Glyphosate and Transgenic Crops: Impacts on Soil Biology and Ecology and the Environment

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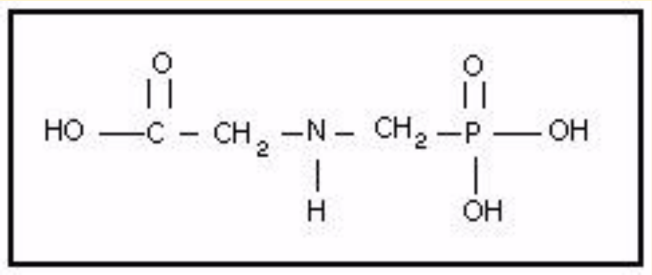


BIOTECHNOLOGY VS GENETIC ENGINEERING

- A matter of terminology and mis-information -

“Traditional definition” - “the integrated use of microbiology, biochemistry and engineering sciences in order to achieve the technological application of the capacities of microorganisms and cultured tissue cells” (Lynch, 1983).

“Soil biotechnology” - study and manipulation of soil microorganisms and their metabolic processes to optimize crop production.

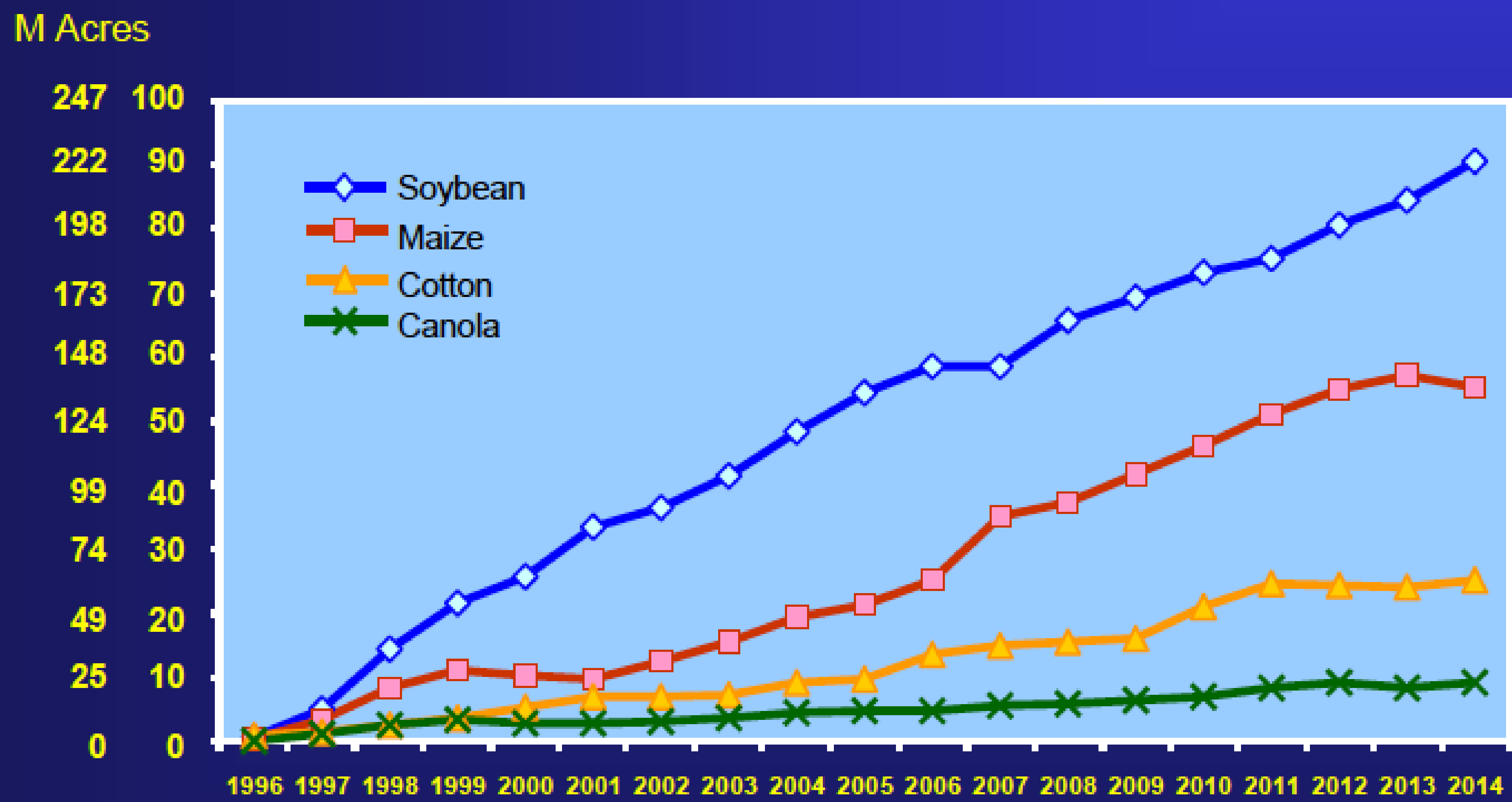
“Modern biotechnology”- (evolved in the latter twentieth century) - intentional manipulation of genes:

a)from different organisms to generate changes in the genetic makeup of a target organism using recombinant DNA or direct injection of nucleic acid into cells, resulting in Genetically Modified or Genetically Engineered, or transgenic organisms (Mosier and Ladisch, 2009). [GMO or GE]

b)By “gene editing” of the genome (DNA) directly within the target organism

GM, GMO, GE

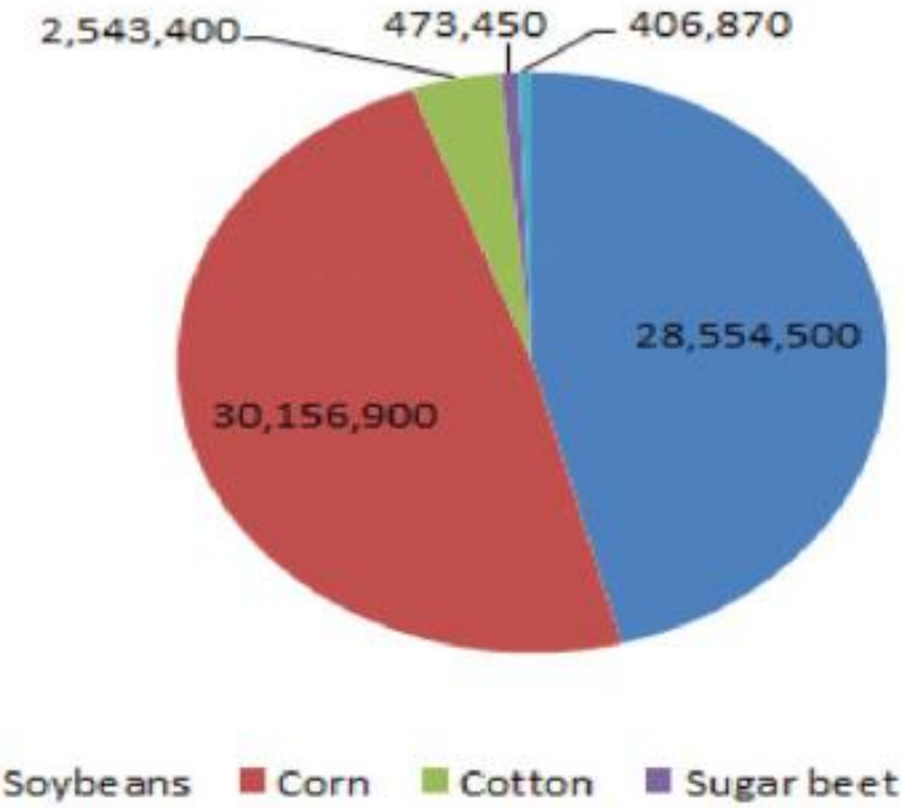
Global Area of Biotech Crops, 1996 to 2014: By Crop (Million Hectares, Million Acres)



James, Clive. 2014. Global Status of Commercialized Biotech/GM Crops: 2014. *ISAAA Brief* No. 49. ISAAA: Ithaca, NY.

The International Service for the Acquisition of Agri-biotech Applications (ISAAA).

U.S. GE - Herbicide Resistant Crop Area



U.S. Glyphosate use, 2007: ≤ 185 million pounds U.S. Atrazine use, 2007: 78 million pounds

Figure 1. United States GM HT crop plantings 2013 by crop (hectares).Sources: derived from USDA, ISAAA, GfK Animal and Crop Health
Note: Base area of the 5 crops 62.1 million ha.

Brookes, G. (2014) Weed control changes and genetically modified herbicide tolerant crops in the USA 1996–2012 . GM Crops & Food: 5:321-332

National Academy of Science. 2016. Genetically Engineered Crops: Experiences and Prospects. The National Academies Press. Washington DE.

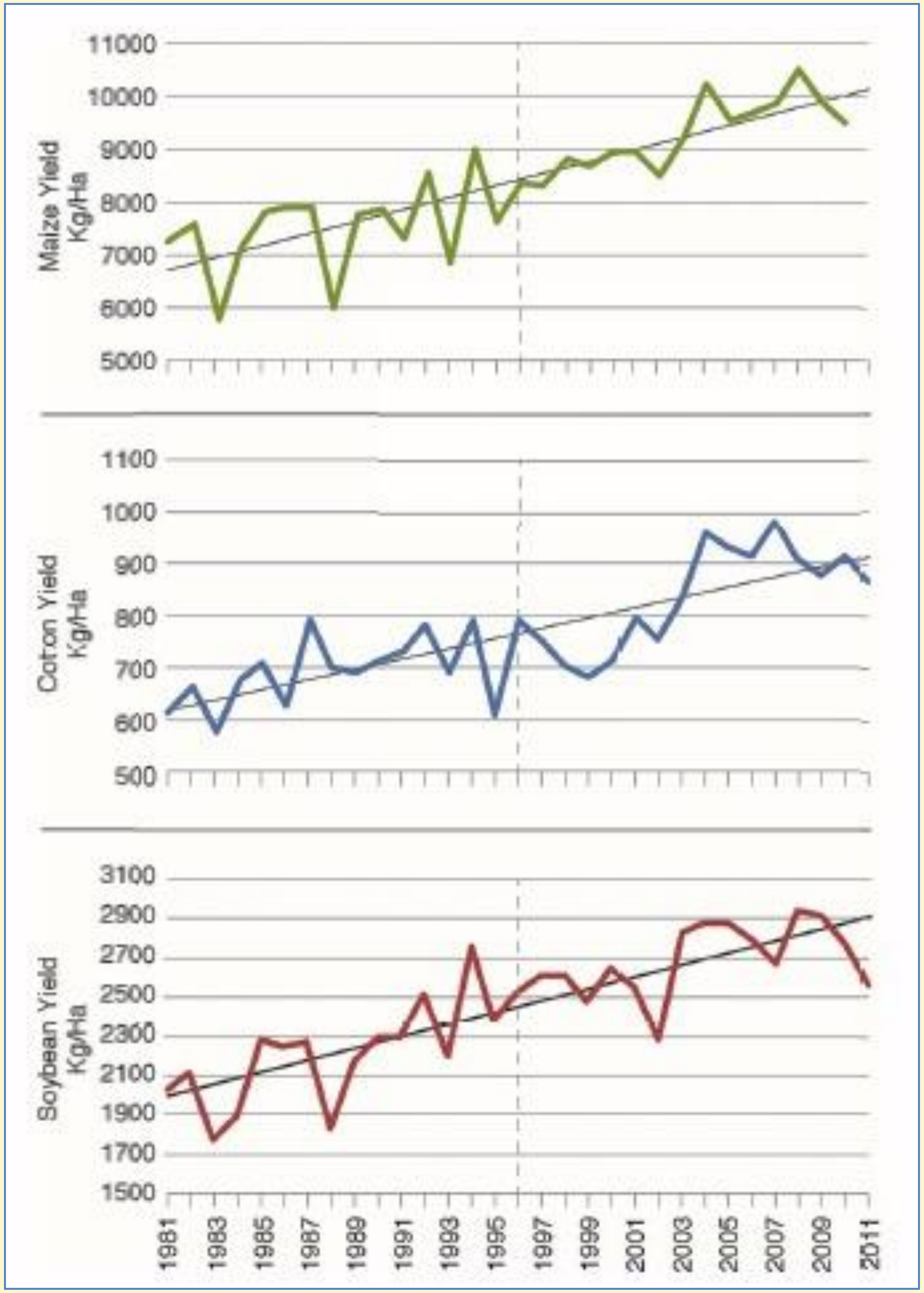


FIGURE 4-2 Yields of maize, cotton, and soybean in the United States, 1980–2011. SOURCE: Duke (2015). NOTE: Dashed line indicates when GE varieties of these crops were first introduced in the United States.

Impacts of "GMO" Cropping Systems on Soil Function or Soil Health*

*Impacts of GE plant alone due to *pleiotropic* or unintended effects of transgenic transformation

NOTE: Based on limited number of studies; generalizations that effects apply to ALL GE crop varieties used in crop production *cannot be made.*

KEY

GMO - Genetically-modified organism

GE - Genetically engineered

Bt - plant engineered to express production of insecticidal protein via incorporation of genes originating from the bacterium *Bacillus thuringiensis*

GR - plant engineered to express resistance to herbicide glyphosate via incorporation of genes that code for glyphosate-resistant enzyme in amino acid synthesis pathway, originating from selected bacteria

Mycorrhizae - symbiotic, beneficial fungi associate with most plants that aid in nutrient and water uptake; disease suppression; contribute to soil structure

NOTE: Supporting literature available.

GE Trait Alone (Bt & GR)
Alter root exudates; **GE-DNA released into environment**
Alter root microbial symbioses
Soil fauna suppression (Bt)
Shift root-associated microbial community structure & function
Alter plant nutrient uptake
Increase susceptibility to soybean cyst nematode
Alters nutrient uptake; photosynthetic patterns; mycorrhizae (GR weeds)
Higher water requirement (poor water-use efficiency) - GE soybean
Bt rice affects soil microbial biomass
Bt toxin increases glyphosate persistence in soil
Bt cotton inferior disease resistance; exude higher amino acids from roots
Bt traits alter N-transforming bacteria & archaea (bacterial-like microbes)
Bt toxins leached from crop residues; affect aquatic organisms

Impacts of GE, Glyphosate-based Cropping Systems on Soil Function or Soil Health

NOTE: Listing of indicators of environmental impacts; may not be able to generalize due to conditions of tests (soil, application methods, etc.)

