INTEGRATED WEED AND SOIL MANAGEMENT

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Microbial Interactions with Weed Seeds and Seedlings and Its Potential for Weed Management

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I. Introduction

Soil microorganisms are responsible for many key processes including nutrient cycling, plant nutrition, xenobiotic transformations, and interacting (either beneficially or antagonistically) with other organisms inhabiting the soil. Recent concerns about possible detrimental effects on the environment by agrichemicals have generated interest in developing alternative pest management strategies including biological control using microorganisms occurring in nature. Some success has been achieved using insects and fungi for controlling specific weeds in certain agroecosystems (Harley and Forno, 1992). However, development of microbial agents for widespread use in managing economically important weeds in cropping and pasture/rangeland systems has not advanced as rapidly as expected. Causes for delays in establishing successful strategies for biological control of these weeds include variable (inconsistent) levels of efficacy, host specificity constraints, and lack of persistence in the field of the selected microorganisms (Kremer and Kennedy, 1996).

At least two important limitations impact the development of environmentally safe, alternative weed management strategies. First, such strategies require detailed quantitative understanding of the ecological and biological factors affecting the dynamics of weed growth and populations (Lindquist et al., 1995). Secondly, knowledge of the ecology and biology of microorganisms that interact with weeds at various stages of a weed's life cycle is needed to properly fit biological approaches in a weed management system. Little research has been conducted to quantify the relationships between soil and rhizosphere microorganisms and weed seeds and seedlings. These relationships are complex and likely are unique to each weed family, if not species, and must be described so that fundamental hypotheses can be made and applied in developing practical weed management systems integrated with biological control strategies. The purpose of this paper is to assess the relationships between soil and rhizosphere microorganisms and weed seeds and seedlings and describe potential weed management strategies that can be developed based on an understanding of these relationships.

II. Weed Seeds and Seedlings and Microorganisms

A. Soil Microbial Diversity

The biological component of soil has largely been ignored as an important aspect in weed seed and seedling growth dynamics and as a resource for potential nonchemical control systems. Soil microorganisms have been implicated in weed seed depletion or seedling growth inhibition, but these effects have not been extensively quantified (Kremer, 1993). When the biological diversity or the variety of microbial species occurring in an ecosystem is considered, the tremendous potential for discovery of organisms with significant impact for managing weeds or other agricultural pests is readily apparent. Kennedy and Smith (1995) estimate that one gram of soil contains one million to ten billion bacteria, which may comprise up to 10,000 different species. Of the approximately one million microbial species on earth, only about 110,000 are known and most of these have yet to be fully described. It is concluded that only a minuscule portion of the total soil microbial potential is currently known, and a wealth of bioactivity and genetic information is waiting to be discovered (Kennedy and Smith, 1995).

Despite the presence of vast numbers of diverse microorganisms, seeds of most weed species persist viably in soil for many years with only a small portion succumbing to microbial attack. Seeds retrieved from soil often possess intimate microbial associations. This is illustrated in Figure 1, which depicts the extent of colonization of giant foxtail (*Setaria faberii* Herrm.) and redroot pigweed (*Amaranthus retroflexus* L.) seeds from the upper 10 cm of a silt loam in central Missouri. High proportions of all seed viability categories [nondormant (imbibed or germinating), viable (dormant, nongerminating), and dead] revealed fungal associations when surface-sterilized seeds were cultured using standard microbiological procedures (Kremer et al., 1984). Bacterial associations were also detected to a lesser extent in all seed categories for both weed species. Microbial associa-

