The Multi-Functional Brassinosteroid Biostimulant, *Vitazyme*, Improves Plant Growth, Soil Health and Tolerance to Glyphosate Herbicide Stress

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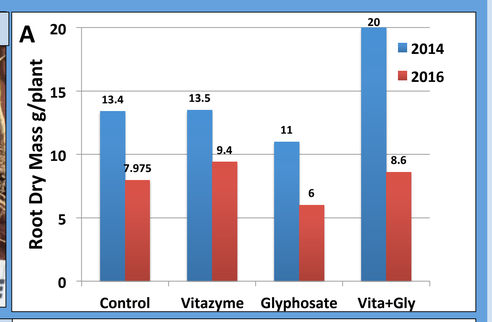
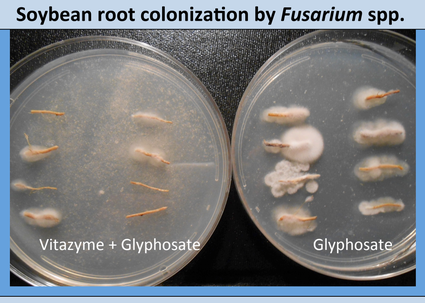
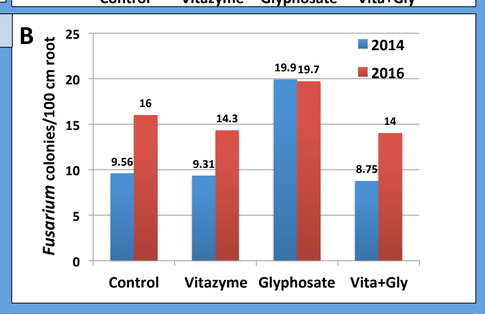
Figure 1. Soil health biological indicators for soybean production with glyphosate herbicide and *Vitazyme* biostimulant

Soybean root biomass enhanced by Vitazyme

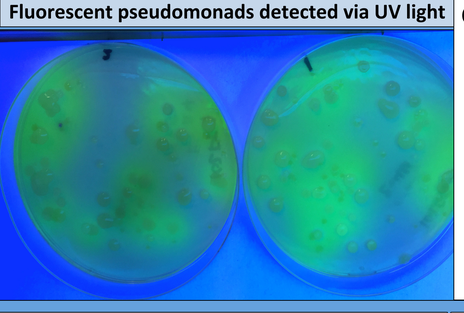


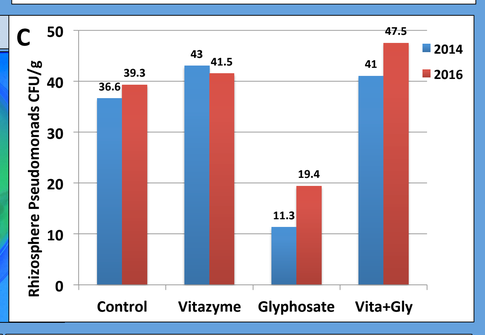
Soybean root colonization by *Fusarium* spp.



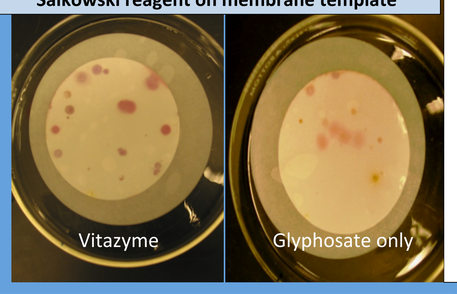


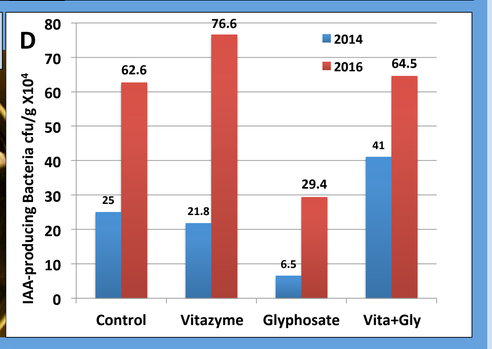
Fluorescent pseudomonads detected via UV light