KEY

Rhizobia - symbiotic, beneficial bacteria associate with leguminous plants (soybean, pea, clovers) that make atmospheric nitrogen available for plant nutrition

Mycorrhizae (AMF) - symbiotic, beneficial fungi associate with most plants that aid in nutrient and water uptake; disease suppression; contribute to soil structure

N, P, K - Nitrogen, Phosphorus, Potassium

**Effects of Glyphosate alone due to direct contact with soil during application to GE crop or burndown (knockdown)

NOTE: Supporting literature available.

GE Trait + Glyphosate (GR)	Glyphosate Alone**
Alter microbial structure/diversity	AMF germ., infection suppressed
Suppress antagonistic microbes	Reduce earthworm activity
Increase *potential* root pathogens **<**	Increase potential pathogens/Induce Antibiotic Resistance
Increase nutrient immobilization	Nutrient immobilization
Residues in soils/sediments/water<	**Residues: soil/sediment/water <**
Decrease soil N uptake	Increase foliar/seed fungi
Rhizobia infection, nodulation, N fixation decrease	Potential increased mycotoxin production - grain, roots (?)
Mycorrhizae affected; alters P, K, water uptake; pathogen protection **<**	Glyphosate absorbed by by non-target plants/crops in soil
Stimulate 'sudden death syndrome' disease (soybean)	Negative effects on rhizosphere microrganisms
Glyphosate released thru roots	Reduce beneficial leaf bacteria
Glyphosate re-absorbed by roots lead to root damage, pathogen attack	Reduce/suppress several classes of soil fauna; **Affects Respiration<**
Alter C metabolism & transport; carbohydrate accum. in roots, leaves	Detrimental metabolic effects some invertebrates- **soil & aquatics**
Glyphosate-laced plant materials decrease activity/function in some	Mobilize adsorbed xenobiotics from soil (DDT) & sediments

How do GE crops “Alone” affect soil and environmental health?

Limited documented evidence on effects of genetic transformations alone on alterations of crop physiological activity (including nutrient accumulation in grain)

=> “Pleotropic Effects” (Indirect or non-intended)

Information on effects in Genetically Engineered **(GE)** crops is slowly accumulating

Continued trend of modern cultivar selection?

***Increasingly difficult to discuss impacts of GE crops without also considering contribution of Glyphosate to overall effect*

Are differences in seed viability, germination, seedling vigor due to <u>pleiotropic effects</u>?

Conventional

Transgenic

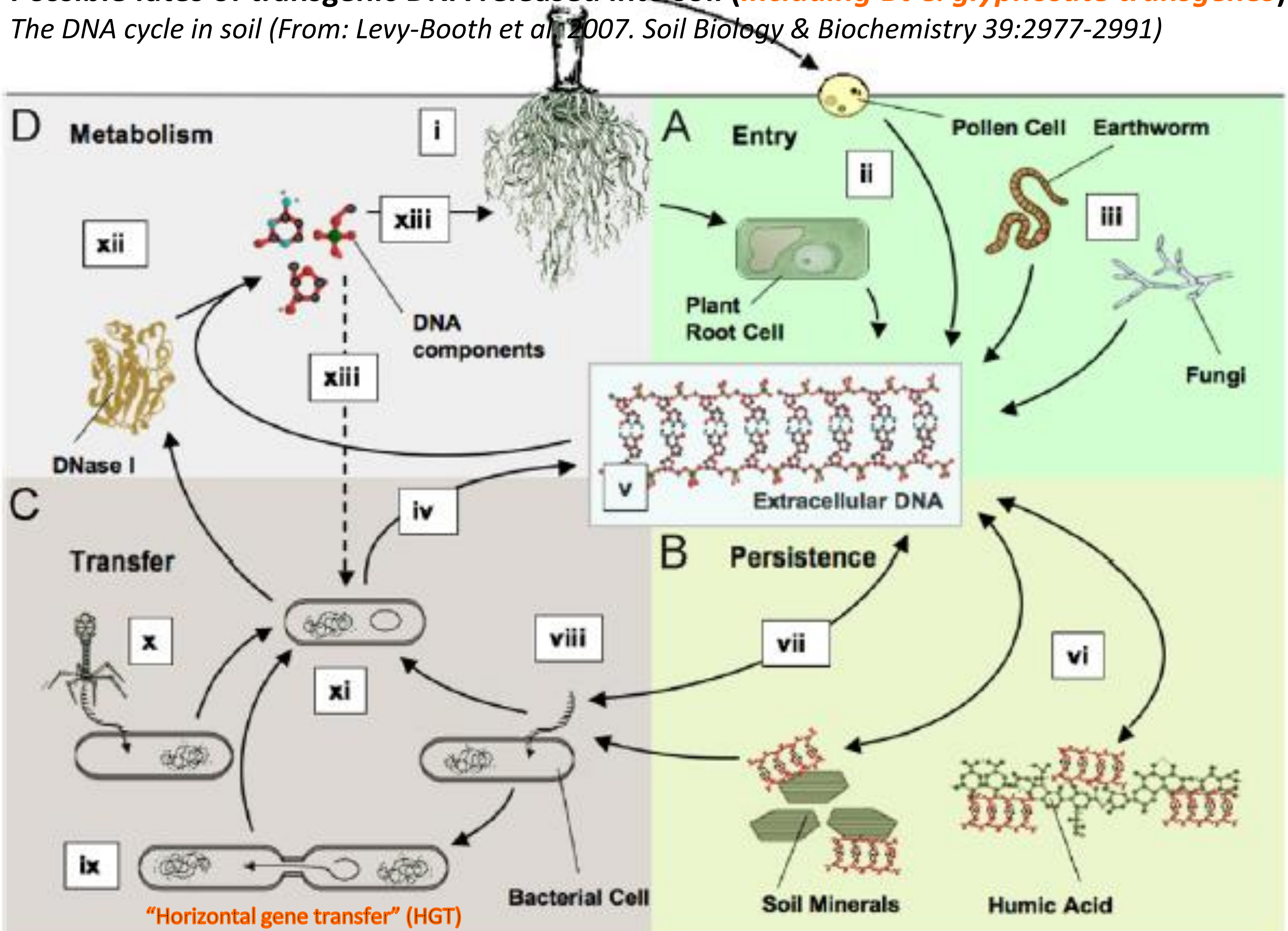
Genes coding for resistance to glyphosate and for Bt insecticidal toxins can be released into soil from roots of transgenic plants

Detected “large concentrations” of transgenic DNA in the soil food web in fields after 3 years of Roundup Ready corn – Roundup Ready soybean rotation; provides an opportunity for “horizontal gene transfer” (HGT) of transgene into native soil bacteria. Whether these genes would be functional or pose other risks within the soil ecosystem is not known.

Hart, M.M., J.R. Powell, R.H. Gulden, D.J. Levy-Booth, K.E.Dunfield, K.P. Pauls, C.J. Swanton, J.N. Klironomos, J.T. Trevors. Detection of transgenic cp4 epsps genes in the soil food web. Agron. Sustain. Dev. 29:497–501.

Possible fates of transgenic DNA released into soil (*Including Bt & glyphosate transgenes*)

The DNA cycle in soil (From: Levy-Booth et al. 2007. Soil Biology & Biochemistry 39:2977-2991)

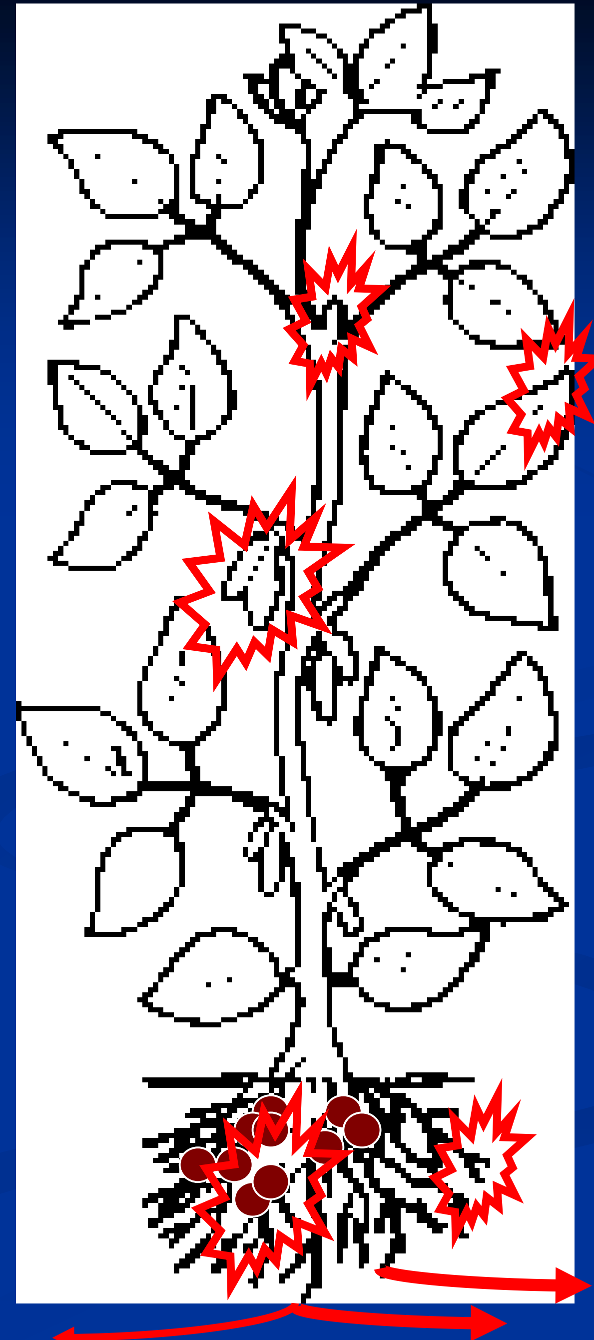


(A) Entry of DNA into Soil; (B) Persistence of DNA in Soil; (C) Transfer of DNA in Soil; (D) Degradation of DNA in Soil

All plant DNA is transient in soil – rapid degradation suggests limited opportunity for natural transformation of transgenic DNA into soil microbes – Gulden et al. 2008. Weed Science 56:767-774

Roundup-susceptible

GE - Roundup-resistant



Key Concept: Glyphosate contributes to overall impact of GE crops with herbicide resistance (GR) --

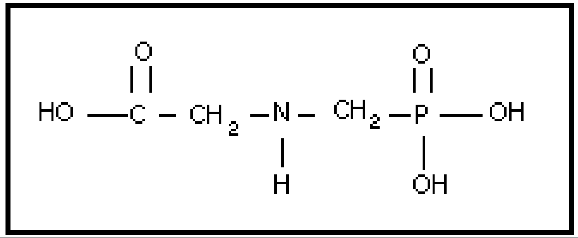
Glyphosate *systemic* – transported throughout the plant.

In RR soybean, glyphosate has been found *localized* in meristematic tissues of growing points, leaves and roots; pod and seed; nodules.

Also glyphosate is *actively released through roots* into rhizosphere soil (root zone) likely with high amounts of carbohydrates & N-compounds in RR soybean -

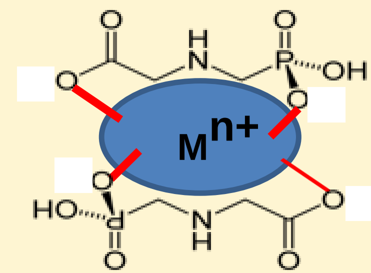
**May affect nearby non-GE plants

(Kremer et al. Int. J. Environ. Anal. Chem. 85:1165, 2005)

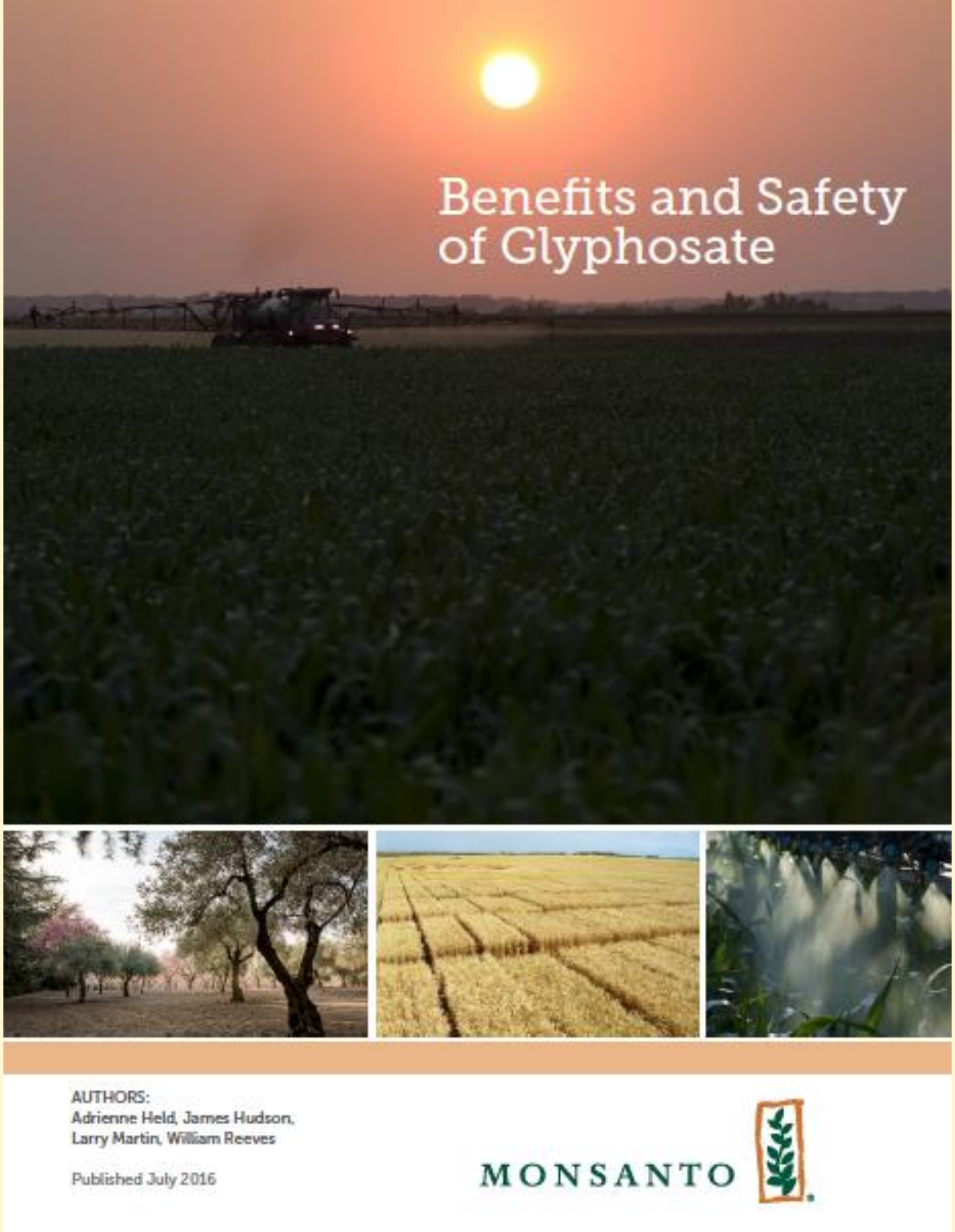


Factors affecting persistence, availability, degradation of glyphosate in soils:

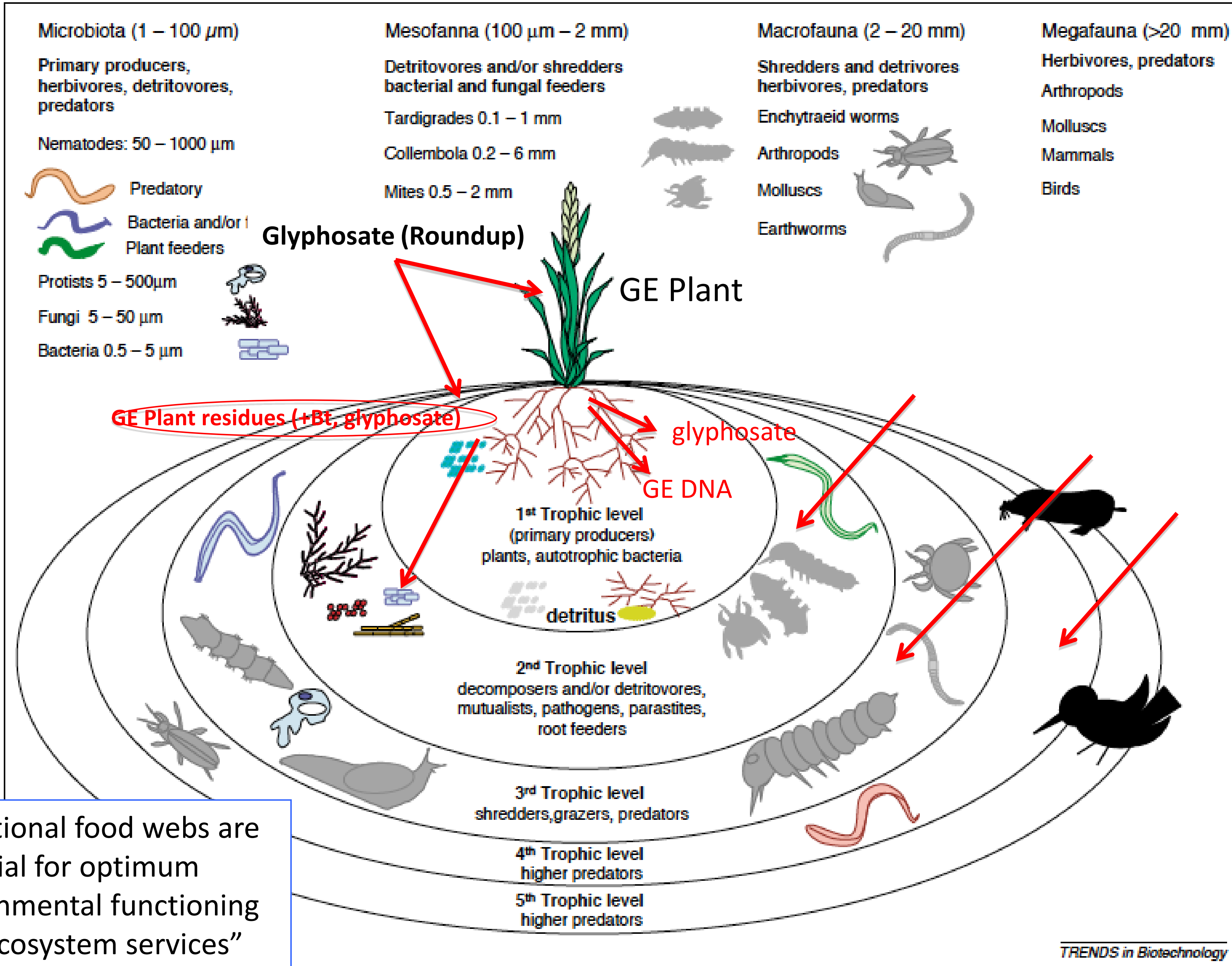
1.Soil pH
2.Soil mineralogy - composition i.e., MnO_4 content
3.Soil texture (>20,000 soil series in U.S.!)
4.Soil organic matter content
5.Soil P content, sorption
6.Plant root reabsorption thru exchange on soil sites with P
7.Soil nutrient status (cationic nutrient content)
8.Herbicide formulation and components (i.e., *surfactants*)
9.Soil surface vegetative residue cover
10.Type of crop management system (crop sequence, transgenic crops, cover crops)
11. Soil oxygen status; compaction (anoxic conditions prolongs persistence)*
12. Resistant to degradation at temperatures ≤ 200C (not degraded by composting)
13. Composition of soil microbial community (adequate diversity [consortium] to completely degrade molecule?)* - metabolic diversity & soil P drive AMPA
14. Bioaccumulation in some soil/sediment meso- & macro-fauna
15. Offsite movement in surface runoff, contaminate surface waters
16. Offsite movement of sediment, accumulation in catchments
17. Leaching into groundwater
18. Formulation composition of the commercial glyphosate product



Much of this information is dismissed; routine use of Glyphosate continues to be defended



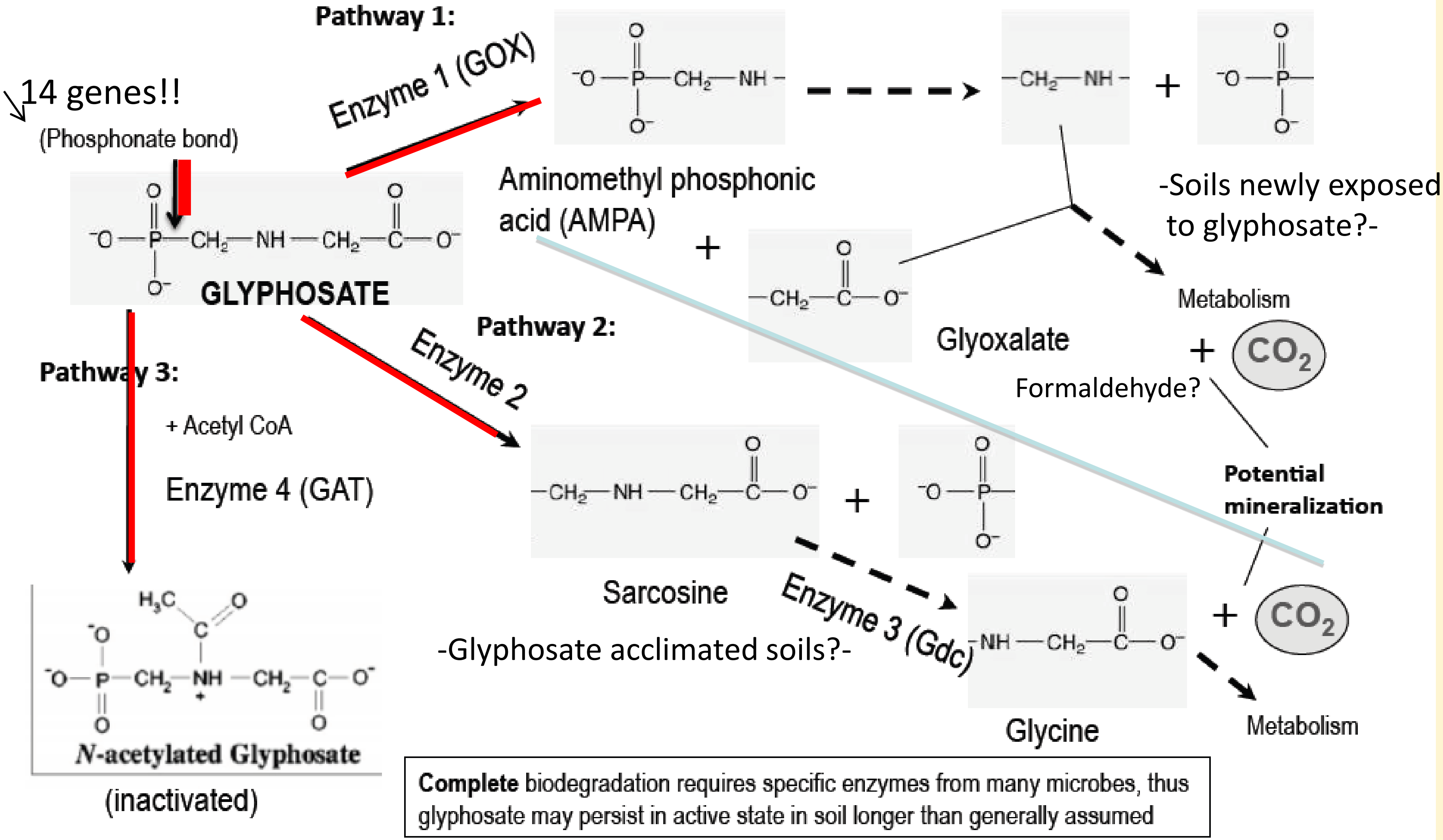
GE crops & Glyphosate may negatively affect trophic (cycling) levels of environmental food webs



Operational food webs are essential for optimum environmental functioning and "ecosystem services"

Figure 1. Soil food web showing an outline classification of the soil biota based on type, body size and trophic level. These components have a variety of interactions ranging from the competitive or predatorial to the cooperative and symbiotic. Size class is based on the width of the organism according to Swift [38].

Highly diverse microbial community (consortia) needed to completely degrade glyphosate in the soil environment - well-managed soils is key to success -



Enzymes: 1, Glyphosate oxidoreductase; 2, C-P Lyase; 3, Glyphosate decarboxylase complex; 4, Glyphosate acetyl transferase

Glyphosate and Acetochlor (GTZ; ‘Harness’, ‘Surpass’) shifts balance of bacterial community composition in rhizosphere soils of corn

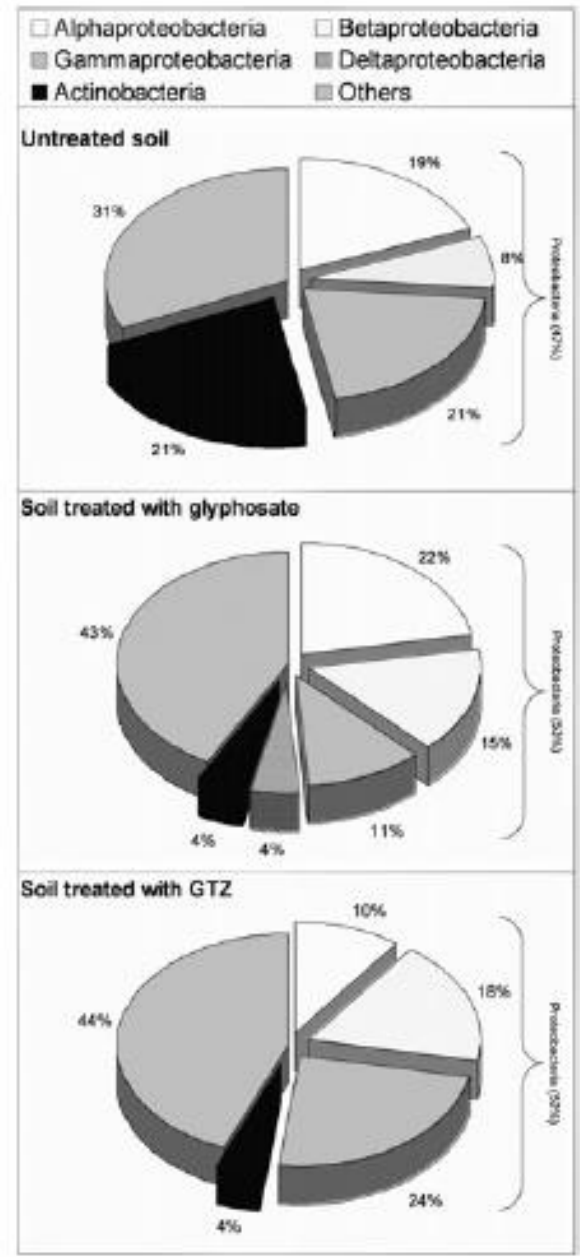


Fig. 1. Taxonomic breakdown of the more relevant phyla from the three soils. The percentages of *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria* and *Actinobacteria* are indicated. The total percentage of *Proteobacteria* from each soil is indicated on the right margin.

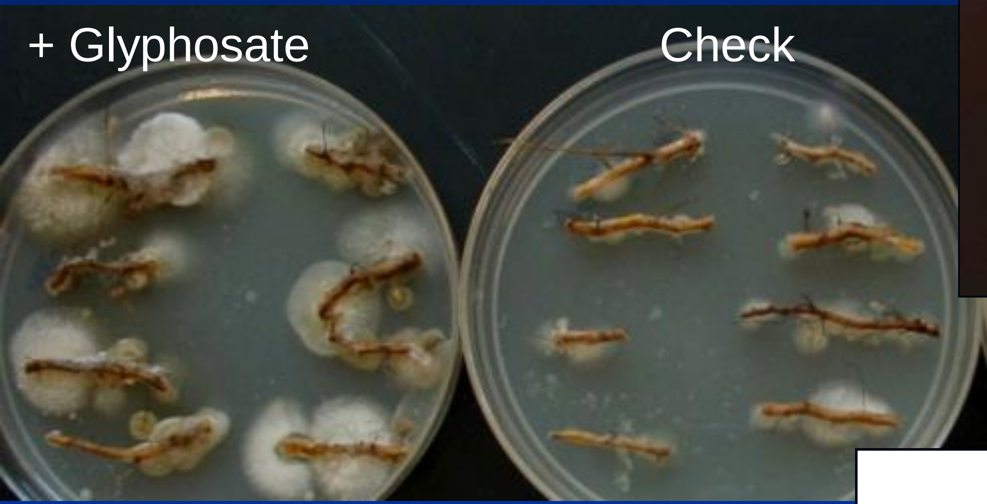
Barriuso et al. 2010. Environmental Microbiology 12:1021-1030.

