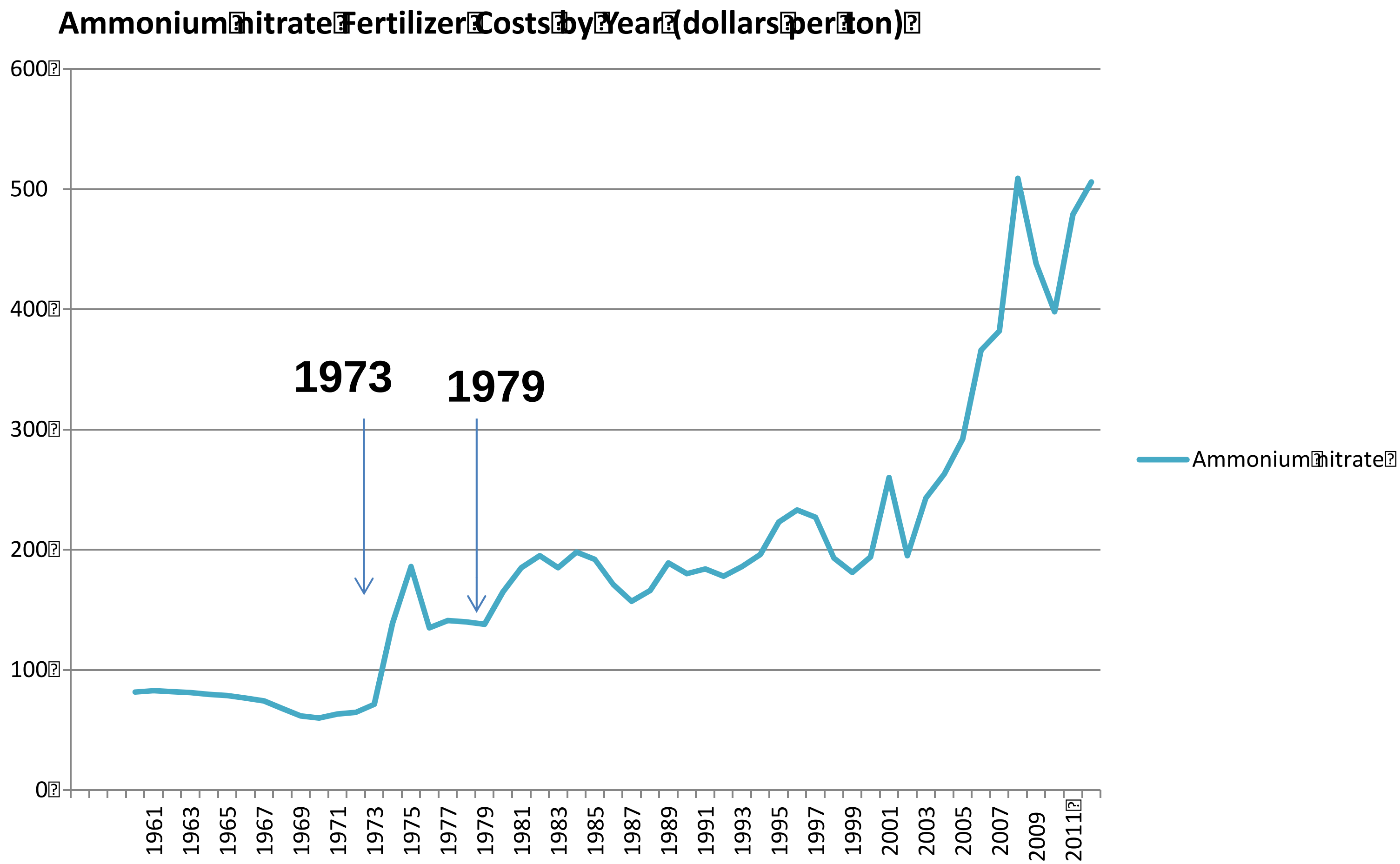
"Biological nitrogen fixation: Innovative approaches to address global challenges”

An NSF task force’s report identified two significant hurdles the NSF needed to overcome to support high risk/high return proposals: 1) the conservatism of the peer review process, and 2) the reluctance of NSF program officers to fund research with a high potential for failure in an environment in which proposals with a high probability of success were not being funded because of limited budgets.

OPEC induced oil shortages in 1973-74 and 1979-80 led to the US



Source: Agricultural Prices, National Agricultural Statistics Service, USDA.

Mosaic Science Magazine, Fall, 1973

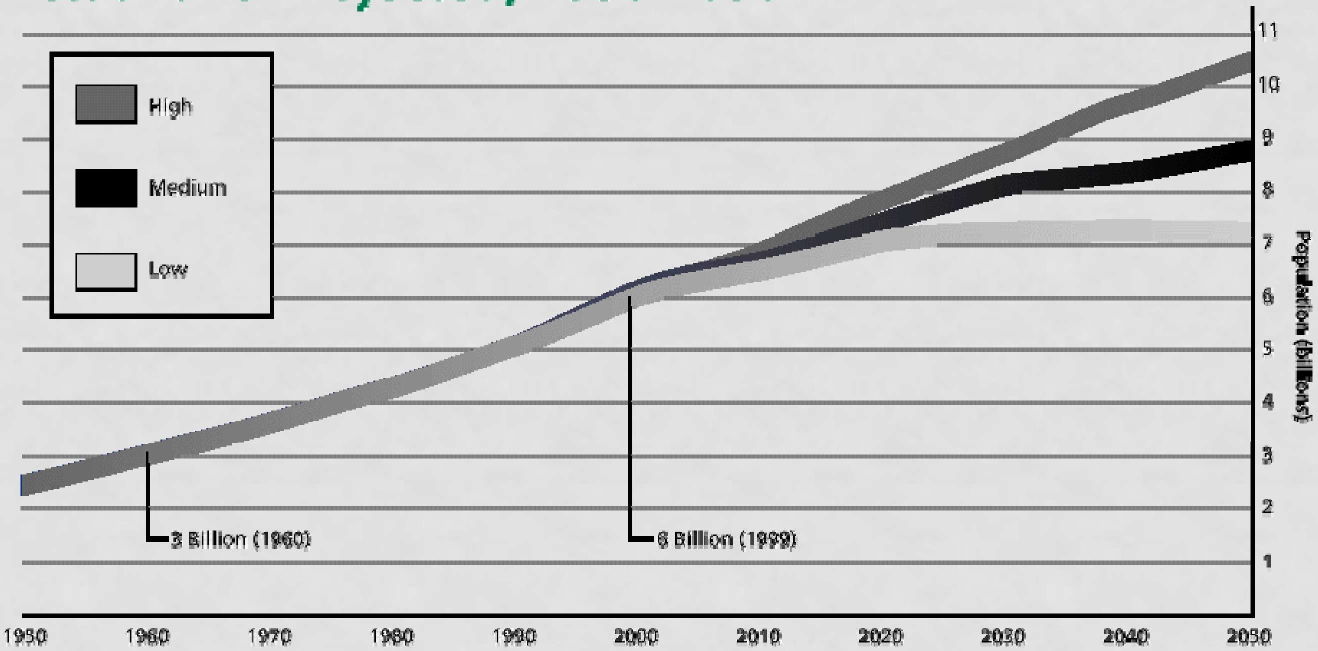
"In view of the many research studies in plant sciences, biology, and chemistry, and in view of the proliferation of new

Plant Physiol. (1982) 70, 1564–1567
0032-0889/82/70/1564/04/$00.50/0

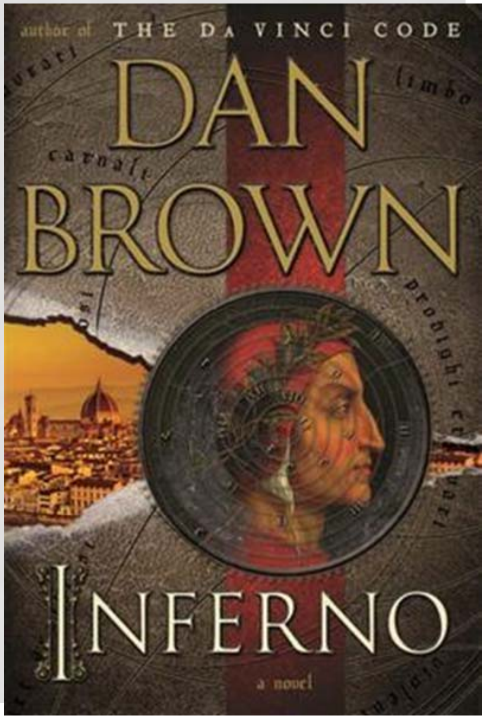
1982

21st CENTURY 'FASHION'

World Population Growth, Actual and Projected, 1950-2050



Source: United Nations. 1998. *World Population Prospects (The 1998 Revision).*



FUTURE PROJECTIONS

	2000	
POPULATION	6 Billion	9 Billion (maybe 10)
HUNGRY PEOPLE	0.8 Billion (17%)	1.4 Billion (17%)
N FERTILIZER	90×10^6 MT	165×10^6 MT
P FERTILIZER	42×10^6 MT	75×10^6 MT
FOOD PRODUCTION	3.5×10^9 MT	6.5×10^9 MT
WATER-STRESSED COUNTRIES	23	52 (10x flow Nile)

D Cordell et al., 2009. Gbl. Clt. Change 19:292;
S Postel. 2010. Water: Adapting to a New Normal. The Post Carbon Reader Series: Water Santa Rosa, CA
PE Fixen. 2009. Perspective on Current and Future Agricultural and Environmental Need for Enhanced Efficiency Fertilizers Plant Management Network.

WORLD AGRICULTURE CRISIS

HUNGRY PEOPLE	>	800 million - 1 billion
POVERTY	>	1.8 billion less than $1 per day
POPULATION	>	160 people every minute, 8 - 10 billion 2040

GLOBAL CLIMATE CHANGE Faster than anticipated

WOMEN	>	70% work in agriculture in low income food deficit countries
FERTILIZER (Non Renewable)	>	12-fold increase in N 6-fold increase in P
CEREALS	>	provide 60% of caloric intake
LEGUMES	>	provide 35-50% of protein intake
MEAT	>	40% of all grain fed to animals
WATER	>	75% of all water use is for agriculture by 2040 need 10X Nile
FOOD	>	By 2030 cereal demand = 3.1 billion tons cereal production = 3.0 billion tons

Many recent reports from the government and other organizations point out the importance of agricultural research to meet future global challenges and call for increased funding…..

- ✓ The 2009 "A New Biology for the 21st Century", a National Research Council report recommended increased support for agriculture.
- ✓ 2013 The Plant Science Decadal Vision, American Society for Plant Physiology, again called for increased support for interdisciplinary, plant-driven science.
- ✓ The National Bioeconomy Blueprint, released by OSTP, pointed out the potential of plant based bio-products.

- ✓ The Dec., 2012 report by the President's Council of Advisors on Science and Technology (PCAST), "Report to the President on Agricultural Preparedness and the Agricultural Research Enterprise", concludes that the nation is not prepared for future agricultural challenges and recommends major R&D investments achieved through expanding the role of competition at USDA and increasing support through NSF.

WHY NITROGEN [N_2]?

- PLANT N IS THE UNDERLYING SOURCE OF ALL HUMAN NUTRITIONAL N
- HIGHLY ABUNDANT BUT UNAVAILABLE DUE TO TRIPLE BOND $N \equiv N$
- PRODUCTION OF N FERTILIZER REQUIRES 2-4% OF EARTH'S NATURAL GAS YEARLY OUTPUT (NONRENEWABLE) AND IS NOT EFFICIENTLY USED BY PLANTS
- OVERUSE IN DEVELOPED WORLD POSES ENVIRONMENTAL PROBLEMS
- LACK OF AVAILABILITY IN DEVELOPING WORLD LIMITS CROP PRODUCTION
- LEGUME SYMBIOTIC N_2 FIXATION RENEWABLE AND SUSTAINABLE

CONSEQUENCES OF TOO MUCH N IN ENVIRONMENT

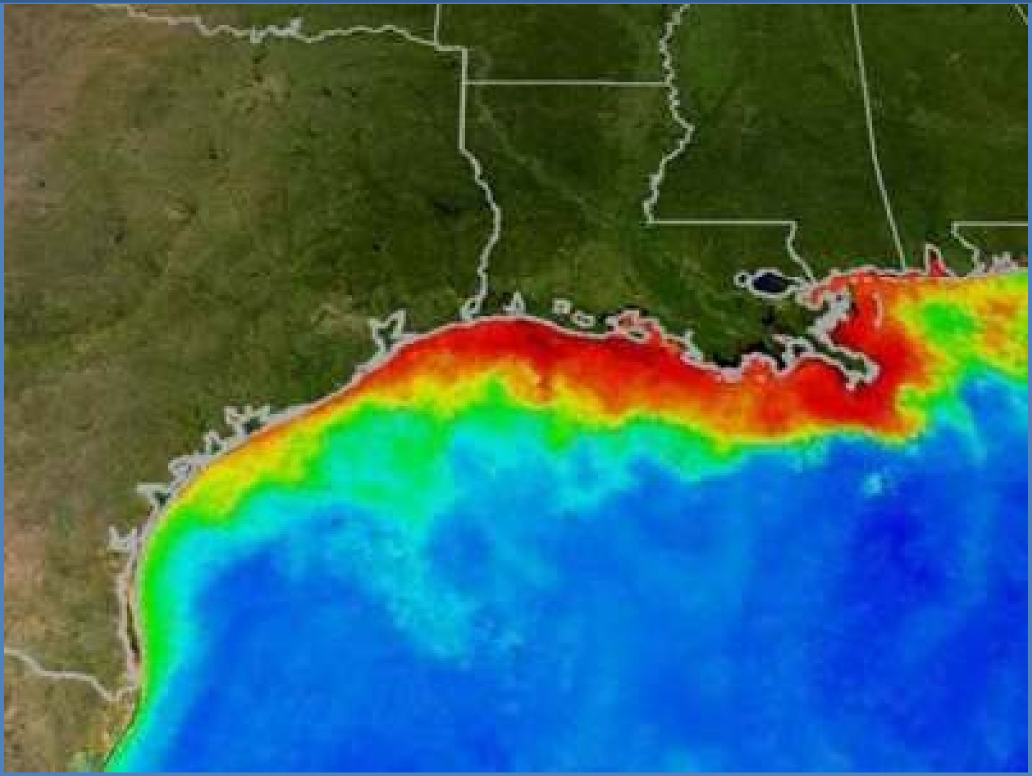


Photo courtesy of NASA/Goddard Space Flight Center Scientific Visualization Studio The above map shows concentrations of phytoplankton, the algal blooms that contribute to dead zones, in Gulf Coast waters.

