HERBICIDAL IMPACTS ON CROP-SOIL MICROBIAL INTERACTIONS AND POTENTIAL PLANT DISEASE

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"Standard" field trials conducted with Roundup Ready soybean.
Field trial on Tiptonville silt loam, Pemiscot County, Missouri 1999

SIMPÓSIO SOBRE
RELAÇÕES ENTRE NUTRIÇÃO MINERAL E INCIDÊNCIA DE DOENÇAS DE PLANTAS

PIRACICABA-SP, 28/02 - 01/03/2005

POTAFOS Tecnologia para Agricultura

Greetings from the State of Missouri!

“Non-target” Impacts of Herbicides:

SCN reduced in conventional soybean:

Blazer (acifluorfen)
Basagran (bentazon)

SCN and *Fusarium* root disease reduced in conventional soybean: Cobra (lactofen)

***Fusarium* root disease enhanced in susceptible soybean cultivars*: Pursuit (imazethapyr)**
Roundup (glyphosate)
***growth chamber study**

Foliar diseases decreased:

“white mold” by Cobra

Potential bacterial root diseases increased on wheat:

Mecoprop, 2,4-D

Development, Commercialization, Adoption of Genetically-Modified (GM), Transgenic, or "Biotech Crops"

1996 - 1.7 million ha - global area

2004 - 81 million ha - global area (47-fold increase)

"Grown by 8.35 million farmers in 17 countries" 2004

14 countries growing ≥ 50,000 ha - 2004

Source: James. 2004. Preview: Global status of commercialized Biotech/GM crops: 2004. ISAA Briefs no. 32.

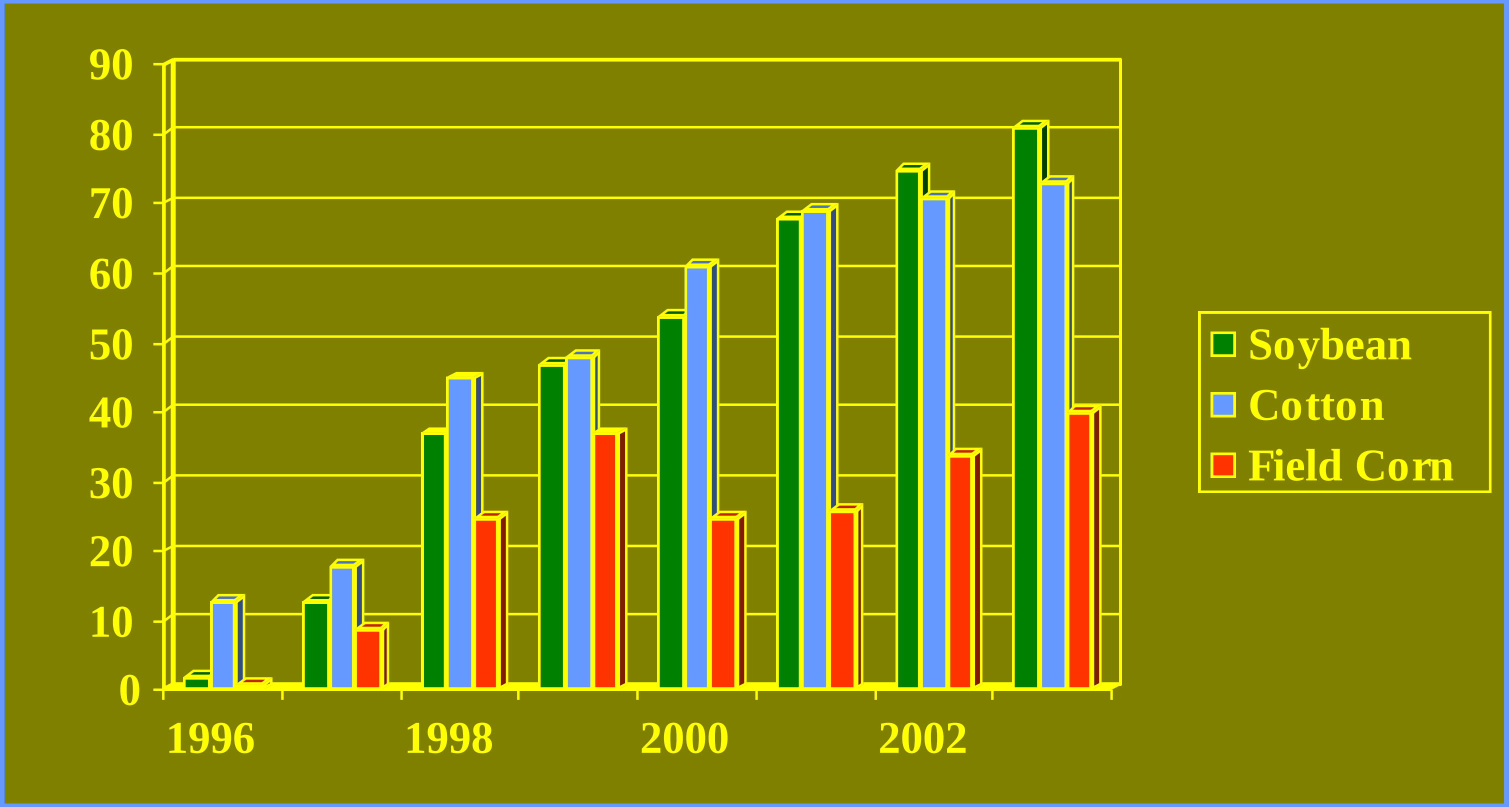
GLOBAL AREA- ROUNDUP READY SOYBEAN PRODUCTION

2003	41.4 million Ha	55%
2004	48.4 million Ha	63%

Source: James. 2004. Preview: Global status of commercialized Biotech/GM crops: 2004. ISAA Briefs no. 32.

Growth of Biotech Acres

% of Total U.S. Acres



Why the Rapid Adoption?
Herbicide Tolerant Crops

- Lower cost of weed control, even with technology fees
- Greatly simplified control procedures
- Higher degree of weed control
- Fewer chemical applications = less trips
- Promotes more sustainable cultural practices
  - Less tillage, less compaction, narrower rows
- Societal aspects (pride, landowner acceptance)

Roundup Ready® Soybean

- contain gene for producing glyphosate-resistant EPSPS
- allows timely POST Roundup® applications during season
- no apparent plant injury or yield reductions

Statements from *"The contribution of agricultural crop biotechnology to American farming"* K. Nill, American Soybean Association (2002)

"The switch in crop production methods . . . allows the natural fungi that grow on plant roots to produce glomalin, a protein that naturally sequesters carbon and keeps it within the soil." {have mycorrhizae been examined?}

"Biodiversity is maintained in biotechnology-derived herbicide-tolerant soybean fields. Soil microbes . . . in conservation tillage biotechnology-derived herbicide-tolerant and conventional soybean fields were similar in number and variety."

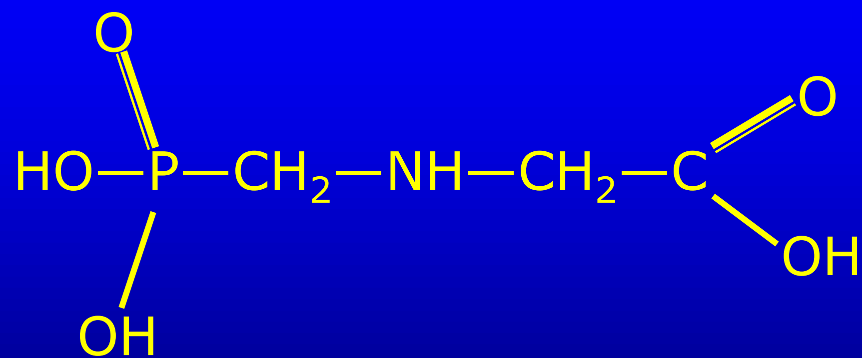
{very broad assumptions; need to consider activity}

Soil conservation benefits?

Soil conservation benefits? Soybean on loess soils of Missouri River uplands - 2004

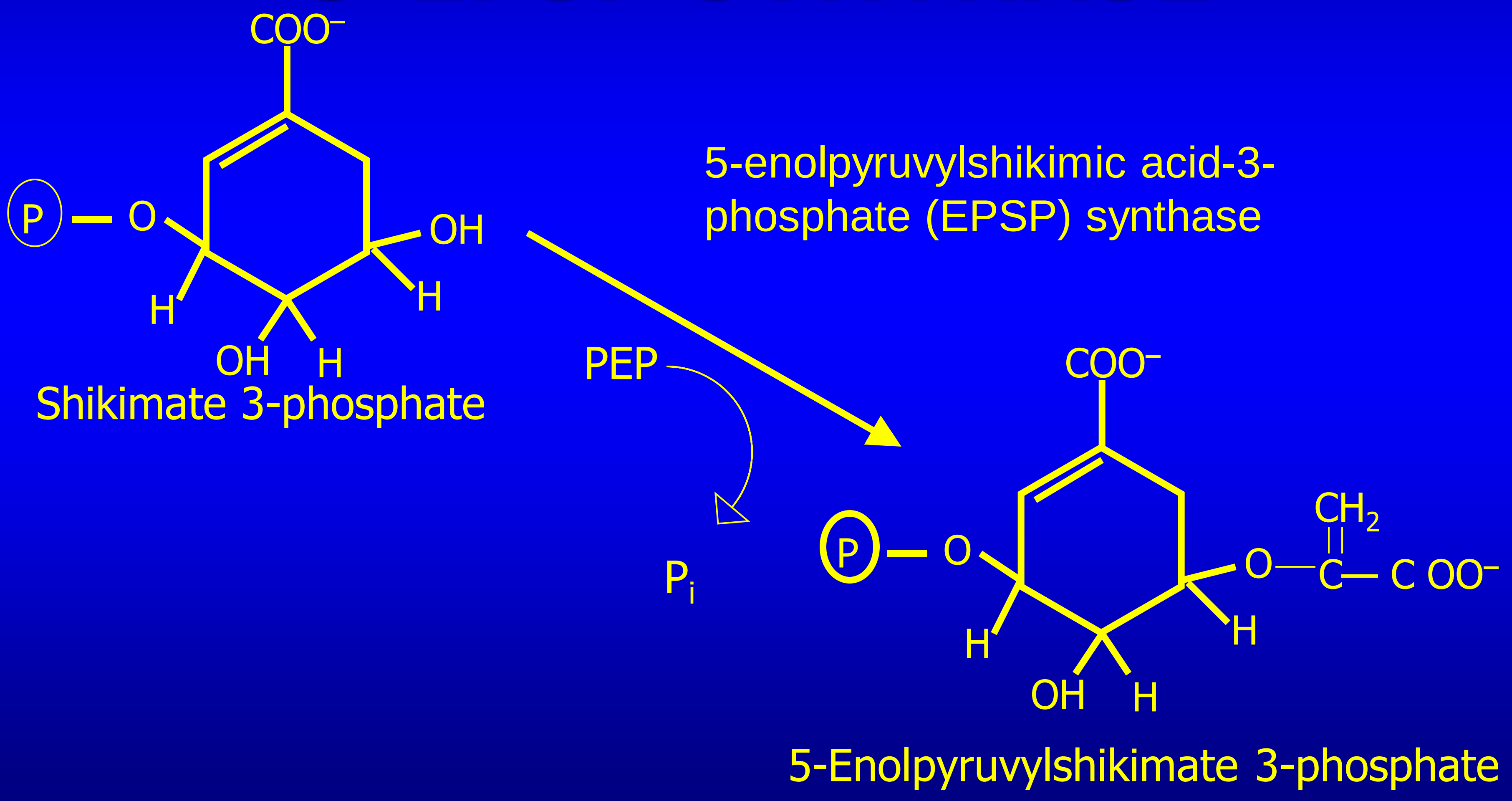
WHAT IS GLYPHOSATE?

- Glyphosate *N*-(phosphonomethyl)glycine active ingredient in **Round-Up®**, best selling herbicide world-wide
- Round-Up® is a nonselective systemic herbicide

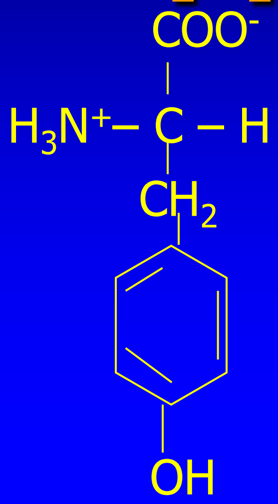


Glyphosate, Active Ingredient of Roundup

GLYPHOSATE IS A COMPETITIVE INHIBITOR 5-EPSP SYNTHASE

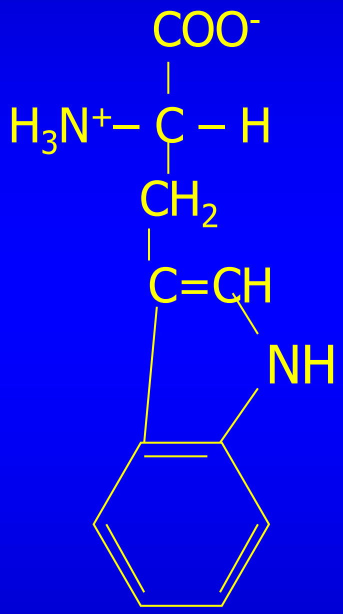


AROMATIC AMINO ACID PRODUCTION IS STOPPED



Tyrosine

Phenylalanine



Tryptophan

- Hetrocyclic compounds are not produced including phytoalexins
- Growth and cell maintenance stops and the plant dies

PHYTOALEXINS

- Plant's defense response to infection
- Antimicrobial, low-molecular-weight secondary metabolites capable of stopping pathogen development (Hammerschmidt 1999)
- Chemically diverse including simple phenylpropanoid derivatives (often products of the shikimic acid pathway), flavonoid- and isoflavonoid-derived, sesquiterpenes and polyketides
- Infection by a pathogen induces accumulation of phytoalexins in plants at the infection site (Hammerschmidt 1999)

Why Study Roundup Ready® Crops and Soil Ecology?