Also an apparent “secondary mode of action” -- soil microbes attack and infect plant roots -- plant-defense compounds derived from aromatic amino acids are not synthesized

Glyphosate-treated plants do not die if grown in a sterile medium, and tend to resume growth over time as the herbicide leaches out or is metabolized. Johal & Rahe 1984,Johal & Huber 2009, Levesque & Rahe 1992

Representative GR soybean root colonization assay results observed consistently, 1997-2007

Fusarium colonization – 2007 study

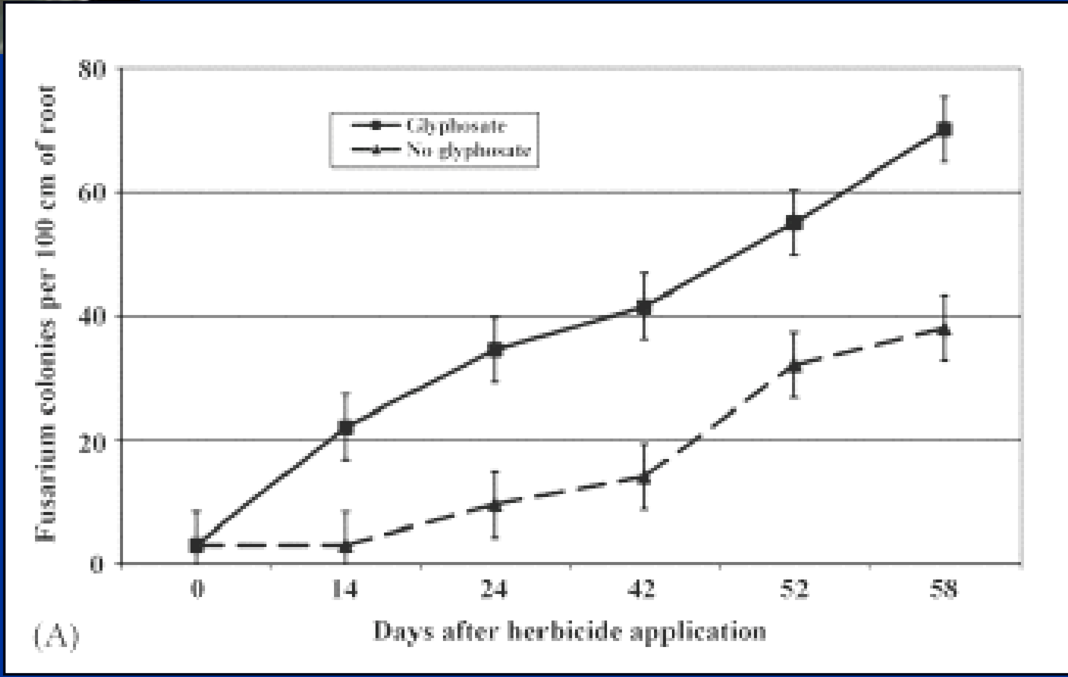


Glyphosate applied at 0.84 kg a.e./ha (0.75 lb/A) on Pioneer '94B01' at growth stage V4, June 1998 in Pemiscott County, MO

Presence of *Fusarium* is indicator of microbial imbalance in rhizosphere and only *potential for pathogenicity.*

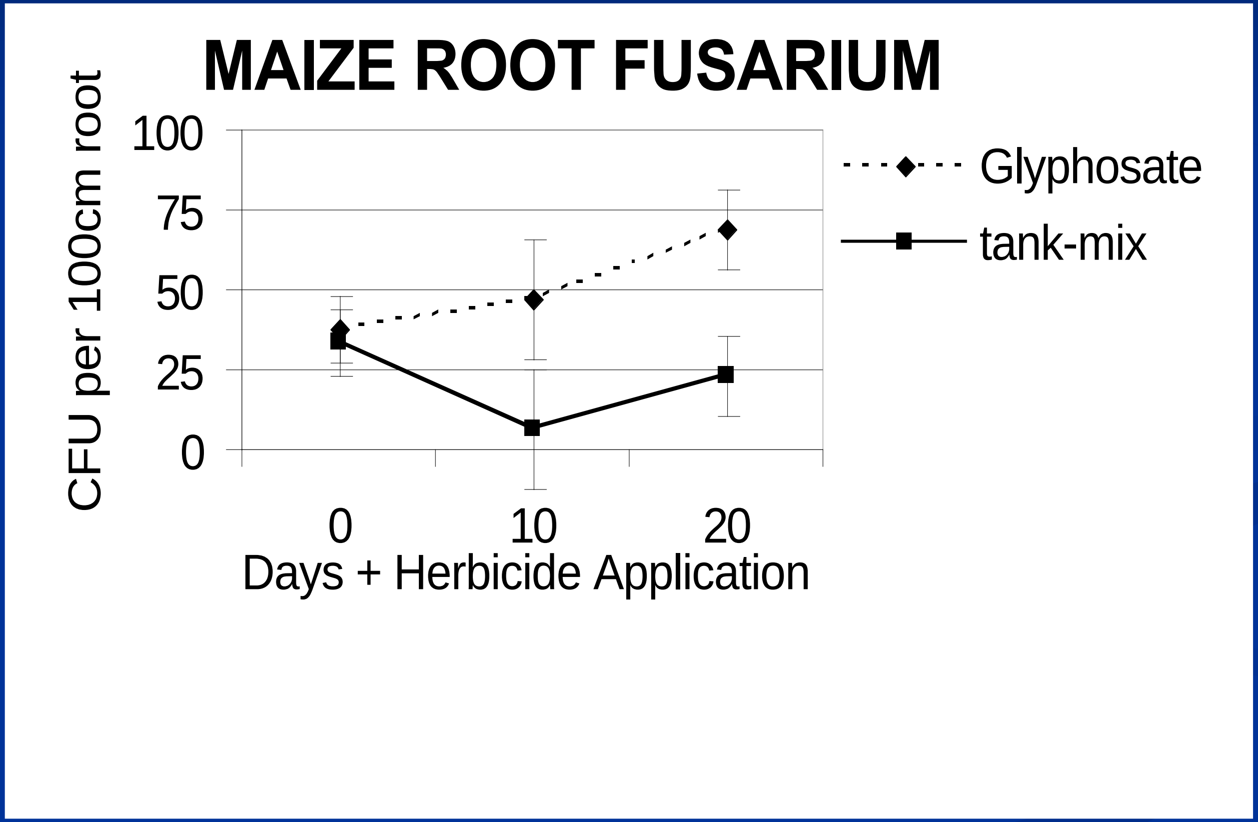
Kremer, R.J., Means, N.E. 2009. Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. Eur. J. Agron. 31:153-161.

Typical trend in *Fusarium* colonization of GR soybean roots



Fusarium root colonization on GR corn ('DeKalb DKC60')

Boone County field trial, 2003



Kremer & Means 2009

2014 Soybean and Corn – Boone County, MO

Fusarium Root colonization (per 100 cm root):

Corn Organic	Corn GR + Gly	Soybean GR + Gly	Soybean Organic
25.0	220.5	146.8	22.8

How does soil health influence fates of herbicides in agroecosystems?

Example herbicide - Glyphosate

Enters soil – Burndown applications, direct contact (spray misses foliage), spray drift, released through roots of both susceptible and resistant (including transgenic crops) plants

Glyphosate is simple molecule but difficult to dissipate

2010 Soybean (Asgrow RR2Y) – Osage County, MO

Fescue burndown

Fescue – no burndown

Scenarios -
Substrates & glyphosate release from grass roots; soil fungi proliferate on grass AND soybean seedling roots; effects of soil residual glyphosate?

Potential impact of glyphosate on beneficial microorganisms

- Soybean-*Bradyrhizobium* relationship:
  Reductions in root nodulation, nitrogen fixation. Data collected on field-grown soybean, 2009. (Zobiole et al. 2012. J. Plant Nutr. Soil Sci. 175:319-330.)

Most cultivars still require 20-25% “non-fixed” N

Soybean nodulation at R1, 2010

Check - No glyphosate applied

Glyphosate applied (WeatherMax, .75 lbs/A)

- Reduced fluorescent pseudomonad bacteria:

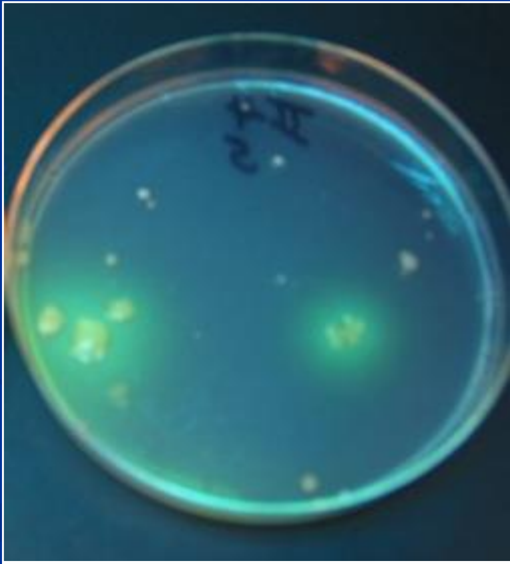
Responsible for suppressing fungal growth in root zone, mobilizing nutrients for plant uptake, producing plant-growth promoting substances, potential biodegradation of synthetic chemicals including herbicides

Include *Pseudomonas fluorescens, P. putida, P. aeruginosa*

Kremer, R.J., Means, N.E. 2009. Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. Eur. J. Agron. 31:153-161.

Selective culture for rhizosphere pseudomonads, Soybean growth stage R1, 2010

Check - no glyphosate + Glyphosate



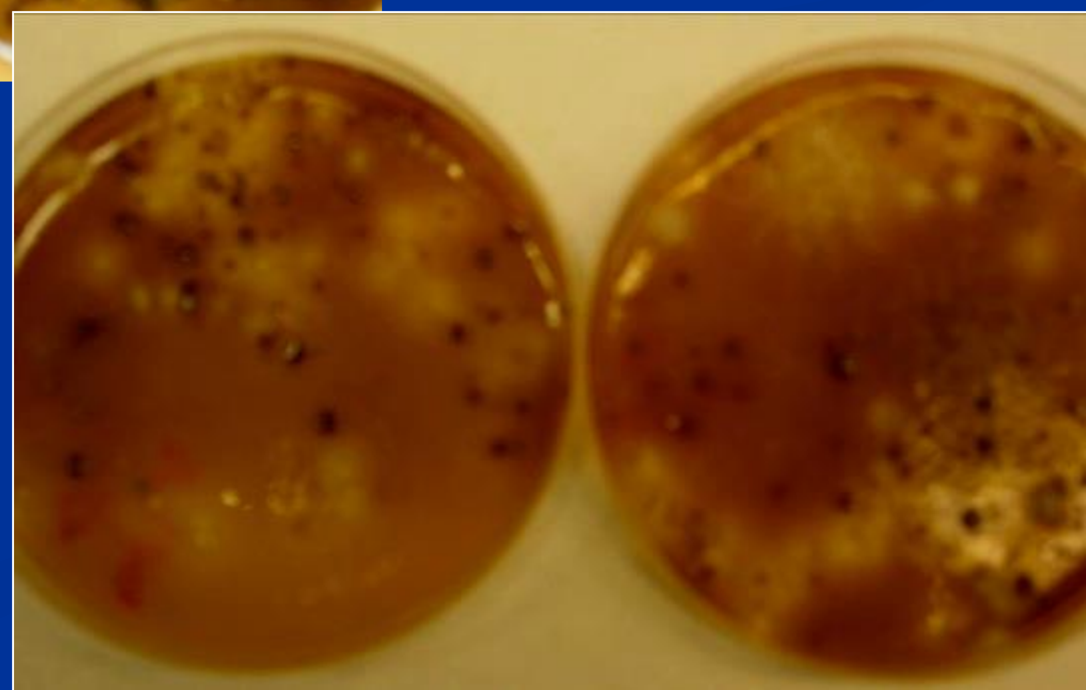
Fluorescence of pseudomonad colonies under UV light

Manganese Interactions - an illustration

Mn transformations primarily microbially-mediated, thus have major impact on plant nutrient availability

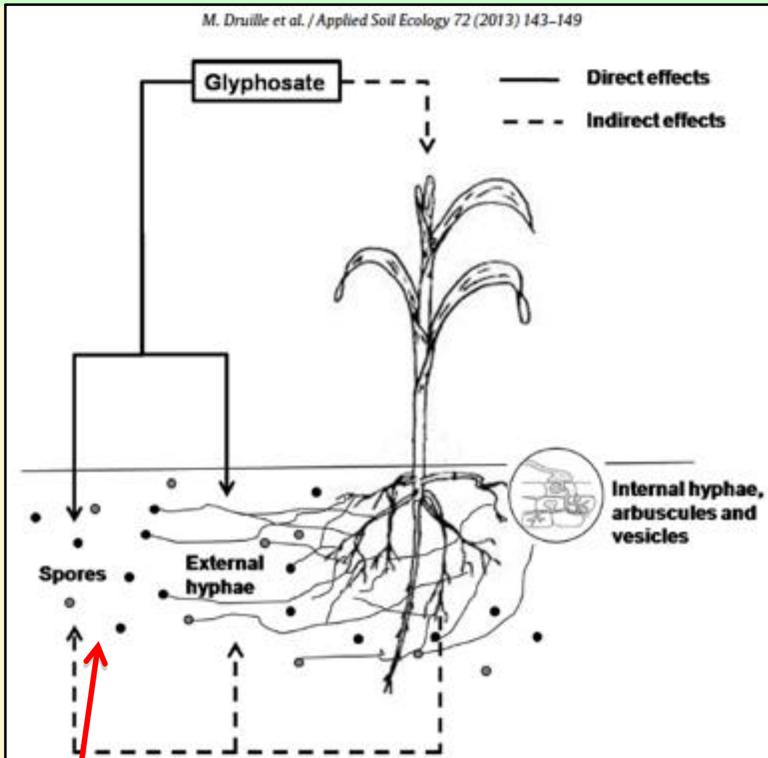
Is glyphosate released into soil involved in Mn availability?