A. Giant foxtail
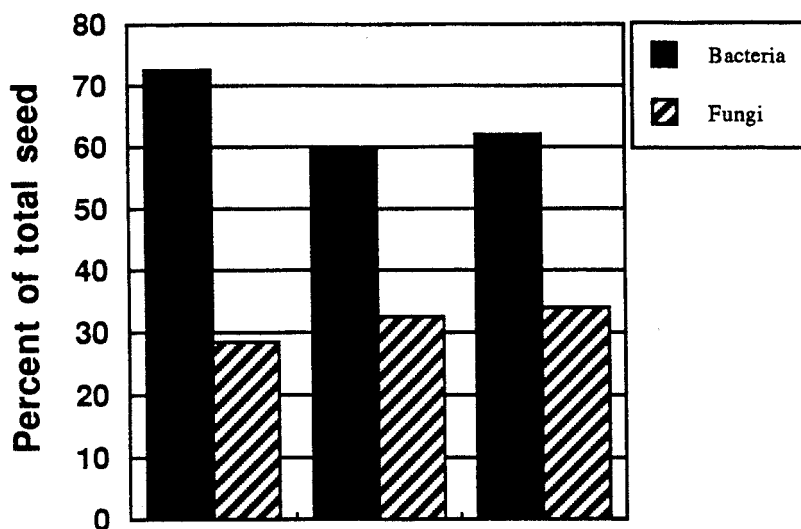
B. Redroot pigweed
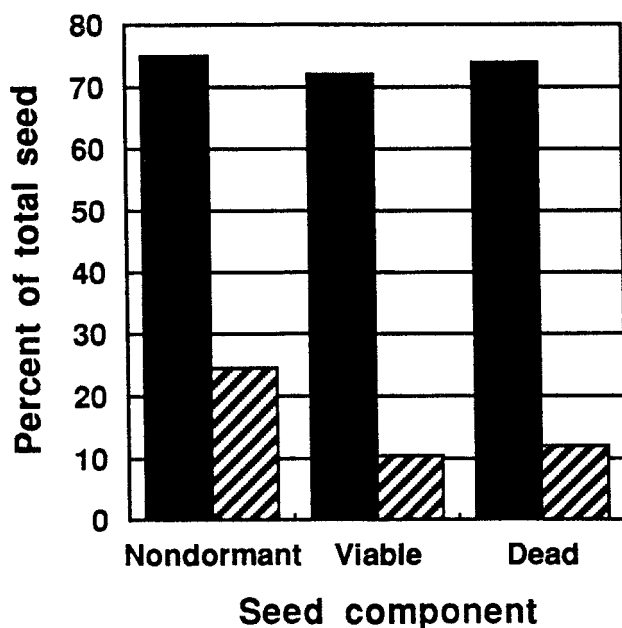
Figure 1. Microorganisms associated with different components of weed seeds retrieved from the surface 10 cm of a Mexico silt loam in central Missouri in 1993. (From R.J. Kremer, unpublished data.)

Table 1. Phytoxicity of microbial isolates from weed seeds retrieved from a Mexico silt loam in central Missouri; reduction in root growth based on lettuce seedling bioassays (each value represents an individual microbial isolate)

Bacteria		Fungi
Velvetleaf	Giant foxtail	Giant foxtail
------------------------------% reduction in root growth--------------------------------		
23.0	15.4	7.7
30.0	40.0	36.4
39.5	53.4	54.0
67.4	67.0	54.5
78.8	73.4	61.5
80.0	84.6	69.2

(From R.J. Kremer, unpublished data.)

tions often comprised up to three different species per seed. Differences in relative proportions of seeds colonized by fungi or bacteria may be due to differences in seed structures between the two species. Although many of the seeds were colonized by microorganisms, high proportions of giant foxtail and redroot pigweed seeds in the seedbank were also viable and emerged as seedlings the following spring. Poor emergence of redroot pigweed from the seedbank commonly occurs (Forcella et al., 1992), which may be related to the high incidence of seedborne bacteria. Interestingly, several microorganisms cultured from seeds of giant foxtail and velvetleaf (*Abutilon theophrasti* Medic.) recovered from soil were highly inhibitory to seedling growth (Table 1) causing up to 85 and 69% reductions in root growth for bacteria and fungi, respectively, based on lettuce seedling bioassays (Alstrom, 1987). The persistence of viable and dormant seeds in the presence of potentially detrimental microorganisms is primarily due to several inherent deterioration resistance mechanisms, which are difficult to overcome (Kremer, 1993). These mechanisms and other factors affecting microbial relationships with weed seeds and seedlings in the soil environment and agroecosystems require further investigation to provide critical information for devising effective approaches for incorporating weed-attacking microorganisms in alternative weed management strategies.

B. Factors Influencing Relationships

The population dynamics of weeds have been typically depicted in simple models to aid in understanding competition effects on crops at various stages during the life cycle of a weed (Kropf and Lotz, 1992; Lindquist et al., 1995; Maxwell and Ghersa, 1992). Occurrence of microbial associations at stages of the weed's life cycle and factors influencing these associations can be superimposed on a generic model of an annual weed to indicate the complex microorganism-weed plant relationships
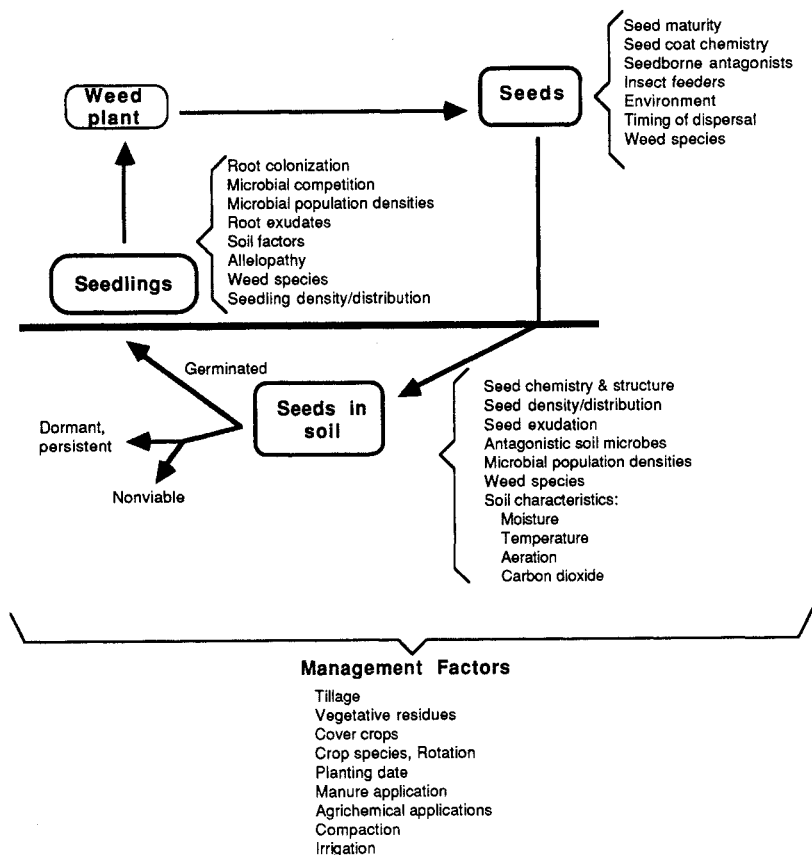
Figure 2. Factors influencing interactions of microorganisms with weed seeds and seedlings.

