Weed Terminator20 Field Trials Report

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Investigator: R.J. Kremer, University of Missouri, Columbia, MO

Objective: Determine effects of Weed Terminator20 treatments applied as weed management practice on soil biology.

Summary:
Soybean (Roundup Ready) was planted at Bradford Farm in May 2017 and 2018 at two different sites. Weed Terminator20 plus the surfactant Boost was applied as two treatments, label and 2X label rates, periodically after weed emergence. Soybean and weed plant roots and soils were excavated on July 7, 2017 and July 19, 2018 when soybeans were in the full-flower to initial pod formation growth stage (R2). The 2017 site had considerable weed pressure allowing complete collection of weeds from all sampled experimental plots and were grouped into waterhemp and grass categories for analysis. The 2018 site had a variable weed density and control by all treatments was improved relative to 2017, which provided very few surviving weeds for sampling. Thus, due to lack of samples representing each treatment and/or each replicate plot, no biological analyses for weeds in 2018 are available. The lack of weeds in 2018 allowed sampling of the "weed-free" check, which was not sampled for biological analysis in 2017. Rainfall patterns between years differed by distribution and with considerably more rainfall occurring during the growing season in 2017 compared with 2018 (Figure 1). Growing season total rainfall was 24.6 and 14.7 inches in 2017 and 2018, respectively. Effects of subsequent differences in soil moisture are reflected in somewhat different biological values noted when comparing yearly results (for example, some microbial component sizes decreased in 2018 compared to 2017, Tables 3 and 4). Due to the differences in rainfall patterns and weed pressure, the results are presented separately for each year rather than combined and averaged over the two years. Regardless, it is readily apparent that Weed Terminator20 treatments resulted in no detrimental effects on biological parameters measured in this study compared with the Roundup (glyphosate) treatment. Effects of Weed Terminator20 did not differ from non-herbicide treatments ("weedy" and "weed-free") on biological parameters.

Compared with Roundup (glyphosate), significant decreases in soybean root colonization by potential pathogenic *Fusarium* spp. were noted for Weed Terminator20 treatment; numbers of beneficial root bacteria (fluorescent pseudomonads) also increased - enhancement of this group of bacteria generally suggests an increased suppression of fungal antagonists in the rhizosphere and potentially reduced root infection. Numbers of Mn-oxidizing bacteria on roots were generally reduced with Weed Terminator20; more importantly, Weed Terminator20 was associated with favorable proportions (high ratio) of Mn-reducing to Mn-oxidizing bacteria, which was maintained on soybean roots compared with RR soybean+Roundup (Tables 1 and 2). Weed Terminator20 did not change or improved total soil microbial biomass and specific microbial components (Tables 3 and 4) thereby maintaining soil microbial diversity and functionality, compared with suppressive effects of glyphosate on some microbial components

and on microbial biomass. Furthermore, Weed Terminator20 did not affect soybean nodulation (Table 5) or suppress soil biological activity, represented by soil glucosidase enzyme (Table 6). The use of Weed Terminator20 in this study did not disrupt overall soil biology and maintained the benefits of an active biological component that contributes to vigorous plant growth and soil health.

Soil/rhizosphere microbiological assays:
Rhizosphere microbial activity generally differed among treatments.
We have consistently documented considerable increases in *Fusarium* root colonization and decrease in beneficial microorganisms in glyphosate-resistant soybean and corn with use of Roundup (Kremer and Means, 2009; Means and Kremer, 2007; Zobiole et al., 2010). Although *Fusarium* is a ubiquitous group of soil fungi, many species are opportunistic phytopathogens and may cause economically important diseases including wilts, root rots under optimum or stressed environmental conditions. Thus, a high root colonization by *Fusarium* species indicates a high potential for detrimental effects on plant growth. Both 2017 and 2018 soybean studies confirmed that glyphosate treatment leads to high *Fusarium* root colonization relative to soybean not receiving glyphosate (Tables 1 and 2). Weed Terminator20 treatments showed significantly (p<0.05) reduced root colonization similar to non-herbicide -treated soybean, suggesting these did not predispose roots to fungal colonization. Enhancement of beneficial microorganisms that suppress *Fusarium* growth and root colonization is due to increases in the beneficial rhizobacteria, fluorescent pseudomonads, Mn-reducing bacteria and indole acetic acid (IAA)-producers, confirm this interaction (Tables 1 and 2). The IAA-producing bacteria are important in providing plant growth promoting substances that stimulate root growth, which counteracts the root growth inhibitory effects of available glyphosate in the rhizosphere (Helander et al. 2019). The Mn-reducing bacteria are important in mobilizing this micronutrient for plant uptake; reductions in this bacteria group may impact multiple metabolic pathways in the plant including photosynthesis and respiration, especially on soils that are insufficient or deficient in Mn for adequate plant growth. Results clearly show that Mn reducer/oxidizer ratio were disrupted with glyphosate use (likely due to chelating properties of glyphosate) while Weed Terminator20 appeared to sustain a favorable ratio of Mn reducer/oxidizer bacteria (Tables 1 and 2). The IAA producing bacteria stimulate root growth and aid in plant nutrient uptake. These bacterial groups were suppressed by glyphosate in 2017 and 2018, confirming previous studies on the Mexico silt loam at the experimental sites.

