis and inhabiting internal portions of the root were considered endorhizal bacteria and were obtained according to the following procedure. Roots were removed from initial dilution bottles, blotted dry, weighed, cut into 1-cm segments, surface sterilized in 10% H_2O_2 for 2 min, rinsed three times in sterile water, and macerated in 5 ml sterile PBS for 1 min using a Stomacher Lab-Blender.[1] The homogenates were serially diluted, plated, and incubated as described above. The same criteria for selection and subculturing representative colony types used for rhizobacteria were used.

Because weed seedlings typical of the cultivated cropping systems were absent from the pasture (CS-6) and prairie sites, soils were collected in June 1997 and 1998 from the upper 10 cm of the soil profile using a soil probe. Each sample was a composite of six soil cores (5 cm diam) that were sieved and dispensed into cone-tainers[2] (16 cm deep, 2.5 cm diam). The soils served as media to grow weeds as "bait plants" that were not present at the uncultivated sites to select for rhizobacteria. A similar baiting procedure has been successfully used to collect rhizobacteria on *Hordeum vulgare* L. (barley) planted in various soils (Olsson et al. 1999). *Ipomoea hederacea, Xanthium strumarium* L. (common cocklebur), *A. retroflexus,* and *Setaria viridis* (L.) Beauv. (green foxtail) were planted in separate cone-tainers containing soil from each site. Six replicates were prepared for each weed species × soil treatment. Weed seedlings were harvested 30 d after planting and rhizobacteria and endorhizal bacteria were isolated as described above.

Lactuca sativa Seedling Bioassays

Bioassays devised using pregerminated *L. sativa* were used to quantify the phytotoxic effects of bacterial isolates (Alström 1987). *Lactuca sativa* seeds were surface sterilized (1.5 min in 1.25% sodium hypochlorite, rinsed twice with sterile water, immersed in 70% ethanol for 1.5 min, and rinsed five times in sterile water), blotted on sterile paper towels, and germinated overnight on 1.0% agar. Pregerminated *L. sativa* seeds with uniform radicles (≤ 2 mm long) were evenly distributed on the agar surface (20 seeds plate^{-1}). Two-day-old cultures of each bacterial isolate on King's B plates were suspended in 5 ml of 0.1 M $MgSO_4$ and a 30-μl suspension containing approximately 10^6 cfu was inoculated on each seed. Control seeds received 0.1 M $MgSO_4$ without bacteria. Two replicates for each treatment were used. After

a 2-d incubation at 27 C in the dark, radicle lengths were measured. The growth inhibition symptoms or injuries were also evaluated. Those isolates causing 50% or higher growth inhibition and/or obvious root injury were selected for repeated or secondary screening on *L. sativa* seedlings to verify the growth inhibition effects of DRB detected in the primary screening. Replication in the secondary screening was increased to four plates.

Host Plant Screening

Those isolates that retained growth-suppressive activities in the secondary screening were tested on the host plants from which they were originally isolated. The plants tested included *S. viridis, I. hederacea, A. retroflexus,* and *Convolvulus arvensis* L. (field bindweed). A procedure similar to that of the *L. sativa* seedling bioassay was followed. The number of seedlings of each weed tested per agar plate was adjusted based on seed size. The seeds were surface sterilized and pregerminated on 1.0% agar. After 48 h incubation, bacterial cultures were suspended in 0.1 M $MgSO_4$ to a concentration of 10^8 cfu ml^{-1} and dispensed onto seeds. To assure contact of bacteria with weed seeds, the volume of inoculum was adjusted based on seed size. Thus, 30 µl (for *S. viridis* and *A. retroflexus*) or 50 µl (for *I. hederacea* and *C. arvense*) of bacterial suspension were used to inoculate each pregerminated weed seed. Root lengths were measured after 48 h incubation. Root injury symptoms including necrosis, discoloration, root hair inhibition, root stunting and other abnormalities were also recorded. The host plant screening was repeated one time.

Characterization of Bacteria

Those isolates showing significant growth-suppressive activity toward host weed seedlings were identified using the API-20NE diagnostic kit.[3] Each kit consists of 20 substrates that are inoculated with a suspension of a bacterial isolate. After 24- and 48-h incubations, reactions are scored positive or negative and, along with the oxidase reaction, used to determine a numerical code, which is matched to profiles in the identification codebook accompanying the kit. Classification of isolates representing distinct morphological groups was verified with gas chromatography–fatty acid methyl ester analysis (Sasser 1990).