Potential Mn-oxidizing (black) & Mn-reducing bacteria (white, clear) and fungi on roots, in rhizosphere soils



Microbial growth on Gerritsen's medium (Huber & Graham 1992)

Potential impact of ***glyphosate introduced into rhizosphere*** on mycorrhizal spore germination and root colonization *[glyphosate = Roundup active ingredient]*

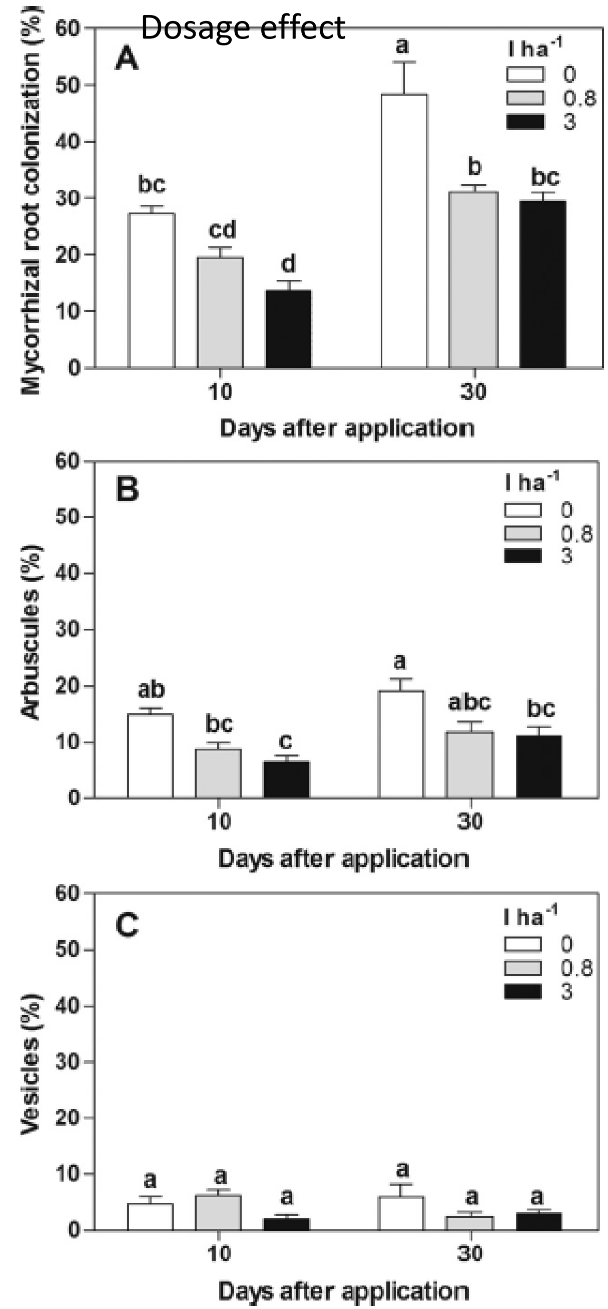


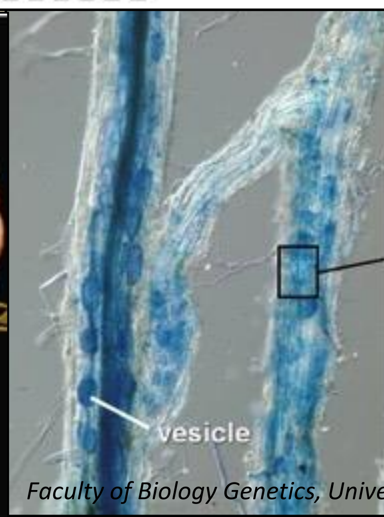
Demonstrated in grassland ecosystems

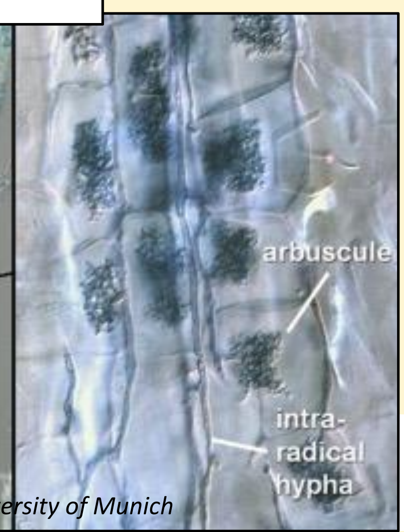
Variable results with transgenic crops
(Powell et al. 2009. Appl. Soil Ecol. 41:128)

Considerations in transitioning??

Dosage effect







Faculty of Biology Genetics, University of Munich

Druille, M., Cabello, M.N., Omacini, M., Golluscio, R.A. 2013. Glyphosate reduces spore viability and root colonization of arbuscular mycorrhizal fungi. Appl. Soil Ecol. 64:99-103.

Glyphosate-resistant soybean showing chlorosis post-glyphosate application – 2009
“Yellow flash”

Insufficient nutrient availability and poor herbicide management often confounds overall effects of glyphosate use
Soybean – 2010

Hydroponic system for collecting root exudates and evaluation of root nodulation in soybean.

Kremer et al. 2005

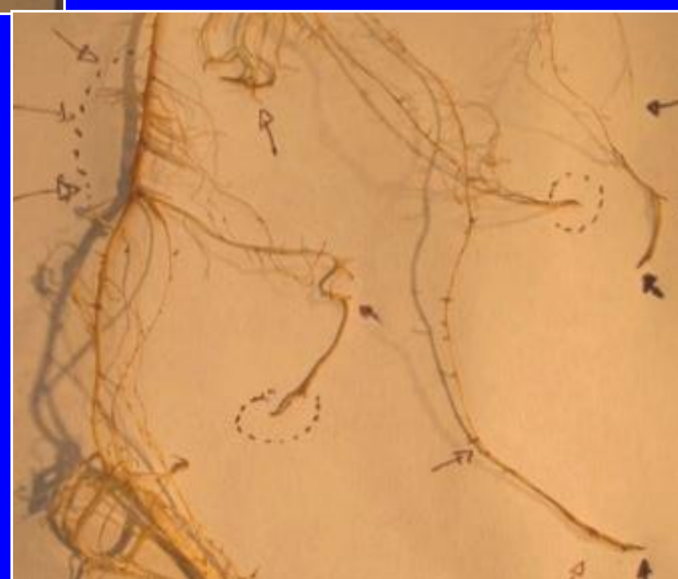


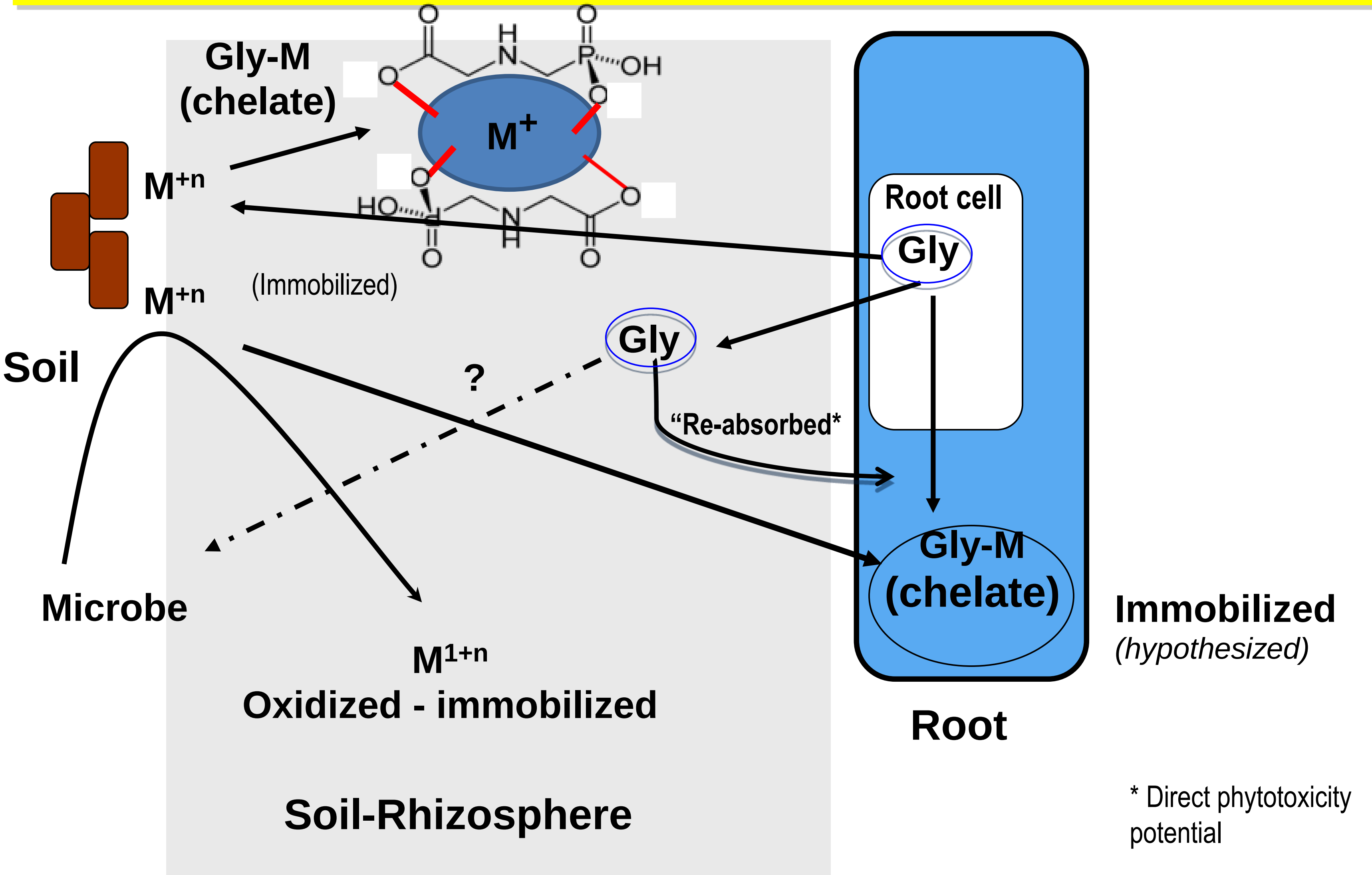
Figure 5. Root development of 28-day-old GR soybean in growth pouch system. **A**. Root on Right from plant with no glyphosate, note abundant nodulation; root on Left from plant receiving glyphosate, note stunting and reduced nodulation. **B**. Root growth abnormalities associated with glyphosate treatment. Note: Blackened root tips and stunted root growth, suppressed nodulation.

Kremer et al. 2010

Importance of maintaining adequate soil fertility/plant nutrition in GR crop production:

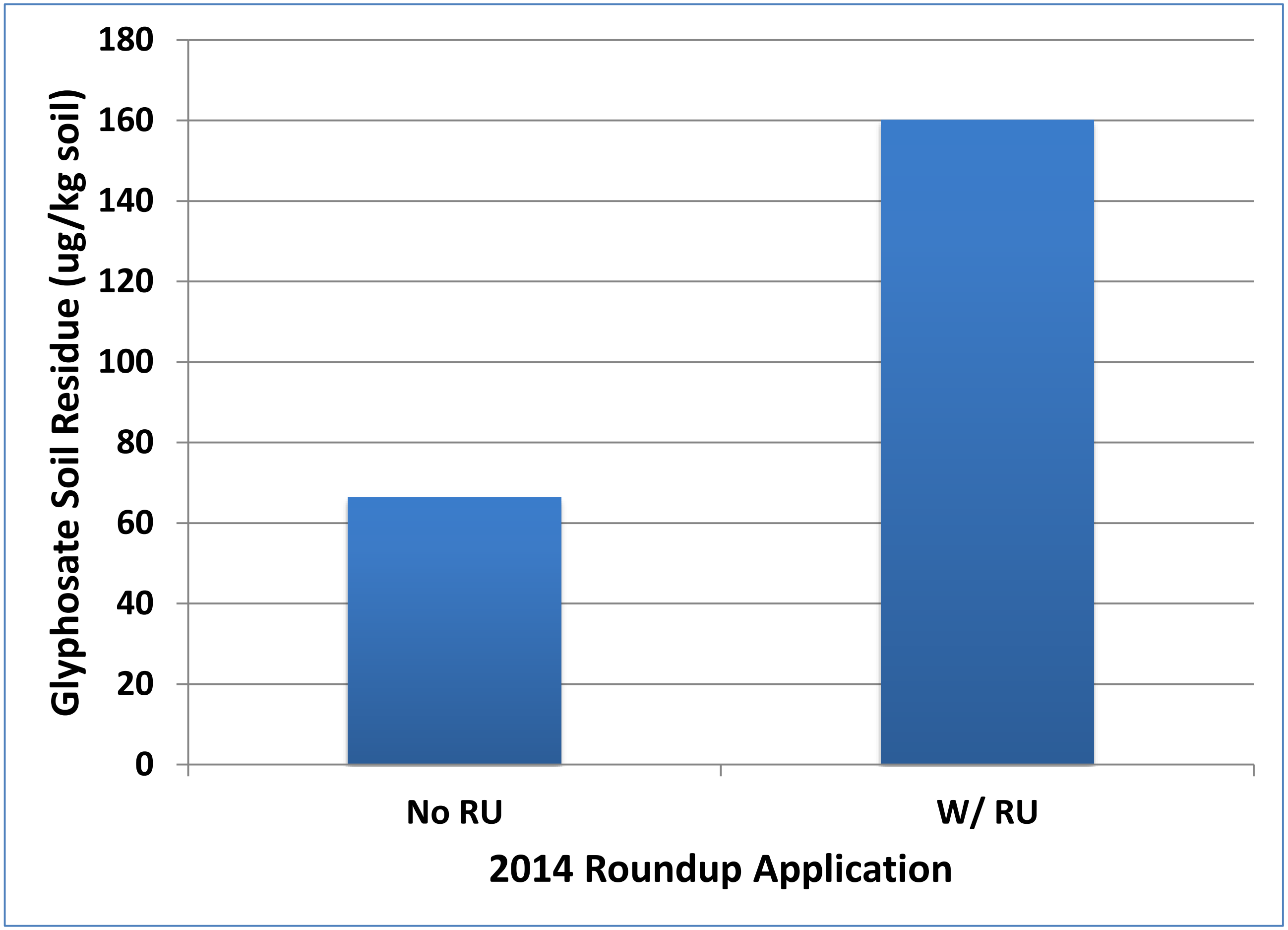
Understanding plant-soil-microbial relationship with GR crops -