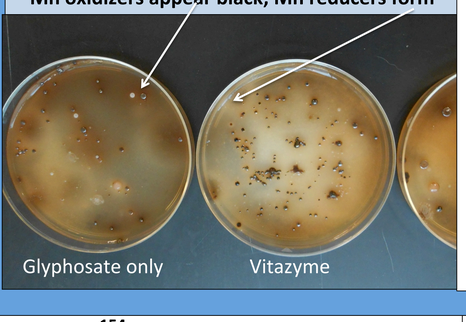


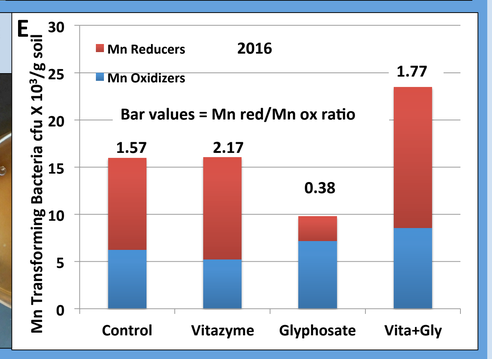
IAA bacteria detected via pink color reaction with Salkowski reagent on membrane template

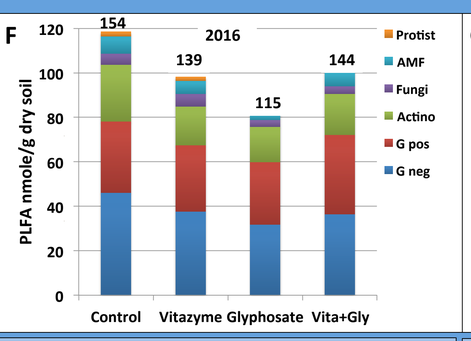


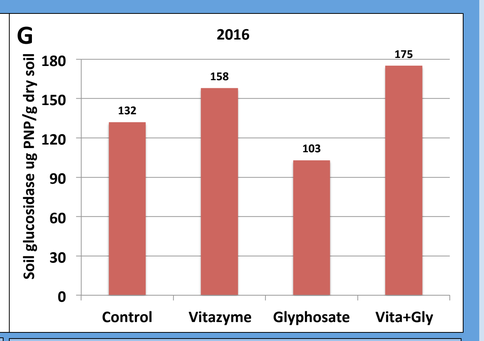


Mn-transforming bacteria on selective medium; Mn oxidizers appear black; Mn reducers form









Soybean rhizosphere soil microbial diversity expressed as soil microbial PLFA components. Bar values = Total PLFA (microbial biomass)

Soybean rhizosphere soil microbial activity indicated by soil glucosidase, a C-cycling enzyme.

Experimental

- Field trials conducted at Columbia (2014) and Novelty (2016-17) Missouri on Mexico sil soil (fine, smectitic, mesic Vertic Epiaqualfs), maize and soybean planted conventionally using minimum tillage; plants and soils collected at R2 soybean & V10 maize growth stage
- Root-colonizing *Fusarium* assessed by selective culture technique (Levesque et al. 1993)
- Rhizosphere pseudomonads determined using S1 agar medium (Gould et al. 1985)
- Indoleacetic acid-producing (IAA) bacteria detected on nitrocellulose membranes reacted with Salkowski reagent for color development (Bric et al. 1991)
- Mn-transforming bacteria detected on Gerretsen's medium (Huber & Graham 1992)
- Glucosidase activity detected using enzyme assay of Eivazi & Tabatabai (1988)
- Soil microbial components and biomass determined using phospholipid fatty acid (PLFA) analysis (Buyer & Sasser 2012; Pritchett et al. 2011)

Introduction

Biostimulants enhance nutrient uptake and crop quality, however, knowledge of effects on soil health is limited (Kremer 2017a). The biostimulant *Vitazyme* provides several modes of action mediated by multiple active brassinosteroids. Attributes of brassinosteroid-based biostimulants include enhanced root development, improved nutrient uptake efficiency, disease suppression, and environmental stress tolerance (Hayat & Ahmad 2011). Limited research has shown that brassinosteroids may regulate pesticide metabolism in plants (Zhou et al. 2015), however, no information is available on potential for overcoming stress of the herbicide glyphosate on rhizosphere biology in transgenic (genetically-modified, [GM]) cropping systems. Glyphosate affects rhizosphere microbial diversity and activity, increased fungal root colonization, decreased beneficial bacterial components, and detrimental effects on crop root growth (Kremer & Means 2009; Wagner et al. 2003). *Vitazyme* was applied in soybean and maize field trials in Missouri USA during 2014 - 2017, with and without glyphosate application.