Statistical Analysis

In the primary screening, bacterial isolates that caused 50% of growth inhibition or more were considered phytotoxic and underwent secondary screening. The growth inhibition results of secondary screening and host screening were subjected to one-way analyses of variance. The least significant differences were calculated according to Tukey's test, whereby the upper significance bounds of the studentized range distribution P = 0.05 were used.

Results and Discussion

General Characterization of Weed Seedling Bacteria

Average population sizes of rhizobacteria and endorhizal bacteria on different weed seedling roots from each sampling ranged from 10^6 to 10^8 cfu g^{-1} fresh root for rhizosphere bacteria and 10^4 to 10^7 cfu g^{-1} fresh root for endorhizal bacteria (Table 3). Weed seedlings from the uncultivated prairie (TP), continuous *Z. mays* (SF-6), and *Z. mays–T. aestivum–T. pratense* rotation (SF-26) had smaller rhizosphere population sizes, suggesting that lower soil pH at these sites may have depressed numbers of culturable rhizosphere bacteria. Weed seedling roots from the organic farming (OF) and integrated crop management (CS-5) systems had relatively large rhizosphere populations. Bacterial populations in rhizospheres of various crop plants are generally higher under conservation tillage compared to conventional tillage (Höflich et al. 1999). Rhizobacteria isolated from all weed seedling roots were composed mainly of fluorescent and nonfluorescent pseudomonads, *Xanthomonas, Agrobacterium,* and *Aeromonas* species (Table 4). These bacterial groups are typical rhizosphere bacteria with high root-colonizing abilities and represent species that have demonstrated growth suppression of several weeds (Kremer and Kennedy 1996).

TABLE 3. Bacterial populations[a] in weed rhizosphere of different cropping systems.

Site	Host plant	Rhizosphere	Endorhizal bacteria
		— $\log_{10}$ cfu g^{-1} fresh root —	
SF-6	Field bindweed	6.2 ± 0.2	4.8 ± 0.2
SF-6	Giant foxtail	6.9 ± 0.3	5.8 ± 0.2
SF-6	Ivyleaf morningglory	6.2 ± 0.2	4.3 ± 0.1
SF-26	Field bindweed	6.5 ± 0.1	4.8 ± 0.2
SF-26	Pricky sida	7.4 ± 0.2	5.4 ± 0.2
CS-1	Common cocklebur	6.6 ± 0.0	5.4 ± 0.1
CS-1	Giant foxtail	7.3 ± 0.2	6.3 ± 0.2
CS-1	Ivyleaf morningglory	7.0 ± 0.1	4.6 ± 0.1
CS-1	Redroot pigweed	7.1 ± 0.1	5.2 ± 0.3
CS-5	Giant foxtail	8.2 ± 0.4	6.8 ± 0.3
CS-5	Pigweed	7.3 ± 0.2	4.8 ± 0.1
CS-6	Ivyleaf morningglory	7.6 ± 0.1	6.8 ± 0.2
CS-6	Common cocklebur	7.3 ± 0.1	5.4 ± 0.1
CS-6	Giant foxtail	8.3 ± 0.3	5.3 ± 0.2
CS-6	Redroot pigweed	8.0 ± 0.2	5.3 ± 0.4
OF	Giant foxtail	8.1 ± 0.1	5.5 ± 0.3
OF	Ivyleaf morningglory	7.9 ± 0.2	5.5 ± 0.3
OF	Redroot pigweed	7.8 ± 0.1	6.7 ± 0.2
OF	Smartweed	7.8 ± 0.1	6.5 ± 0.2
TP	Ivyleaf morningglory	6.8 ± 0.2	6.0 ± 0.1
TP	Common cocklebur	6.7 ± 0.2	5.9 ± 0.2
TP	Giant foxtail	7.7 ± 0.1	6.5 ± 0.3
TP	Redroot pigweed	7.7 ± 0.1	5.4 ± 0.3

[a] Mean ± standard deviation of four replications.

L. sativa Seedling Bioassays

Over 1,400 isolates of rhizobacteria from weed rhizospheres were tested, yielding a range of effects on root growth of *L. sativa* seedlings. The growth response of *L. sativa* seedling roots ranged from 80% growth inhibition to no effect or growth stimulation. In primary screening, phytotoxic rhizobacteria distributed among different cropping systems ranged from 13 to 25% of the total for each cropping system (Table 5). The standard of 50% for selecting growth-suppressive rhizobacteria was very strict to limit the number of isolates for in-depth study and to effectively de-

TABLE 4. Characterization of deleterious rhizobacteria yielding greatest growth-inhibitory activity as determined in lettuce seedling bioassays.