(Figure 2). Interactions of pathogens with actively growing weeds have been described previously and serve as bases for development of classical and mycoherbicide biocontrol strategies. The focus of the present paper is on interactions of weed seeds and seedlings with soil and rhizosphere microorganisms. Overviews on the development and deployment of pathogens for biological control of weeds are available in previous publications (Charudattan, 1991; TeBeest et al., 1992; Watson, 1991).

Several factors affect interactions of soil and rhizosphere microorganisms associated with seeds and seedlings at the various demographic stages of an annual weed (Figure 2). For example, the extent and composition of bacteria and fungi inhabiting the seed coat surface and interior seed structures while seeds develop on

the plant are affected by weed species, seed maturity, and several seed coat characteristics (Kirkpatrick and Bazzaz, 1979; Kremer, 1987; Kremer et al., 1984).

Weed seeds in soil, the seedbank, are exposed to multitudes of soil microorganisms, yet only a few studies are available on seed-microbial relationships in soil. Seedborne fungi of green foxtail [*S. viridis* (L.) Beauv.] and giant foxtail persisted as colonists of seeds along with two soilborne fungi with phytopathogenic properties after the seeds were incorporated into soil in the field (Pitty et al., 1987). Seed germination of wild oat (*Avena fatua* L.) was depressed by both soil and seedborne microorganisms in soils at moisture levels of 50% or more of water holding capacity (Kiewnick, 1964). Soil and seedborne microorganisms also affected germination of bull thistle [*Cirsium vulgare* (Savi) Tenore] seeds (van Leeuwan, 1981). Fungi associated with velvetleaf seeds on plants persist after dispersal to the soil surface and seem to antagonize potential soilborne seed pathogens thereby preventing or delaying seed deterioration (Kremer, 1986). However, velvetleaf seeds previously penetrated by seed-feeding insects while on the plant prior to dispersal are readily invaded by microorganisms, and seed viability is drastically reduced (Kremer and Spencer, 1989b). Similarly, the majority of seeds of puncturevine (*Tribulus terrestris* L.) attacked by a selective seed-feeding weevil were infected and viability destroyed by soil microorganisms when the seeds were dispersed to soil (Goeden and Ricker, 1973).

Based on limited studies, rhizospheres of weed seedlings support high numbers of diverse microorganisms similar to those documented for crop and horticultural plant species (Curl and Truelove, 1986). Fungal associations reported on weed seedlings have largely resulted from empirical observations of detrimental effects of soilborne fungi on specific weed species. Soilborne fungi examined for biological control potential inhibited seedling emergence or suppressed plant growth when the fungi were established in soil at relatively high densities (approx. 100 million or more propagules per m^2) (Boyette et al., 1984; Grey et al., 1995; Jones et al., 1988). Different soils were included as factors in one study, which revealed that the fungal-weed seedling association was not affected by soil texture (Boyette et al., 1984). Similarly, actinomycetes able to produce compounds phytotoxic to barnyardgrass (*Echinochloa crusgalli* Beauv.) seedling root growth were isolated from a diverse collection of soils with no clear indication of particular edaphic factors favoring development of inhibitory isolates (Heisey et al., 1985). The abundance and composition of bacteria colonizing the rhizospheres of weed seedlings vary among weed species (Kremer et al., 1990). Rhizobacteria are those components of rhizosphere bacteria able to colonize root surfaces, some of which detrimentally affect plant growth and vigor and are often host-specific. It was suggested that the distinctive rhizobacteria-weed species associations were influenced by exudation of specific root compounds or controlled by certain genes in the plant. A subsequent study demonstrated that specific bacteria were attracted toward the seeds or seedlings in response to exudates from both imbibed seeds and seedlings of velvetleaf (chemotaxis) in soil (Begonia and Kremer, 1994). Currently, several research projects are investigating deleterious rhizobacteria (DRB) originating from weed seeds or seedlings for potential biological control of at least 18 weed species (Kremer and Kennedy, 1996). Few studies, however, have

investigated factors that influence efficacy of DRB in soils and rhizospheres. Among these is a report indicating that cool, moist soils are most conducive to colonization of downy brome (*Bromus tectorum* L.) seedlings by the DRB *Pseudomonas fluorescens* D7 (Johnson et al., 1993).