1. Impact of Roundup reaching soil surface.
2. Impact of glyphosate within the plant
   Incidence of soil fungi increases on roots of Roundup-treated, non-genetically altered crops and weeds – "secondary mode of action"
   [Rahe et al. 1990]

3. Concerns by producers of apparent "unexplained" production problems in Roundup Ready® Soybean

4. Potential "risk factor" with introduction of GM crops into the soil environment → possible unanticipated or detrimental effects on native organisms and biological processes [Angle, Molec. Ecol. (1994) 3:45-50]

High incidence of *Fusarium* Head Blight (FHB) levels in wheat in Saskatchewan

"Risk Production Factors" associated with FHB:

Environment (rainfall, temperature)

Crop Production Factors –

****Roundup applied 18 – 36 prior to wheat planting had <u>most consistent</u> relationship to FHB development across all years studied.**

M. Fernandez (2003). Report to Saskatchewan Agriculture Development Fund.

Reports on Consequences of Roundup Ready Crops

- **Development of Roundup-resistant weeds**

  (Heap & LeBaron, 2001)
- **Reductions in nodulation, leghemoglobin, chlorophyll in soybean under stress**

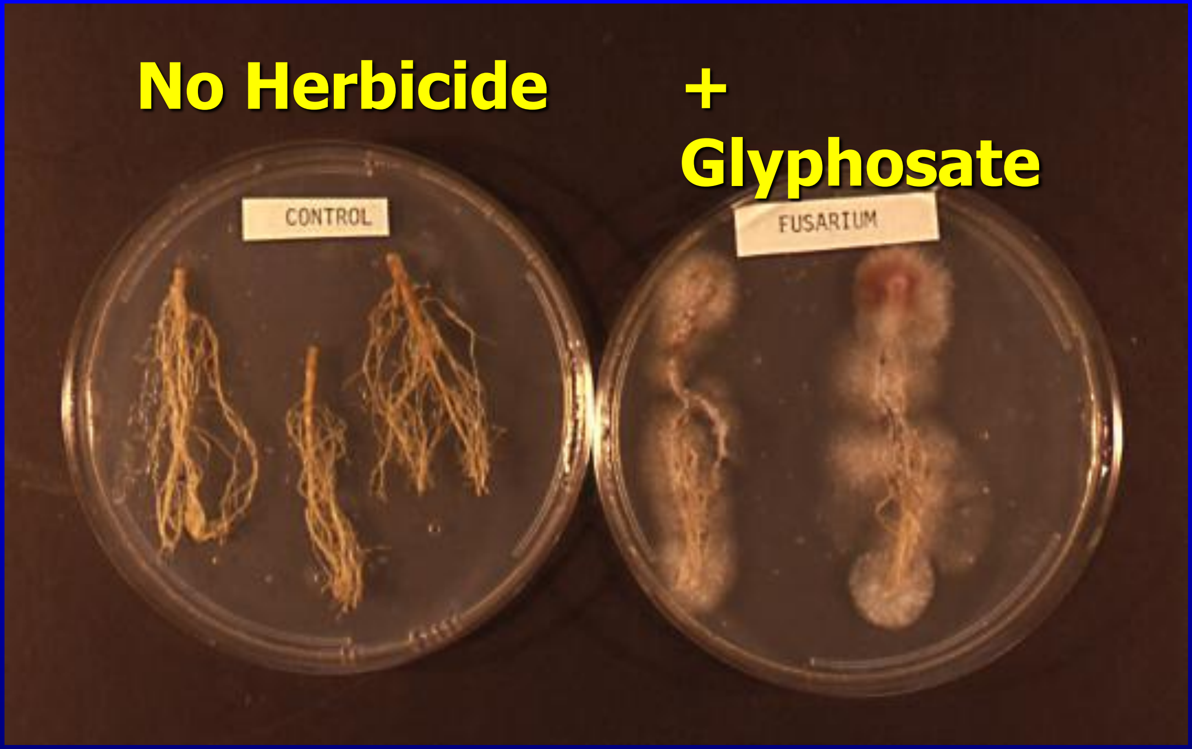
  (King & Purcell 1998; Reddy et al. 2000)
- **Fruit (boll) abortion in Roundup Ready cotton**

  (Pline et al. 2002. Abstr. Weed Sci. Soc. Am., p. 29)
- **Increased severity of "take-all" disease in winter wheat crop following Roundup Ready soybean (Indiana) - caused by soilborne fungal pathogen -**

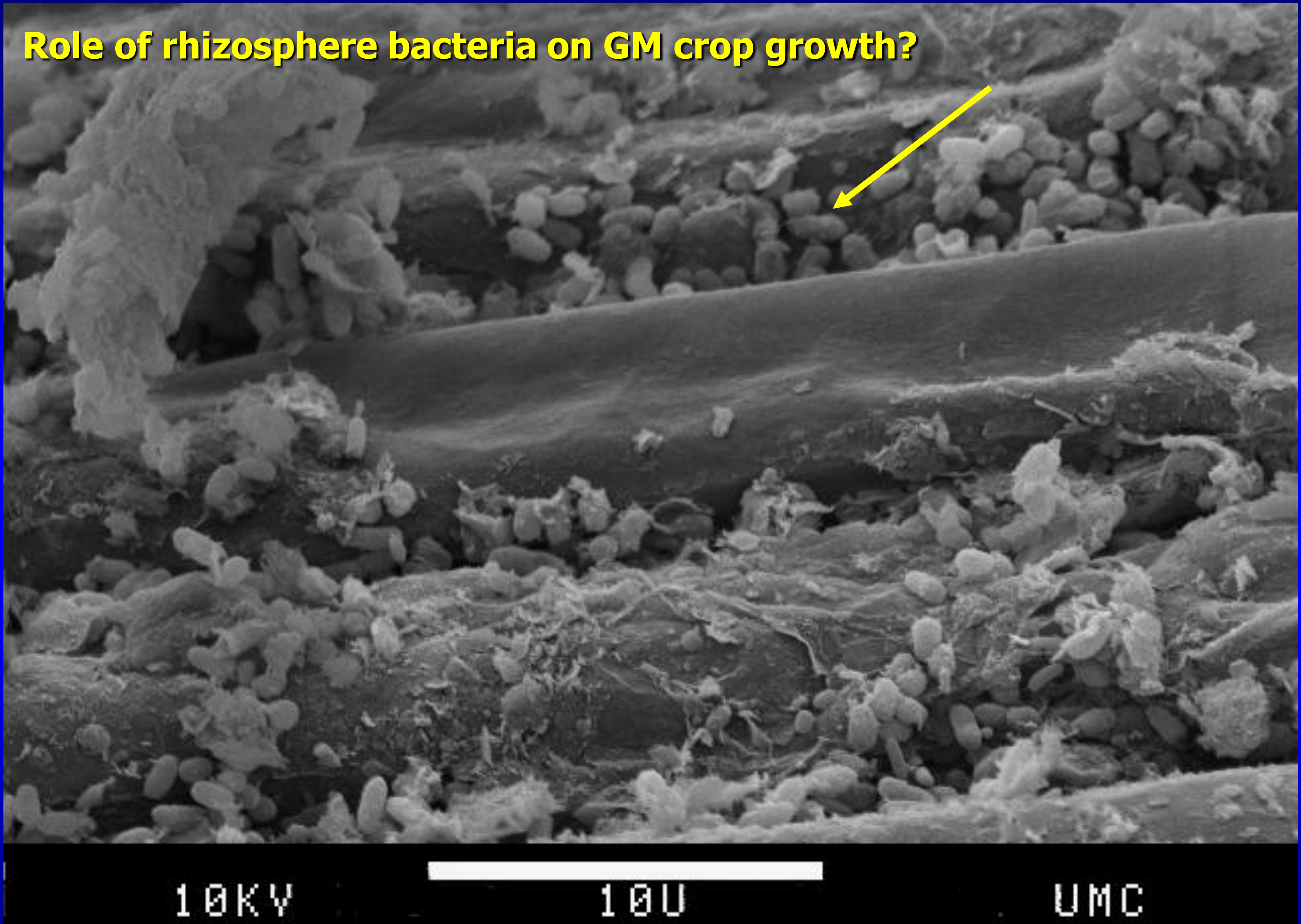
  (Hickman et al. 2002. Abstr. Weed Sci. Soc. Am., p.7)

Previous Research – Glyphosate-Rhizosphere Interactions

- High root colonization in susceptible plants by soilborne fungi => 'secondary mode of action' – possibly due to shutdown of phytoalexin production [Rahe et al. 1990]
-



Role of rhizosphere bacteria on GM crop growth?



Deleterious rhizobacteria on root surface of giant foxtail

Soil and Root Fungi

***Fusarium* spp.**

- **indicators of microbial ecology of soybean rhizosphere**
- **potential for pathogenicity to plants i.e., response to root exudates**
- **some members cause economically important diseases**
  - **root rots**
  - **sudden death syndrome (SDS; *Fusarium solani* fsp *glycines*)**
- **may associate with SCN to increase disease severity (SDS)**

Soybean Cyst Nematode (*Heterodera glycines*)

- Microscopic, parasitic roundworm
- Infects and parasitizes soybean roots
- Causes poor nutrient and water utilization
- Infestations in 28 states and Ontario, Canada
- Estimated soybean yield loss $1.67 billion in 1998

SCN Adult (veriform) and eggs

SCN Cysts on Soybean Roots

ROUNDUP READY SOYBEAN, WESTERN ILLINOIS, 2001

Does glyphosate applied to GR soybean enhance certain diseases?

Roundup Ready soybean planted using typical management practices

Study 1: MU Delta Center (1997 – 2000)
MU Bradford Farm (1999 – 2000)

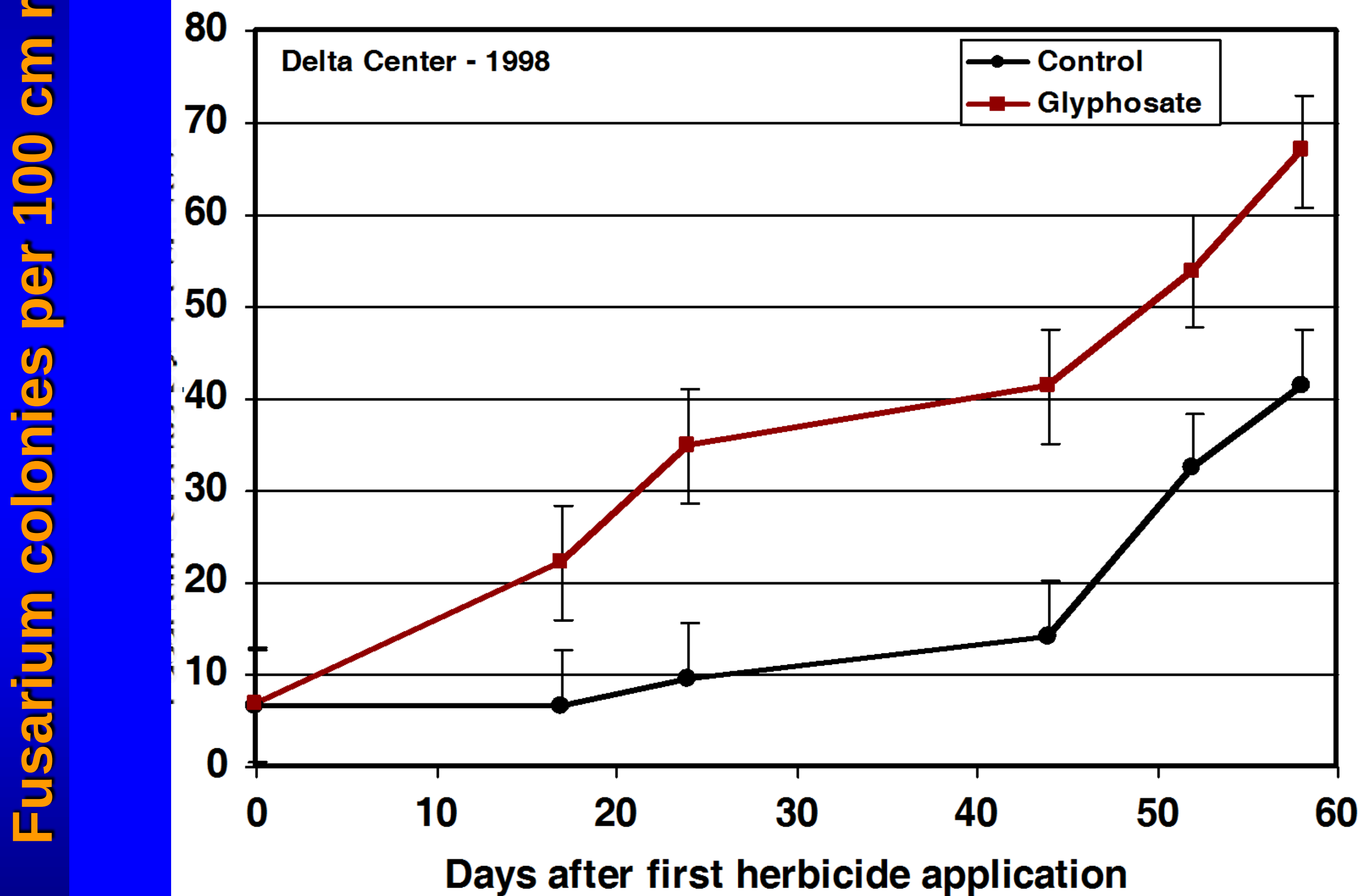
Herbicide: Roundup Ultra® at 2 and 4 wk POST
No herbicide