Isolate code	Host	Origin	Identification[a]
A1-1	Field bindweed	SF-6	*Pseudomonas aureofaciens*
A1-10	Field bindweed	SF-6	*Flavobacterium indologenes*
A1-15	Field bindweed	SF-6	*Pseudomonas fluorescens*
B1-7	Giant foxtail	SF-6	*Pseudomonas putida*
CCH27	Common cocklebur	CS-6	*Agrobacterium radiobacter*
CFH15a	Giant foxtail	CS-6	*Chryseomonas luteola*
CFH33	Giant foxtail	CS-6	*Aeromonas hydrophila*
CMH2	Ivyleaf morningglory	CS-6	*A. hydrophila*
CMH3	Ivyleaf morningglory	CS-6	*A. hydrophila*
CMR2	Ivyleaf morningglory	CS-6	*A. hydrophila*
D2-10	Field bindweed	SF-26	*A. hydrophila*
D2-11	Field bindweed	SF-26	*A. hydrophila*
D2-26	Field bindweed	SF-26	*C. luteola*
G1-1	Giant foxtail	CS-1	*P. fluorescens*
G1-16	Giant foxtail	CS-1	*Chromobacterium violaceum*
G2-10	Giant foxtail	CS-1	*A. radiobacter*
G2-11	Giant foxtail	CS-1	*P. fluorescens*
I2-12	Redroot pigweed	CS-1	*A. radiobacter*
J1-44	Giant foxtail	CS-5	*P. fluorescens*
J1-45	Giant foxtail	CS-5	*P. fluorescens*
J2-4	Giant foxtail	CS-5	*Aeromonas caviae*
K1-15	Redroot pigweed	CS-5	*P. aureofaciens*
K1-30	Redroot pigweed	CS-5	*Vibrio* sp.
L1-12	Giant foxtail	OF	*A. radiobacter*
L1-41	Giant foxtail	OF	*P. aureofaciens*
L2-12	Giant foxtail	OF	*P. fluorescens*
L2-19	Giant foxtail	OF	*P. fluorescens*
M1-10	Ivyleaf morningglory	OF	*P. fluorescens*
M2-3	Ivyleaf morningglory	OF	*C. luteola*
TCH9	Common cocklebur	TP	*A. hydrophila*
TCR34	Common cocklebur	TP	*A. hydrophila*
TCR44	Common cocklebur	TP	Not identified
TFR1	Giant foxtail	TP	*Xanthomonas maltophilia*
TMH16	Ivyleaf morningglory	TP	*P. aureofaciens*
TMR13	Ivyleaf morningglory	TP	*A. hydrophila*
TPH10	Redroot pigweed	TP	*A. hydrophila*
TPH2	Redroot pigweed	TP	*C. luteola*
TPH4	Redroot pigweed	TP	*Burkholderia cepacia*
TPR15	Redroot pigweed	TP	*B. cepacia*
TPR16	Redroot pigweed	TP	*P. aureofaciens*

[a] Deleterious rhizobacteria identification based on results from API diagnostic kits (BioMerieux Vitek, Hazelwood, MO) and gas chromatographic analyses of bacteria cellular fatty acid methyl ester extracts.

TABLE 5. Proportions of rhizobacteria and endorhizal bacteria originating from weed seedlings determined to be deleterious rhizobacteria in different cropping systems.[a]

Site[b]	Lettuce seedling bioassays		
	Primary	Secondary	Host screening
	%		
OF	22.9	22.4	3.9
CS-1	18.3	18.3	3.8
CS-5	25.3	24.1	4.8
CS-6	13.0	6.8	3.1
SF-6	20.8	19.2	4.0
SF-26	22.0	22.0	3.0
TP	21.0	15.9	13.6
LSD (0.05)	4.4	7.1	2.0

[a] Based on proportion of bacterial isolates significantly inhibiting ($P < 0.05$) indicator species in specified bioassay.
[b] Site codes are listed in Table I.

tect the most virulent bacteria. The basis for using the rigid selection criterion was a previous statistical analysis of root lengths that indicated significant inhibition was typically detected at about 75% of the control (Kremer et al. 1990). Root growth inhibitory values for representative isolates from weed seedlings across cropping systems were variable (Figure 1). Root damage symptoms included necroses, blackened root tips, discoloration of root surfaces, stunted lateral root development, and reduced root hair development. Secondary screening verified the phytotoxic effects detected in the primary screening because similar proportions of DRB associated with the cropping systems were observed (Table 5). Results indicated that the strict standard of 50% root inhibition for selecting deleterious rhizobacteria in the primary screening was very effective in initial detection of phytotoxic bacteria. In summarizing the primary screening, the highest proportions of DRB were detected among isolates from the integrated crop management (CS-5) and the organic farming (OF) systems. The lowest proportion of