MAJOR LEGUMES: PRODUCTION AND N2 FIXATION

- HA GROWN
- Mt PRODUCED 843 MILLION
- Mt N2 FIXED 24 MILLION
- N FERTILIZER $ VALUE $20-30 BILLION

“It is shocking—not to mention short-sighted and potentially dangerous—how little money is spent on agricultural research.” – Bill Gates

The problems that limit the ability of federal agencies to fund long-range, risky innovative research (i.e., 1 the conservatism of the peer review process, and 2) the reluctance of program officers to fund research with a high potential for failure in an environment in which proposals with a high probability of success were not being funded because of limited budgets.) are not found in philanthropic organizations, which have stepped up to fill this gap and to drive innovation in biological, physical and social sciences.

Gates Meeting: "Enhancing biological nitrogen fixation in crop plants" April 19-21, 2012

Three topic areas discussed:

1. Developing a rhizobial symbiosis in cereals
2. The introduction and encouragement of diazotrophic bacteria and cereal crop interactions
3. Synthetic biology, the design of a new organelle to fix N in crop plants

The research needed to achieve a practical, field level application of any of these technologies is likely to require a long term commitment.

<u>Two of three topics to be discussed:</u>

1. **Developing a rhizobial symbiosis in cereals**
2. **The introduction and encouragement of diazotrophic bacteria and cereal crop interactions**
3. **Synthetic biology, the design of a new organelle to fix N in crop plants ---Dr. Luis Rubio (Madrid), Ray Dixon (Norwich)**

Goals more tightly defined:

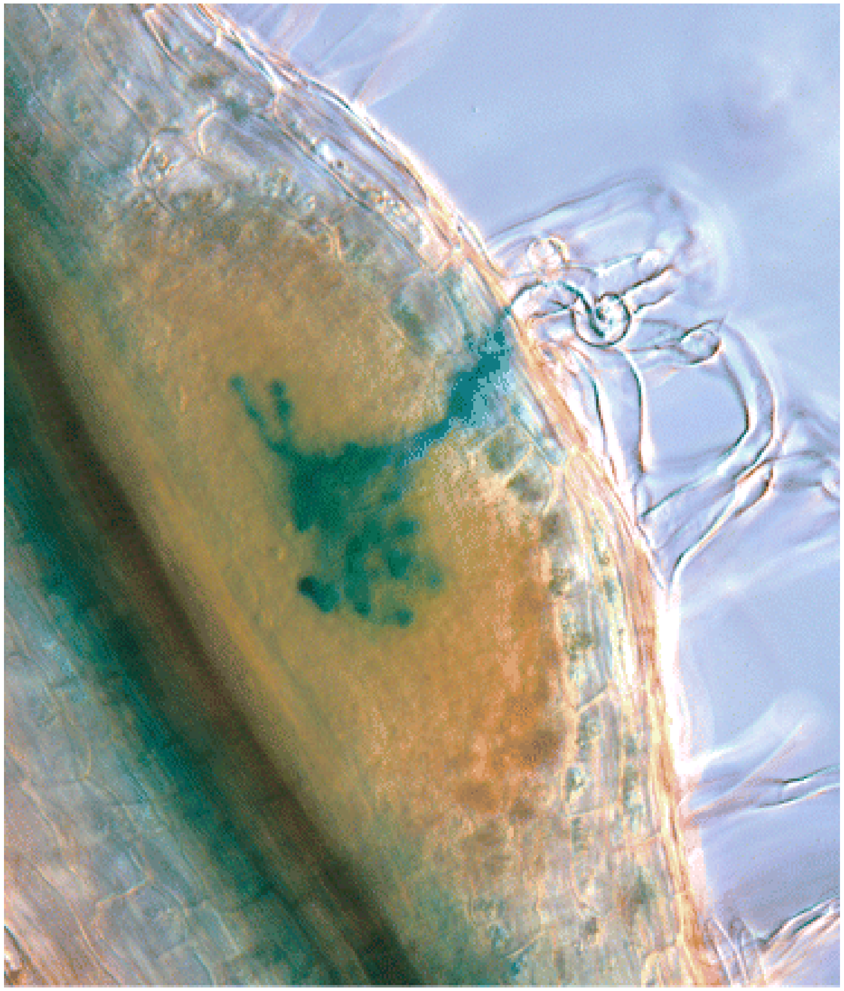
"Engineering the Sym pathway of cereals for recognition of nitrogen fixing bacteria"—John Innes Institute, Dr. Giles Oldroyd, lead investigator

Engineering the Sym pathway of cereals for recognition of nitrogen fixing bacteria

Questions:

- **What is the sym pathway?**
- **What would one engineer into plants?**
- **The title appears to presuppose that cereals and other non-legume plants lack the ability to recognize nitrogen fixing bacteria, is this true?**
- **If successful, how far would this get you toward achieving the original goal of**

“Developing a rhizobial symbiosis in cereals”?

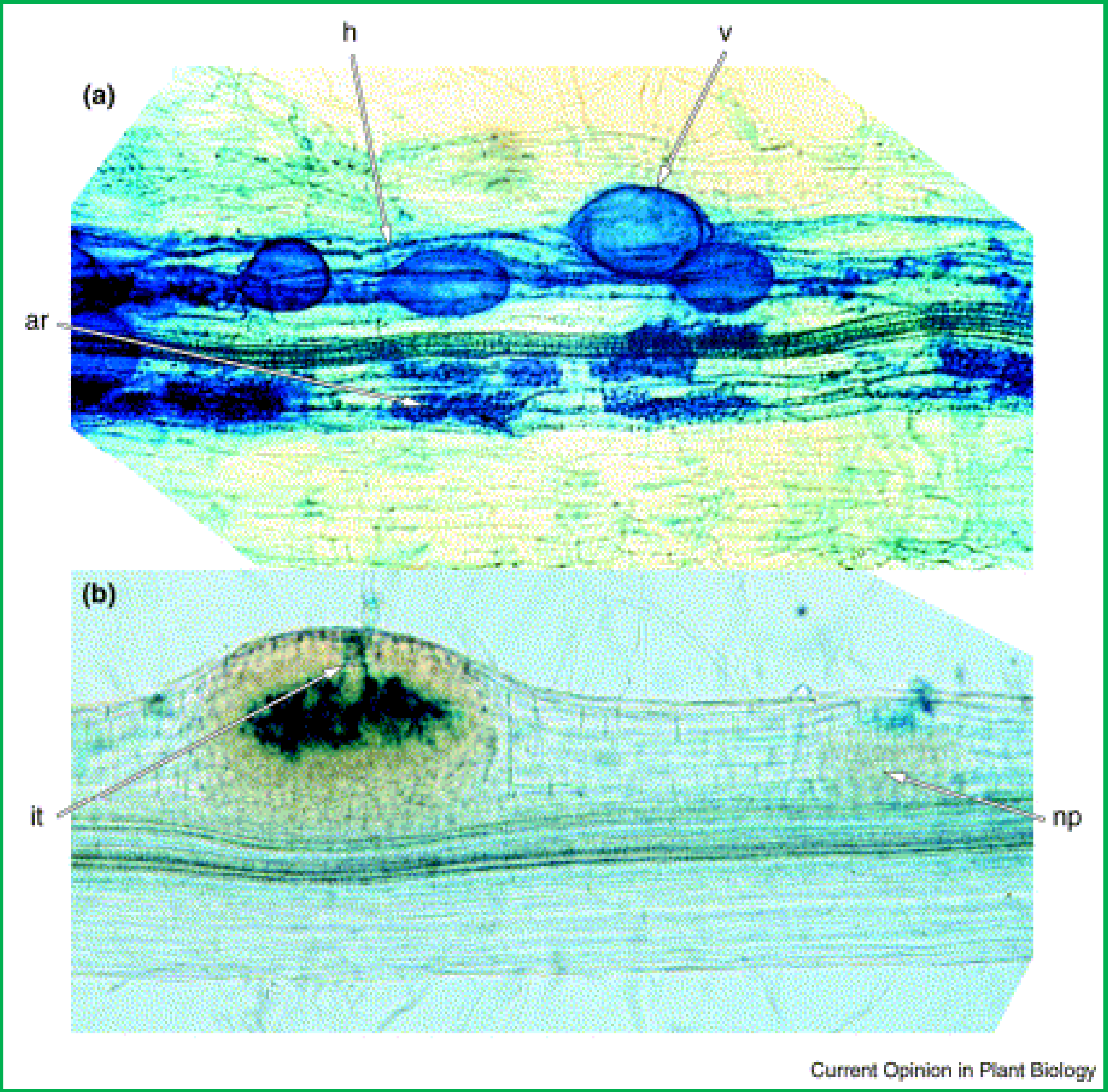


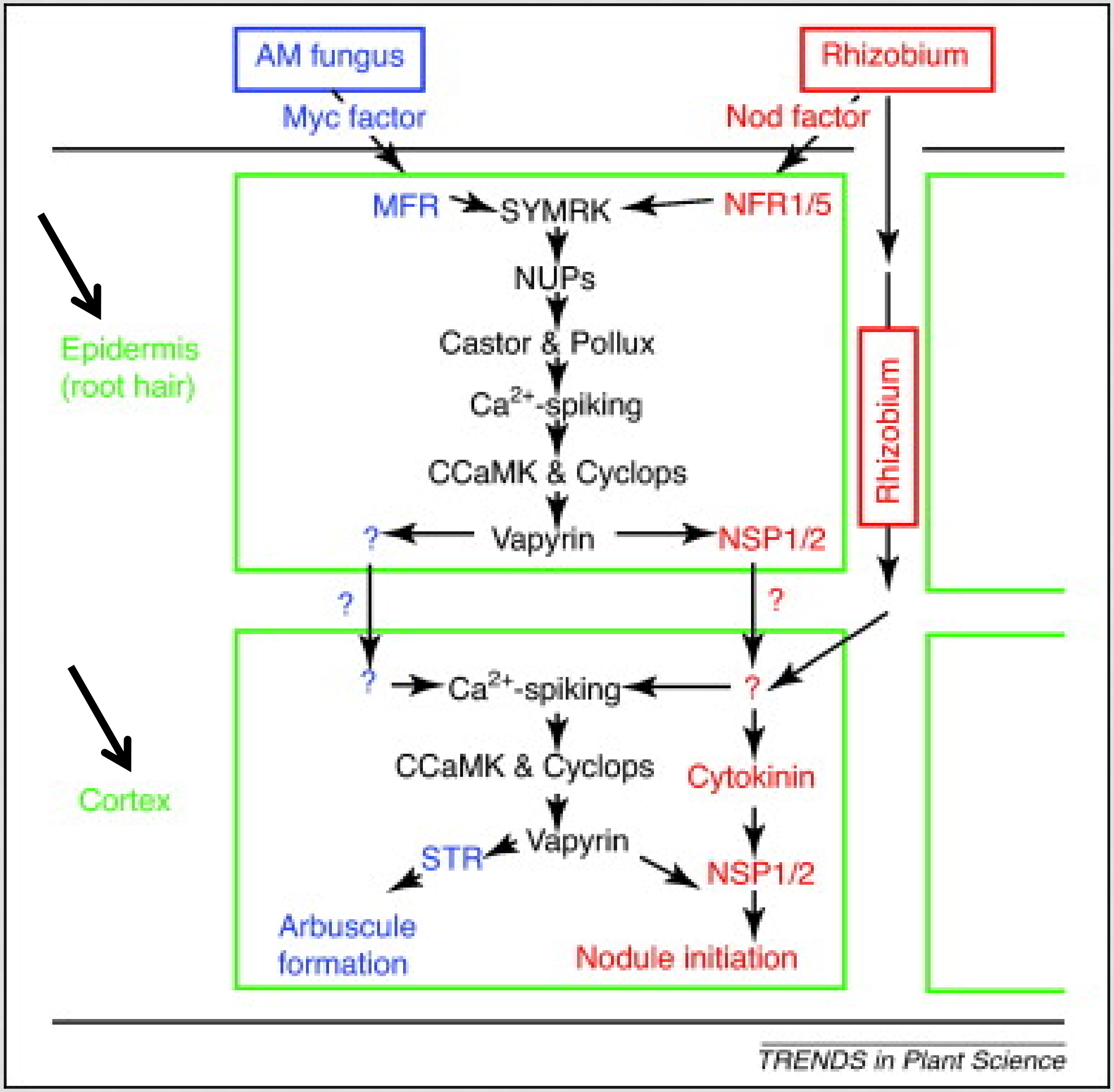
Chen et al., (2009) J. Bacteriol. 191: 6833-6842