Microbial relationships between weed seeds and seedlings are further impacted by several management factors imposed in agroecosystems (Figure 2). Nearly all research on the influence of management on soil microorganisms has dealt with those associated with crop plants. Soil continuously cropped to potato (*Solanum tuberosum* L.) caused a shift in the rhizosphere microbial equilibrium toward increasing activities of the soilborne pathogens *Verticillium dahliae*, *Rhizoctonia solani* and *Streptomyces* spp. detrimental to root development (Schippers et al., 1986). More phytotoxic rhizosphere bacteria were isolated from corn (*Zea mays* L.) grown continuously compared to corn in a corn-soybean [*Glycine max* L. (Merr.)] rotation (Turco et al., 1990). A similar situation may develop in soils under cultivation with continuous or annual velvetleaf infestations in which populations of wilt pathogens (*Verticillium* spp.) increase to cause significant reductions in velvetleaf seedling growth, competition, and seed production (Lindquist et al., 1995). Agrichemicals including herbicides and insecticides often increase rhizosphere microbial populations primarily due to stimulation of root exudates by affected plants (Curl and Truelove, 1986). Crop residues remaining on the soil surface in minimum-tillage systems can serve as substrates for specific bacteria able to produce metabolites inhibitory to seedlings (Stroo et al., 1988). Soil surface layers in long-term no-till fields contain accumulated organic and inorganic substances that may provide optimum environments for intense biological activity ideal for proliferation of weed seed predators and pathogens causing shifts in weed compositions (Cardina et al., 1991).

Development of approaches to exploit the interactions for practical application in weed management is only now being pursued by a few research projects, most of which are in the exploratory or preliminary testing stages (Kremer and Kennedy, 1996). The work summarized above illustrating microorganism-weed seed/seedling interactions and related effects of various environmental and management factors involved a limited number of weed species or was based on results from studies with crop species. It is precarious to suggest that similar relationships would occur with other weed species under the same set of factors. A more complete understanding of the ecology and biology of microorganisms that interact with specific weeds is needed not only to define their usefulness as potential biological control agents, but also to develop practices to manipulate the environment to favor either the development of naturally-occurring weed-suppressive agents or those agents introduced into the agroecosystem as part of alternative weed management approaches.

III. Potential of Soil and Rhizosphere Microorganisms in Weed Management

A recent review of research on weed seedbanks (Kremer, 1993) noted that weed infestations continue to occur despite the use of advanced weed management technologies, and that complete control of weed growth practiced over several years would not eliminate weeds in the field. Subsequent weed infestations are due to a small but highly persistent proportion of the seedbank that is not affected by conventional practices nor associated microorganisms (Figure 1). Some progress has been made in promoting the decline in weed seed numbers in soil through various cultural practices that enhance weed seed-decaying microorganisms. Weed management involving biological control may ultimately rely on several organisms each of which possesses unique mechanisms of action for controlling weeds at one or more demographic stage of development.

Although our understanding of the factors influencing the relationships between microorganisms and weed seeds and seedlings (Figure 2) is limited, enough information is available to devise strategies for exploiting these relationships for consideration in alternative weed management. Some strategies have previously been proposed (Kremer, 1993; Kremer and Kennedy, 1996) and will be summarized and amended with recent developments in this area.

A. Application of Selected Microorganisms

In studies conducted to date, selected microorganisms have typically been applied directly to soil or vegetative residues to attack germinating seeds and emerging seedlings and for eventual suppression of weed growth. This strategy seeks to regulate development of specific weeds before or coincident with emergence of crop plants. Thus, the problem weed is not eradicated, but early growth is significantly suppressed to allow the developing crop plants to effectively compete for growth requirements with the weakened weed seedlings. This strategy is most effective when weed growth coincides with environmental factors conducive to microbial growth and plant-suppressive activity, as illustrated by *P. fluorescens* D7 selected for biocontrol of downy brome in winter wheat (Kennedy et al., 1991). Selection of seed and seedling microorganisms possessing certain key properties greatly improves chances for effectiveness in soil. An ideal combination includes selective attraction of microorganisms (chemotaxis) to weed seeds and seedlings by exudates diffused from seeds released during germination or from seedling roots, rapid seed and seedling root colonizing ability, and toxin production. Chemotaxis has been demonstrated for bacterial isolates toward velvetleaf seeds and seedlings, which was related to effective seedling growth suppression (Begonia and Kremer, 1994). Aggressive root colonization was a major factor in establishing and sustaining growth suppression of downy brome by *P. fluorescens* D7 through the winter wheat growing season (Kennedy et al., 1991). Little is known about the colonizing ability of other microbial weed biocontrol agents, thus it is suggested

that this important trait be assessed in screening programs searching for effective agents (Kremer and Kennedy, 1996).