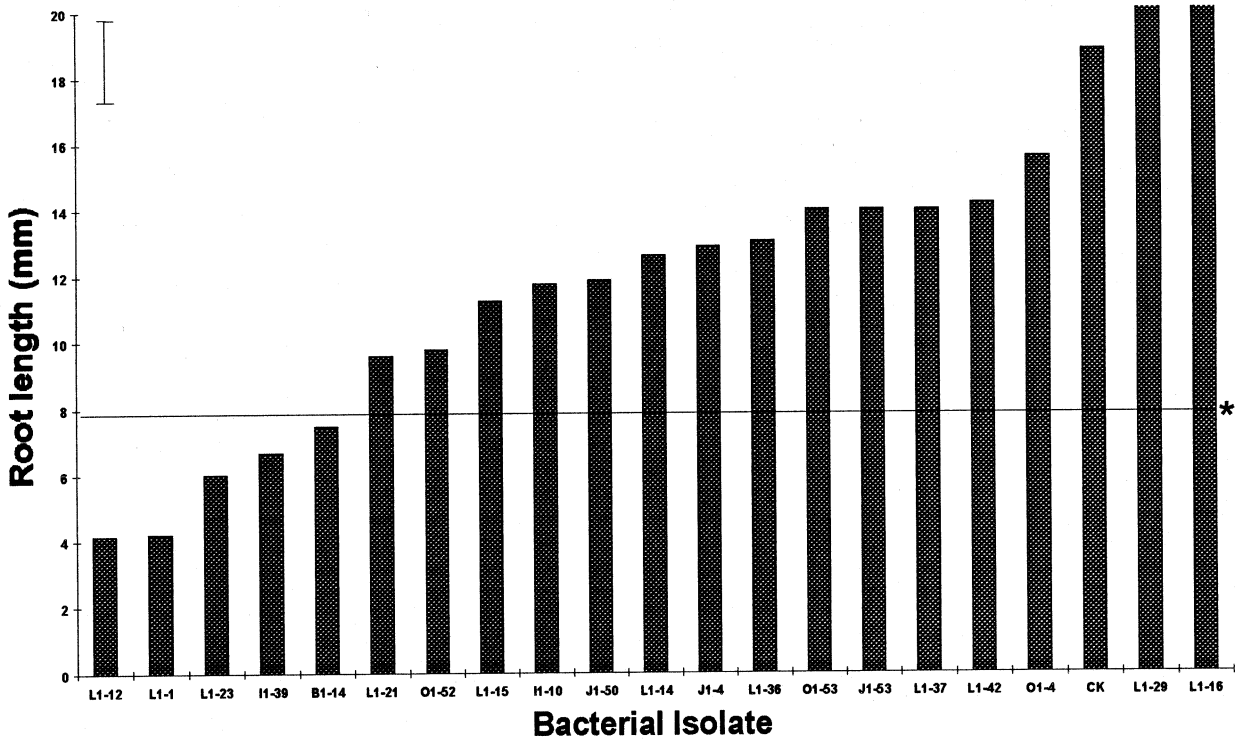

FIGURE 1. Activity of selected rhizobacterial isolates toward root growth of *Lactuca sativa* seedlings. Isolates selected as DRB had values ≤ 50% of control, as denoted by horizontal line (*). Vertical bar represents LSD ($P < 0.05$). Codes for each isolate indicate accession numbers; CK, noninoculated check.

DRB was detected among isolates from the continuous cool-season grass pasture system (CS-6). The highest proportions of bacteria phytotoxic toward *L. sativa* seedlings originated from the rhizosphere compared to inside the roots represented by the endorhizal bacteria (data not shown).

The presence of a large number of inhibitory bacteria in association with weed rhizospheres has been reported in other studies. Kremer et al. (1990) found that 35 to 65% of rhizobacterial isolates inhibited seedling growth of several broadleaf weeds. Only 8.1% of rhizobacteria tested in the laboratory inhibited root elongation of *Bromus tectorum* L. (downy brome) without affecting growth of *T. aestivum* (Kennedy et al. 1991). In a study of biocontrol agents of weeds in *T. aestivum,* over 25% of the rhizobacteria isolated from *B. tectorum, Bromus japonicus* Thumb. *ex* Murr. (Japanese brome), and *Aegilops cylindrica* Host. (jointed goatgrass) inhibited root growth of winter annual grasses (Harris and Stahlman 1996).