- **What is the sym pathway?**

Endomycorrhiza fungal infection

Rhizobium-induced nodule





Ercolin and Reinhardt (2011) Trends in Plant Science

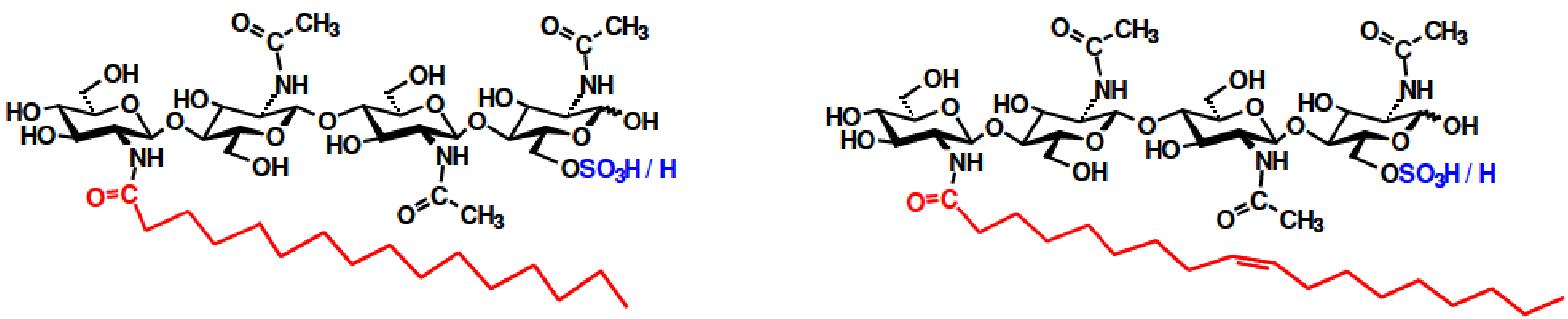
We now know that the several of the signaling steps are shared between the endomycorrhizal and rhizobial symbiosis.

Since the endomycorrhizal symbiosis is very widespread and arose some 400 mya, we assume that the mechanism of rhizobial infection evolved from the endomycorrhizal symbiosis, which does occur in cereals

Endomycorrhizae and Rhizobia Produce Chemically Related LCO signals.

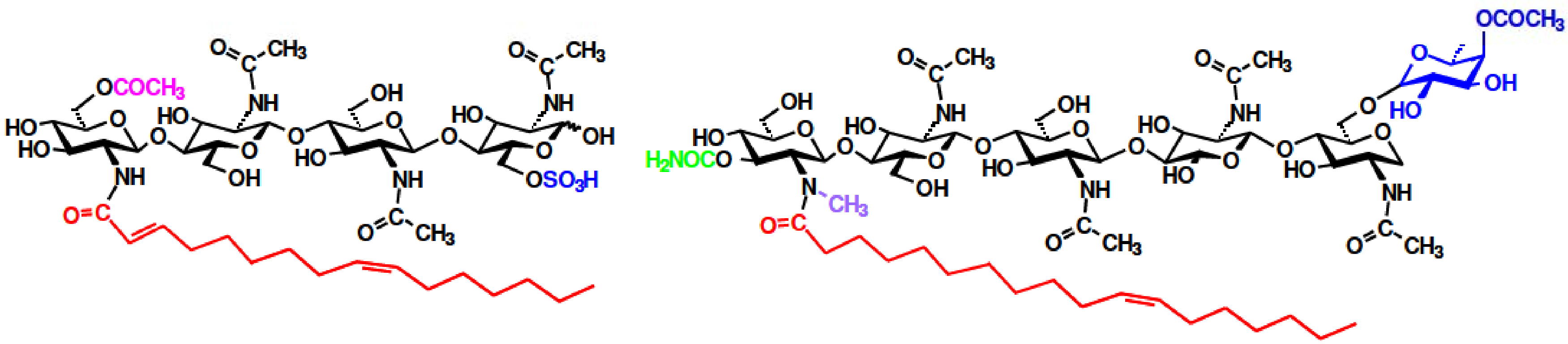
C Myc-LCOs

Glomus intraradices



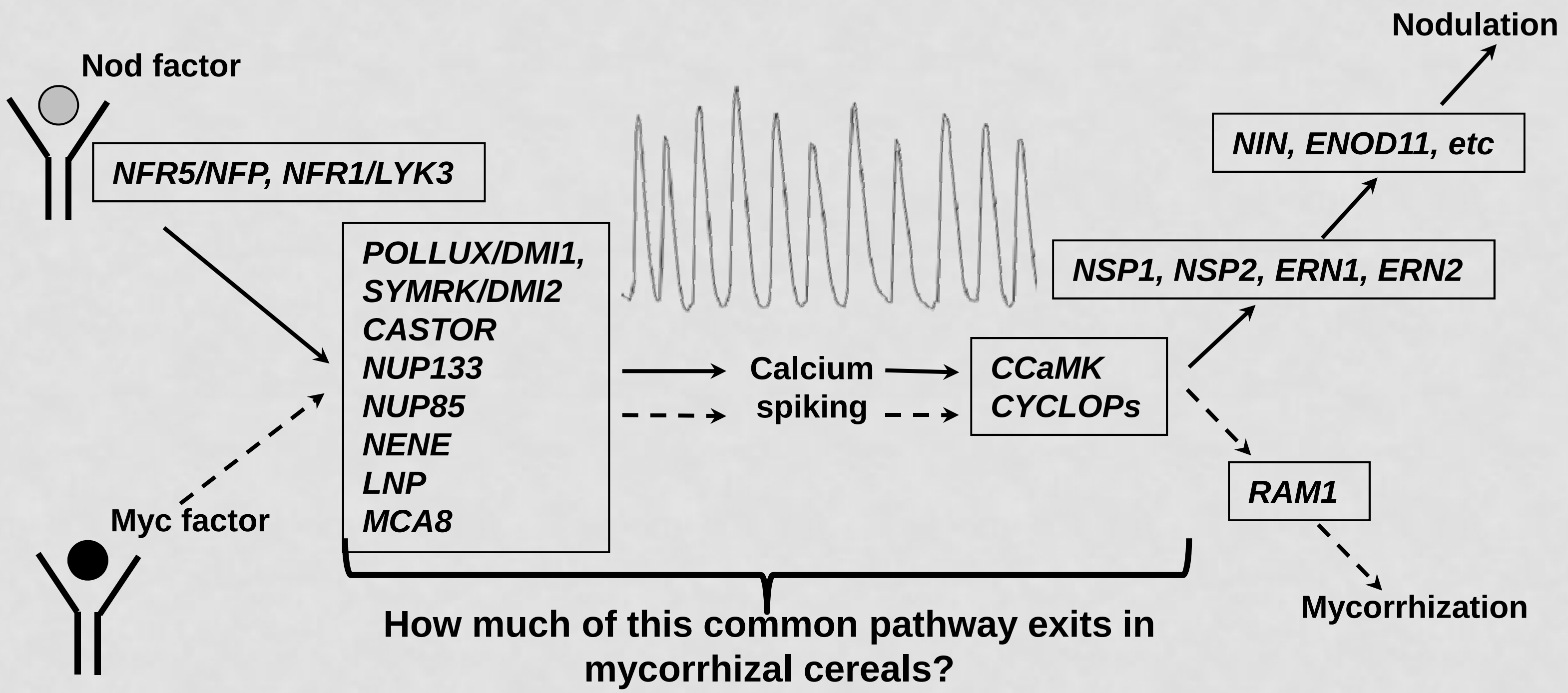
D Nod factors

Sinorhizobium meliloti | *Mesorhizobium loti*

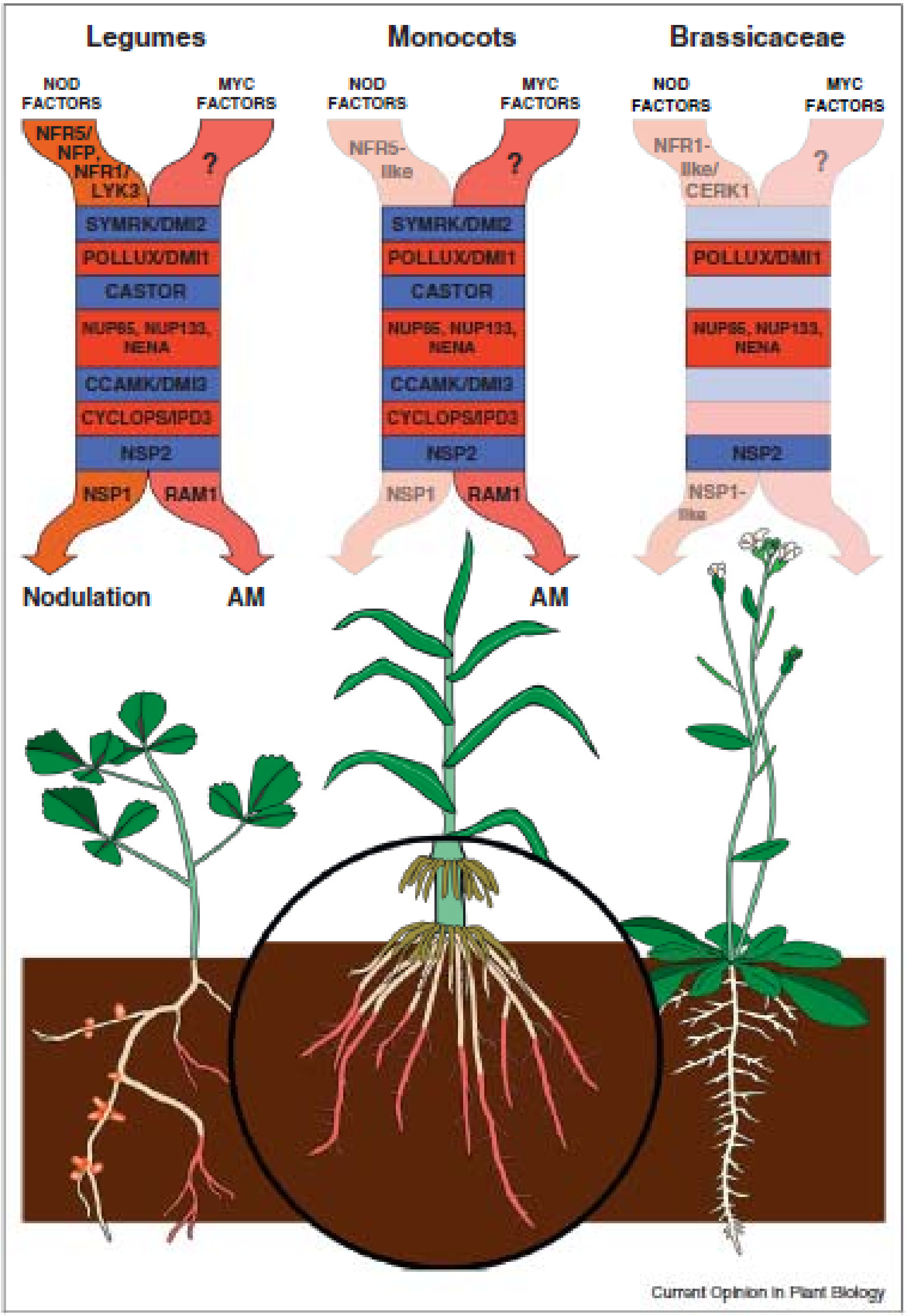


Gough and Cullimore (2011) Mol. Plant-Microbe Interactions

- **What would one engineer into plants?**



The symbiosis signaling pathway of legumes. The common Sym pathway is required for nodulation and mycorrhization. Nodulation specific receptor kinases and transcription factors lie upstream and downstream of the common Sym pathway. Nod factor induced calcium oscillations are indicated. Where appropriate the names of orthologous genes from *Lotus* and *Medicago* are shown.



In two recent reviews, Venkateshwaran et al. (2013) and Delaux et al (2013) argue that it is the conservation of a core set of symbiotic genes that determines whether plants are capable of entering into a symbiosis with either rhizobia or mycorrhizae. For example, they argue that Brassicaceae (e.g., Arabidopsis) lack many of these genes.

Venkateshwaran, et al, Symbiosis and the social network of higher plants, Current Opinion in Plant Biology, Volume 16, Issue 1, February 2013, Pages 118-127
Delaux et al. Evolution of the plant-microbe symbiotic ‘toolkit’. Trends Plant Science 18: 298-304.