Sampling: Soybean plants sampled frequently up to 60 days post-Roundup application ("Intensive sampling")

Measurements: Root *Fusarium*
Soil *Fusarium*
SCN egg reproduction

Figure 2. Incidence of rhizosphere *Fusarium* on RR soybean.

Vertical bars denote LSD ($p<0.05$)



Observations of higher root colonization of glyphosate-treated GM soybean by *Fusarium* spp. compared to no glyphosate treatment [Kremer 2003]

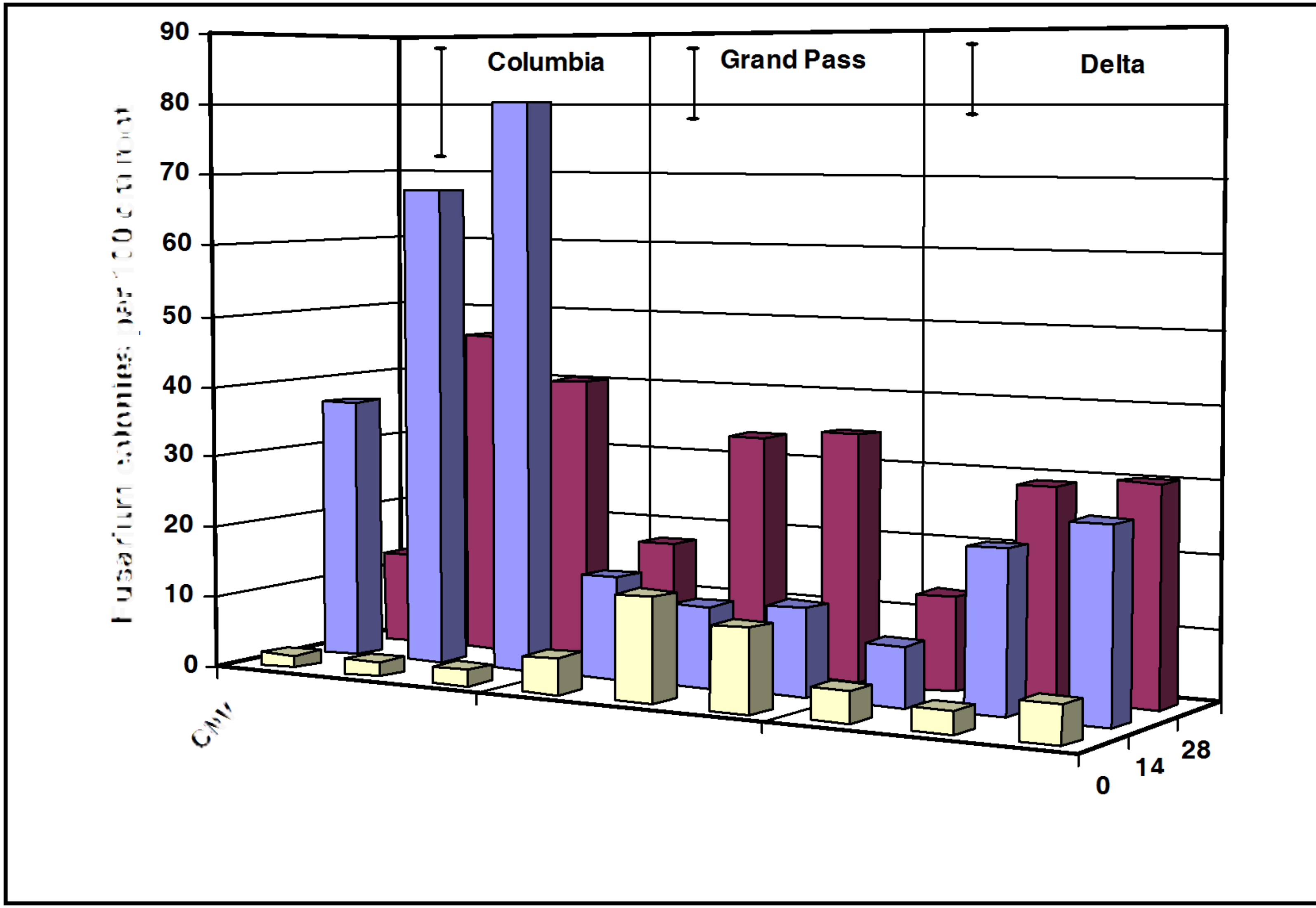
Study 2: Seven Missouri locations – 1999

Herbicide: Roundup Ultra® at 2 and 4 wk POST
Squadron® (Prowl + Scepter)
Roundup Ultra® + Squadron®

Sampling: Soybean plants sampled PRE, and
2 and 4 weeks post-Roundup application

Measurements: Root *Fusarium*
Soil *Fusarium*
SCN egg reproduction
Soybean yield
Seed fungi

Figure 4. Effects of herbicides on *Fusarium* root colonization of soybean at three Missouri locations averaged across cultivars, 1999[a]



[a] Days = "days after initial glyphosate application;" vertical bars are LSD (p<0.05) for within location comparis
Gly=glyphosate; CNV=conventional herbicide; GLY+CNV=glyphosate+conventional herbicide

Soybean cyst nematode egg reproduction factor, 1999.

		Herbicide treatment		
Location	Cultivar	Conventional	Roundup	Roundup + Convent.
Bradford	Asgrow	7.5	18.7	4.9
	Pioneer	1.4	1.6	4.8
	Novartis	1.2	1.4	2.7
	Dekalb	2.2	18.8	12.0
Delta Center	Asgrow	0	0	0
	Pioneer	0.1	0	0
	Novartis	0	0.2	0
	Dekalb	0	0	0
Grand Pass	Asgrow	6.0	0	15.8
	Pioneer	0.4	0.5	0.8
	Novartis	12.9	2.7	10.2
	Dekalb	0.6	4.8	4.5

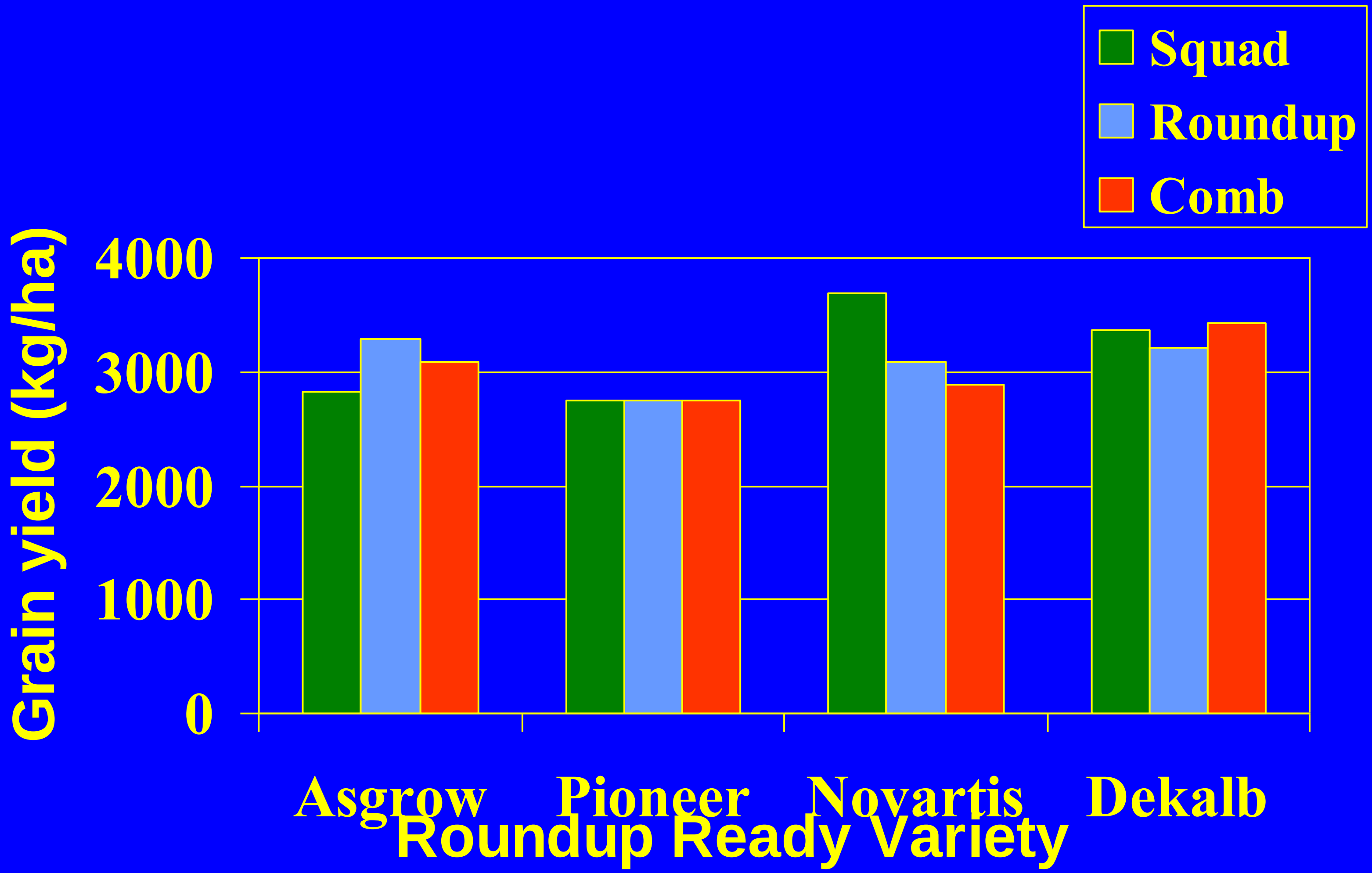
Reproductive Factor = (harvest egg count / planting egg count)