Possibilities for nutrient deficiency in plant when soil test indicates sufficient levels



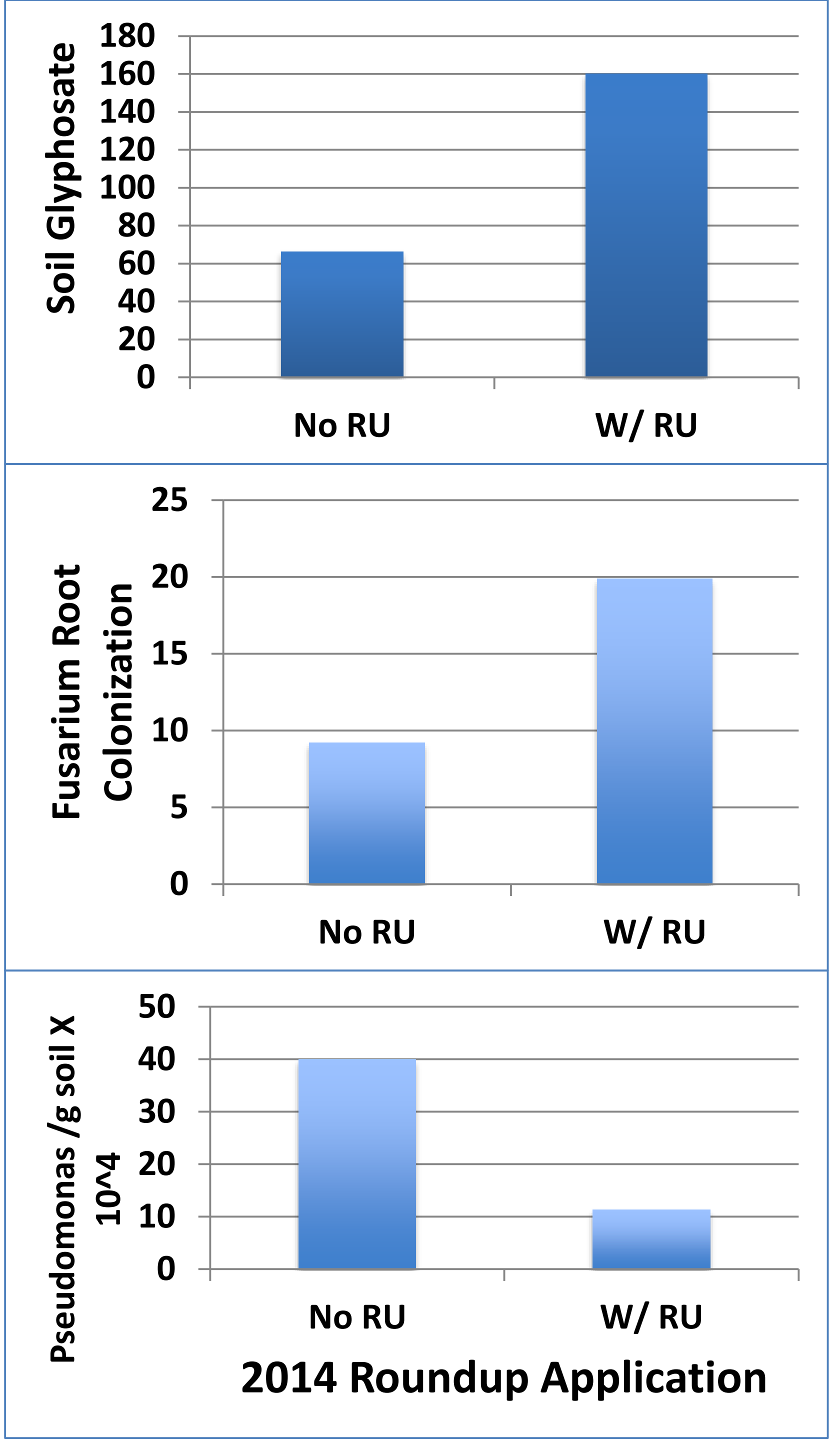
Kremer & Means 2009, Bott et al. 2008,Cakmak et al. 2009, Bernards et al. 2005

Residual glyphosate in rhizosphere soil of GE soybean with and without Roundup* in 2014 (Mexico silt loam, Boone County, MO)**



* Application rate = 0.75 lb a.i./A

**Roundup applied at field site in periodically thru 2013

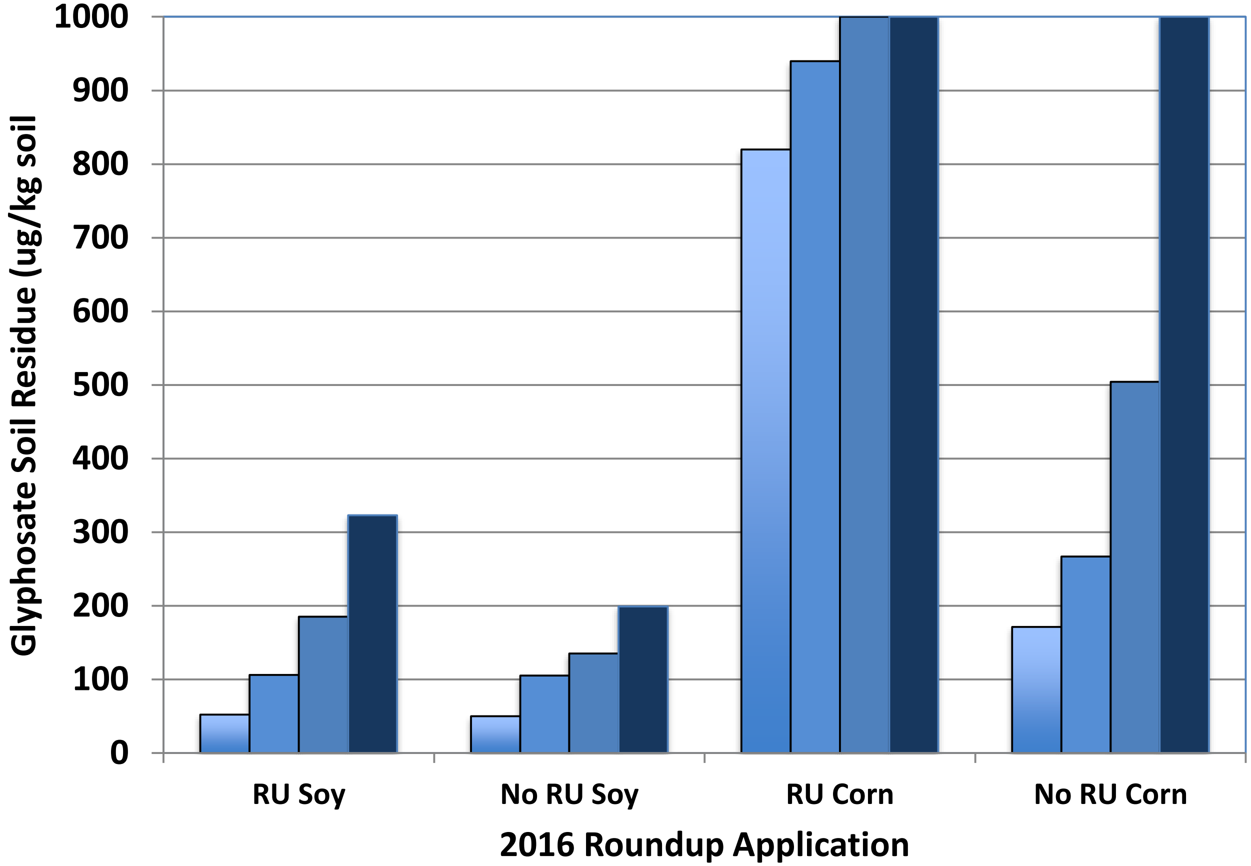


Relationship of selected soil (rhizosphere) measurements in GE soybean with and without Roundup (glyphosate)* in 2014 (Mexico silt loam, Boone County, MO)**

* Application rate = 0.75 lb a.i./A

**Roundup applied at field site periodically thru 2013

Residual glyphosate in rhizosphere soil of GE soybean and corn with and without Roundup* from different plots in 2016 (Mexico **silt loam**, central Missouri)** (high clay content; 2.0 – 2.5% SOM)



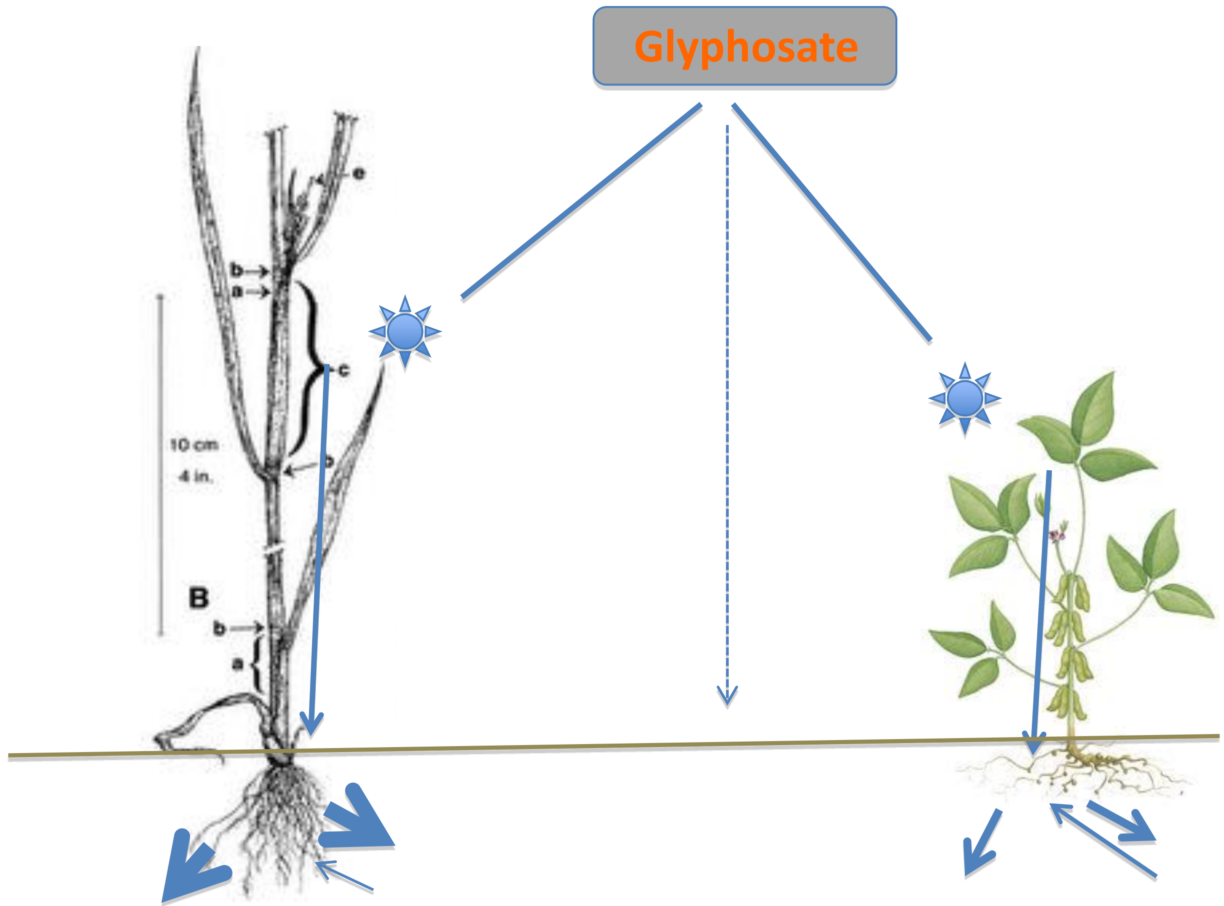
* Application rates = 0.75 – 2.0 lb a.i./A

**Roundup applied at field site in periodically thru 2015 either burndown or as weed management tool during season

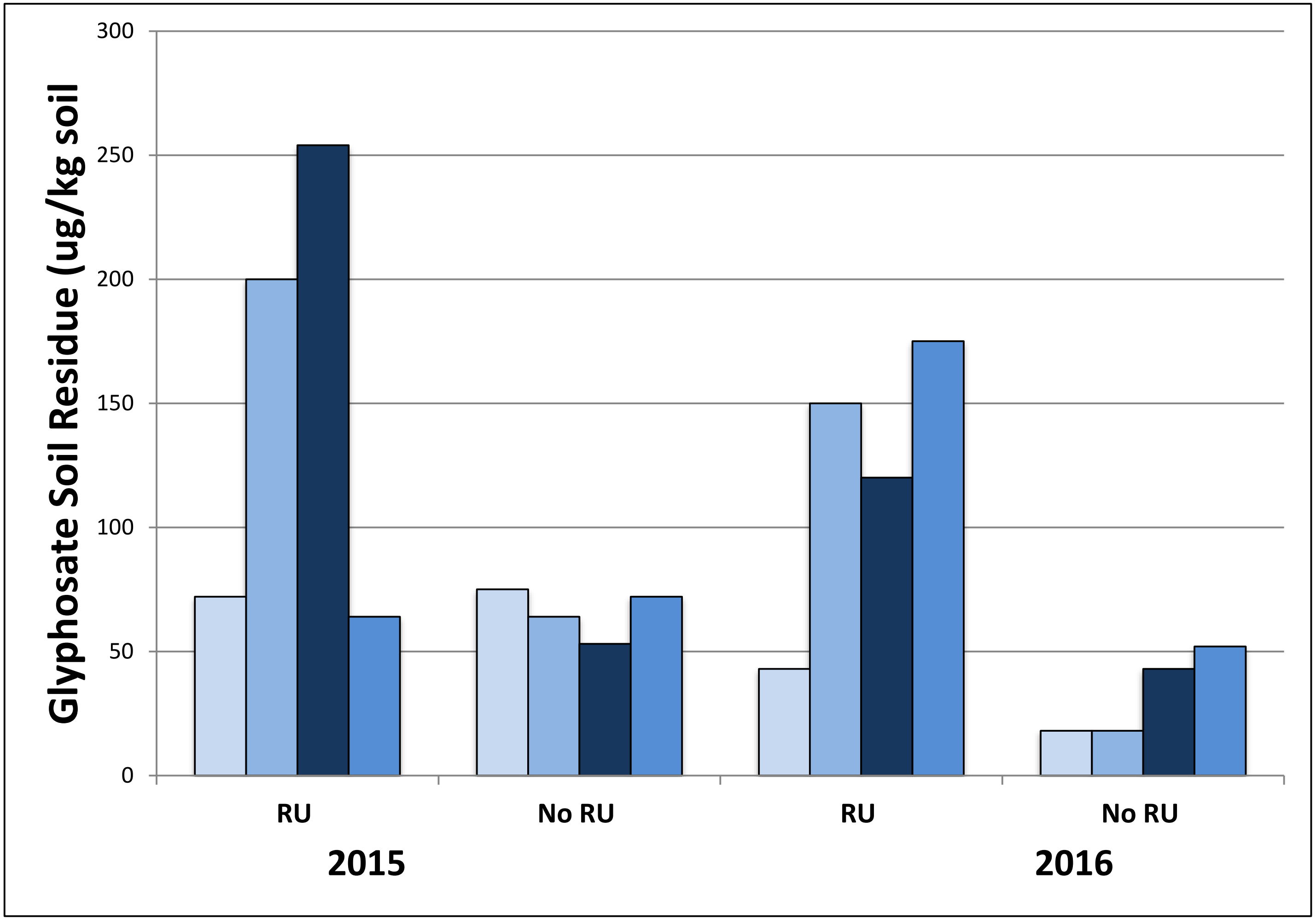
Why more residual glyphosate in soil cropped to corn compared with soybean?