B. Integration of Multiple Agents for Weed Management

Several recent studies indicate that combinations of biocontrol microorganisms are more effective in controlling target pests in soils, spermospheres, and rhizospheres than if inocula are comprised of individual microorganisms (Fukui et al., 1994; Pierson and Weller, 1994). Increased biocontrol of soilborne diseases by combinations of biocontrol bacteria is likely due to greater diversity of introduced phenotypes able to more thoroughly colonize roots and survive the biological, chemical, and physical changes that occur in soils and rhizospheres. Thus, multiple strains of biocontrol microorganisms result in a greater variety of traits for pest suppression, which can be expressed over a wide range of environmental conditions and a broad range of microhabitats. Likewise, potential problems of inconsistent control of weeds from site to site might be overcome by devising different strain combinations for different sites to account for differences in soil properties, weed biotypes or management system. This approach has been proposed for improving the performance of bacterial control agents for take-all disease in wheat (*Triticum aestivum* L.) (Cook, 1993). The multiple strain or organism approach will aid in managing a greater diversity of weed species and biotypes, involve a wider array of biocontrol mechanisms, and be effective under a broader range of environments. These approaches may be adaptable for systems designed for site-specific management of spatially distributed aggregates or clumps of weeds, typical of many fields in row-crop production (Mortensen et al., 1993). As suggested for biocontrol of take-all disease in winter wheat (Pierson and Weller, 1994), biocontrol of weeds with DRB may ultimately require the use of many (≥ 10) "core strains" in a mixture based on weed species composition, soil type, crop cultivars, and tillage system. Preliminary research has indicated that multiple DRB strains increased suppression of downy brome in winter wheat compared with single strains (A.C. Kennedy, personal communication).

Enhancement of detrimental activity of seedborne fungi by the selective seed-feeding insect *Niesthrea louisianica* on velvetleaf seed viability has been described (Kremer and Spencer, 1989a,b). This combined biocontrol agent strategy for preventing or reducing seed production by weeds escaping early-season control could be integrated as part of a total weed management program for optimizing use of both chemical and biological approaches (Kropf and Lotz, 1992). The most practical application of DRB and insect combinations would be in situations where the insect agent feeds on roots or crowns of target weeds. Indeed, it has been suggested that leafy spurge (*Euphorbia esula* L.) control resulting from feeding by root-boring larvae of flea beetles (*Aphthona* spp.) may be enhanced due to secondary invasion by plant pathogens naturally present in soils (Rees and Spencer, 1991). Exploitation of flea beetle larvae as vectors of DRB selective for suppression of leafy spurge could contribute an additional strategy for control of this

noxious range weed and serve as a model for integration of root insect-DRB combinations on other weeds.

C. Phytotoxins

Deleterious activity toward weed seed viability and seedling growth by most microorganisms under study for biological control is due to the production of phytotoxins. The fungus *Gliocladium virens* applied to soil on a rice (*Oryza sativa* L.) grain substrate produced the herbicidal metabolite viridiol that prevented pigweed emergence without harming emerging cotton (*Gossypium hirsutum* L.) seedlings (Howell and Stipanovic, 1984). Rhizobacteria for biological control of weeds likely metabolize phytotoxins at root surfaces where they are readily absorbed by the plant. It is not known how widespread phytotoxin production is among weed biocontrol rhizobacteria, but evidence is accumulating showing that phytotoxins play a causal role in deleterious activity (Souissi and Kremer, 1994; Tranel et al., 1993). There is currently some question as to whether phytotoxins produced in culture and applied as a bioherbicide are as effective in controlling weeds compared to application of the intact organism. Durbin (1983) points out that some bacterial pathogens are unable to produce phytotoxins in culture but only produce them *in planta*, possibly due to the requirement of specific seed or seedling exudate as substrates for the inhibitory compounds. Therefore, before considerable research efforts are devoted to *in vitro* production of 'natural products' for weed control, a complete understanding of the conditions required for optimum and effective phytotoxin production is necessary. It is likely that successful establishment of rhizobacterial inocula that produce high levels of phytotoxin in the rhizosphere would be more economical than chemical synthesis of the compound (Arshad and Frankenberger, 1991).