The occurrence and magnitude of DRB on weed seedlings are likely affected by multiple factors in a cropping system. DRB on crop plants affected by individual factors have been reported for crop rotations vs. continuous monoculture, conventional tillage vs. no-till, or organic vs. inorganic nutrition. Rovira et al. (1990) found that populations of DRB increased on the *T. aestivum* rhizoplane with increasing frequency of *T. aestivum* in a crop rotation. *Triticum aestivum* and, especially, *S. tuberosum* were sensitive to cropping frequency, and low yields were attributed to the increase of DRB when those crops were planted more frequently in the rotation (Schippers et al. 1987). Low yields of *Z. mays* frequently planted in "short rotation" were due partly to a buildup of certain inhibitory rhizobacteria specifically associated with *Z. mays* roots (Turco et al. 1990). In the present study, the incidence of DRB based on *L. sativa* seedling bioassays did not differ between the *Z. mays–T. aestivum–T. pratense* rotation (SF-26) and continuous *Z. mays* (SF-6) receiving the same fertilization regime.

The lack of significant differences in DRB as a proportion of isolates from all weed seedlings within cropping systems was most likely due to combinations of fertilization, tillage, and crop rotations. The effects of each single practice may be confounded so that the final influence of each cropping system did not significantly affect DRB distribution. Comparison of the uncultivated prairie (TP) and the cool-season pasture (CS-6), which were similar in terms of permanent grassland, yielded dramatically different proportions of DRB (Table 5), which were possibly related to differences in organic matter content and soil pH. The uncultivated prairie soil has higher levels of microbial biomass C, bacterial and fungal biomass, and soil microbial activity compared to cultivated cropping systems (Jordan et al. 1995). The combination of low soil pH and high soil organic matter content might also contribute to selection of a higher proportion of DRB in the rhizosphere of weeds in uncultivated prairie soil compared to the cool-season pasture soil. The higher incidence of DRB on seedlings from the organic farming (OF) system compared to that from the high agrichemical input, minimum tillage *Z. mays–G. max* rotation (CS-1) suggests that regular addition of organic amendments resulting in a buildup of soil organic matter enhances the development of high populations of DRB in weed rhizospheres. Based on the high rhizosphere populations of weed seedlings from the organic farming and integrated cropping (CS-5) systems (Table 3), the populations of DRB associated with weed seedlings in these two systems would also be higher than for those in the other cropping systems.

When DRB detected by *L. sativa* seedling bioassays were separated relative to their original weed hosts, patterns