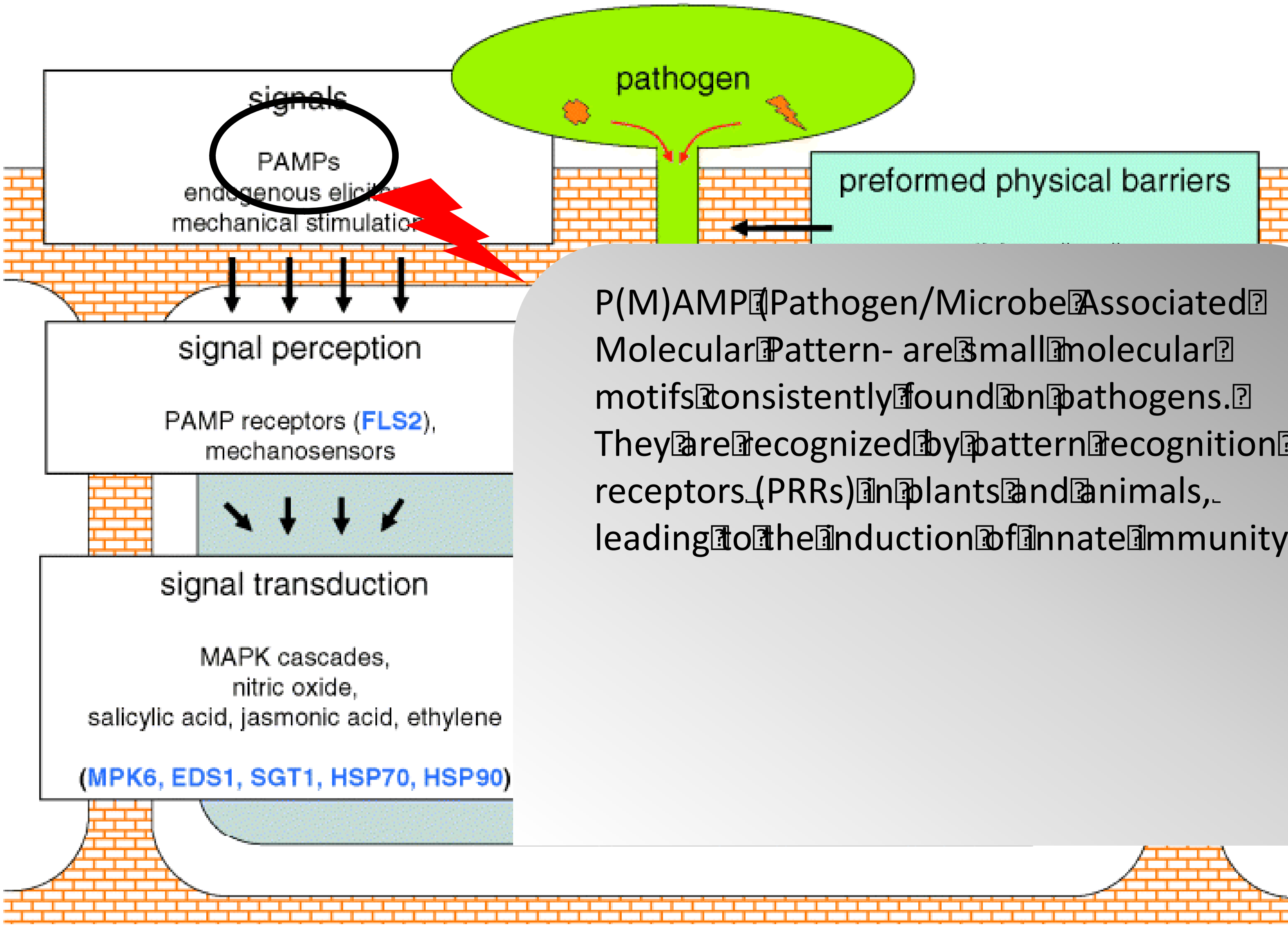
Two possible hypotheses to explain the lack of rhizobial symbiosis in some plants (e.g., monocots) are that

- **They lack the ability to recognize the Sym signals (e.g., Nod factor)**

and/or

pathways

Yan Liang, Yangrong Cao, Sandra Thibivilliers, Jinrong Wan, Kiwamu Tanaka, Jeongmin Choi, Changho Kang, Gary Stacey (2013) Non-legumes respond to Rhizobial Nod Factors by suppressing MAMP-triggered innate immunity. Science 341: 1384-1387



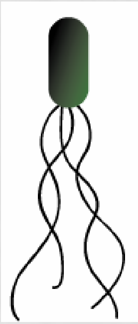
P(M)AMP (Pathogen/Microbe Associated Molecular Pattern- are small molecular motifs consistently found on pathogens. They are recognized by pattern recognition receptors (PRRs) in plants and animals, leading to the induction of innate immunity.

Mechanisms of Plant Defense

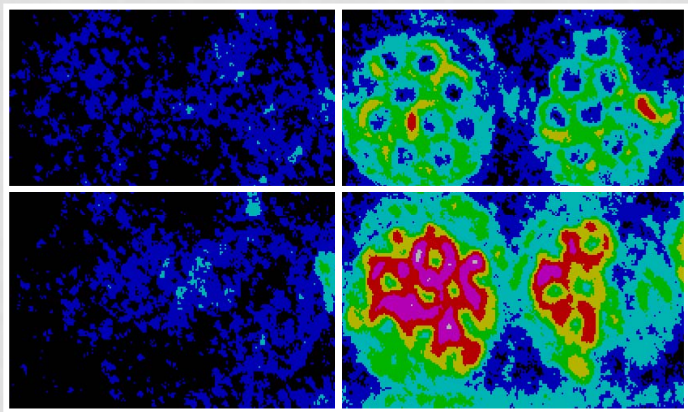
Nürnberger and Lipka. 2005. Mol. Plant Pathol. 6: 335

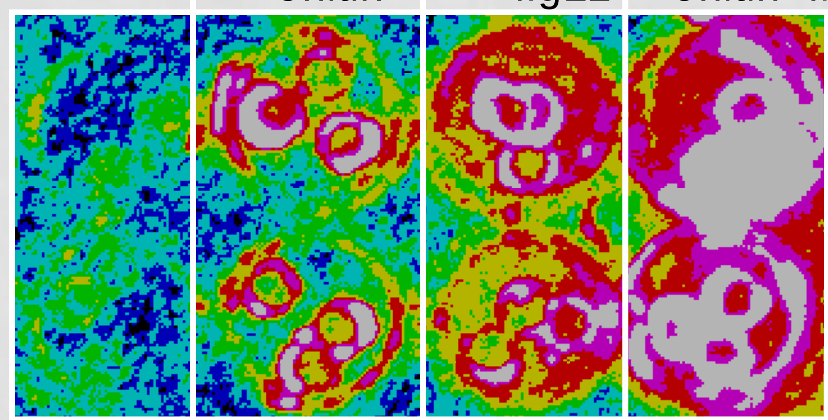
Myriad of MAMPs triggering plant defense (MTI)

Name	Origin	reference
Ergosterol	Fungi	Emumera et al., 2004
Xylanase (TKLGE)	Fungi	Ron et al., 2004
Pep-13	Oomycetes	Brunner et al., 2002
EF-Tu (Elf 18)	Bacteria	Zipfel et al., 2006
LPS	Bacteria	Erbs et al., 2003
Flagellin (Flg22)	Bacteria	Gomez-Gomez et al., 2002; Meziane et al., 2005
Chitin	Fungi	Felix et al., 1993 Ramonell et al., 2002



ROS assays on soybean leaf discs from 2 weeks old plants

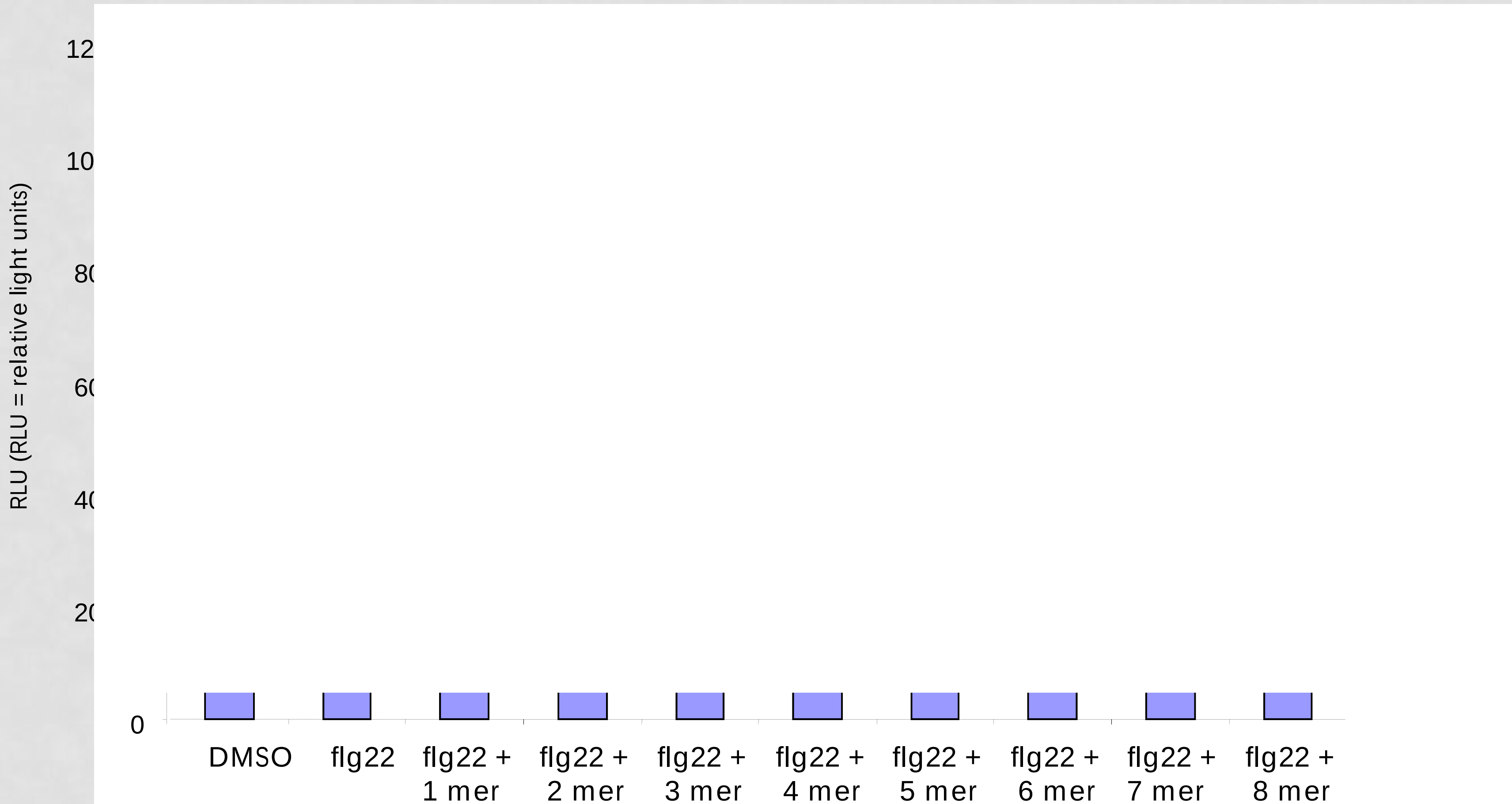




Use a combination of MAMPs to elicit a stronger response and to activate MTI with both bacterial (flg22) and fungal (chitin) MAMPs

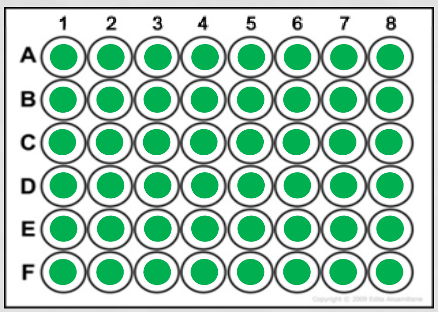
(synergistic effect; Aslam et al., 2009)

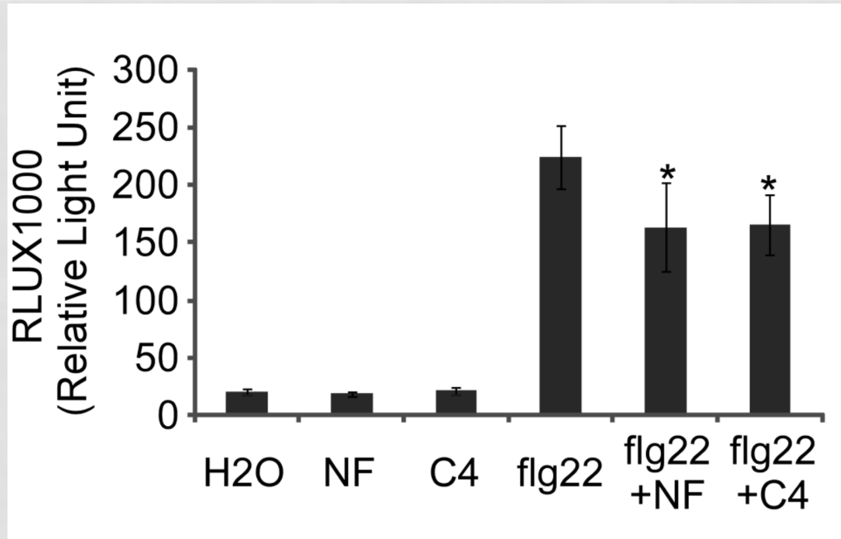
Identification of chitooligomers involved in synergistic effect with flg22