Objectives

- Determine effects of *Vitazyme* on selected soil health indicators
- Determine effects of Vitazyme on rhizosphere biology in transgenic soybean and maize receiving glyphosate in field trials
- Determine potential effects of Vitazyme on crop growth in soils with residual glyphosate contents

Results

***Vitazyme* - improved soil health indicator and overcame glyphosate effects on maize and soybean at all sites across years:**

- Root growth (dry biomass) improved (Figs. 1A & 2A) while colonization by potentially pathogenic *Fusarium* fungi was significantly ($p<0.05$) reduced (Figs. 1B & 2B), root nodulation on soybean improved or did not decrease with glyphosate + *Vitazyme* (data not shown)
- Rhizosphere fluorescent pseudomonads, the majority contribute beneficial plant growth-promoting functions, and IAA-producing bacteria, significantly increased in both glyphosate-treated crops (Figs. 1C & 2C and Figs. 1D & 2D, respectively)
- Maintained high Mn reducers to Mn oxidizers ratio, notably in presence of glyphosate, suggesting adequate available Mn (reduced) for plant and microbial uptake and metabolism, and limiting unavailable, immobilized Mn (oxidized) associated with glyphosate and oxidizing bacteria (Figs. 1E and 2E)
- Maintained soil microbial community composition (PLFA groups) and total microbial biomass in glyphosate treated crops at similar levels of control, non-glyphosate soils, which tended to improve mycorrhizal component (Figs. 1F & 2F)
- Enhanced glucosidase activity, indicator of soil microbial function, in both control and glyphosate treatments (Fig. 1G)

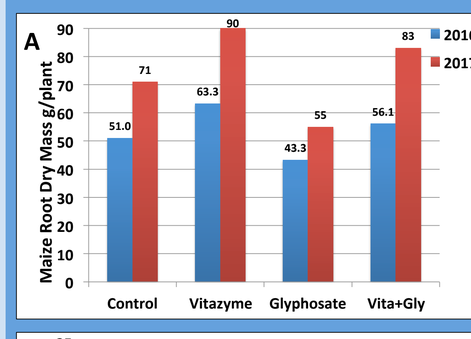
Glyphosate Soil Residues and Grain Yields:

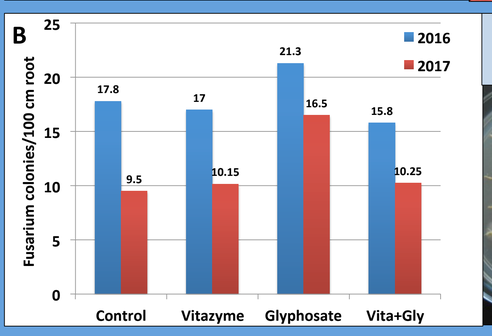
- Residual glyphosate contents in soil ranged from 0 to >1000 µg/kg (ppb) of soil, which was randomly distributed among plots at field sites, regardless of glyphosate application timing
- Soybean grain yields increased 1 to 4% with *Vitazyme* while maize yields increased 1 to 4% (data not shown)
- No relationship between yields of individual plots with soil glyphosate content was found for maize (2016)
- Apparent relationship between *soybean* yield in individual plots with soil glyphosate - tendency for lower yield with higher soil glyphosate; *Vitazyme* may offset potential yield decline due to glyphosate buildup in a Mexico silt loam (6 of 8 plots; Fig. 3)

Conclusions

- A polyphasic microbial analysis entailing multiple assessments of sensitive soil health indicators, previously used to evaluate GM crop effects on soil biology and ecology (Kremer & Means 2009), was successfully adapted for evaluating the biostimulant *Vitazyme* as a means of suppressing effects of glyphosate on root growth and rhizosphere biology in (GM) cropping systems and maintaining or improving soil health.
- Soil microbial diversity based on PLFA analyses was restored by *Vitazyme* in soils planted to maize and soybean treated with glyphosate; a high microbial diversity is essential to maintain a stable ecosystem and crop productivity (Grayston et al. 1998).