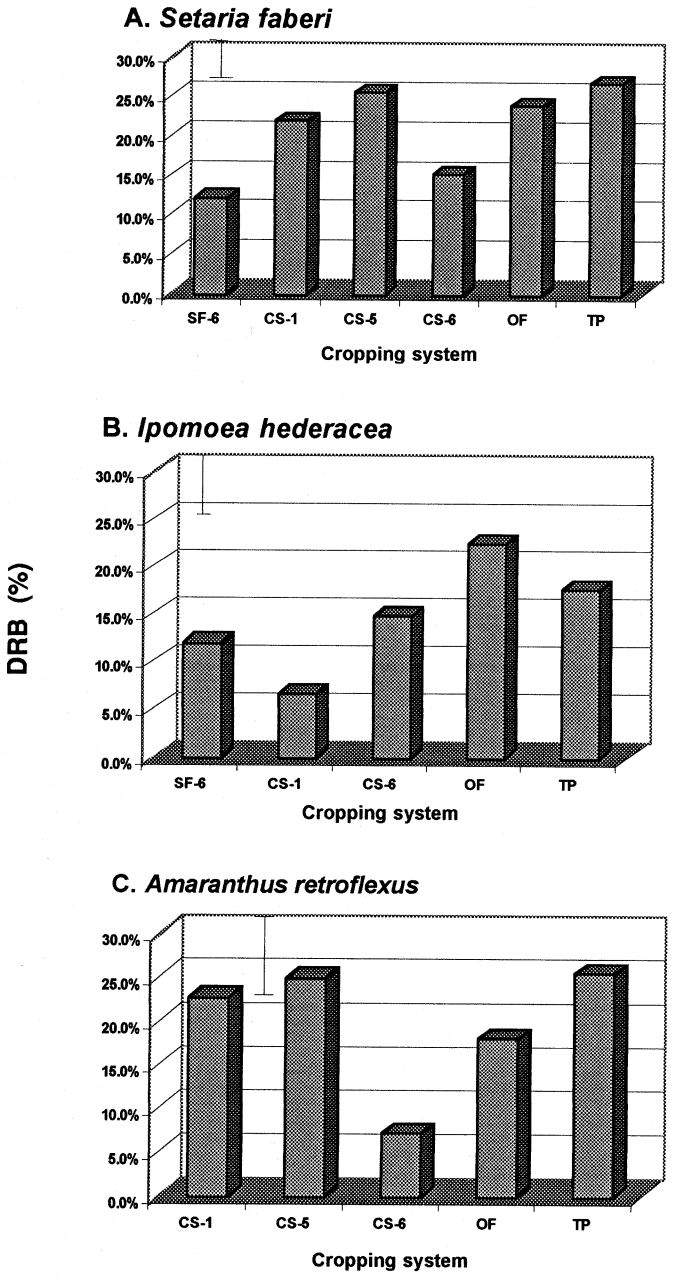

FIGURE 2. Proportions of rhizobacteria and endorhizal bacteria from *Setaria faberi* (A), *Ipomoea hederacea* (B), and *Amaranthus retroflexus* (C) within cropping systems that significantly ($P < 0.05$) inhibited seedling root growth relative to noninoculated checks. Vertical bars represent LSD ($P < 0.05$) for comparison of cropping systems within weed species. Cropping system codes are listed in Table 1.

emerged indicating the influence of cropping system on the DRB–weed association. For example, occurrence of DRB in *S. faberi* rhizospheres was highest in uncultivated prairie soil and lowest in the *Z. mays–T. aestivum–T. pratense* (SF-26) rotation and cool-season pasture (CS-6) (Figure 2A). The highest frequency of DRB associated with *I. hederacea* was found in the organic farming system followed by the uncultivated prairie, whereas the lowest frequency was associated with the *Z. mays–T. aestivum–T. pratense* rotation (SF-26) and cool-season pasture (CS-6) (Figure 2B). The highest proportions of DRB associated with *A. retroflexus* (Figure 2C) occurred in the uncultivated prairie soil, whereas that in the cool-season pasture was clearly lower than in the other cropping systems. Interestingly, high proportions of DRB in the rhizosphere of *X. strumarium* occurred in the two nondisturbed sites, the prairie and cool-season pasture (15.0 and 15.2%, respectively), relative to the *Z. mays–G. max* rotation (CS-1; 13.0%), which had a field infestation of *X. strumarium.* Proportions of DRB in the rhizospheres of *C. arvense* from continuous *Z. mays* (32.0%) and *Z. mays–T. aestivum–T. pratense* rotation (SF-26; 18.2%) suggested that continuous *Z. mays* encouraged the incidence of rhizobacteria phytotoxicity toward *C. arvense.* The comparisons of DRB associated with specific weed species indicated that the uncultivated prairie and the organic farming and integrated cropping (CS-5) systems, all with relatively high soil organic matter, had higher proportions of DRB. These results suggest that cropping systems that include organic farming practices or integrate management practices including interrow cultivation, cover crop use, or reduced agrichemical inputs may have advantages in promoting naturally occurring DRB that could be exploited in alternative weed management systems.

Host Weed Bioassays

Based on *L. sativa* seedling bioassays, the highest levels of DRB were detected in the cropping system under no-tillage (CS-5), which agrees with previous findings of high populations of growth-inhibiting rhizobacteria associated with plants in no-tillage systems (Fredrickson and Elliott 1985). However, host plant screening further reduced the proportion of detectable DRB in each cropping system ranging from 3 to 5% except for the uncultivated prairie, which remained high at 13.6% (Table 5). Although no significant differences were apparent among some cropping systems in terms of the occurrence or proportion of DRB detected in weed seedling bioassays, the integrated crop management (CS-5) and organic farming (OF) systems may promote weed-suppressive DRB, based on the higher numbers of bacteria present in weed seedling rhizospheres (Table 3). Elsherif and Grossman (1990) demonstrated that influences of different crop rotations, crop-growing intensities, and plant protection practices on occurrence of fluorescent pseudomonads could be detected as differential populations but were less pronounced compared to the natural fluctuations observed during different growth stages of *T. aestivum.* The uncultivated prairie supported a high proportion of deleterious rhizobacteria, possibly because of factors unique to this natural ecosystem, including less soil perturbation and erosion, a continuous presence of dense root systems in the soil, and a low soil pH (4.9). Differences in the prevalence of DRB among the ecosystems studied cannot be attributed to any one factor. Harris and Stahlman (1996), in describing the effects of DRB on *Bromus* spp. and *A. cylindrica,* suggested that the physical, chemical, and biological properties of soil associated with a particular production regime probably regulate the abundance of inhibitory rhizobacteria in the plant rhizosphere. Although *L. sativa* seedling bioassays are useful in preliminary screening for phytotoxicity, they may overestimate the proportion of DRB or may detect little if any activity toward specific weeds, as was reported for *Euphorbia esula* L. (leafy spurge) (Souissi and Kremer 1998). It is necessary to test selected bacterial isolates on their host