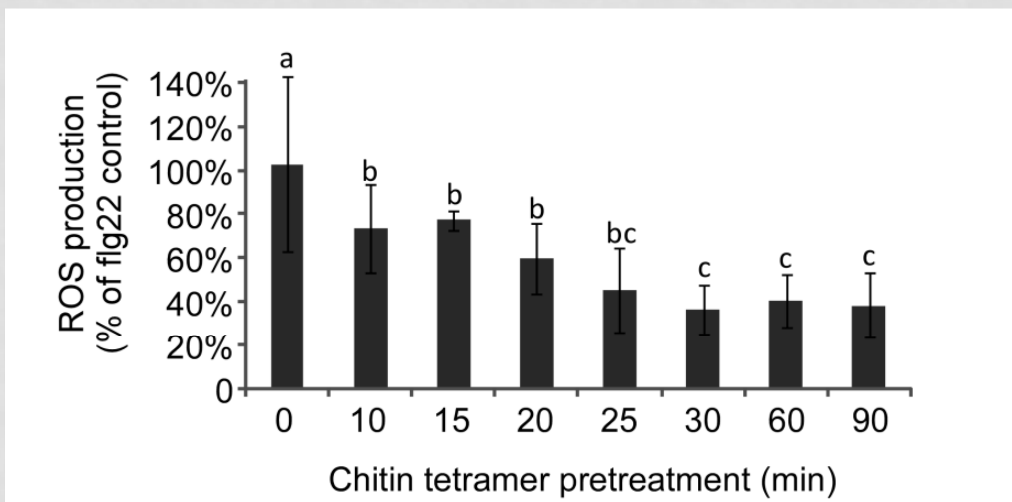


Histogram representing ROS production after 20 minutes of flg22 and diverse chitin oligomers (1µM) treatments.

Different letters represent the statistical difference between the treatments with a p-value=0.05