Soil microbiome characterization:

The soil microbiome (microbial community) was characterized using phospholipid fatty acid (PLFA) analysis. This analysis detects lipids of microbial membranes as “biomarkers” for specific groups of microorganisms producing a profile or “fingerprint” of the community structure. Biomarkers specific to functional groups of microorganisms include bacteria, actinobacteria, fungi, arbuscular mycorrhizal fungi (AMF), and protists. Thus, rapid changes in the microbial community structure are based on changes in PLFA patterns, which we expected to occur in the rhizospheres of soybean and weeds. Also, the total PLFA concentration is a measure of viable microbial biomass. Based on the present studies, soybean rhizosphere soils from the non-

glyphosate treatment including Weed Terminator20 supported higher total microbial biomass (Tables 3 and 4). Although total PLFA does not provide information on microbial community composition, higher biomass suggests a more complex community structure relative to rhizospheres with lower total PLFA, which was apparent from detrimental effects of glyphosate. Most microbial components were maintained at levels higher in non-glyphosate treatments, however, significantly higher densities of arbuscular mycorrhizal fungi (AMF) and protozoa were consistently detected for Weed Terminator20 in soybean and weed rhizospheres (Tables 3 and 4). Higher densities of AMF associated with roots suggest that an effective symbiosis between the plants and fungus was established and maintained for efficiently exchanging plant-available nutrients and water extracted from root-free soil by AMF hyphae as well as contributing to soil aggregation and structure. Higher protozoa component suggests that an effective soil food web remained in place for enabling optimum nutrient cycling within the rhizosphere.

Soil biological activity:

Soybean nodulation (Table 5) measured as nodule mass per plant is an indicator of nitrogen-fixation ability and efficiency. We have observed detrimental effects of glyphosate on soybean nodulation in many previous studies (Kremer et al. 2009), which was demonstrated in this study. Weed Terminator20 treatments consistently supported the highest soybean nodulation in both study years and obviously does not cause suppression of nodule development associated with glyphosate. In some cases, it seems Weed Terminator20 might stimulate nodulation when compared with the weed-free and weedy control treatments. However, any possible direct treatment influence would require further studies to confirm this observation.

Soil glucosidase activity is considered a good indicator of management-induced changes in soil health and is sensitive for differentiating effects of crop management on biological activity and soil health. The substrate for glucosidase, cellobiose, is a product of cellulose decomposition and is involved in carbon cycling. Weed Terminator20 treatments did not suppress soil glucosidase activity in contrast to the lower activity found for glyphosate. This suggests that microbial activity represented by soil glucosidase would not be detrimentally affected by Weed Terminator20 used as a weed management practice.

References:

Helander, M., Pauna, A., Saikkonen, K., Saloniemi, I. 2019. Glyphosate residues in soil affect crop plant germination and growth. Scientific Reports *(2019) 9:19653* | https://doi.org/10.1038/s41598-019-56195-3

Kremer, R.J., Means, N.E., and Kim, S. 2005. Glyphosate affects soybean root exudation and rhizosphere microorganisms. Int. J. Environ. Anal. Chem. 85:1165-1174.

Means, N.E., Kremer, R.J. 2007. Influence of soil moisture on root colonization of glyphosate-treated soybean by *Fusarium* species. Comm. Soil Sci. Plant Anal. 38:1713-1720.

Zobiole, L.H., R.J. Kremer, R.S. Oliveira, and J. Constantin. 2010. Glyphosate affects microorganisms in rhizospheres of glyphosate-resistant soybeans. J. Appl. Microbiol. 110:118-127.

Table 1. Root and soil biological parameters under various weed management practices in soybean, 2017.