plants to assess adequately the potential for natural growth suppression of weeds in a particular ecosystem.

Communities of specific soil and rhizosphere bacteria develop in ecological niches formed under a particular set of crop production practices, which offers opportunities to manage the population of rhizosphere-colonizing bacteria to favor suppressive bacteria (Rovira 1994). Although management of agroecosystems for natural weed suppression in soils has been suggested (Boyetchko 1996; Kennedy 1999), this is the first study demonstrating the relationship of DRB on selected weed seedlings with management practices in agro- and natural ecosystems. Information from this study suggests that uncultivated prairie and no-tillage systems harbored high numbers of DRB and may be useful in selecting the type of tillage practice for a crop production field. However, further studies on diverse soils, different crops, and other environmental factors are required to expand our knowledge of the ecological relationships required for developing soil environments for weed suppression in a variety of agroecosystems. This knowledge base can then be applied to modify existing cropping systems or to design novel systems that can reduce weed growth and competition while optimizing crop yields with minimal reliance on chemical control measures.

Sources of Materials

[1] Stomacher Lab-Blender laboratory homogenizer, Tekmar, P.O. Box 429576 Cincinnati, OH 45242-9576.

[2] Cone-tainers, plastic plant growth containers, Stuewe & Sons, Inc., 2290 SE Kiger Island Dr., Corvallis, OR 97333-9461.

[3] API-20NE diagnostic kit, containing a series of substrates for identification of gram-negative bacteria, BioMerieux Vitek Inc., 591 Anglum Rd., Hazelwood, MO 63042-2320.

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Literature Cited

Alström, S. 1987. Factors associated with detrimental effects of rhizobacteria on plant growth. Plant Soil 102:3–9.

Araujo, M.A.V., L. C. Mendonca-Hagler, A. N. Hagler, and J. D. van Elsas. 1996. Selection of rhizosphere-competent *Pseudomonas* strains as biocontrol agents in tropical soils. World J. Microbiol. Biotech. 12:589–593.

Bolton, H., L. F. Elliott, R. I. Papendick, and D. F. Bezdicek. 1985. Soil microbial biomass and selected soil enzyme activities: effect of fertilization and cropping practices. Soil Biol. Biochem. 17:297–302.

Boyetchko, S. 1996. Impact of soil microorganisms on weed biology and ecology. Phytoprotection 77:41–56.

Campbell, J. N., K. Conn, L. Sorlie, and F. D. Cook. 1986. Inhibition of growth in canola seedlings caused by an opportunistic *Pseudomonas* sp. under laboratory and field conditions. Can. J. Microbiol. 32:201–207.

Cherrington, C. A. and L. F. Elliott. 1987. Incidence of inhibitory pseudomonads in the Pacific Northwest. Plant Soil 101:159–165.

Doran, J. W. and D. M. Linn. 1994. Microbial ecology of conservation management systems. Pages 1–27 *in* J. L. Hatfield and B. A. Stewart, eds. Soil Biology: Effects on Soil Quality. Boca Raton, FL: Lewis Publishers.

Duffy, B. K., B. H. Ownley, and D. M. Weller. 1997. Soil chemical and physical properties associated with suppression of take-all of *T. aestivum* by *Trichoderma koningii.* Phytophathology 87:1118–1124.

Elliott, L. F. and D. E. Stott. 1997. Influences of no-till cropping systems on microbial relationships. Adv. Agron. 60:121–147.

Elsherif, M. and F. Grossmann. 1990. Effects of different cropping systems on the occurrence of fluorescent pseudomonads. Phytopathology 130: 65–81.