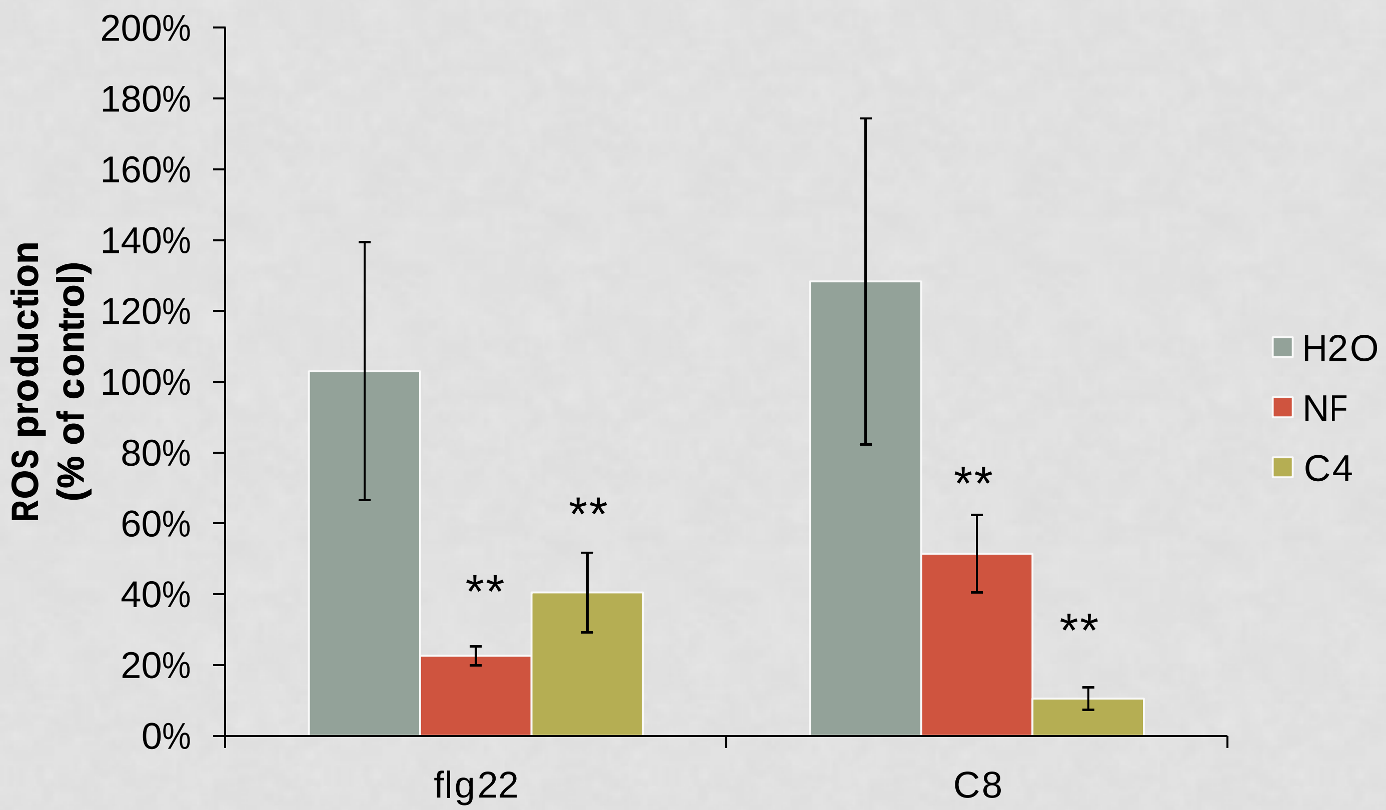


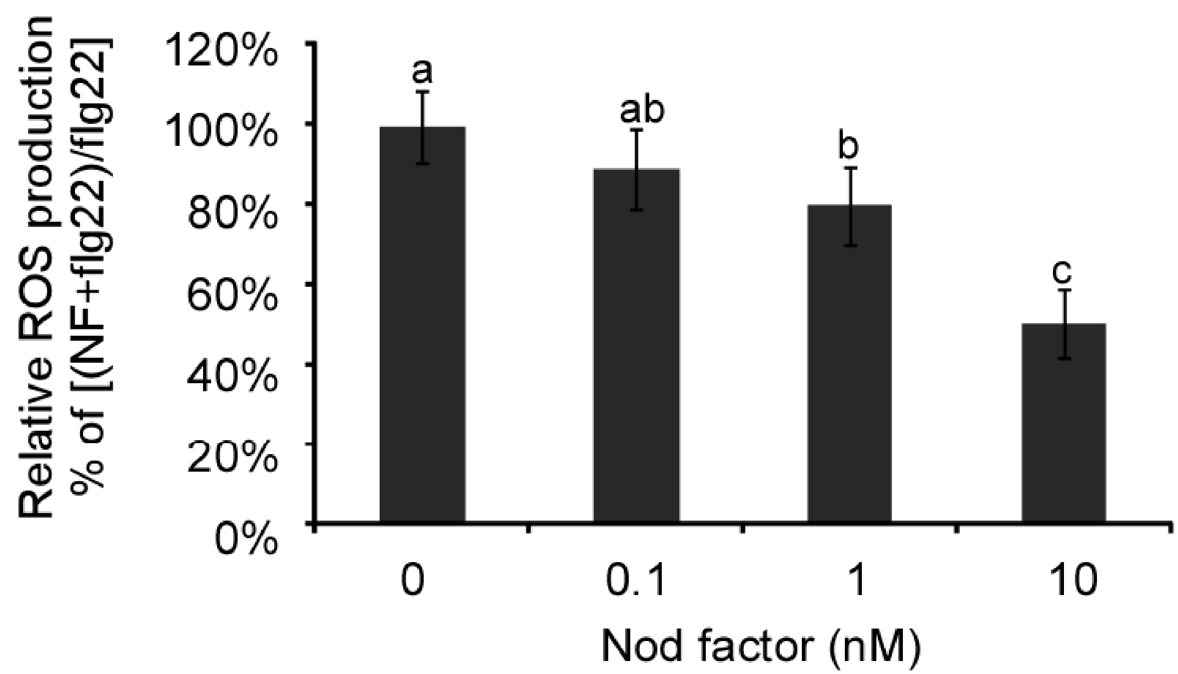




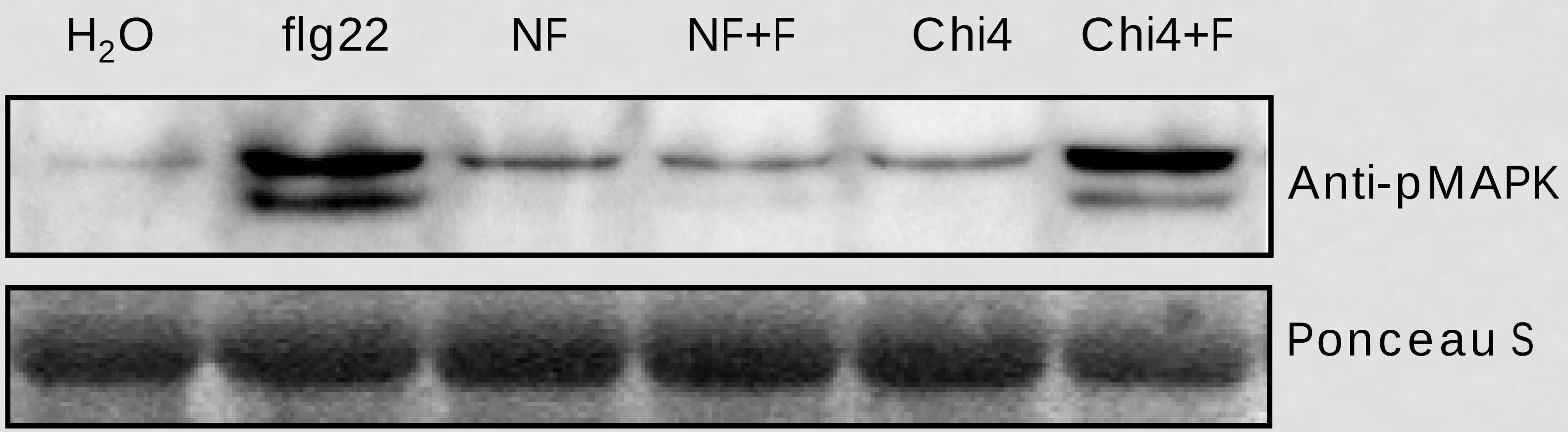
PRETREATMENT ENHANCED THE SUPPRESSIVE EFFECT

Soybean leaves

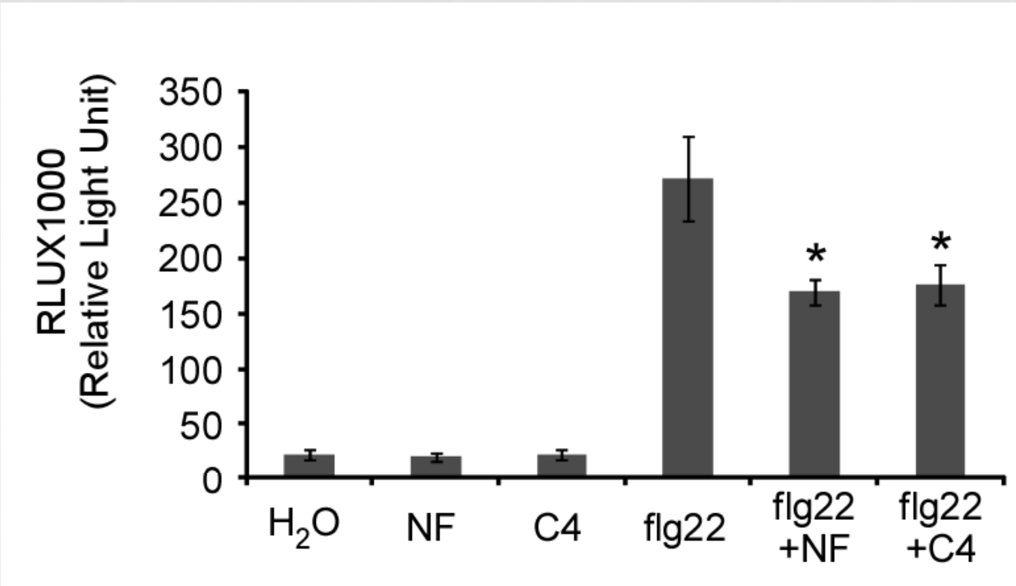


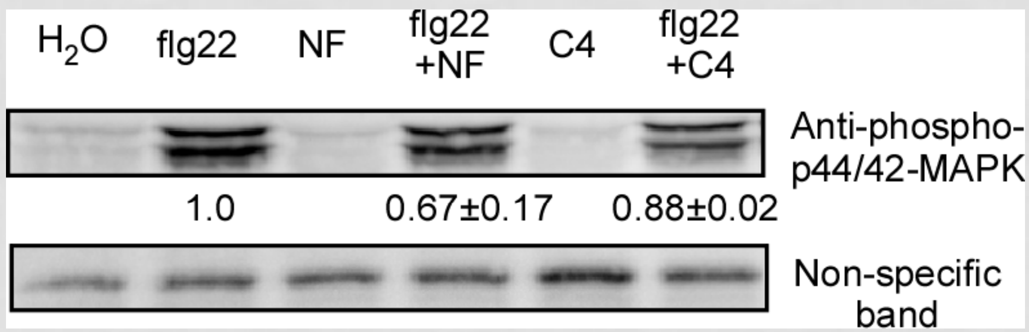


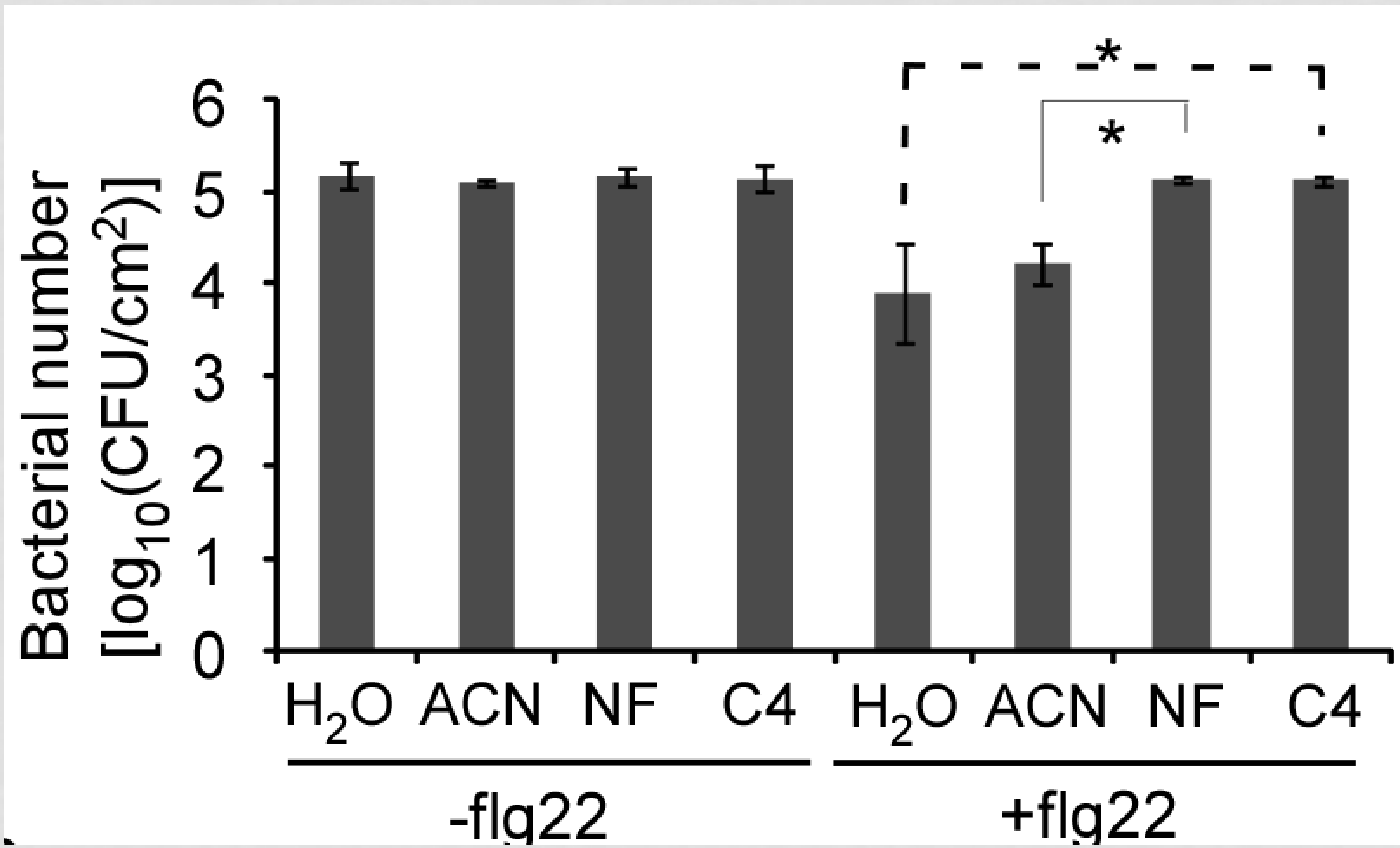
Relative ROS production
% of [(NF+flg22)/flg22]
120%
100%
80%
60%
40%
20%
0%
a
ab
b
c
0
0.1
1
10
Nod factor (nM)



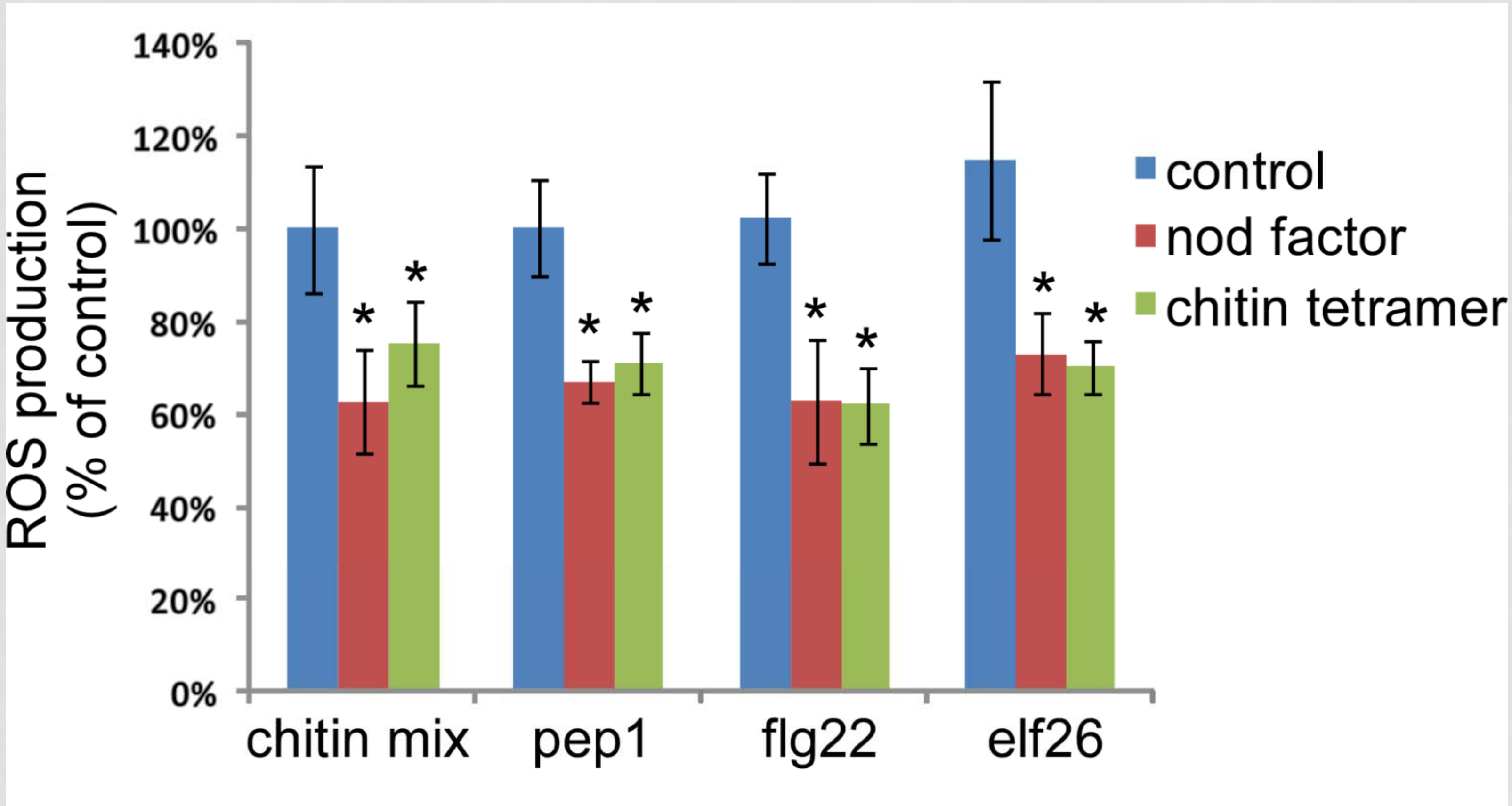
H_2O
flg22
NF
NF+F
Chi4
Chi4+F
Anti-pMAPK
Ponceau S


RLUX1000
(Relative Light Unit)
350
300
250
200
150
100
50
0
*
*
H_2O
NF
C4
flg22
flg22
+NF
flg22
+C4


H_2O
flg22
NF
flg22
+NF
C4
flg22
+C4
Anti-phospho-
p44/42-MAPK
1.0
0.67±0.17
0.88±0.02
Non-specific
band

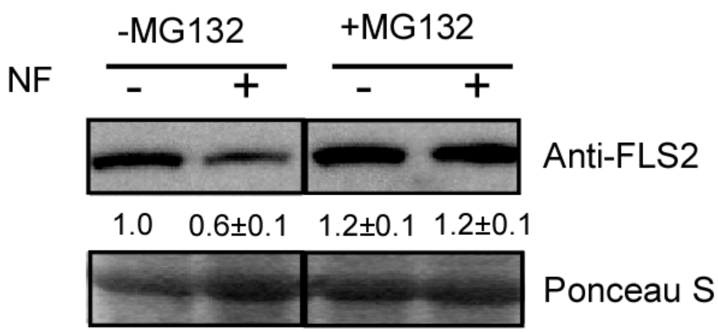


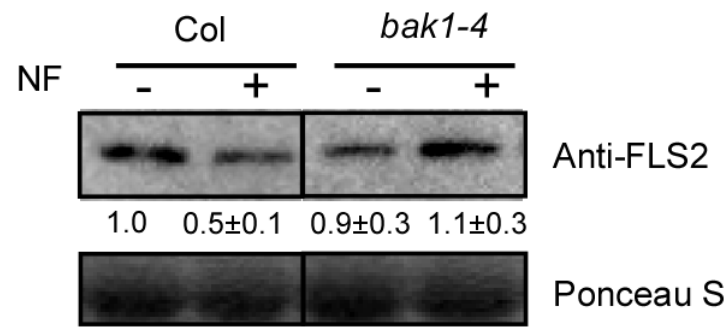
Bacterial number [log10(CFU/cm2)]
6
5
4
3
2
1
0
*
*
H2O
ACN
NF
C4
H2O
ACN
NF
C4
-flg22
+flg22

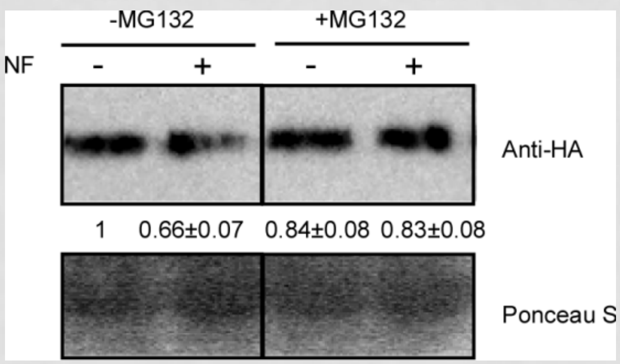


ROS production (% of control)
140%
120%
100%
80%
60%
40%
20%
0%
chitin mix
pep1
flg22
elf26
control
nod factor
chitin tetramer