Bradford field count at planting = 1318 eggs

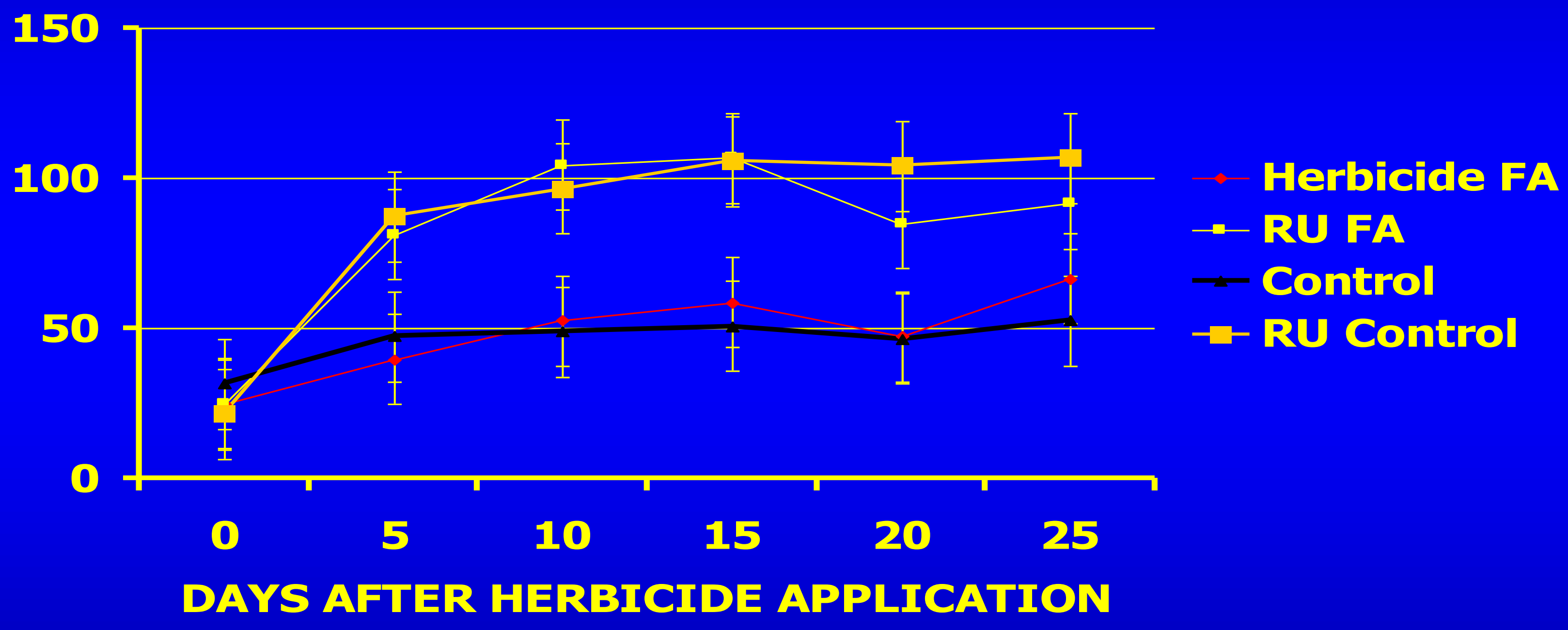
Delta field count at planting = 143 eggs

Grand Pass field count at planting = 707 eggs

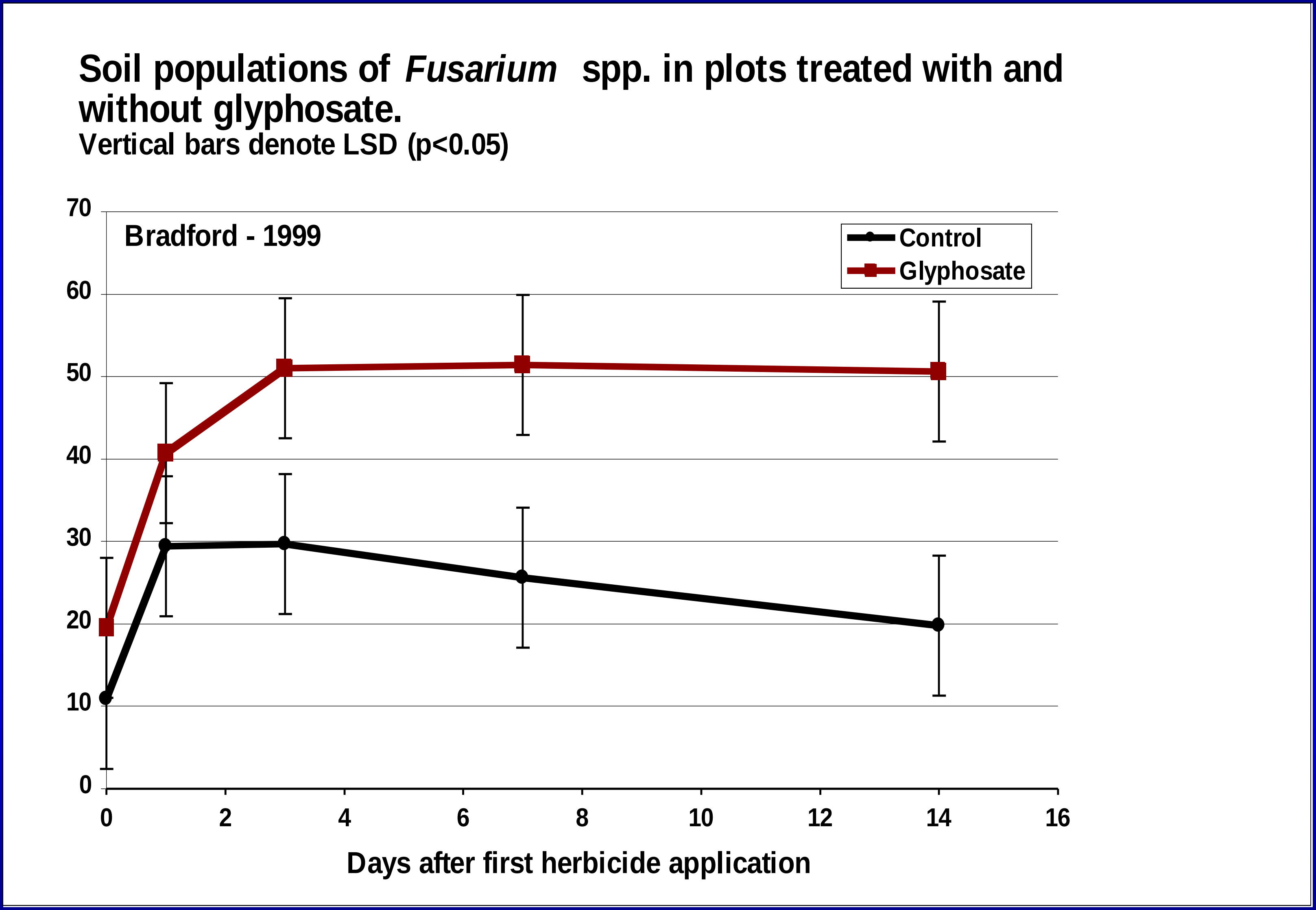
Soybean yield 1999 Grand Pass



ROOT FUSARIUM - 2002



Soil *Fusarium* responded similarly as root *Fusarium*



Identification of Root Fusarium Isolates

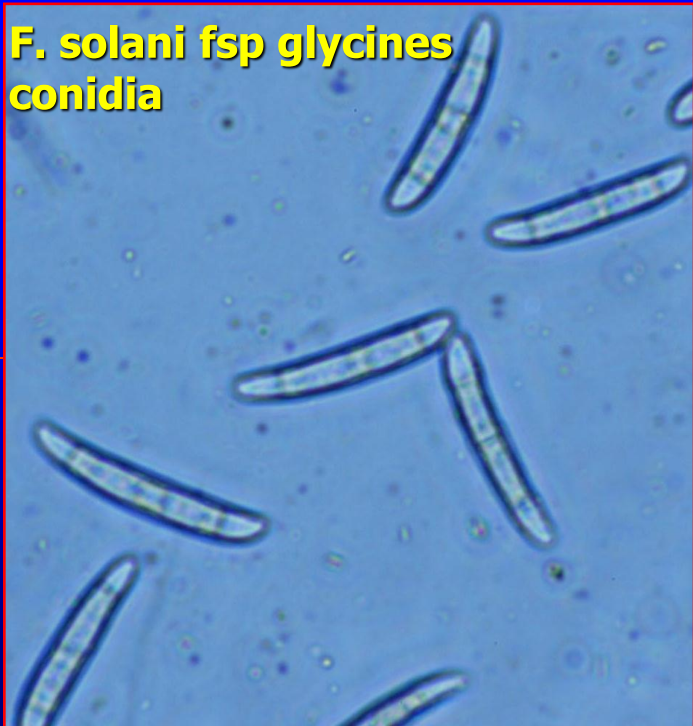
1. Subculture, characterize based on cultural and microscopic morphology; key based on Nelson et al. (1983)
2. Verified using nuclear DNA translation elongation factor sequence primer and Penn State database (Skovgaard et al. (2001)*

Identifications Over All Treatments:

Fusarium oxysporum **complex – 72%**

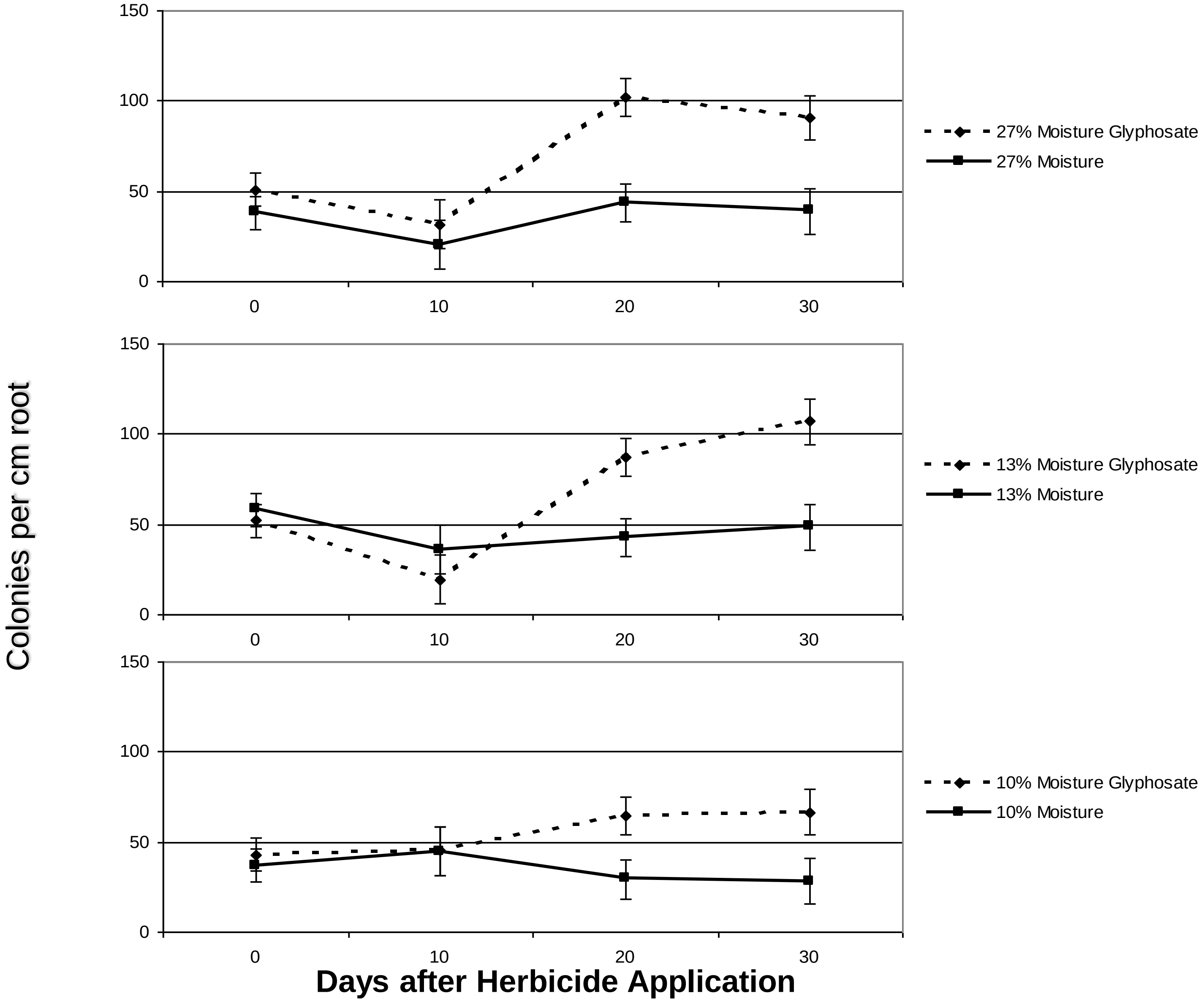
Fusarium solani **complex – 18%**

Fusarium equiseti **– 9%**

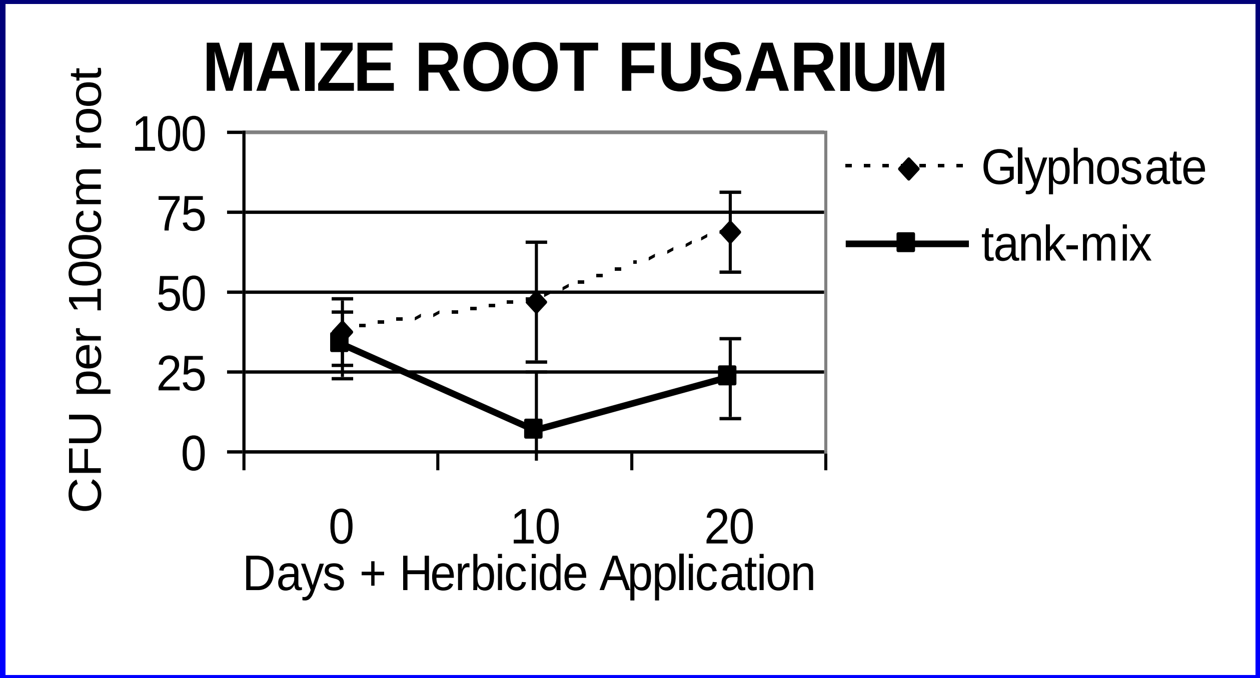


*Analyses conducted at ARS Mycology Lab, Peoria, IL by Kerry O'Donnell, 2004

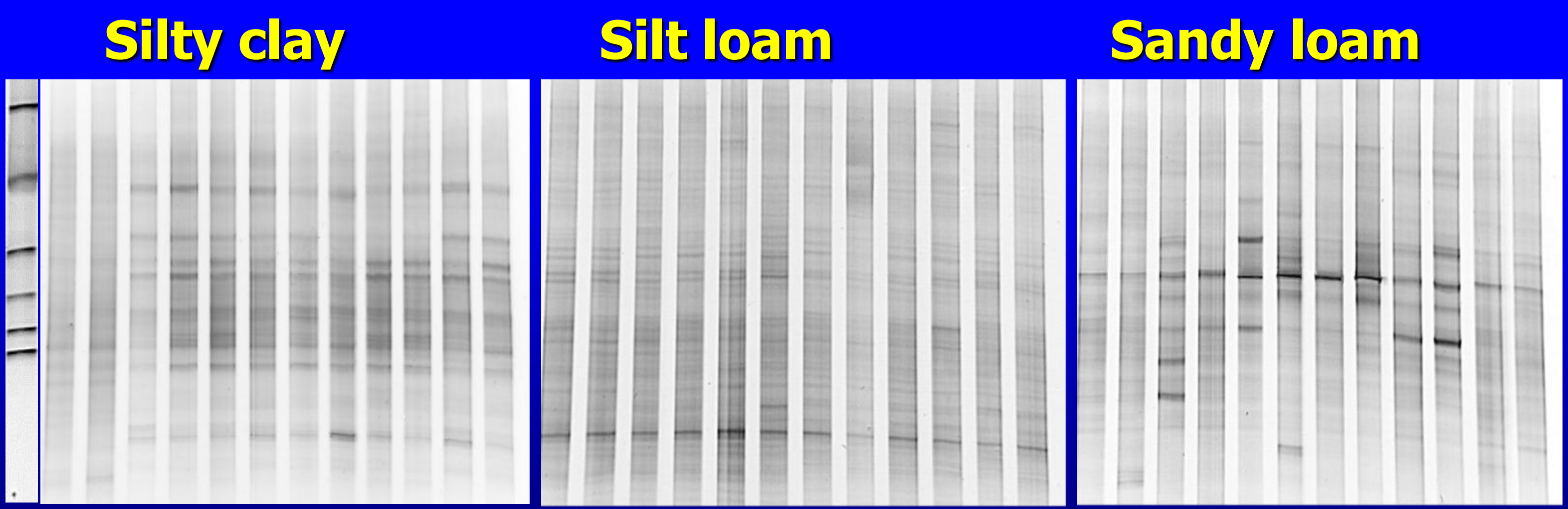
FIGURE 1
EFFECTS OF GLYPHOSATE ON SOYBEAN ROOT *FUSARIUM* AT 3 SOIL MOISTURES



Vertical Bars in Graphs Represent Line Differences (LSD at the 0.05 Level)

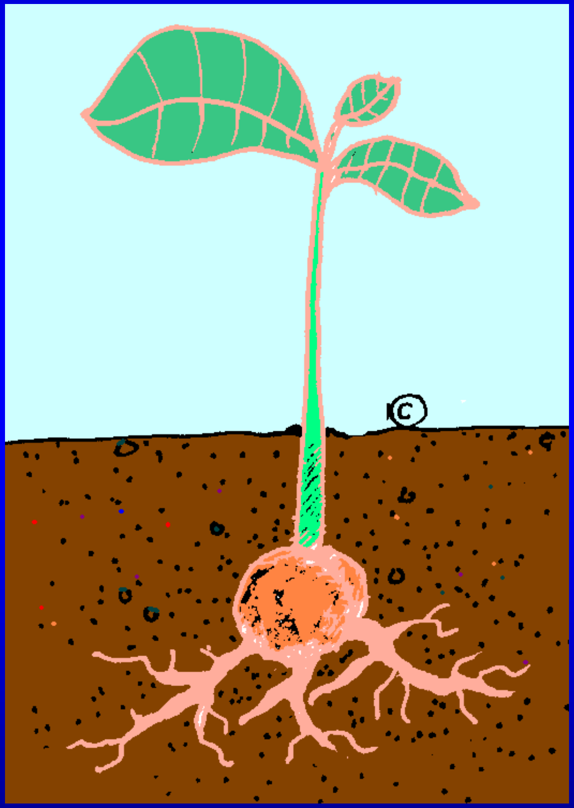


Bacterial diversity differed among soil textures, not maize variety



GLYPHOSATE IS SYSTEMIC

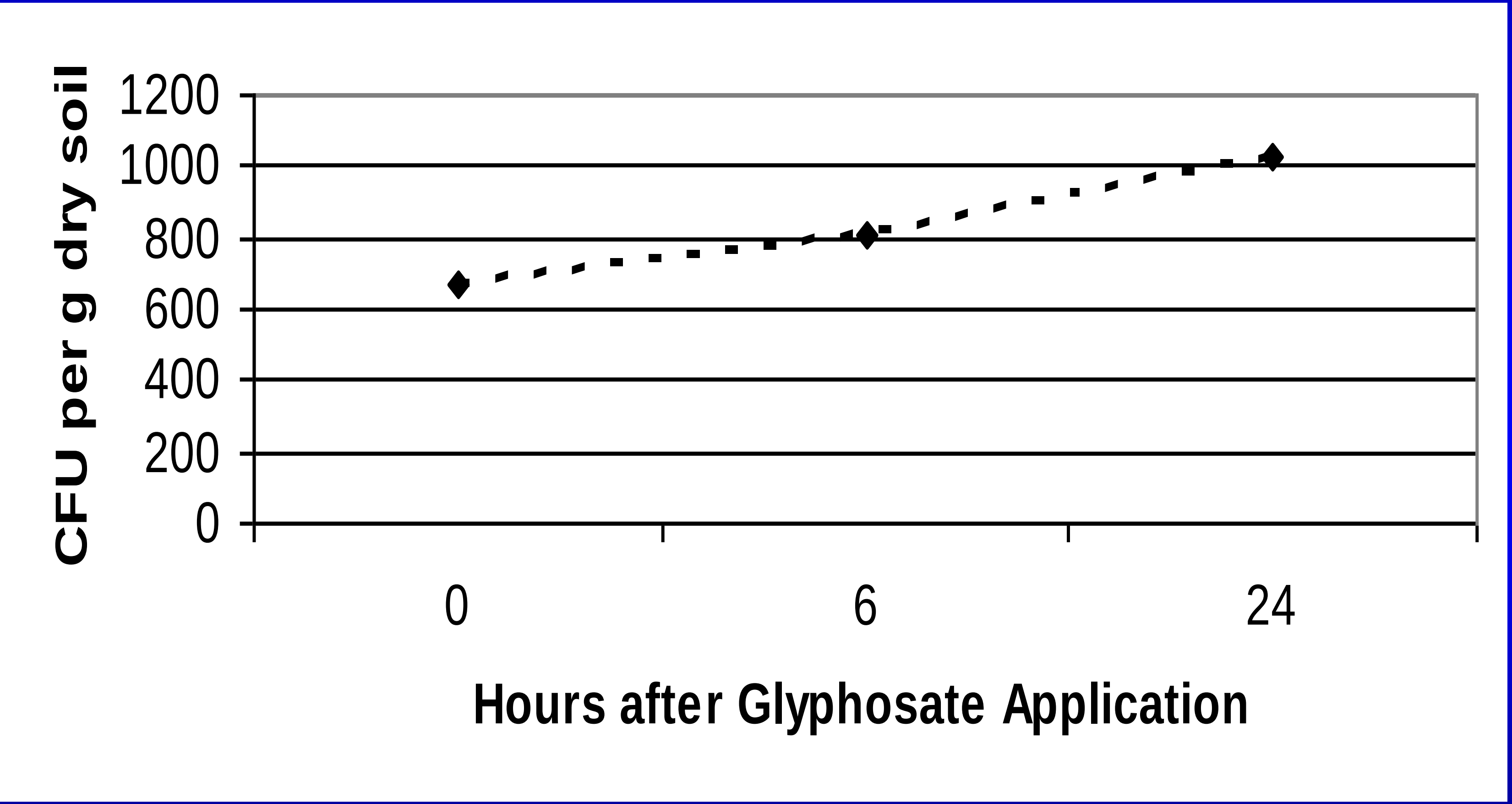
- Glyphosate is translocated in the symplast and accumulates in roots and meristematic regions (Duke, 1988, Hernandez et al., 1999)



GLYPHOSATE FATES

- Incidental soil contact: Glyphosate rapidly and tightly adsorbed to soil (K_d=33-660 ml g^{-1}); has shown little potential for runoff and mobility (Vencill 2002)
- However, glyphosate is directly and rapidly degraded by soil microorganisms (Haney et al., 2000)
- Applied to foliage: Low levels of glyphosate appear in the rhizosphere of <u>susceptible plants</u> (Coupland and Caseley 1979, and Rodrigues *et al*., 1982)

Glyphosate Applied to Surface of Mexico sil



Fate of Glyphosate in Agroecosystems Planted to "Roundup Ready" Crops

- Bound to EPSPS (both susceptible and resistant forms)
- Very small amount degraded in plant – aminomethylphosphonic acid [AMPA] – phytotoxic?
- Systemic movement to metabolic sinks
  - Nodules (soybean) – Reddy & Zablotowicz, 2003
  - Seeds – Duke et al., 2003
  - Roots – Duke, 1996
- Little information on release from roots of GM plants
  - Time course of release; Environmental fate
  - Rhizosphere effects; Impact on Rhizosphere microbes

GLYPHOSATE IN THE RHIZOSPHERE

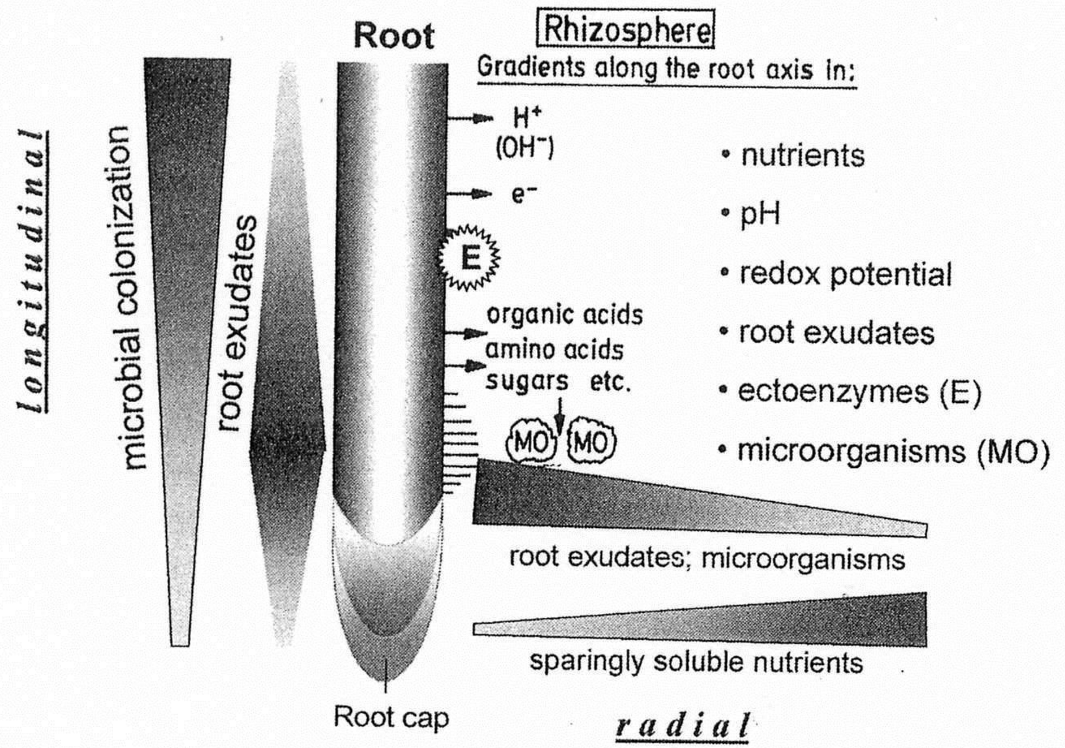
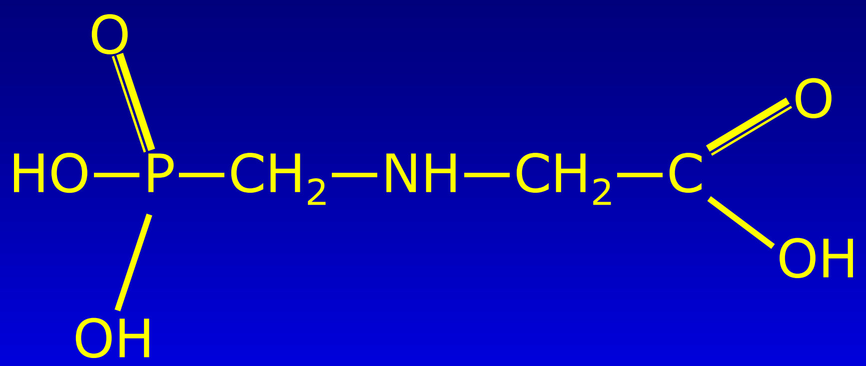


Figure 1 Gradients in the rhizosphere.