•Multiple in-season applications vs soybean
•Translocation of more glyphosate to roots for release into soil
•Different microbial community – fewer "glyphosate degraders"
•Less re-absorption of glyphosate by roots

Preliminary



Residual glyphosate in soil from Iowa farm with and without Roundup under transition from GE to non-GE crops (**sandy slit loams**, Delaware County)
(Coarse soil texture; SOM 1.8 – 2.3%)



Glyphosate and Glyphosate-Resistant Crops (‘Roundup-Ready’ [RR] Production System)

- **Advancement in effective weed management**

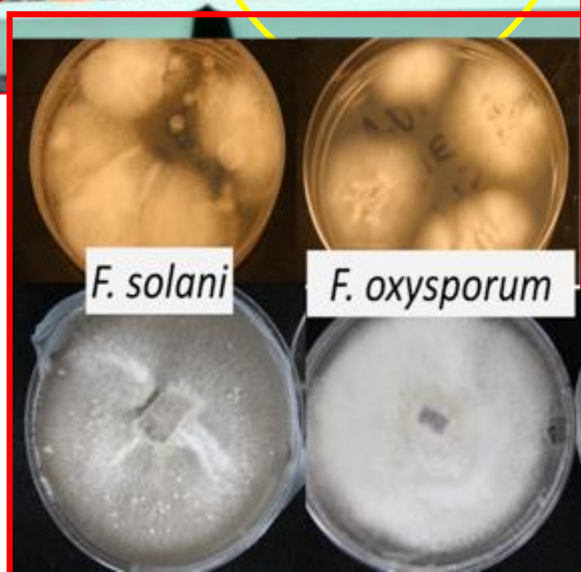
(despite the development of glyphosate-resistant weed biotypes)

Soybean with glyphosate-resistant waterhemp, Boone County MO, 2010

Response of Resistant & Susceptible Waterhemp to Soil Sterilization Following Treatment with Glyphosate

Fresh Soil: Resist+ Gly Suscept+ Gly

BOTH glyphosate-susceptible and -resistant biotypes colonized by *Fusarium* fungi
Glyphosate-resistant biotypes may also be resistant to fungal infection
Overall microbial diversity differed little regardless of biotype or glyphosate use.
(Rosenbaum et al. 2014)



Management Considerations -

If glyphosate must be continued in your farming operation:

- **Reduce use** *(Challenge when GR corn – GR soybean)*
- **Use properly – burndown guidelines**
- **Use alternative herbicides**
- **Rotate with non-GE crops**
- **Build soil biology, microbial diversity (soil health)**
  - **Organic amendments**
  - **Cover cropping**

Soybean no-tilled too soon after fescue treated with Roundup burndown application –
Osage County, MO 2010

Better management of crop residues and soil surface - Benefit microbes & nutrient retention

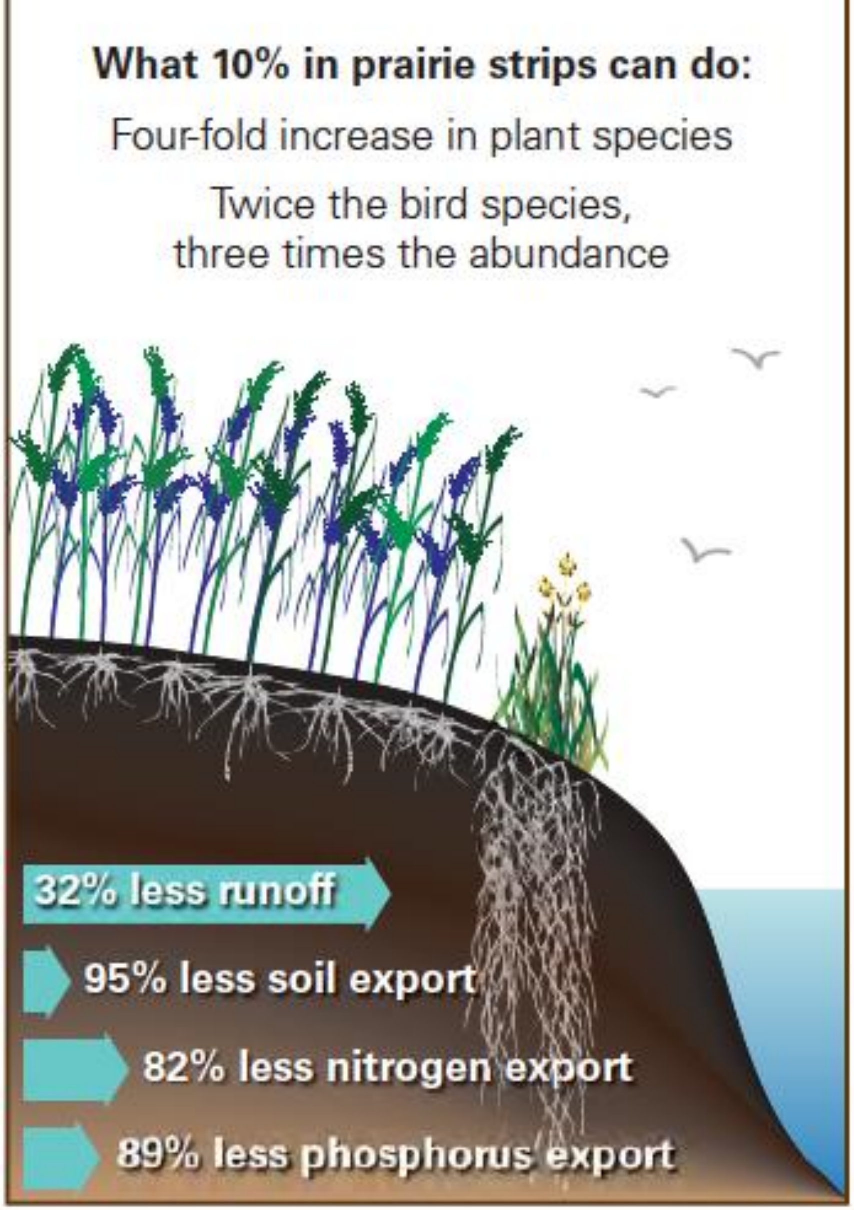
Applies to management in both commodity production systems AND transitional systems

Minimize water erosion in production fields

Management Considerations - Limit pesticide impacts; Improve soil health

reduce offsite movement of glyphosate

www.leopold.iastate.edu/strips-research-team



Iowa "Prairie Strips" System:

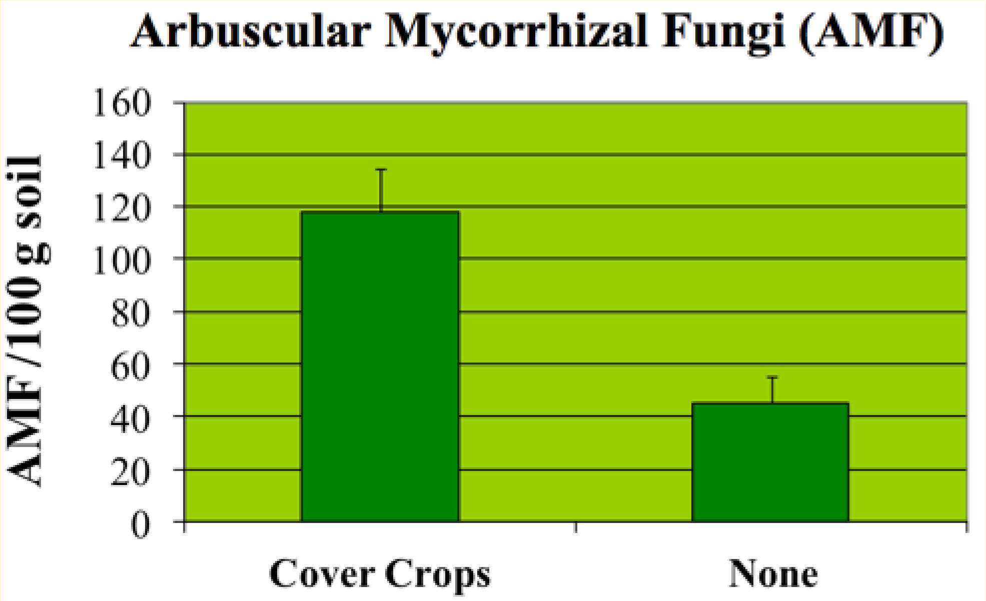
Prairie strips integrated with cropland at the Neal Smith National Wildlife Refuge

Environmental benefits of native plants in waterways, buffer and riparian strips

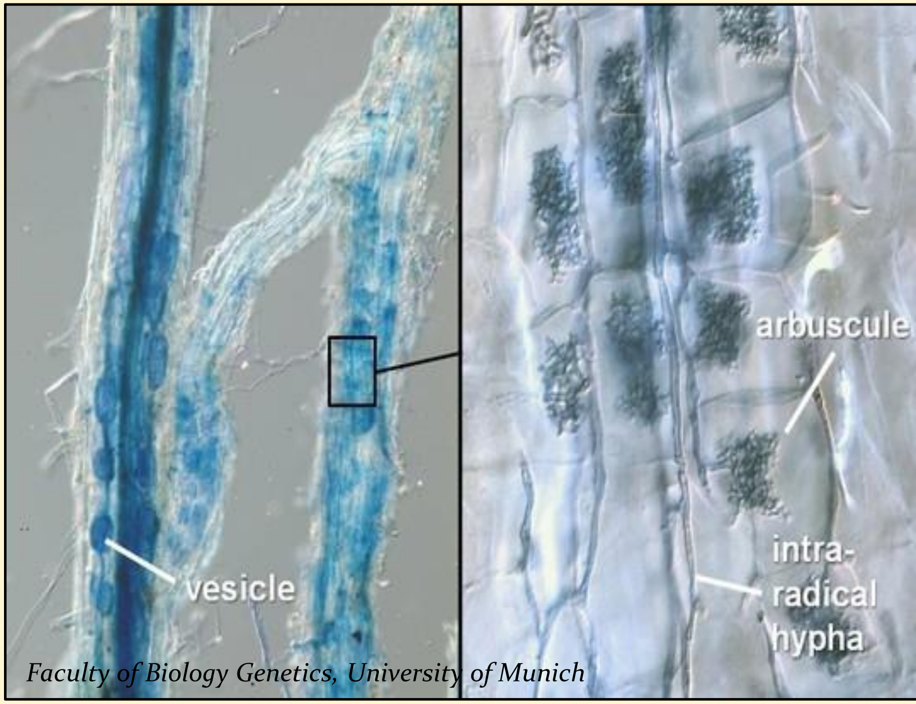
Cover crops reduce use of glyphosate or eliminate need for herbicides

Austrian winter pea, Boone County, MO

Mycorrhizae: soil health indicator
Implications: Cover Crop System vs No Cover Crop



A five-way cover crop mix (winter wheat-clover-vetch-turnip-radish) significantly increased AMF



>*Increased AMF propagules specific for numerous crop host plants due to cover cropping
Thus cover crop management would score higher than no cover crops Lehman et al., 2012

>**Abundant and effective mycorrhizal community can aid in suppressing weeds including herbicide-resistant biotypes. Li et al. 2016. Weed Science 64:642-652

Cover crops for reducing or eliminating inputs of glyphosate -
Rye rolled fb no-till corn -
2013 (Boone County, Missouri)

Management to reduce glyphosate impacts (continued)

Include alternative non-transgenic crops in crop rotation -
Grain sorghum 2013 (Osage County, Missouri)

Be aware of possible consequences of glyphosate use for terminating cover crop

Soil Health root bioassays detect potential seedling pathogens associated with cover crop termination.