Known metabolites produced in the rhizosphere of plants that can be phytotoxic at higher than physiologic concentrations include the auxins and hydrogen cyanide. Rhizosphere-inhabiting microorganisms likely synthesize and release auxins as secondary metabolites because of rich supplies of substrates exuded from plant roots. Some microorganisms produce auxins in the presence of a suitable precursor such as tryptophan (TRP) (Arshad and Frankenberger, 1991). The prospect of using rhizobacteria that produce excessive amounts of growth-regulating substances in the rhizosphere to suppress growth of weed seedlings has received little attention. Such rhizobacteria could selectively colonize weed seedling roots, localize excess auxin production, and minimize potential deleterious effects on crop growth. In a preliminary study, different plant seedlings reflected a wide range in response (measured as root length) to application of a selected DRB alone and in combination with TRP (Table 2). Root length inhibition ranged from 90.5% for field bindweed (*Convolvulus arvensis* L.) to 24.8% for soybean. Inoculation with the isolate alone resulted in strong inhibition of root growth in field bindweed, green foxtail, and morningglory (*Ipomoea* sp.) while lesser inhibition was observed for seedlings of wheat and soybean. The enterobacterial strain used as inoculum in this study is representative of others typically found in plant rhizospheres, which readily

Table 2. Effect of tryptophan (TRP; 10 μM) and isolate 3.8.12.7 applied alone or combined on seedling root growth of different plant species in agar bioassay

Treatment	Wheat	FBW	VL	RRP	GFT	MG	SB
Control	57.8d[a]	47.5d	59.7d	31.2c	30.9c	48.2b	25.8b
TRP alone	48.6c	38.8c	53.2c	31.5c	28.4c	49.6b	29.3b
Isolate 3.8.12.7	33.5b	10.0b	36.6b	14.6b	8.5b	13.9a	25.4b
TRP + isolate 3.8.12.7	19.1a	4.5a	17.1a	7.3a	3.8a	9.1a	19.4a

[a]Mean values within columns followed by the same letter are not significantly different at $p < 0.05$; plant species codes: FWB = field bindweed; VL = velvetleaf; RRP = redroot pigweed; GFT = green foxtail; MG = morningglory; SB = soybean. (Modified from Sarwar and Kremer, 1995.)

colonize plant roots and actively produce auxins (Sarwar and Kremer, 1995). This finding demonstrated that live inocula delivered to the rhizosphere of weeds produced effective concentrations of auxins causing phytotoxicity. Efficacy of the biocontrol organisms was further enhanced when TRP was provided as a precursor for producing phytotoxic concentrations *in situ*.

D. Formulations and Delivery of Microbial Agents

Formulation and delivery systems that promote survival and colonization of weed seeds and seedlings by DRB in the field are critical in attaining an acceptable level of efficacy. Formulations that enhanced bioactivity of biocontrol microbes against soilborne pathogens (Roberts and Fravel, 1993) and those developed for plant growth-promoting rhizobacteria (Caesar and Burr, 1991) can be adapted for use in developing prototype inoculants for weed biocontrol agents. Zorner et al. (1993) stressed the importance of utilizing formulations that alter the soil or rhizosphere environment to favor establishment and optimum activity of biological control agents. The use of exotic carbon sources can specifically enhance establishment and phytotoxic activity in the weed rhizosphere. Inoculum containing a unique carbon source that is not widespread in nature will enhance the establishment of selected agents in soil or rhizosphere and out-compete native bacteria, simultaneously producing phytotoxins to be absorbed by the weed seedling. Inocula can be prepared to contain slow-release formulations such as starch-encapsulated alginate granules (Daigle and Connick, 1990) so that the exotic carbon source will be released over time thereby extending the biological activity. Formulations can also be used to provide precursors of known phytotoxic compounds produced by the agent. For example, addition of L-TRP with the excess auxin-producing rhizobacterium *E. taylorae* 3.8.12.7 in a starch formulation applied to soil caused significant suppression of green foxtail root growth but had no effect on soybean growth (Table 3). Although preliminary, these results are encouraging and indicate the potential for manipulating weed rhizospheres by providing suitable precursors

Table 3. Effect of L-tryptophan (L-TRP) and selected rhizobacteria on root growth of soybean and green foxtail planted together in soil

Isolate	L-TRP (µM)	Root length (mm)	
		Soybean	Green foxtail
None	0	26.1	9.3
3.8.12.7	0	25.4	8.6
None	10	24.4	9.6
3.8.12.7	10	24.2	6.4[a]