Low levels of glyphosate in the rhizosphere of treated plants (Coupland and Caseley 1979 and Rodrigues et al., 1982)

Figure from Neumann and Romheld, 2002 in Waisel et al. (eds.) Plant Roots The Hidden Half. Marcel-Dekker.



Glyphosate, Active Ingredient of Roundup

Potential source of C, N, P for growth and activity of soil and rhizosphere microorganisms

Scenarios - Glyphosate Impacts in Rhizosphere

- Phytoalexin production decreased in GM plants
  - Enzyme weakened indirectly due to genetic modification of plant [Gressel, 2001]
  - Root disease development a consequence of genotype, not use of glyphosate [Njiti et al., 2003]
- Glyphosate may serve as nutrient source for soil microorganisms [Haney et al., 2000]
- Glyphosate may stimulate growth of certain fungi [Krzysko-Lupicka & Orlik, 1997]

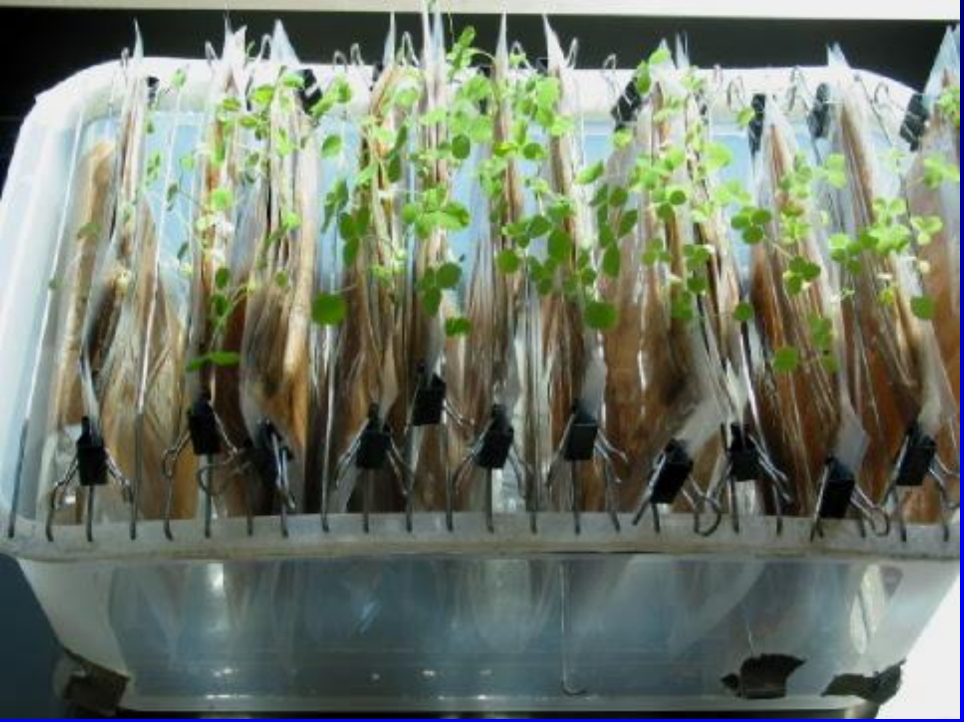
- Therefore, Glyphosate released into rhizosphere of plants genetically engineered for glyphosate resistance may affect microbial populations or activity in the rhizosphere --

OBJECTIVES

- Demonstrate glyphosate release from roots of glyphosate-resistant (GR) soybean
- Determine changes in concentrations of other major root exudates due to glyphosate treatment
- Determine effects of glyphosate in root exudates on selected rhizosphere microorganisms

EXPERIMENTAL

- Soybean cultivars:
  - Glyphosate-resistant (GR): ‘Pioneer 94B01’
  - Glyphosate-susceptible (W82): ‘Williams 82’



Hydroponic system for collecting root exudates

Growth pouches + nutrient solution

Soybean seedlings allowed to develop

Glyphosate applied based on field rates – 14 days

Nutrient solution + root exudates collected periodically for 16 d

ANALYSES

- Glyphosate – Enzyme-linked immunosorbent assay

  Detection limit = 1.0 ppb

- Soluble carbohydrates – Anthrone reaction, based on glucose equivalents

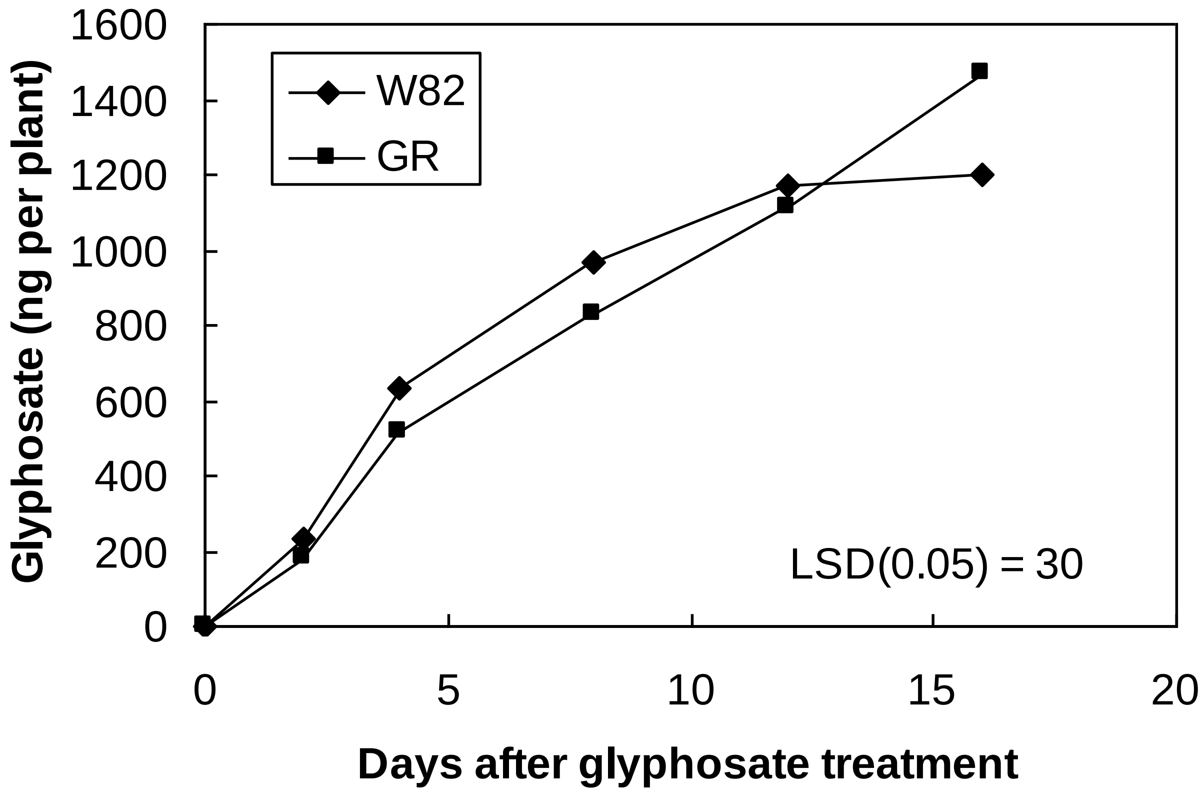
- Amino acids – Ninhydrin-nitrogen reaction

MICROBIAL BIOASSAYS

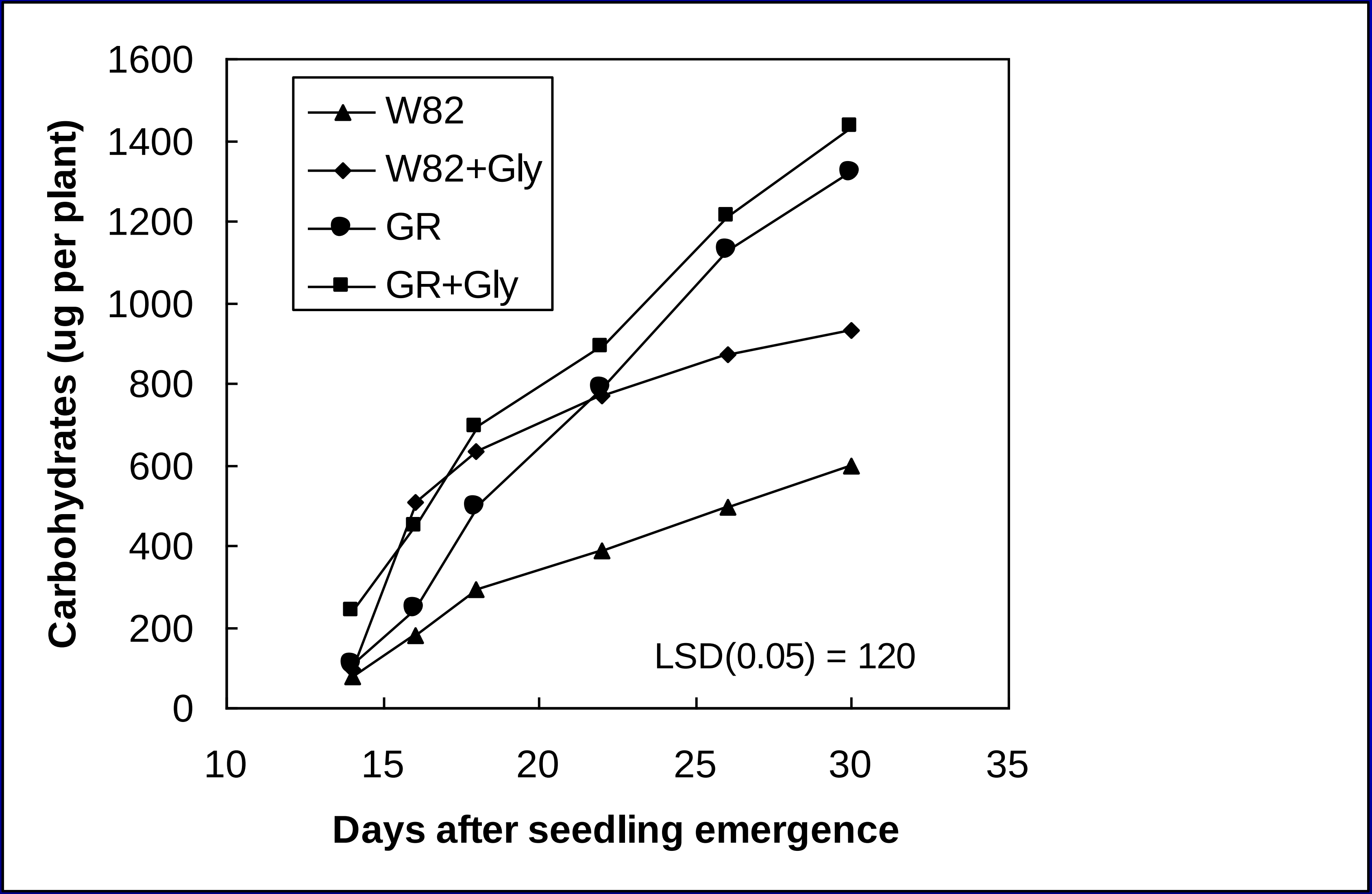
- Selected Fusarium spp. and rhizosphere bacteria from soybean rhizospheres cultured in 24-well microtiter plates on root exudates