Fredrickson, J. K. and L. F. Elliott. 1985. Colonization of winter *T. aestivum* roots by inhibitory rhizobacteria. Soil Sci. Soc. Am. J. 49:1172–1177.

Gallandt, E. R., M. Liebman, and D. R. Higgins. 1999. Improving soil quality: implications for weed management. J. Crop Prod. 2:95–121.

Harris, P. A. and P. W. Stahlman. 1996. Soil bacteria as selective biological control agents of winter annual grass weeds in winter *T. aestivum.* Appl. Soil Ecol. 3:275–281.

Höflich, G., M. Tauschke, G. Kühn, K. Werner, M. Frielinghaus, and W. Höhn. 1999. Influence of long-term conservation tillage on soil and rhizosphere microorganisms. Biol. Fertil. Soils 29:81–86.

Jordan, D., R. J. Kremer, W. A. Bergfield, K. Y. Kim, and V. N. Cacnio. 1995. Evaluation of microbial methods as potential indicators of soil quality in historical agricultural fields. Biol. Fertil. Soils 19:297–302.

Kennedy, A. C. 1999. Soil microorganisms for weed management. J. Crop Prod. 2:123–138.

Kennedy, A. C., L. F. Elliott, F. L. Young, and C. L. Douglas. 1991. Rhizobacteria suppressive to the weed downy brome. Soil Sci. Soc. Am. J. 55:722–727.

Kennedy, A. C. and K. L. Smith. 1995. Soil microbial diversity and the sustainability of agricultural soils. Plant Soil 170:75–86.

Kremer, R. J., M.F.T. Begonia, L. Stanley, and E. T. Lanham. 1990. Characterization of rhizobacteria associated with weed seedlings. Appl. Environ. Microbiol. 56:1649–1655.

Kremer, R. J. and A. C. Kennedy. 1996. Rhizobacteria as biocontrol agents of weeds. Weed Technol. 10:601–609.

Olsson, S., S. Alström, and P. Persson. 1999. Barley rhizobacterial population characterised by fatty acid profiling. Appl. Soil Ecol. 12:197–204.

Ramsay, A. J., R. E. Stannard, and G. J. Christensen. 1986. Effect of conversion from ryegrass pasture to *T. aestivum* cropping on aggregation and bacterial populations in a silt loam soil in New Zealand. Aust. J. Soil Res. 24:253–264.

Rovira, A. D. 1994. The effect of farming practices on the soil biota. Pages 81–87 *in* C. E. Pankhurst, B. M. Doube, V.V.S.R. Gupta, and P. R. Grace, eds. Soil Biota: Mangement in Sustainable Farming Systems. East Melbourne, Australia: CSIRO.

Rovira, A. D., L. F. Elliott, and R. J. Cook. 1990. The impact of cropping systems on rhizosphere organisms affecting plant health. Pages 389–436 *in* J. M. Lynch, ed. The Rhizosphere. Chichester, Great Britain: J. Wiley.

Sasser, M. 1990. Identification of bacteria through fatty acid analysis. Pages 199–204 *in* Z. Klement, K. Rudolph, and D. C. Sands, eds. Methods in Phytobacteriology. Budapest: Akadamiai Kiado.

Schippers, B., A. W. Bakker, and A.H.M.B. Peter. 1987. Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. Ann. Rev. Phytopathol. 25:331–358.

Skipper, H. D., A. G. Ogg, Jr., and A. C. Kennedy. 1996. Root biology of grasses and ecology of rhizobacteria for biological control. Weed Technol. 10:610–620.

Smibert, R. M. and N. R. Krieg. 1994. Phenotypic characterization. Pages 607–654 *in* P. Gerhardt, R.G.E. Murray, W. A. Wood, and N. R. Krieg, eds. Methods for General and Molecular Bacteriology. Washington, DC: American Society for Microbiology.

Souissi, T. and R. J. Kremer. 1998. A rapid microplate callus bioassay for assessment of rhizobacteria for biocontrol of leafy spurge (*Euphorbia esula* L.). Biocontrol Sci. Technol. 8:83–92.

Stroo, H. F., L. F. Elliott, and R. I. Papendick. 1988. Growth, survival and toxin production of root-inhibitory pseudomonads on crop residues. Soil Biol. Biochem. 20:201–207.

Turco, R. F., M. Bischoff, D. P. Breakwell, and D. R. Griffith. 1990. Contribution of soil-borne bacteria to the rotation effect in *Z. mays.* Plant Soil 122:115–120.

Wardle, D. A. 1992. A comparable assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biol. Rev. 67: 321–358.

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