Exhibit 5b, #94

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Growth Suppression of Annual Weeds by Deleterious Rhizobacteria Integrated with Cover Crops

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Abstract

Development and rapid acceptance of biological control is challenged by factors limiting the spectrum of activity, efficacy, and reliability. Effectiveness of biological control may be best demonstrated as a component in an overall biological weed management system. Cover crops as components of biological weed management may be used for integrating biological control agents by promoting establishment in soils for attack of weed seedlings prior to planting the main crop. The objective of this study was to evaluate several cover crop species alone and integrated with soilborne deleterious rhizobacteria (DRB) for weed management potential. In each year of the study, cereal grain cover crops reduced weed biomass 90% compared to weedy checks. *Brassica* cover crops and sweetclover (*Melilotus officinalis* [L.] Lam.) reduced weed biomass to greater extent when combined with soil-applied DRB. DRB were detected on roots of cover crops and established on roots of adjacent weed seedlings for subsequent growth suppression. In 1998 soybean (*Glycine max* [L.] Merr.) planted in several cover crop residues without herbicide gave seed yields higher than weedy checks and equivalent or higher than conventionally grown soybean. Weed suppression was further enhanced when DRB were included. Integration of nonchemical weed control methods has potential in reducing herbicide use and enhancing efficacy of biological weed management.

Keywords: allelopathy, biological weed management, deleterious rhizobacteria, integrated biological control, seedbanks

Public concerns about environmental contamination, soil erosion and degradation, pesticide residues in foods, development of herbicide-resistant weed biotypes, development of new weed problems formerly of secondary importance due to single control approaches, and other social, economic and health-related impacts of conventional agriculture has increased interest in agricultural sustainability. Thus, there is a growing trend toward development of agroecosystems that rely on the management of ecological interactions rather than on dependence on agrichemical use to maintain productivity (Liebman and Ohno 1998). Within the sustainable agroecosystem concept, the approach to weed

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control is through "integrated weed management" in which all available strategies including tillage, cultural practices, herbicides, genetic manipulation, allelopathy, and biological control are used to enhance the competitiveness of the crop over the weeds (Aldrich and Kremer 1997, Dieleman and Mortensen 1998). The development of integrated weed management considers all aspects of the particular cropping system where each management component contributes to weed suppression but not necessarily complete control (Dieleman and Mortensen 1998). Weed management viewed in this context illustrates how biological and ecological aspects of weeds can be used as a multiple control strategy to reduce the weed seedbank in soil, prevent weed emergence, and minimize competition from weeds growing with desired plants (Aldrich and Kremer 1997).

Development and rapid acceptance of biological control as a practical management option in cropping systems is challenged by factors limiting their spectrum, efficacy and reliability. Biological control as a single tactic approach is often not effective in long-term weed management. Effectiveness of biological control, therefore, may be best demonstrated as a component of weed management where it could be integrated with other weed control methods (Boyetchko 1996). Further, biological control could be exploited in "biological weed management," the use of living organisms or their products to reduce or prevent the growth and reproduction of weeds (Cardina 1995). One or more organisms (microorganisms, plants, insects, etc.) may be manipulated in ways that enhance means of suppressing development of weeds at one or more phases of the life cycle. Cover crops as components of biologically-based management systems may be used for integrating biological control agents by promoting establishment in soils for attack of weed seeds and seedlings prior to planting the production crop. This approach would meet the requirement of providing an environment that sustains the biological control agents (Boyetchko 1996). Currently cover crop use in many regions is limited and few investigations have examined efficacy of cover crops without using herbicides. For example, rye (*Secale cereale* L.) and hairy vetch (*Vicia villosa* Roth.) under no-tillage could only reduce weed density an average of 78% without supplemental herbicides (Teasdale *et al.* 1991). A wider range of cover crop species under various cultural practices needs study for weed suppressive effects. Weed suppression by cover crops may involve allelopathy, the potential of which in integrated systems is essentially unknown.

Deleterious rhizobacteria (DRB), naturally-occurring segments of rhizosphere microbial communities that are toxigenic but not parasitic, have potential to suppress weed growth through manipulation of rhizosphere ecosystem (Kremer and Kennedy 1996). However, successful performance of soil-applied biological control agents is hampered by inconsistent effectiveness often caused by poor survival or low activity of the introduced organisms (Van Elsas *et al.* 1992). Cover crops offer a synergistic weed management approach by serving as a delivery system for soilborne biotic agents such as DRB, by providing nutrients and a suitable environment for DRB proliferation and activity, and by aiding in establishing DRB in soil prior to weed germination and emergence (Teasdale 1998, Kremer 1998).