Microbiologial Assay Results - 2017												
	Fusarium Root Colonization (Colonies/100 cm root)		Rhizosphere fluorescent pseudomonads X 10,000 cfu/g soil		IAA-producing rhizobacteria X 10,000 cfu/g soil		Mn-Reducing Rhizobacteria X 1000 cfu/g soil		Mn-Oxidizing Rhizobacteria X 1000 cfu/g soil		Mn Reducer/Oxidizer Ratio	
SOYBEAN:												
Glyphosate	26.07	a	6.9	b	50.0	b	8.13	a	26.25	a	0.30	b
Weed Terminator20 - 1X	12.89	b	180.0	a	212.5	a	8.75	a	5.50	b	1.68	a
Weed Terminator20 - 2X	12.11	b	208.8	a	148.8	a	14.13	a	10.43	b	1.41	a
Weedy Check	12.01	b	238.8	a	175.0	a	8.50	a	9.00	b	1.07	a
LSD (0.05)	*6.14*		*82.4*		*80.0*		*10.00*		*5.16*		*0.71*	
GIANT FOXTAIL:												
Glyphosate	33.69	a	14.9	b	21.8	b	4.38	b	14.13	a	0.37	b
Weed Terminator20 - 1X	15.14	c	181.3	a	140.0	a	8.00	b	4.75	b	1.67	a
Weed Terminator20 - 2X	15.72	b	95.0	a	117.5	a	15.75	a	14.25	a	1.14	a
Weedy Check	22.66	b	162.5	a	125.0	a	11.38	a	8.38	a	1.38	a
LSD (0.05)	*7.21*		*35.6*		*28.2*		*5.16*		*6.90*		*0.49*	
WATERHEMP:												
Glyphosate	25.39	a	9.3	c	43.8	b	5.88	b	16.38	a	0.39	b
Weed Terminator20 - 1X	12.89	b	85.0	b	145.0	a	10.13	b	8.63	b	1.22	a
Weed Terminator20 - 2X	12.70	b	106.3	ab	120.0	a	13.38	a	18.50	a	0.72	a
Weedy Check	12.60	b	116.3	a	126.3	a	18.63	a	18.38	a	1.04	a
LSD (0.05)	*5.67*		*29.7*		*27.2*		*6.9*		*3.8*		*0.32*	
Means followed by same lower-case letter do not differ significantly at 5% probability level based on least significance determination (LSD)												
cfu: colony-forming units												
IAA: indole-acetic acid												
Mn: manganese												

Colonization or infection of roots by *Fusarium* fungi expressed as fungal colonies per 100-cm of root

Mn Reducer/Mn Oxidizer Ratio is the Proportion of Mn-reducing bacteria to Mn-oxidizing bacteria

Table 2. Root and soil biological parameters under various weed management practices in soybean, 2018.

Microbiologial Assay Results - 2018												
	Fusarium Root Colonization (Colonies/100 cm root)		Rhizosphere fluorescent pseudomonads X 10,000 cfu/g soil		IAA-producing rhizobacteria X 10,000 cfu/g soil		Mn-Reducing Rhizobacteria X 1000 cfu/g soil		Mn-Oxidizing Rhizobacteria X 1000 cfu/g soil		Mn Reducer/Oxidizer Ratio	
SOYBEAN:												
Glyphosate	28.92	a	27.75	b	3.3	b	16.20	a	55.45	a	0.29	b
Weed Terminator20 - 1X	13.22	b	92.50	a	20.3	a	18.50	a	11.50	b	1.60	a
Weed Terminator20 - 2X	11.45	b	103.50	a	27.5	a	27.56	a	19.34	b	1.42	a
Weedy Check	9.42	b	113.25	a	13.8	a	19.25	a	18.50	b	1.04	a
Weed-free Check	14.00	b	78.00	a	13.12	a	17.00	a	18.00	b	0.94	a
LSD (0.05)	*10.20*		*46.00*		*9.8*		*15.00*		*10.45*		*0.66*	
Means followed by same lower-case letter do not differ significantly at 5% probability level based on least significance determination (LSD); cfu = colony-forming units												
cfu: colony-forming units												
IAA: indole-acetic acid												
Mn: manganese												

Colonization or infection of roots by *Fusarium* fungi expressed as fungal colonies per 100-cm of root

Mn Reducer/Mn Oxidizer Ratio is the Proportion of Mn-reducing bacteria to Mn-oxidizing bacteria

Table 3. Microbial characterization of rhizosphere soils of soybean and selected weeds from plots receiving various weed control treatments, July 2017.