[a]Significantly different ($p < 0.05$) from other treatment combinations within the column based on LSD.
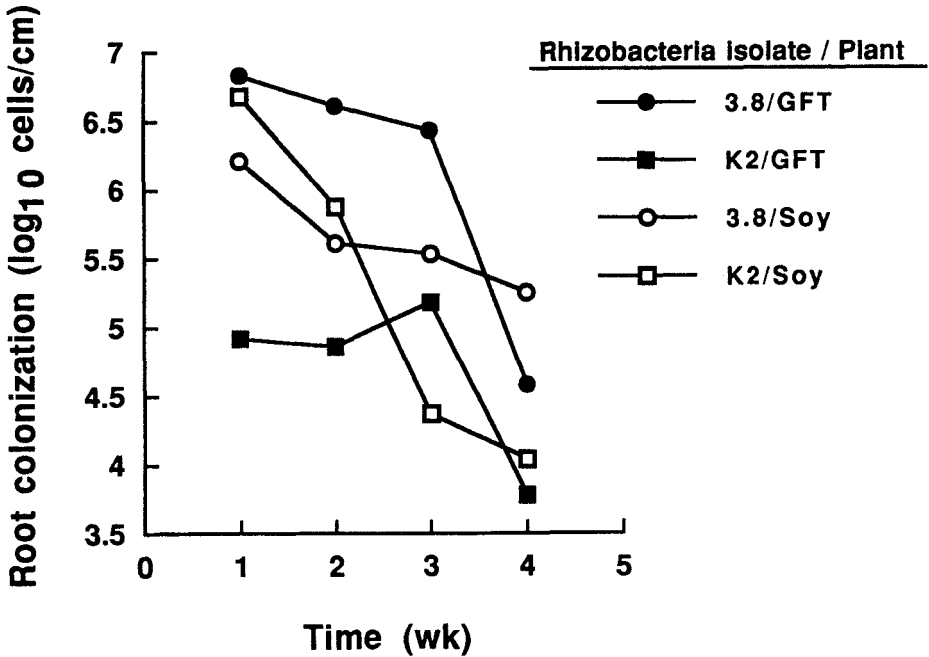(From R.J. Kremer and M. Sarwar, unpublished data.)

for production and delivery of phytotoxic compounds in soil and in the presence of crop roots.

A unique approach for delivery of microbial agents to soil infested with weed seeds is by either direct inoculation of crop seeds with the agents or by promoting colonization of crop roots by the agents in formulations applied at planting. Preliminary studies with a starch-based formulation (Connick et al., 1993) containing rhizobacteria for controlling giant foxtail showed high root colonization was achieved not only on giant foxtail but also on soybean (Figure 3). Giant foxtail growth was suppressed, but soybeans were not affected (R.J. Kremer, unpublished data). Based on this study, crop roots not only may deliver microbial agents to adjacent roots of weeds but also maintain or even enhance the agent's numbers for attack of seedlings emerging later in the season.

E. Integration with Cultural Practices

1. Agrichemicals

Integration of herbicides with biocontrol agents may be necessary to reduce adverse impacts of weeds on the crops and to establish the weed-attacking microorganisms for long-term control. Only limited examples of agrichemicals used in combination with seed and seedling microbial agents are available. Seedling emergence and seed viability of velvetleaf in soil were significantly reduced when the fungus *Fusarium oxysporum* was combined with several agrichemicals (Kremer and Schulte, 1989). Reduction in emergence of Texas gourd [*Cucurbita texana* (Scheele) Gray] seedlings by *Fusarium solani* f. sp. *cucurbitae* was enhanced when the fungus was combined with trifluralin (Weidemann and Templeton, 1988). Greaves and Sargent (1986) found that colonization of wheat roots by *Pseudomonas* spp. was greatly enhanced and resulted in extensive cellular and tissue damage when plants were treated with mecoprop. They suggested that exploitation of plant root-herbicide-microorganism interactions potentially could be an effective strategy

Figure 3. Root colonization of giant foxtail (GFT) and soybean (Soy) by rhizobacteria in starch-base inocula applied to a Mexico silt loam. (From R.J. Kremer, unpublished data.)

for biological control of weeds. Rhizobacteria inhibitory to downy brome and jointed goatgrass (*Aegilops cylindrica* Host.) exhibited higher growth-suppressive activity in soil when combined with herbicides at reduced rates of application (Kremer and Kennedy, 1996). Growth suppression by some DRB combined with metribuzin and diclofop applied at sublethal rates was additive. Diclofop plus bacteria increased root growth suppression of downy brome by 0 to 12% over diclofop alone. Further research into the mechanisms of herbicide-rhizobacteria interactions is needed to develop strategies where DRB selected for activity toward a weed can be paired with a specific chemical that increases susceptibility of the weed to the DRB. Successful development of this integrated strategy will increase efficacy of DRB agents, reduce amounts of herbicides required for weed control, and decrease potential environmental contamination.

2. Cover Crops, Mulches, and Allelopathy

Cover crops and mulches as components of alternative management systems may be used for integrating biocontrol agents by delivering the agents on seeds and promoting their establishment in soils for attack of weed seeds and seedlings prior to planting the main crop. Previous research demonstrating that certain legume cover crops promoted populations of soilborne plant pathogens of cotton (Rothrock et al., 1995) suggests that cover crops may be useful in establishing weed-attacking microorganisms in soil well in advance of weed seedling emergence. A rhizo-bacterium deleterious toward giant foxtail was coated on oat (*Avena sativa* L.) seeds and planted in soil containing giant foxtail seeds prior to planting soybeans as the primary crop. The rhizobacterium readily colonized oat seedling roots as well as those of giant foxtail seedlings (Figure 4). The vegetative growth of oats was removed after eight weeks to accommodate soybean planting. The roots continued to support rhizobacterial populations that established in soil and colonized giant foxtail seedlings emerging with soybeans (R.J. Kremer, unpublished data). Combined effects of potential allelopathic activity by the oats plus the rhizo-bacterium may also impact weed growth suppression. An apparent interaction between allelopathic substances from kochia [*Kochia scoparia* (L.) Shrad.] and the fungus *Rhizopus* sp. on inhibition of sugarbeet (*Beta vulgaris* L.) germination and seedling growth (Wiley et al., 1985) suggests the potential for discovery of similar relationships for microbial agents and crop seeds that may be useful in weed management.