The typical broad spectrum of weeds in production fields has stimulated investigations on the selectivity of weed suppression by various cover crops and on developing approaches to integrate these into weed management for specific, regional cropping systems (Teasdale 1998). Also, biological control alone may only target one or two weed species in cropping systems and cannot be relied on as a single-tactic approach (Cardina 1995). In this study we examine combinations of several cover crop species alone and

integrated with soilborne deleterious rhizobacteria for weed management potential.

Materials and Methods

Various cover crops (Table 1) were established in 1997 and 1998 at two sites (Bradford and Sanborn) in mid-Missouri on Mexico silt loam (fine, montmorillonitic, mesic, Mollic Endoaqualf). The Bradford site, 16 km east of Columbia, MO, has a history of corn (*Zea mays* L.) and soybean (*Glycine max* Merr. [L.]) production under minimum tillage; the Sanborn site at the Sanborn Field long-term experimental field on the campus of the University of Missouri, Columbia, was in permanent meadow for ten years before establishment of the present study. Prior to establishing the studies soil cores were collected from the upper 10-cm of the profile at both sites and processed for weed seedbank analysis (Forcella *et al.* 1992). After separation from soil components, seeds were categorized according to plant genus or species and placed on agar (1%) for 72 h at 28°C. Viable seeds included germinated seeds and seeds that remained hard (non-imbibed) or firm but not decomposed after incubation. Crops were broadcast or drilled at standard seeding rates after shallow roto-tillage in small plots (2-m by 2-m) in randomized complete block designs with three replications. Plots were split with subplots inoculated with or without DRB selected for growth suppression of giant foxtail (*Setaria faberi* Herrm.) and velvetleaf (*Abutilon theophrasti* Medik.).

The DRB isolates, identified using the API-NE20 diagnostic kit (Bio Mericux Vitek

Table 1.
Cover crop species evaluated for weed growth suppression at two field sites in mid-Missouri in 1997 and 1998.

Family	Cover crop species	Common name, variety
Brassicacaea	*Brassica napus* L.	Canola var. 'Victoria'
	Brassica napus L.	Rapeseed var. 'Dwarf Essex'
	Brassica juncea L.	Green mustard var. 'Greenwave'
	Brassica rapa L. subsp. *narinosa*	Green mustard var. 'Tatsoi'
Fabaceae	*Lespedeza stipulaceae* Maxim	Korean lespedeza var. 'Marion'
	Medicago lupulina L.	Black medic var. 'Alliance'
	Medicago sativa L.	Alfalfa var. 'Nitro'
	Melilotus officinalis (L.) Lam.	Sweetclover, yellow-blossom VNS[a]
	Trifolium alexandrinum L.	Berseem clover VNS
	Vicia villosa Roth	Hairy vetch VNS
Poaceae	*Hordeum vulgare* L.	Winter barley VNS
	Lolium multiflorum Lam.	Annual ryegrass VNS
	Secale cereale L.	Winter rye VNS
	Triticum aestivum L.	Winter wheat var. 'Cardinal'
Polygonaceae	*Fagopyrum sagittatum* Gaertn.	Buckwheat VNS

[a]VNS, "variety not stated" by supplier.

Inc., Hazelwood, MO), were *Enterobacter taylorae* 38128 (origin, giant foxtail), *Alcaligenes faecalis* K2 (origin, giant foxtail), and *Pseudomonas* sp. 2129 (origin, velvetleaf). Single cultures of each isolate were grown 36 h in King's B broth, combined in equal volumes and suspended in 0.1M $MgSO_4$ (Kremer *et al.* 1990), and applied with crop seeds at planting to deliver 10^8 colony-forming units (cfu) per m^2 using a backpack sprayer. The mixed inoculum was re-applied at soybean planting after cover crop termination.

In order to document establishment of DRB in the field, spontaneous antibiotic-resistant mutants of the inoculum DRB were developed with resistance to 100 ug/ml nalidixic acid + 80 ug/ml rifampicin. The mutants were individually inoculated on seeds in separate plots adjacent to the main study. At weekly intervals, seedlings were retrieved, root biomass and lengths determined, suspended in 0.01M $MgSO_4$, diluted and plated on antibiotic-containing King's B agar medium following procedures described previously (Kremer *et al.* 1990). Root colonization of seedlings of cover crops and selected weeds by applied DRB was expressed as $\log_{10}$ cfu per cm root.