Schenck et al. 2013 ASA Abstracts

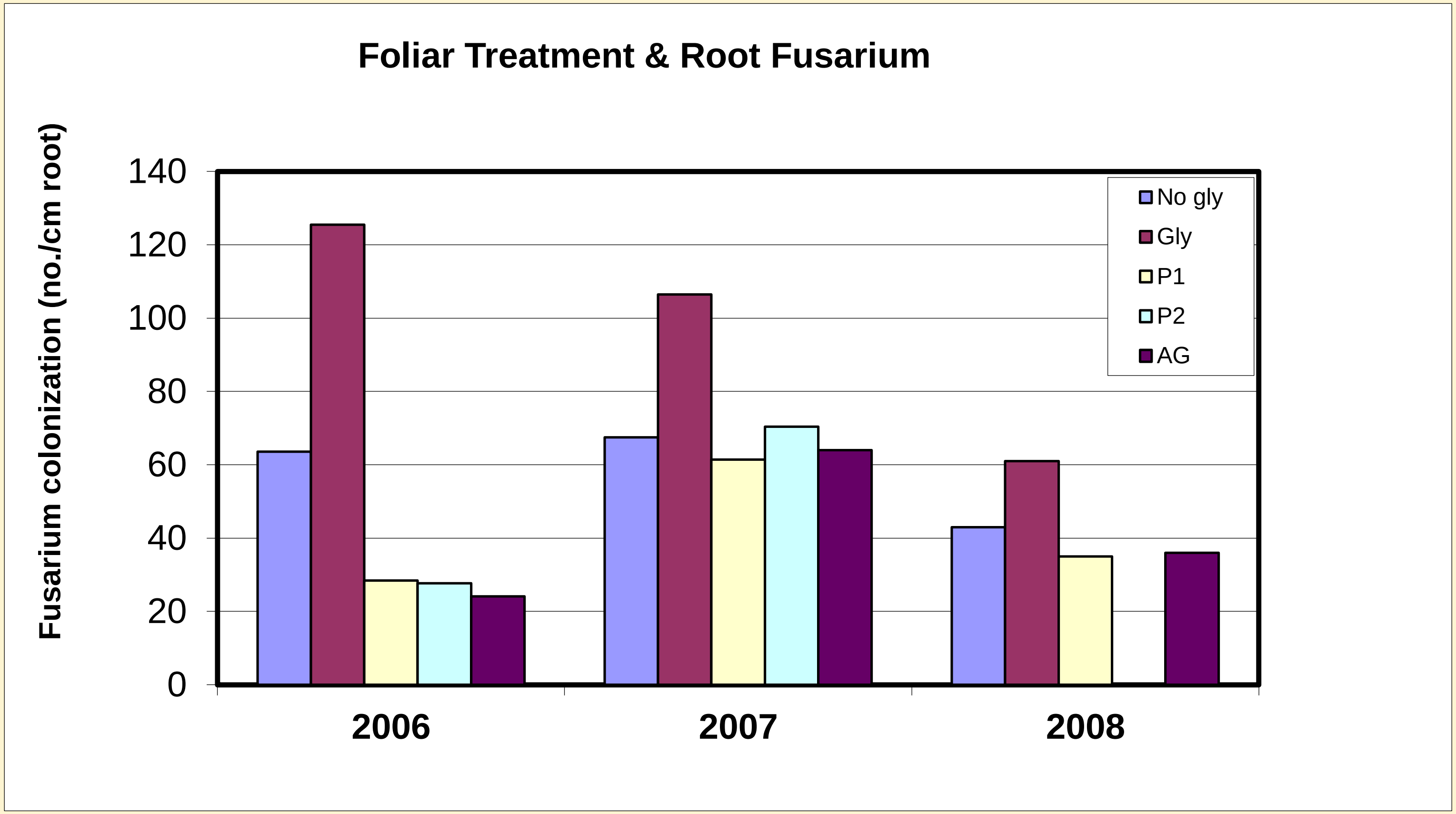
Fig. 1. Corn plant grown following rye cover crop with diseased radicle (red arrow).

Management tactics to overcome adverse effects –
Use of Biologicals:

Salicylate, a precursor of plant-defense compounds, applied as foliar treatment suppressed colonization of soybean roots by *Fusarium* in field study, 2007

+ Glyphosate **Check(No glyphosate)** **Glyphosate+Salicylate**

“Prebiotic-type (AG) and similar **bioproducts** contribute 40 to 80% decrease in *Fusarium* root colonization compared with glyphosate alone; Response at this site depends on weather conditions, soybean variety, initial inoculum level, cropping history



P1= ‘Product 1”
P2= ‘Product 2’
AG= ‘Product 3’

Manage for *sufficient nutrients in soil* for optimum biological activity

Nutrients are essential to physiological and biochemical functions, many of which are common to *both* plants and microorganisms

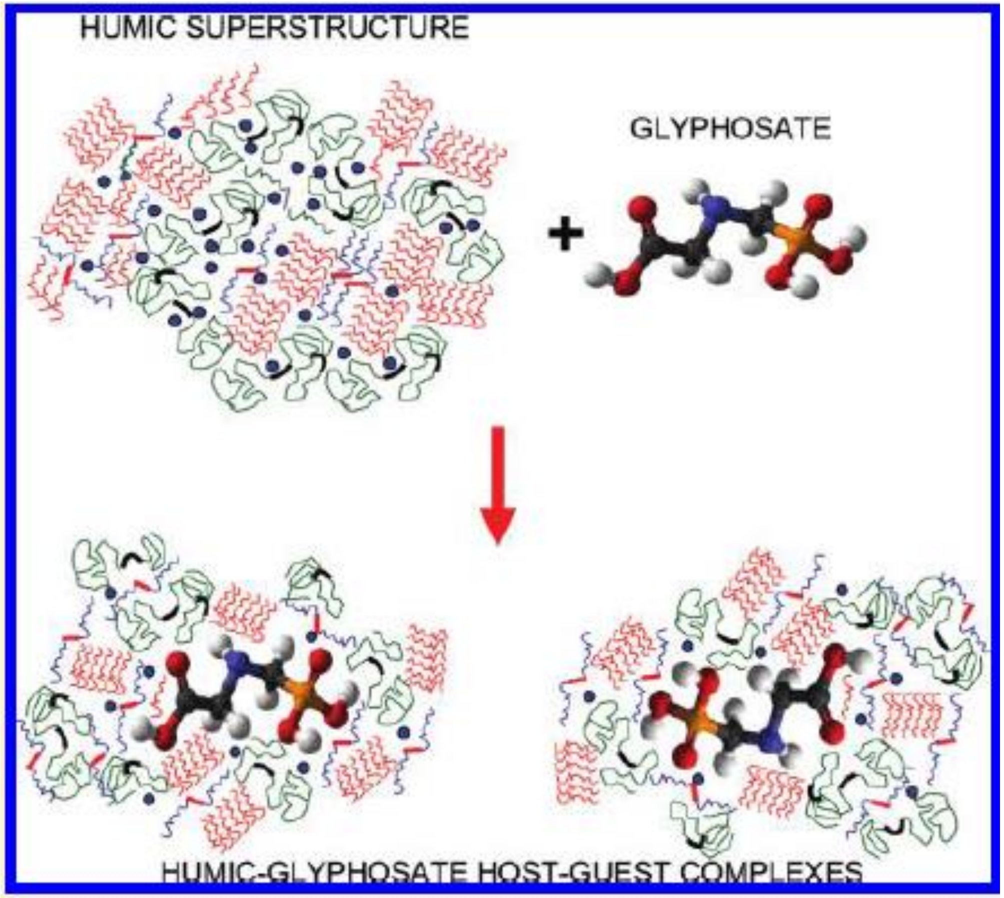
Nutrient elements	Biochemical/Physiological functions
C, O, H, N, S	Major constituents of organic substances (carbohydrates, proteins, lipids, enzymes)
P, **Ca**, **B**	Linkage of major polymers in cell walls and membranes (phospholipids, nucleotides; cellulose); energy transfer
K, Mg,	Cellular ion, pH balance; enzyme activation; photosynthetic activity
Mn, Fe, Cu, Zn, Mo, Ni	Activate/mediate numerous important ***enzymatic reactions*** in various metabolic pathways

Note: *Deficiency or immobilization* of an essential nutrient may affect one or more metabolic pathways, yet have consequences on *overall functioning* of plant or microorganism

Adapted from Datnoff et al. 2007. *Mineral nutrition and plant disease*. APS Press.

Amendments for managing glyphosate residues in soils and water; useful for transition from GE cropping systems

Glyphosate binds to humic substances at slight acid pH to neutralize activity



Mazzei & Piccolo. 2012. Environmental Science & Technology 46:5939-5946.

Biochar or Activated C may adsorb available glyphosate, protect beneficial microbes, enhance microbial activity

Seed coating or in-furrow granular application

Will 2,4-D, dicamba, and other herbicides for new transgenic crops to manage glyphosate-resistant weeds affect soil biology?

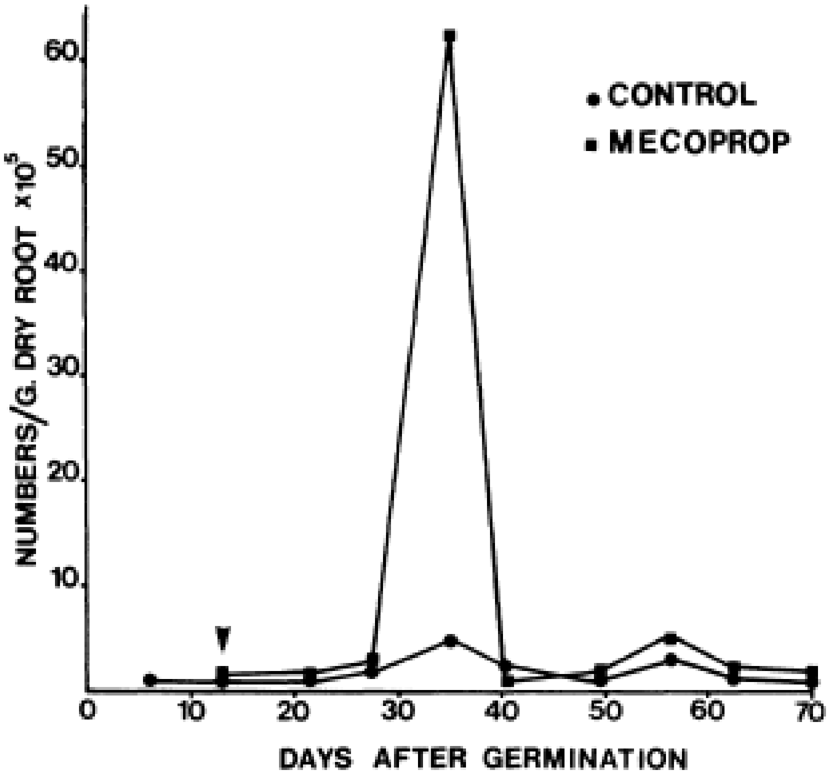


Figure 1. Populations of fluorescent *Pseudomonas* spp. in the cortex of wheat (var. Maris Dove) treated with mecoprop (10 kg ha^{-1}). Plants were grown in sand and populations counted by plate-dilution technique after maceration of washed roots. The arrow indicates time of spraying.

Greaves & Sargent. 1986. Weed Sci. 34 (Suppl. 1):50

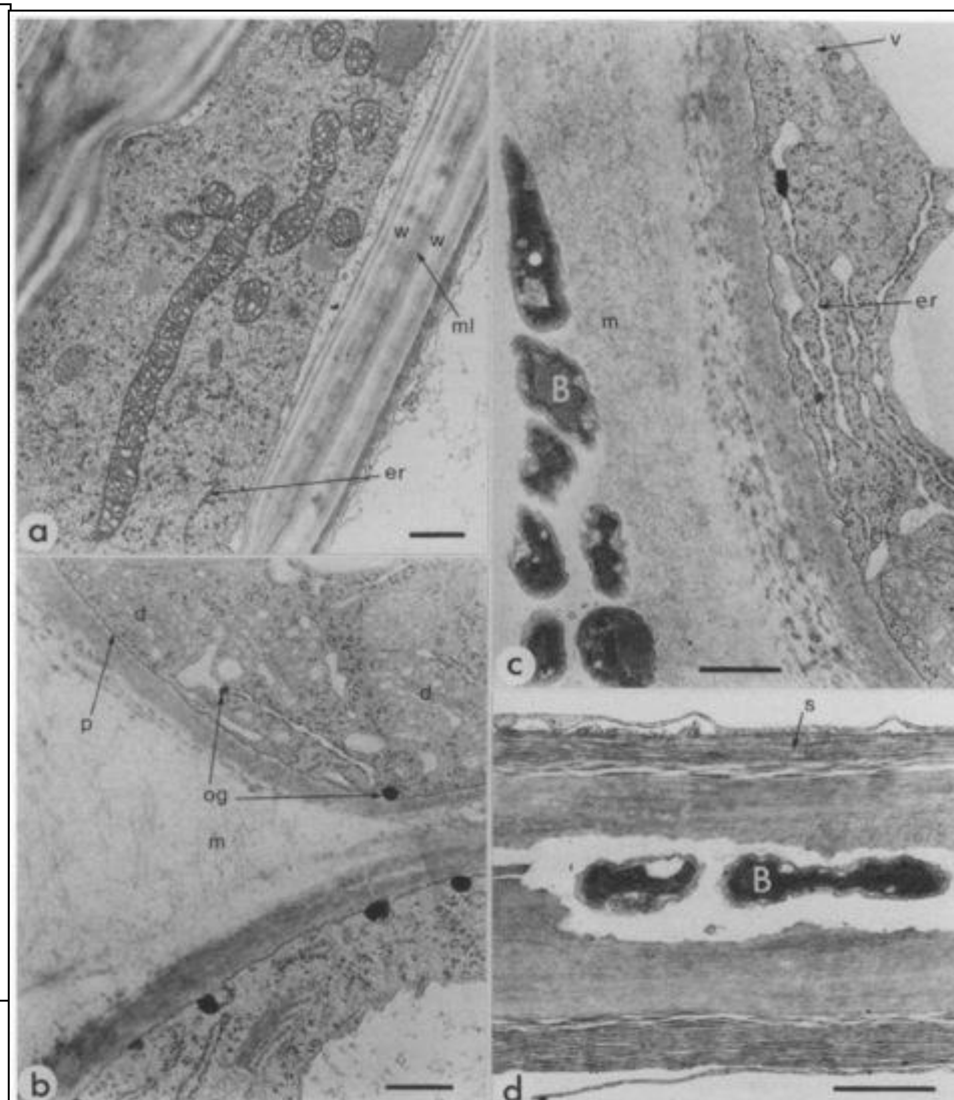


Figure 2. Electron micrographs of sections through roots of wheat (var. Maris Dove) damaged by mecoprop and invaded by bacteria. Sections were cut through cortex tissue approximately four cells below the epidermis and near an emerging lateral root. Plants were grown in organic soil (control) or sand and sampled 70 days after treatment with mecoprop. (a) control; (b, c, d.) mecoprop-treated (10 kg ha^{-1}). B, bacterium; d, dictyosome; er, endoplasmic reticulum; m, microfibril; ml, middle lamella; og, osmiophilic granule; p, plasmalemma; s, secondary wall; v, vesicle; w, cell wall. Scale bar represents 0.5 µm.

Why should we be concerned about GE crops and glyphosate use in agriculture?

If we recognize food production as a system of relationships that intertwine soil, water, air, and the human community, ***food should be produced in a manner that promotes (or respects) environmental quality*** ("*Food Sovereignty*")*.

It is then in our interest to educate consumers about how GE crops and the use glyphosate may impact agricultural production systems to ***develop an awareness of how our food is produced and how food quality might be affected***.

*A. Natsoulas. 2014. *Food Voices: Stories From The People Who Feed Us.* John Natsoulas Press, Davis, CA.


SOIL HEALTH
MATTERS
Natural Resources Conservation Service