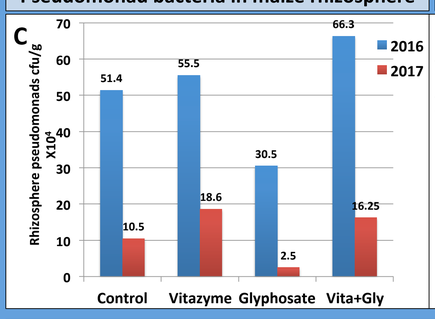
Figure 2. Soil health biological indicators for maize production with glyphosate herbicide and *Vitazyme* biostimulant



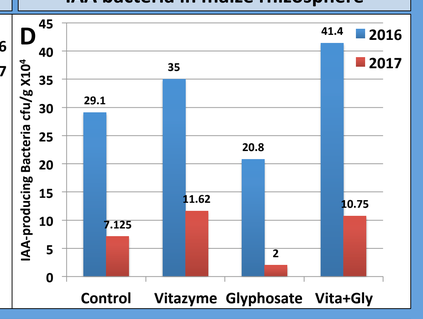


Maize root colonization by *Fusarium* spp.

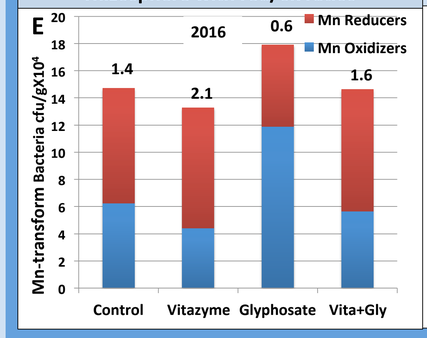
Pseudomonad bacteria in maize rhizosphere



IAA bacteria in maize rhizosphere



Mn-transforming bacteria in maize rhizosphere with red/ox ratios



Maize rhizosphere soil microbial diversity. Bar values = Total PLFA (microbial biomass)

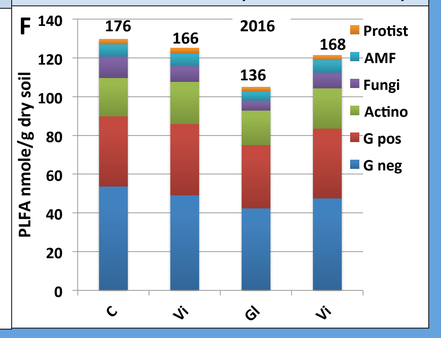
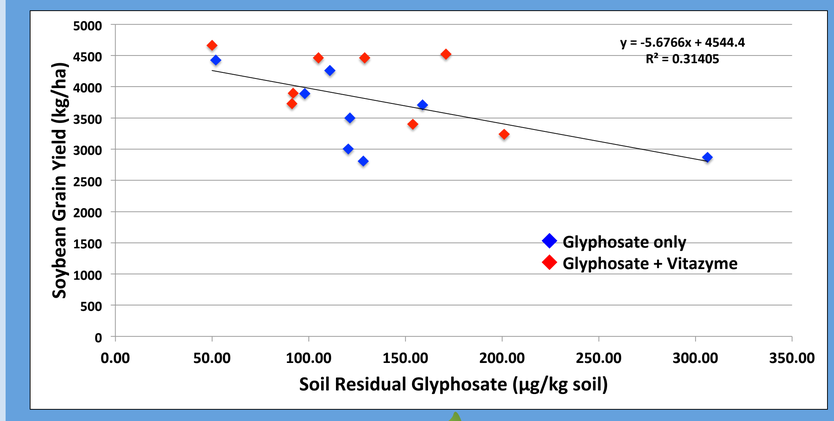


Figure 3. Potential effect of *Vitazyme* on relationship of soil residual glyphosate and soybean grain yield (2014 & 2016). Individual plot data)



Conclusions continued:

- Glyphosate residue buildup in soils under GM crops is a reality (Kremer 2017b) and may affect crop growth and yields in future seasons. Biostimulants such as *Vitazyme* might offset effects of residual glyphosate (Fig. 3), *however more evaluation under rigorous experimental conditions is required to confirm effects*.
- Results from this project demonstrate that biostimulants can be a major management factor for addressing recurrent productivity problems and declining soil health associated with GM crops in current crop production systems

Note: References cited herein are available as hardcopy handouts or as a pdf upon request.