Cover crop and weed above-ground biomass were harvested in June and August 1997 and June 1998 by clipping all growth at the soil surface level within a 0.1 m^2 quadrat randomly placed in each subplot. Biomass samples were separated into cover crop, grass, and broadleaf components and dried at 65°C for 72 h for dry weight determinations.

In 1998 cover crops were terminated on 18 May by mowing followed by glyphosate application at 1.12 kg/ha. Soybean (cv. 'Pioneer 9395') was planted in 38-cm rows with a no-til planter. Soybean was harvested on 15 October by threshing plants removed from 1-m row segments. Soybean grain yields were expressed on a 12% moisture basis.

Data were subjected to appropriate analysis of variance procedures. Treatment means were compared by using Fisher's protected least significance difference (LSD) at 95% level of probability.

Results and Discussion

Weed seedbanks differed between sites with at least seven predominant species at Bradford (reflecting history of cultivated row crops) and only two at Sanborn (reflecting long-term perennial forage management) (Table 2). Seed density among plots varied widely with nearly all species showing spatial distribution.

Total biomass accumulation was greatest for winter rye, winter wheat, winter barley, yellow-blossom sweetclover, and annual ryegrass; total weed biomass was reduced greatest by these cover crops (Tables 3 and 4). Other legumes suppressed weeds to a lesser extent due to low stand establishment when seeded in the fall. Soil inoculation with selected DRB combined with cover crops reduced weed biomass compared to most noninoculated plots. Broadleaf and grass biomass was equally suppressed by winter barley, winter wheat, winter rye, annual ryegrass, and sweetclover regardless of inoculation with DRB (Tables 3 and 4). Brassica crops generally reduced broadleaf weeds moreso than grasses (largely giant foxtail); when combined with selected DRB, each weed component was reduced somewhat. Variable response to cover crops by weed species has been reported previously (Teasdale 1998). Brassica cover crops varied in biomass production (oilseed rape > green mustard > canola > Tatsoi mustard) but appeared equivalent in selectively suppressing *Amaranthus* species throughout the season and suppressing late-season giant foxtail (data not shown). Sustained weed suppression by *Brassica* spp. after mowing sug-

Table 2.
Seedbank densities of weed species in Mexico silt loam at two sites in mid-Missouri.

Weed species	Viable seed	
	Bradford	Sanborn
	(no. per sq. m X 100)	
Amaranthus spp.	22 (4.80)	3 (0.8)
Field pennycress (*Thlaspi arvense*)	9 (2.65)	0
Pennsylvania smartweed (*Polygonum pensylvanicum*)	8 (1.8)	0
Morningglory spp. (*Ipomoea* spp.)	7 (1.5)	1 (0.4)
Common ragweed (*Ambrosia artemisiifolia*)	2 (1.05)	0
Velvetleaf (*Abutilon theophrasti*)	2 (0.5)	0
Giant foxtail (Setaria faberi)	95 (9.6)	3 (0.8)
Various grasses	0	582 (66.7)

Mean with (SEM).

Table 3.
Cover crop and weed biomass (dry weight) with and without selected deleterious rhizobacteria at Bradford in 1997.

Cover crop	Cover crop		Grass weed		Broadleaf weed	
	No DRB	+DRB	No DRB	+DRB	No DRB	+DRB
	g/m^2					
Annual ryegrass	150	190	160	100	90	50
Winter barley	1450	1400	0	0	10	30
Winter rye	1290	1050	0	0	0	0
Winter wheat	1300	1350	0	0	10	20
Sweetclover	700	700	170	130	90	120
Hairy vetch	300	300	100	170	150	210
Canola	270	260	40	30	40	20
Rapeseed	270	450	20	20	10	0
Mustard, green	260	270	20	10	40	20
Mustard, Tatsoi	230	260	80	70	70	30
Weedy check	_	_	150	160	220	260
LSD (0.05)	100	80	25	30	40	20