- Selected microorganisms used in growth response to glyphosate concentrations

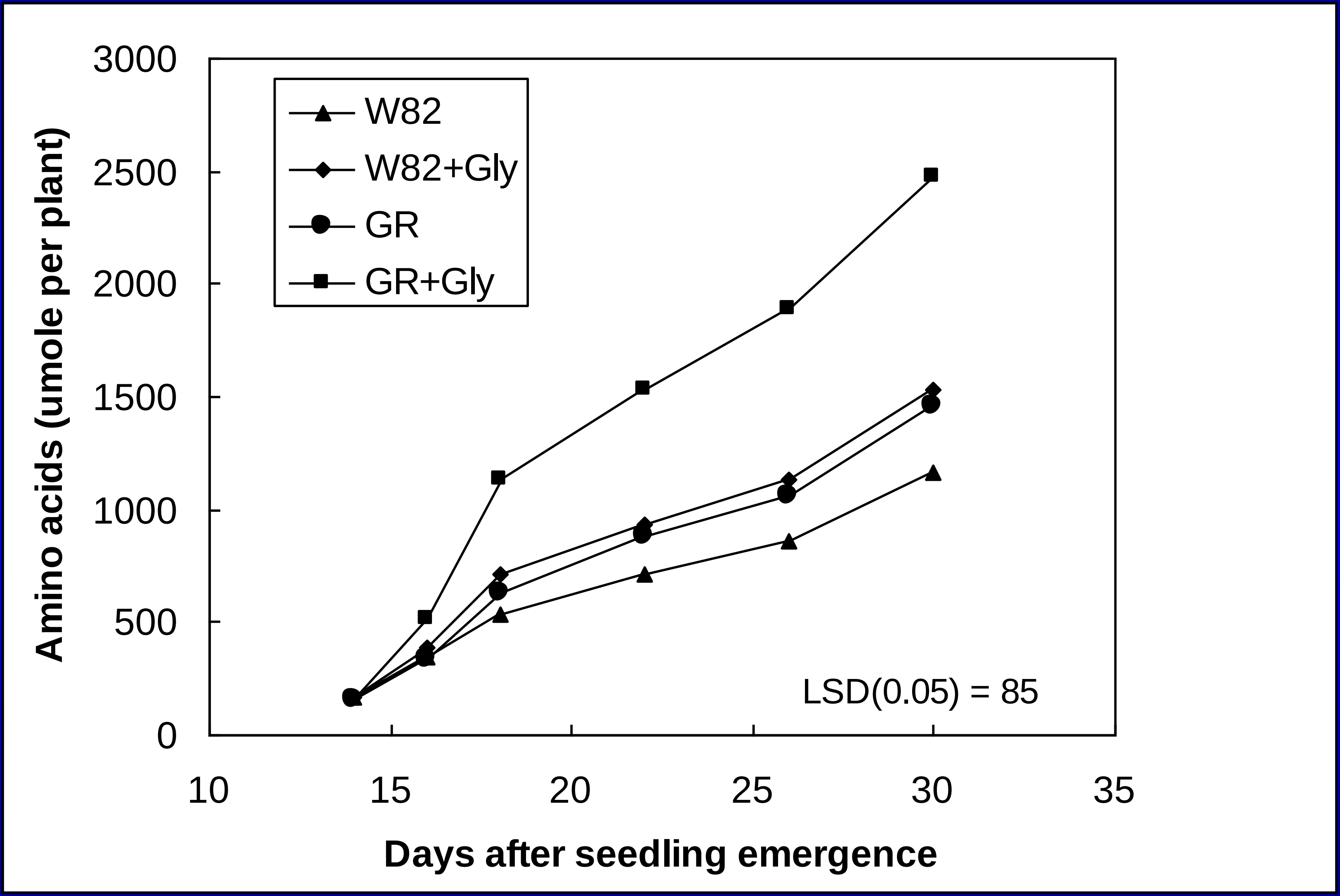
Cumulative glyphosate release into root exudates



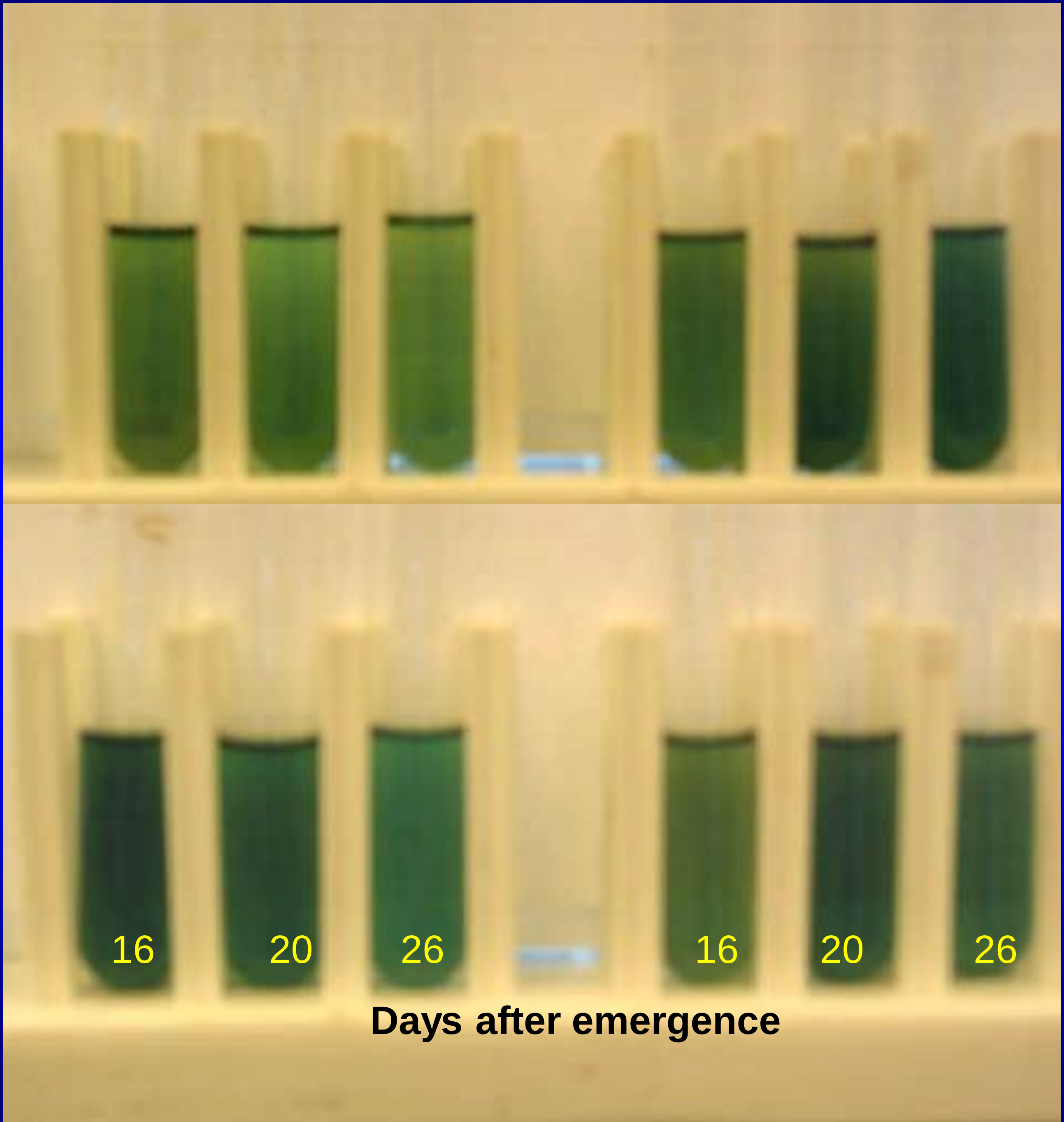
Cumulative carbohydrate released in exudates



Cumulative amino acids released in exudates



Williams 82 Roundup Ready



Carbohydrate content of soybean root exudates

– no herbicide treatment

- After glyphosate treatment

TABLE I Growth of selected fungal and bacterial cultures in root exudates collected from hydroponically-grown soybean 16 d after glyphosate application.
Fungal biomass was determined after 28 d incubation; bacterial populations were determined after 10 d incubation.

	Williams 82		**Glyphosate-resistant**	
	No glyphosate	**+ Glyphosate**	**No glyphosate**	**+ Glyphosate**
Fungal strains:	(mg fungal biomass)			
Fusarium 110	10 a*	10 a	10 a	8 a
Fusarium 206	31 b	29 b	36 a	35 a
Fusarium 301	14 b	18 c	8 d	27 a
Fusarium 304	20 c	24 b	16 d	28 a
	LSD (0.05) = 4			
Bacterial strains:	($\log_{10}$ cfu ml^{-1})			
Pseudomonas 39	6.36 b	7.36 a	6.30 b	5.78 b
Pseudomonas IRB	7.68 a	6.78 b	7.54 a	6.54 b
P. fluorescens G211	7.16 a	6.76 a	6.30 a	6.32 a
	LSD (0.05) = 0.6			

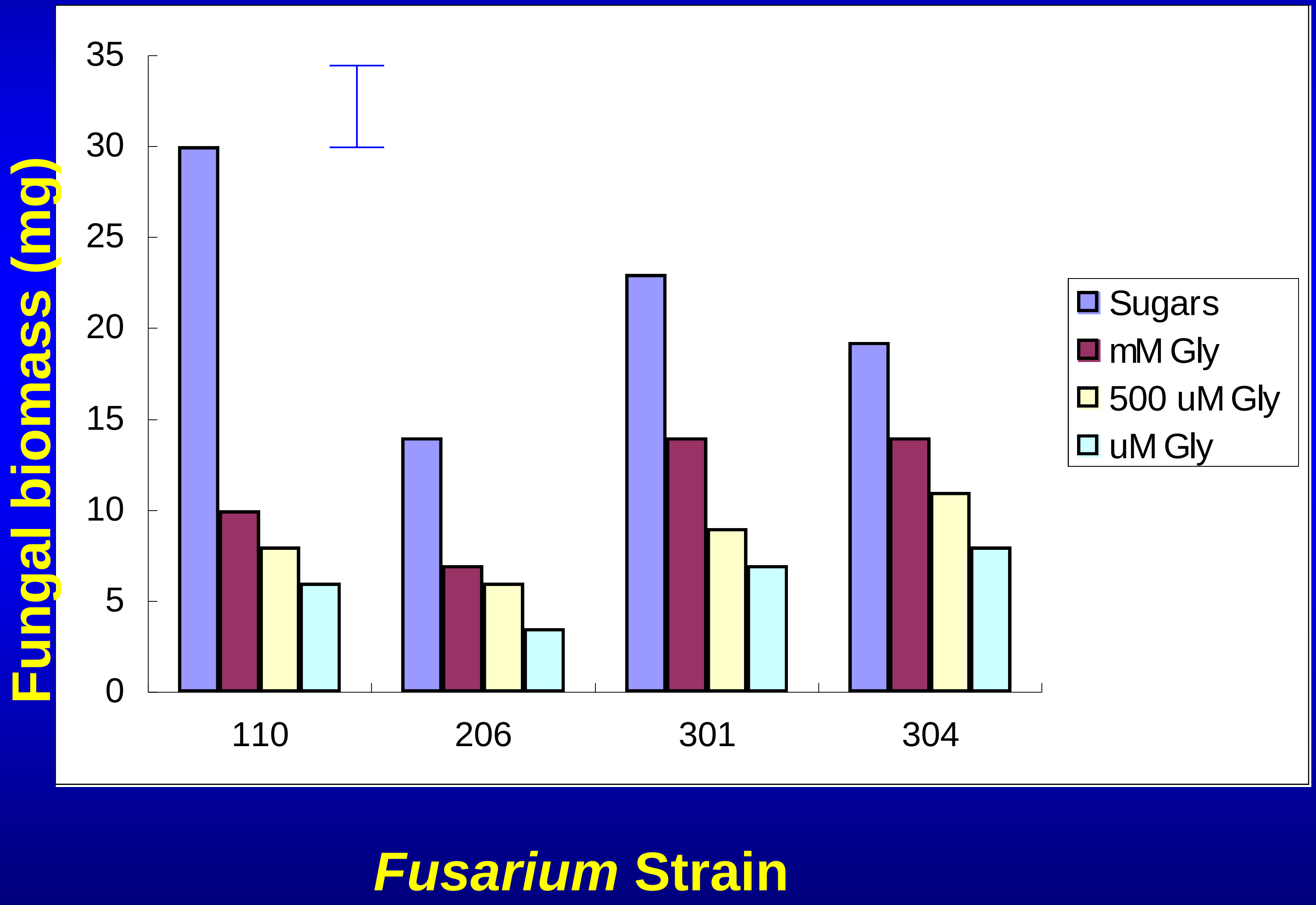
* Values within a row followed by the same letter are not significantly different.