Weed Terminator 20 - 2017 Soybean trial, Bradford Farm, Univ of Missouri. Summary means of soil PLFA results.								
		PLFA, nanomoles/g soil						
Soybean:		G neg	G pos	Actino	Fungi	AMF	Protozoa	Total PLFA
	Glyphosate	113.20a	31.12a	20.14a	6.27a	5.89b	2.54b	146.52b
	Weed Terminator20 1X	140.55a	28.63a	20.017a	4.74a	8.90a	4.17a	235.71a
	Weed Terminator20 2X	121.68a	25.88b	16.20a	3.91a	8.06a	3.68a	225.49a
Weedy	No Herbicide	69.64b	27.46a	24.22a	4.60a	4.25c	1.97b	196.95a
	LSD (0.05)	*38.15*	*4.18*	*10.42*	*3.46*	*1.16*	*1.03*	*47.20*
Giant Foxtail:	Glyphosate	40.36a	26.55a	13.50b	3.47a	3.88b	1.72b	118.44a
	Weed Terminator20 1X	41.68a	26.44a	16.13a	3.69a	5.09a	2.64a	126.55a
	Weed Terminator20 2X	35.66a	26.27a	15.86b	2.455a	4.46a	2.66a	120.68a
Weedy	No Herbicide	40.583a	28.32a	15.54b	3.40a	5.54a	2.88a	127.30a
	LSD (0.05)	*7.98*	*3.70*	*2.60*	*1.24*	*0.58*	*0.45*	*11.07*
Waterhemp sp.:	Glyphosate	40.05a	27.54a	15.42a	3.43a	4.33b	2.36b	118.79b
	Weed Terminator20 1X	48.56a	28.11a	15.92a	3.54a	5.90a	4.26a	139.47a
	Weed Terminator20 2X	44.45a	28.84a	15.62a	3.36a	5.89a	3.71a	128.05b
Weedy	No Herbicide	40.17a	27.58a	16.46a	3.38a	5.02b	2.20b	120.46b
	LSD (0.05)	*28.47*	*4.00*	*1.65*	*1.51*	*1.04*	*1.08*	*14.16*

Means within a column followed by same lower-case letter do not differ significantly based on least significance determination (LSD) at the 5% level of probability.

G neg, Gram-negative bacteria; G pos, Gram-positive bacteria; Actino, Actinobacteria; Fungi, saprophytic fungi; AMF, Arbuscular mycorrhizal fungi; Total PLFA, total extracted soil phospholipid fatty acid contents representing viable soil microbiome (a surrogate for total living soil microbial biomass)

Table 4. Microbial characterization of rhizosphere soils of soybean from plots receiving various weed control treatments, July 2017.

Weed Terminator 20 - 2018 Soybean trial, Bradford Farm, Univ of Missouri. Summary means of soil PLFA results.									
		PLFA, nanomoles/g soil							
Soybean:		G neg	G pos	Actino	Fungi	AMF	Protozoa	Total PLFA	
	Glyphosate	41.23	23.17	13.27	5.64	3.83b	3.83b	115.76b	
	Weed Terminator20 1X	41.60	24.27	14.18	5.53	5.11a	4.78a	126.57a	
	Weed Terminator20 2X	40.24	23.37	12.76	6.97	5.95a	4.96a	129.33a	
Weedy	No Herbicide	46.87	25.05	13.67	6.22	5.42a	4.92a	137.19a	
	Weed-free	52.70	26.52	14.66	6.85	5.24a	5.34a	139.28a	
	LSD (0.05)	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*1.05*	*0.90*	*10.20*	

Means within a column followed by same lower-case letter do not differ significantly based on least significance determination (LSD) at the 5% level of probability.

G neg, Gram-negative bacteria; G pos, Gram-positive bacteria; Actino, Actinobacteria; Fungi, saprophytic fungi; AMF, Arbuscular mycorrhizal fungi; Total PLFA, total extracted soil phospholipid fatty acid contents representing viable soil microbiome (a surrogate for total living soil microbial biomass)

Table 5. Soybean nodulation on root samples of soybean affected by weed control treatment collected at stage R2 (pod initiation to early pod formation).

Soybean Nodulation (Growth Stage R2)				
	Nodule mass, mg/plant			
	2017		**2018**	
Glyphosate	1440	c	1590	d
Weed Terminator20 - 1X	2275	a	2495	a
Weed Terminator20 - 2X	2070	ab	2370	ab
Weedy Check	1960	b	2200	bc
Weed-free	1850	b	2050	c
LSD (0.05)	*285*		*305*	

Means within a column followed by same lower-case letter do not differ significantly based on least significance determination (LSD) at the 5% level of probability.

Table 6. Glucosidase activity detected in soybean rhizosphere soils from various weed management treatments at Bradford Farm in 2017 and 2018.

Soil Glucosidase Activity				
	µg PNP/g soil			
	2017		**2018**	
Glyphosate	75.40	b	61.32	b
Weed Terminator20 - 1X	121.55	a	104.19	a
Weed Terminator20 - 2X	113.60	a	88.44	a
Weedy Check	115.75	a	88.67	a
Weed-free	108.45	a	86.21	a
LSD (0.05)	*28.50*		*30.5*	

The "µg PNP/g soil" is the unit for enzymatic activity based on production of para-nitrophenol in micrograms per gram dry soil from a substitute substrate of para-nitrophenyl carbohydrate.

Figure 1. Rainfall for Bradford Farm, Boone County, Missouri during 2017 and 2018 growing season.