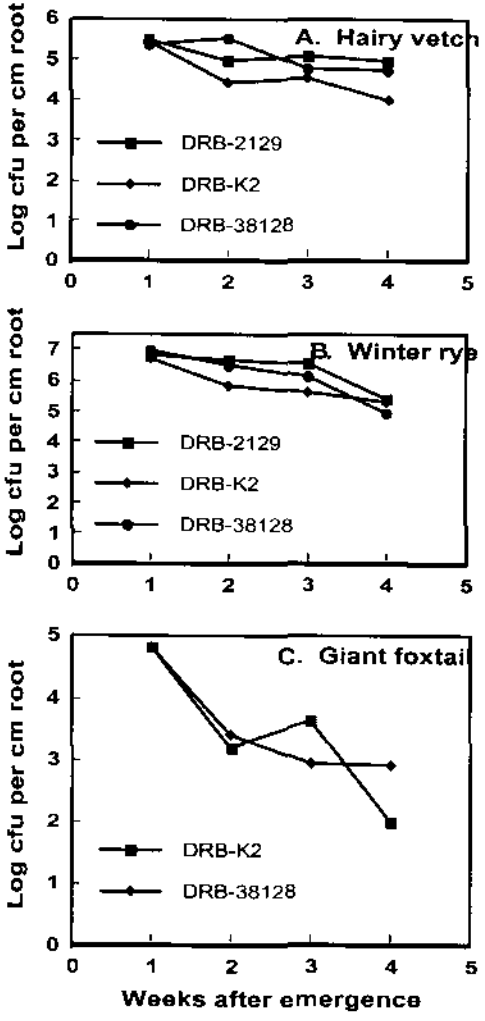

Fig. 2. Colonization of hairy vetch (A) and winter rye (B) seedling roots by selected DRB applied with seeds at planting. Colonization of giant foxtail (C) occurred from DRB established on roots of cover crops growing nearby.

spatial distribution of weeds within experimental area, and small plot sizes contributing to variability among replicates. Interestingly, soybean yields in most cover crop treatments were higher or equivalent to soybean in adjacent field plots grown under "conventional" practices that included herbicides applied at recommended rates for weed control (Table 5).

DRB marked with antibiotic resistance established high and stable populations on roots of cover crops, i.e., hairy vetch and winter rye during the four week sampling period (Fig. 2A and B). The two DRB isolates originating from giant foxtail also colonized roots of noninoculated giant foxtail seedlings adjacent to the inoculated cover crops (Fig. 2C). This indicated that soil-applied DRB survived and established in soil for subsequent colonization on target weeds in the cover crop system. Similarly, a previous report demonstrated a plant-growth-promoting pseudomonad inoculated on various crops colonized roots of the weeds pigweed (*Amaranthus retroflexus* L.) and barnyardgrass (*Echinochloa crus-galli* [L.] Beauv.) by horizontal migration from the inoculated crops up to 60 cm away (Wiehe and Höflich 1995). The present study is the first to report on establishment of DRB from inoculated cover crops on roots of later-germinating weed seedlings for subsequent weed growth suppression in the field. These results also verify a model proposed by Skipper *et al.* (1996) illustrating that rhizobacteria developing on crop roots likely persist in the rhizosphere for sub-

sequent colonization and suppression of emerging weed seedlings.

The results of the present study suggest that integration of nonchemical weed control methods have potential in suppressing weed growth and reducing herbicide use. Cover crops used in this system served multiple important functions that included providing suitable environment for establishment and proliferation of soilborne biological control agents, releasing allelochemicals that contributed to weed suppression, and disrupting the soil environment to minimize weed establishment (Cardina *et al.* 1999). Other attributes of cover crops include promoting naturally-occurring weed suppressive microorganisms (Cardina *et al.* 1999), providing habitats for seed predators for reducing weed seedling emergence (Reader 1991), and enhancing plant-growth-promoting bacteria on the production crop (Sturz *et al.* 1998) that may also complement the interaction with applied weed-suppressive DRB and will be examined in future studies. Cover crops for integrating biological control by delivering the agents on seeds and promoting their establishment in soils for attack of weed seedlings prior to planting the main crop is similar to a "system management" approach recently demonstrated where a crop underseeded with a living green cover and treated with a postemergence bioherbicide resulted in successful control of the target weed and control of other weeds by the cover crop (Pfirter *et al.* 1997). Research efforts are underway to develop biological control agents highly effective in suppressing a broad spectrum of weeds, to refine formulations for optimum survival and field application, and to define conditions under which cover crops stimulate the establishment of weed-suppressive microorganisms.

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