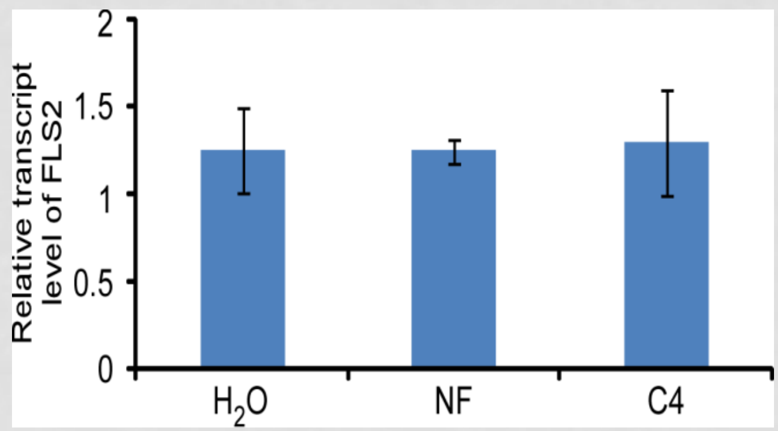
NOD FACTOR INDUCED FLS2 AND EFR PROTEIN DEGRADATION, BUT DID NOT REPRESS THEIR

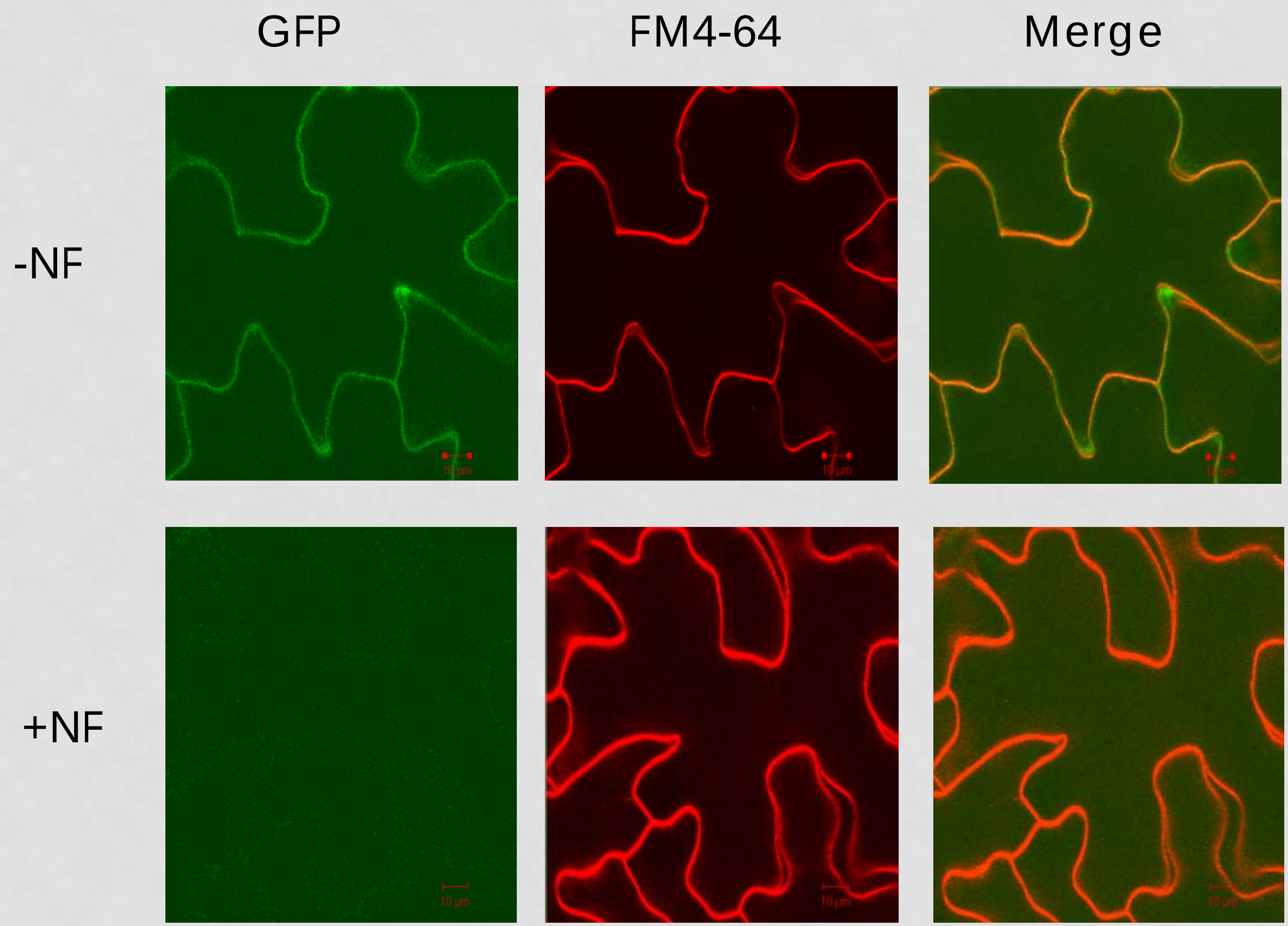


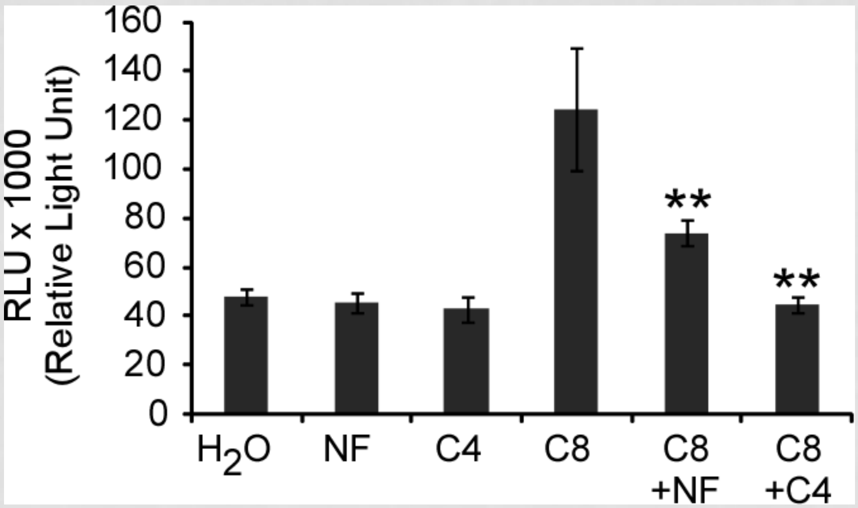




EFR-HA

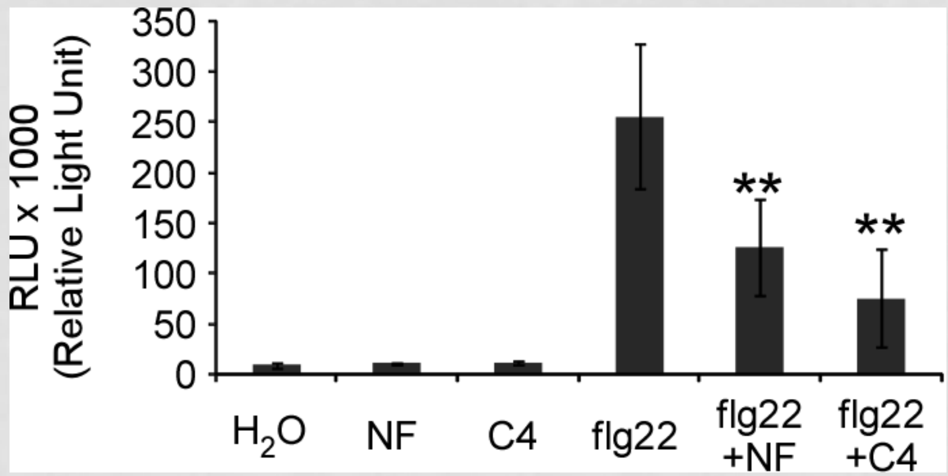



GFP
FM4-64
Merge
-NF
+NF



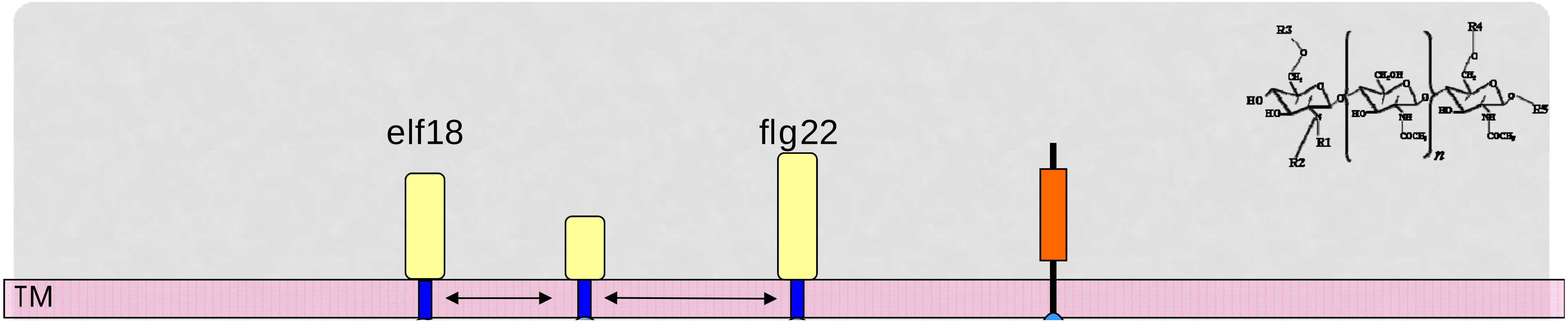
RLU x 1000
(Relative Light Unit)
160
140
120
100
80
60
40
20
0
**
**
H2O
NF
C4
C8
C8
+NF
C8
+C4

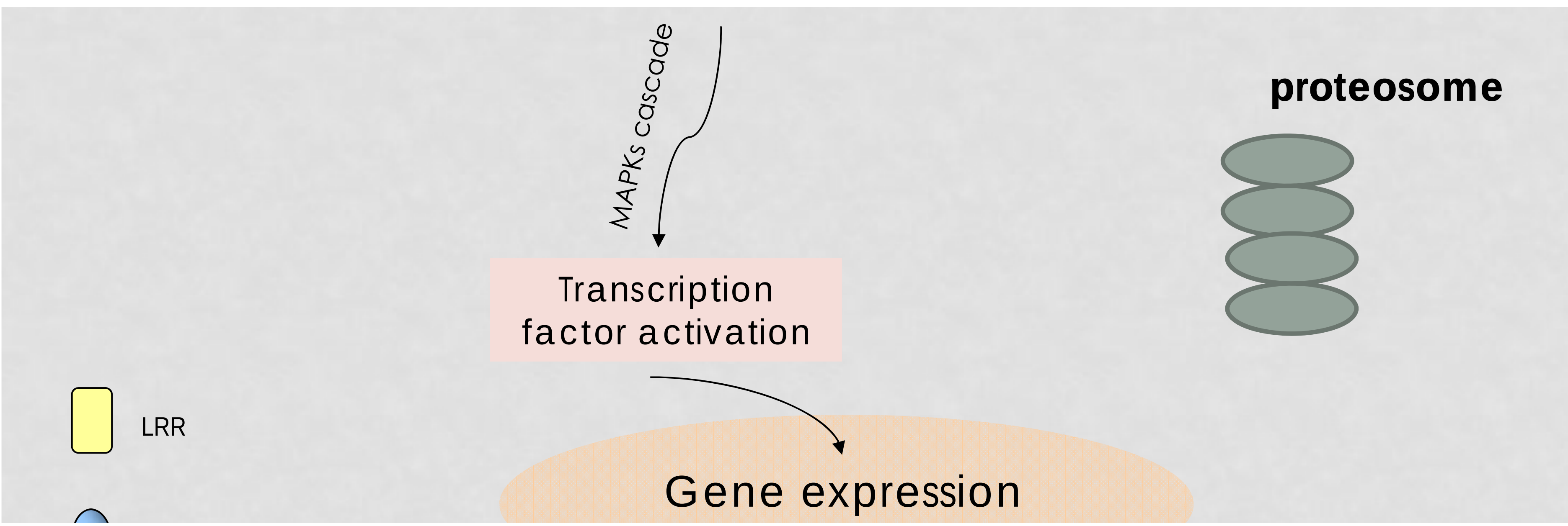
Corn

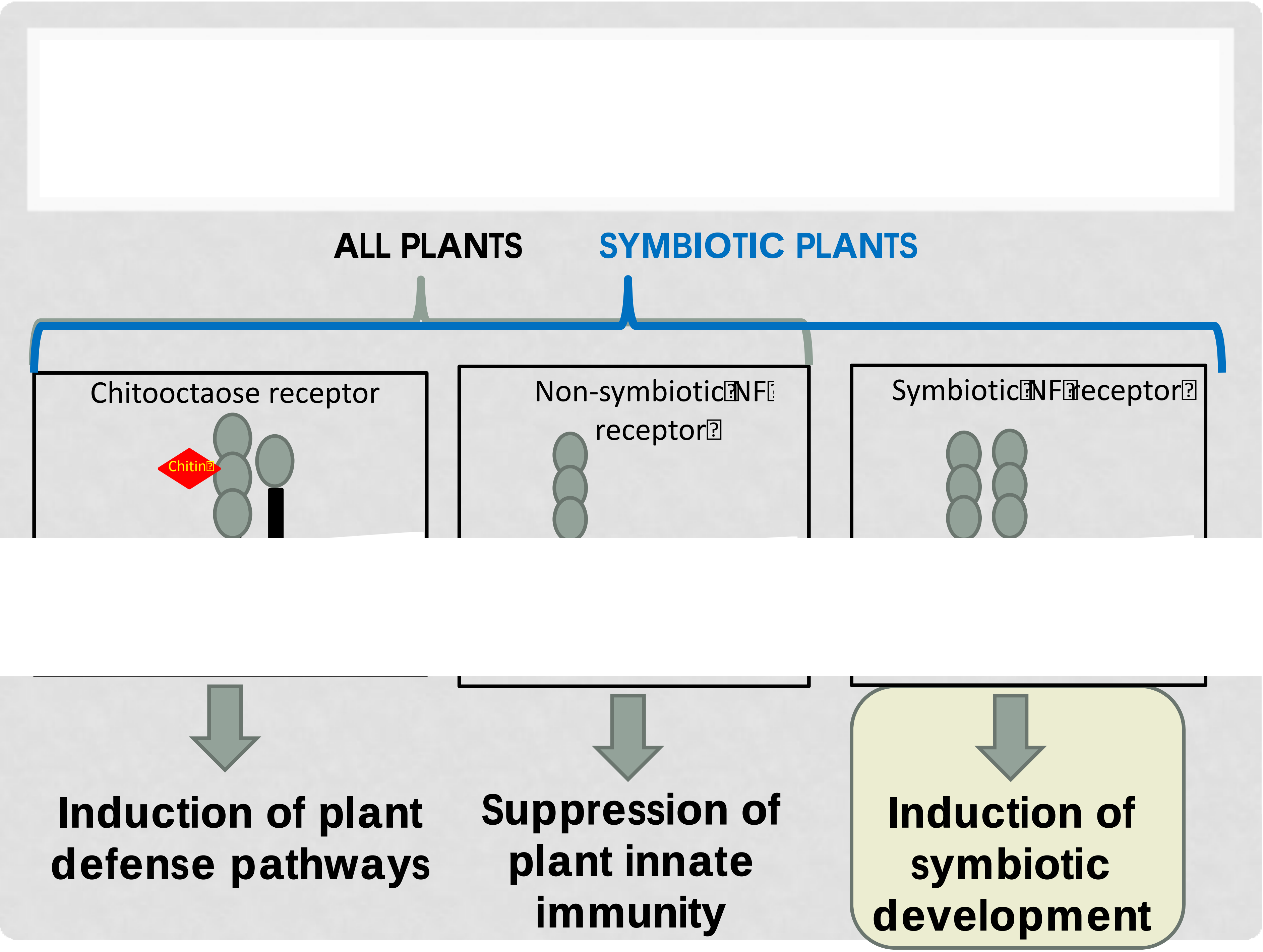


RLU x 1000
(Relative Light Unit)
350
300
250
200
150
100
50
0
**
**
H2O
NF
C4
flg22
flg22
+NF
flg22
+C4