Generally, those fungal isolates able to grow in root exudates of GR soybean + glyphosate treatment was likely due to:

1. **High concentrations of sugars in exudate ["substrate loading effect" favors dominance of fungi in rhizosphere – Griffiths et al., 2004]**
2. **Glyphosate in exudates may selectively stimulate growth of *Fusarium* spp. - Krzysko-Lupicka & Orlik, 1997**
3. **Thus, glyphosate released into rhizosphere combined with release of high concentrations of carbohydrates a/o amino acids influence fungal activity in rhizosphere of GR soybean**

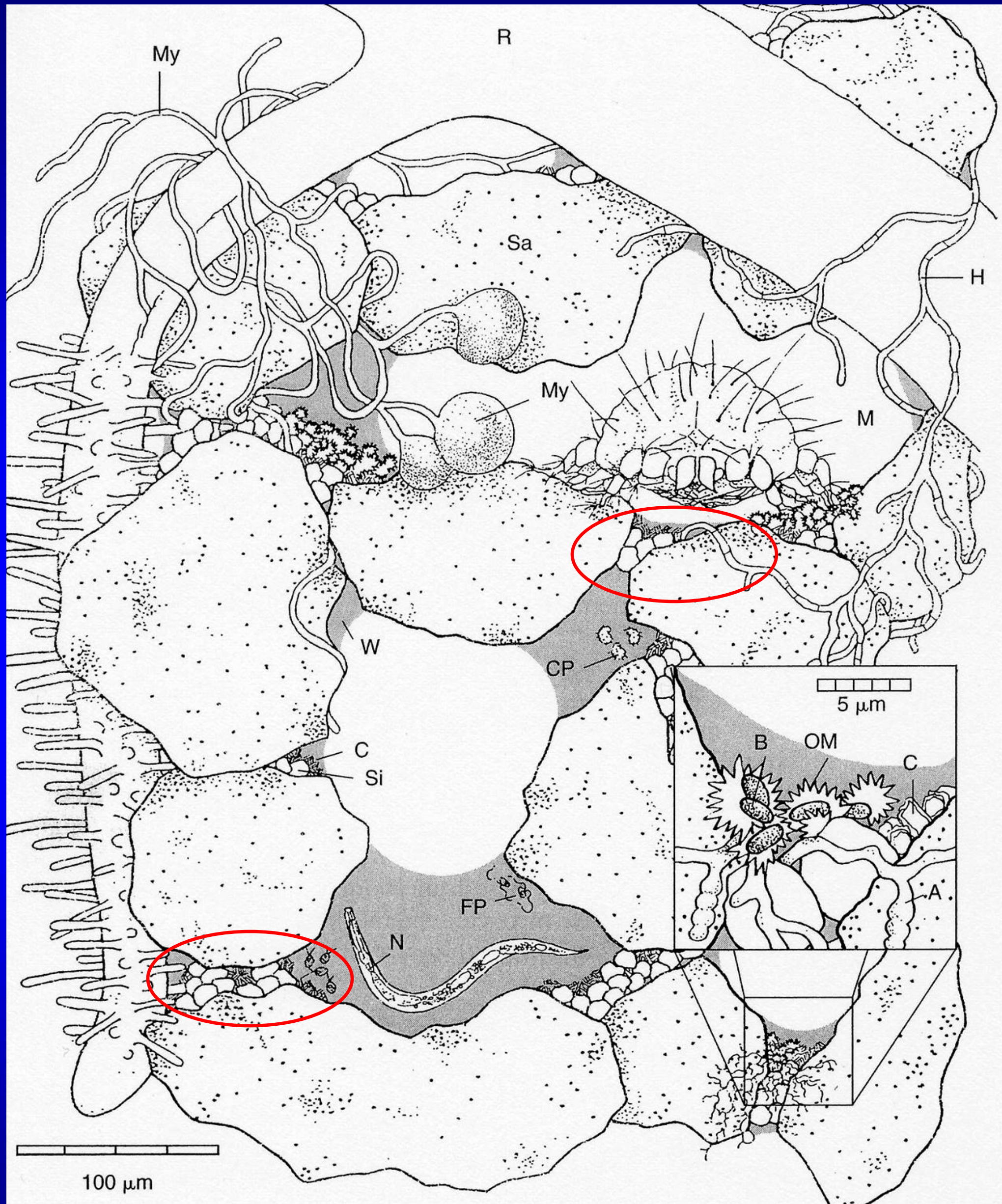
Growth of fungal cultures on glyphosate in 24-well microtiter plates – 28 d growth

Growth response of *Fusarium* on glyphosate



PRELIMINARY CONCLUSIONS

- Glyphosate applied to GR soybean is exuded into rhizosphere
- Glyphosate in combination with higher concentrations of carbohydrates and amino acids favor high colonization of roots by Fusarium compared to bacterial colonization
- A possible indirect effect of genetic modification of soybean for glyphosate resistance appears to increased rates of root exudation of carbohydrates and amino acids
- Glyphosate fate in GR cropping systems may have significant "indirect impact" on soil biology in addition to any direct environmental consequences



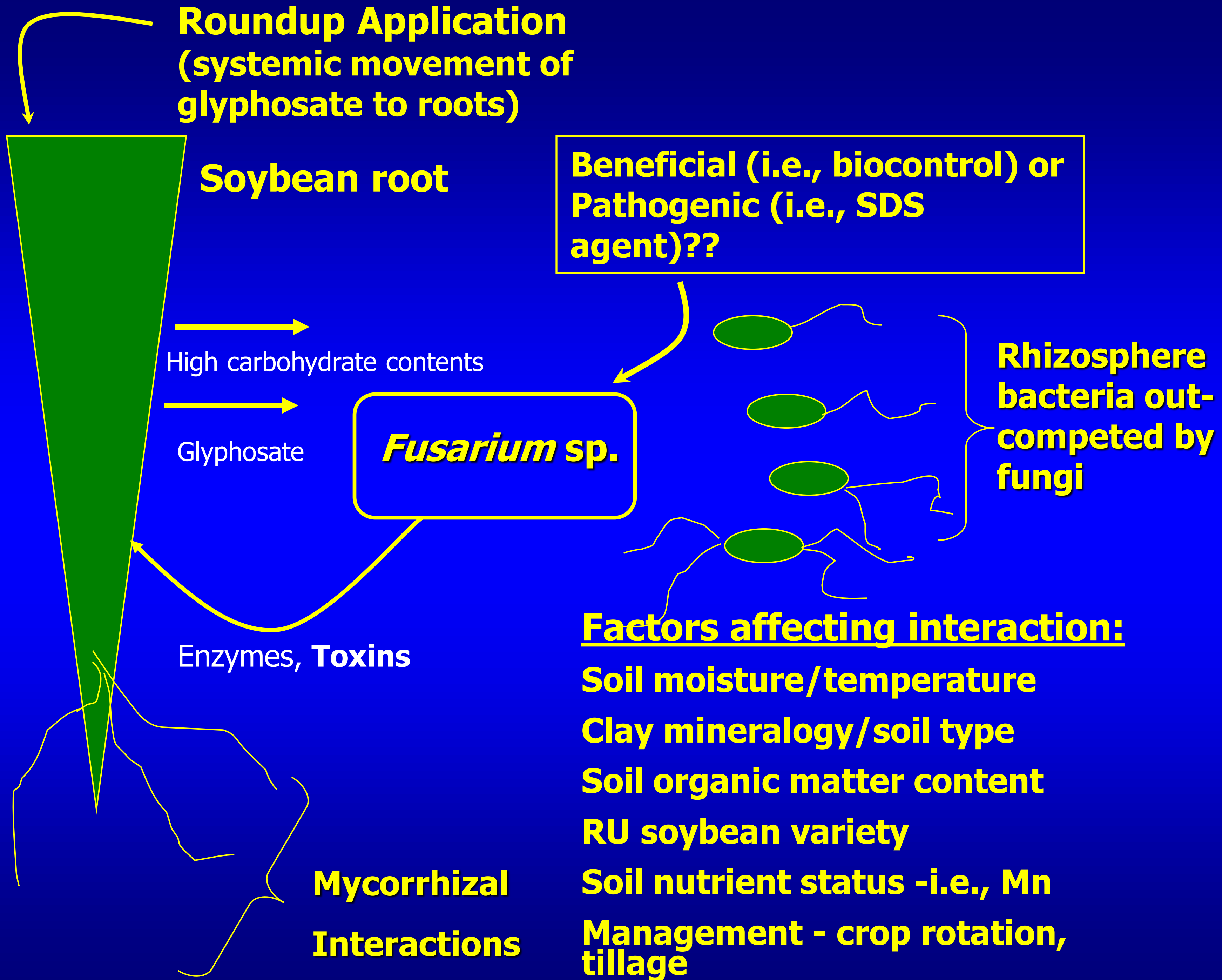
Root exudates:

Direct impact on processes in rhizosphere;

Altered composition, concentration, or rate of exudation of carbohydrates, amino acids, etc. impact microbial communities and processes;

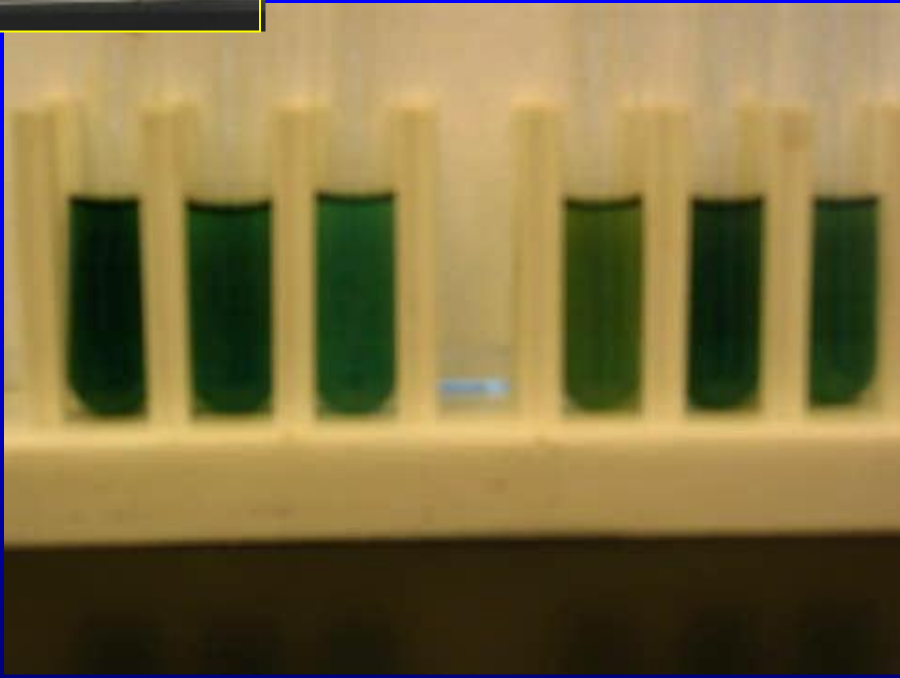
Exuded glyphosate may be readily available to rhizosphere microorganisms

Figure from Sylvia et al. 1998. Principles and Applications of Soil Microbiology


Roundup Application
(systemic movement of
glyphosate to roots)
Soybean root
Beneficial (i.e., biocontrol) or
Pathogenic (i.e., SDS
agent)??
High carbohydrate contents
Glyphosate
Fusarium sp.
Rhizosphere
bacteria out-
competed by
fungi
Enzymes, **Toxins**
Factors affecting interaction:
Soil moisture/temperature
Clay mineralogy/soil type
Soil organic matter content
RU soybean variety
Soil nutrient status -i.e., Mn
Management - crop rotation,
tillage
Mycorrhizal
Interactions

Soybean GR cultivars

Several hundred GR soybean cultivars are available - what is variability in root exudation characteristics?



Soybean seedlings in growth chamber – same age. Physiological differences between RR and “conventional varieties? How do trait differences affect rhizosphere processes, microbiology?

Conditions for Potential Disease Development

- <u>Environmental Factors</u> - Rainfall, Temperature
- <u>Soil Factors</u> - water content, texture, nutrient status
- <u>Soil Biology</u> - other soilborne pathogens, SCN, rhizosphere bacteria, other fungi
- <u>Other Soybean Pests</u> - soybean rust, aphid, weeds
- <u>GM Soybean Physiology</u> - variation across varieties?
- <u>GM Soybean Resistance Traits</u> - SCN, SDS, Root rot, etc.
- <u>Herbicide Management</u> - Rates, Formulations, # of Applications, and timing of Roundup; Integrated with other herbicides (i.e., residuals, specific for certain weeds - resistance, tolerance)
- <u>Cultural Practices</u> - Crop rotation frequency, tillage

ACKNOWLEDGEMENTS

- USDA Special Grant – SCN
- Ag Spectrum Co.
- Soil Microbiology Lab at the University of Missouri – Jenan, Heidi, Atim, Michael, Sara
- **Thank you** for the invitation to participate in the agricultural tour and symposium