Vegetative residues at or near the soil surface could serve as substrates for production of weed-suppressive agents by DRB applied directly to the residues. As previously demonstrated, numbers of wheat-inhibitory DRB increased dramatically when applied on crop residues, which promoted production of toxins inhibitory to wheat (Stroo et al., 1988). An approach is envisioned in which DRB applied on surface residues for producing phytotoxins might suppress weed growth prior to planting the crop, similar to a preemergence herbicide tactic.

3. Cropping Practices

Cultural practices used for nonchemical weed control offer convenient application methods for integrating microorganisms for biological control of weeds in cropping systems. Tillage can influence the frequency of inhibitory bacteria occurring in soil and their growth-suppressive activity. Downy brome and jointed goatgrass were suppressed by rhizobacteria under either conventional or minimum tillage suggesting that application of selected DRB during tillage may be effective in integrated weed management (Kremer and Kennedy, 1996).

Crop rotation is a practice that may also be manipulated to encourage develop-ment of specific inhibitory bacteria on weed roots. Previous work reporting a rotation effect in corn was due partly to certain rhizobacteria specifically associated with corn roots illustrates the potential for using DRB to achieve suppression of weeds in crop rotation systems (Turco et al., 1990).
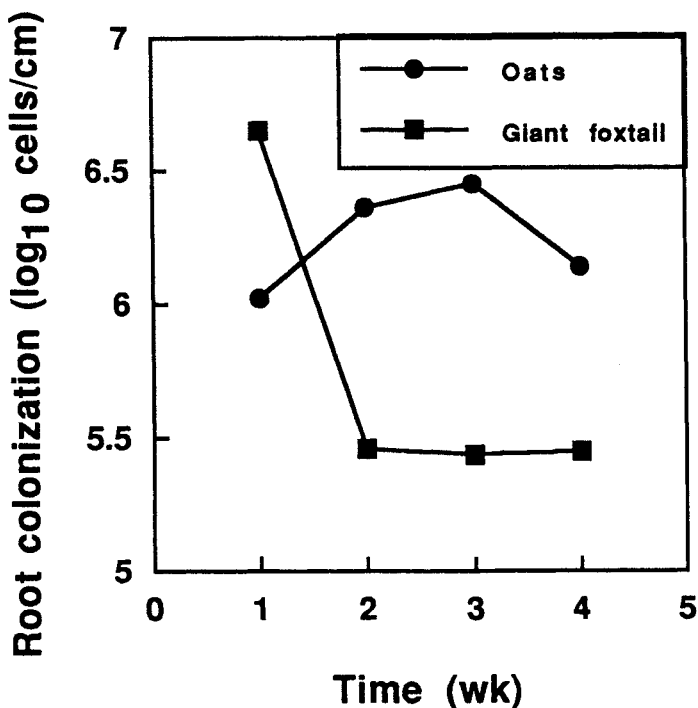
Figure 4. Root colonization of giant foxtail and oats by rhizobacterial isolate 3.8.12.7 inoculated on oat seeds and planted in a Mexico silt loam.
(From R.J. Kremer, unpublished data.)

4. Competition

Efficacy of DRB in field studies has been attributed partly to reduced competitiveness of weeds due to growth suppression by DRB (Kennedy et al., 1991). Increasing crop interference in the field by manipulating row spacing, seeding rates, and other cultural practices to suppress early weed growth has been proposed as a viable component of integrated weed management (Jordan, 1993).   Highly competitive varieties selected through crop breeding could be used in conjunction with weed-suppressive microorganisms and further enhance effectiveness and acceptance of integrated weed management systems emphasizing nonchemical control.

IV. Conclusions and Research Needs

An urgent need exists to develop alternative weed management systems that will reduce herbicide use, provide optimum crop yields, and protect the environment.

The need to provide efficacious weed control with low inputs of agrichemicals will determine research approaches taken to develop alternative weed management programs. For biological control components to be seriously considered in these alternative programs, considerable research in development and implementation of effective agents will be required. Intensive fundamental investigations in the areas of ecology and biological activity of microorganism-weed seed/seedling relationships are necessary for better understanding and exploitation of biocontrol mechanisms, development of suitable formulations for use in the field, and integration of biocontrol into weed management and agricultural practices. As more efficacious microbial-based strategies for consistent suppression of weed growth are developed, prospects for acceptance of these strategies in production systems and commercial development should increase considerably. Microbial approaches that target the seed and seedling stages of weeds are additional biological control strategies that can supplement current mycoherbicides and offer augmentative control options as herbicide use becomes more restricted.

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