Tomato


elf18
flg22
TM


MAPKs cascade
proteosome
Transcription factor activation
LRR
Gene expression


ALL PLANTS
SYMBIOTIC PLANTS
Chitooctaose receptor
Chitin
Non-symbiotic NF receptor
Symbiotic NF receptor
Induction of plant defense pathways
Suppression of plant innate immunity
Induction of symbiotic development

Gates Meeting: “Enhancing biological nitrogen fixation in crop plants” April 19-21, 2012

Three topic areas discussed:

1. **Developing a rhizobial symbiosis in cereals**

2. **The introduction and encouragement of diazotrophic bacteria and cereal crop interactions**

3. **Synthetic biology, the design of a new organelle to fix N in crop plants**

Note that of these three topics, topic 2 was the only one not chosen by the Gates foundation for funding. Primarily because there were few advocates of this approach at the meeting.

The introduction and encouragement of diazotrophic bacteria and cereal crop interactions

I originally ranked this topic as the more achievable goal among the three discussed?

Because agricultural relevant systems already exist in nature, have been harnessed for practical use and support a small but growing commercial inoculant business (much more prevalent in South America)

Special Issue of Plant and Soil Volume 356, July 2012.
"The role of biological nitrogen fixation by non-legumes in the sustainable production of food and biofuels"

N_2-fixation associated with grasses

A brief ‘cyclical’ history

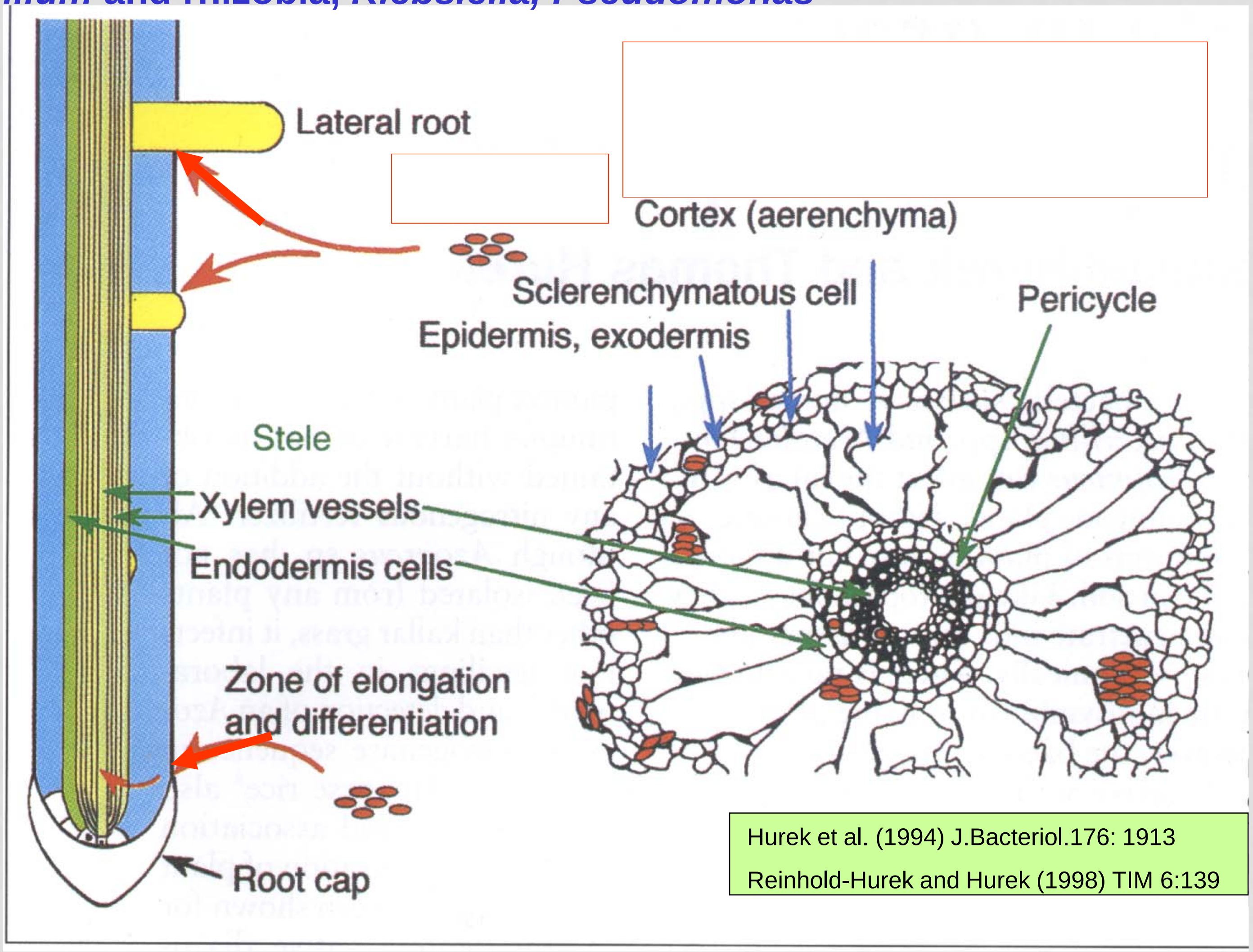
- “Azotobacterin” in Russia / *Azotobacter paspali* associated with *Paspalum* - Döbereiner
  - 1972 Brown - concluded inoculation responses due to hormonal effects of the bacteria
- The rhizosphere acetylene-reduction/ inoculation era
  - 1972 Döbereiner, Day and Dart - ARA associated with roots/*Spirillum lipoferum*
  - 1976 Smith et al. Science - inoculation responses to *Azospirillum* in USA
  - 1979 Tien et al./Okon et al. - inoculation responses due to hormonal effects
- The endophyte/sugar cane era
  - 1986/88 Baldani/ Döbereiner *Herbaspirillum/Gluconacetobacter*
  - 1980’s/1990’s Boddey/Urquiaga et al - large amounts of N_2 fixed
  - 1990’s and later - Other roles of endophytes - hormones- Lebuhn et al 1997, Sevilla and Kennedy, 2000

Recurrent, although sporadic, reports of biological nitrogen fixation supported by ^{15}N incorporation---consensus in Brazil is that sugarcane receives ~20% of its nitrogen from associative nitrogen fixation—some reports in wild grasses up to 60-70%. Rumors of corn genotype with high fixation rates???

Where?	Plant association-Bacteria?	Plant association – Plant?

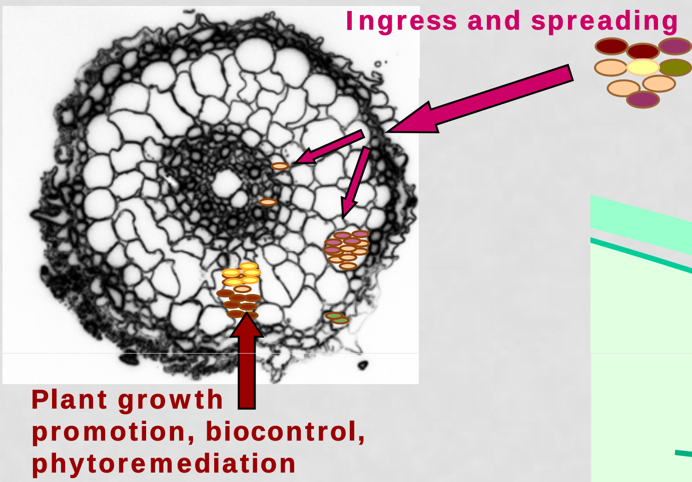
Colonization of grass roots by diazotrophic endophytes

Azoarcus **spp.,** ***Herbaspirillum*** **spp.,** ***Gluconacetobacter diazotrophicus*****, some** ***Azospirillum*** **and rhizobia,** ***Klebsiella, Pseudomonas***

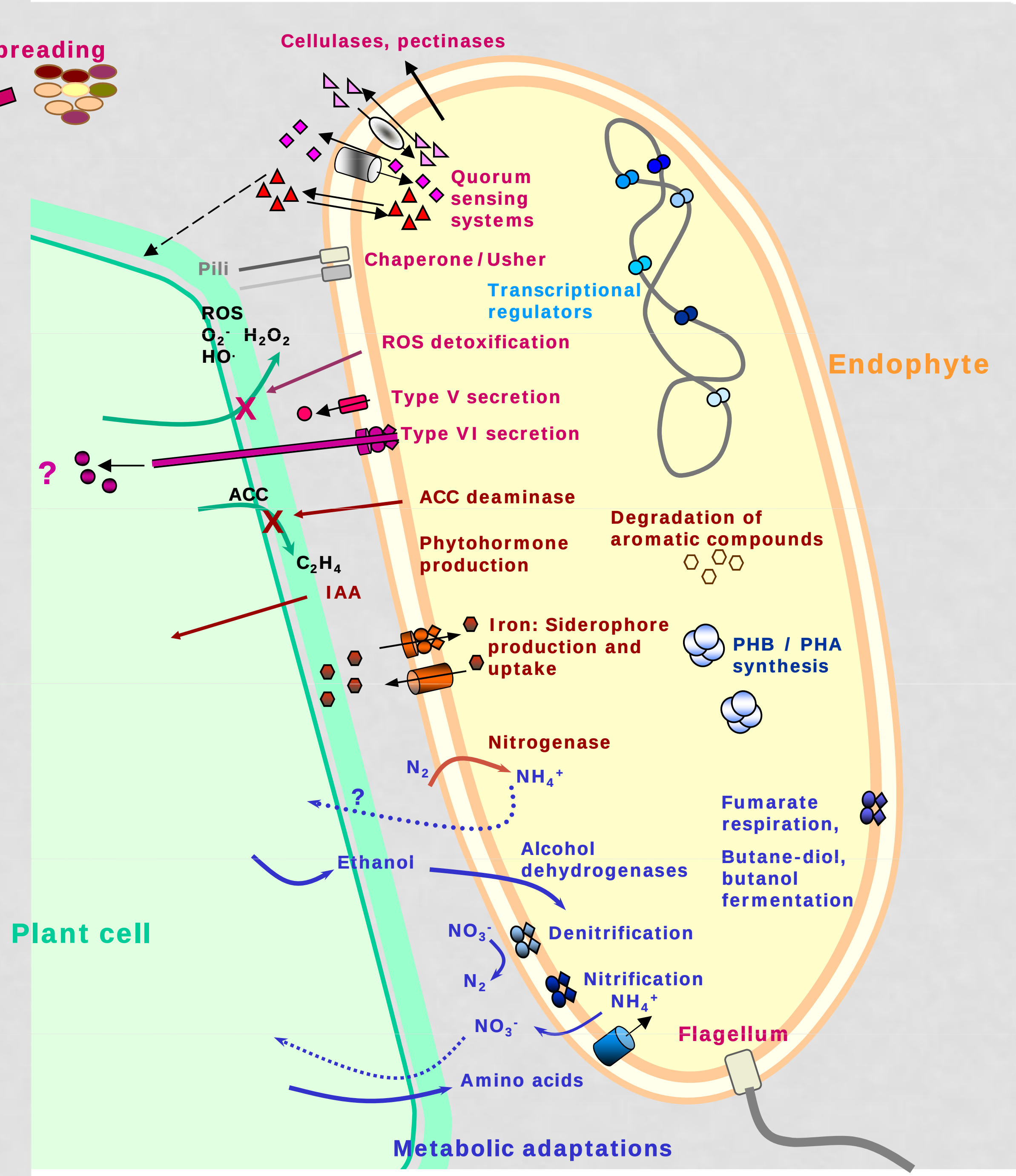


Hurek et al. (1994) J.Bacteriol.176: 1913

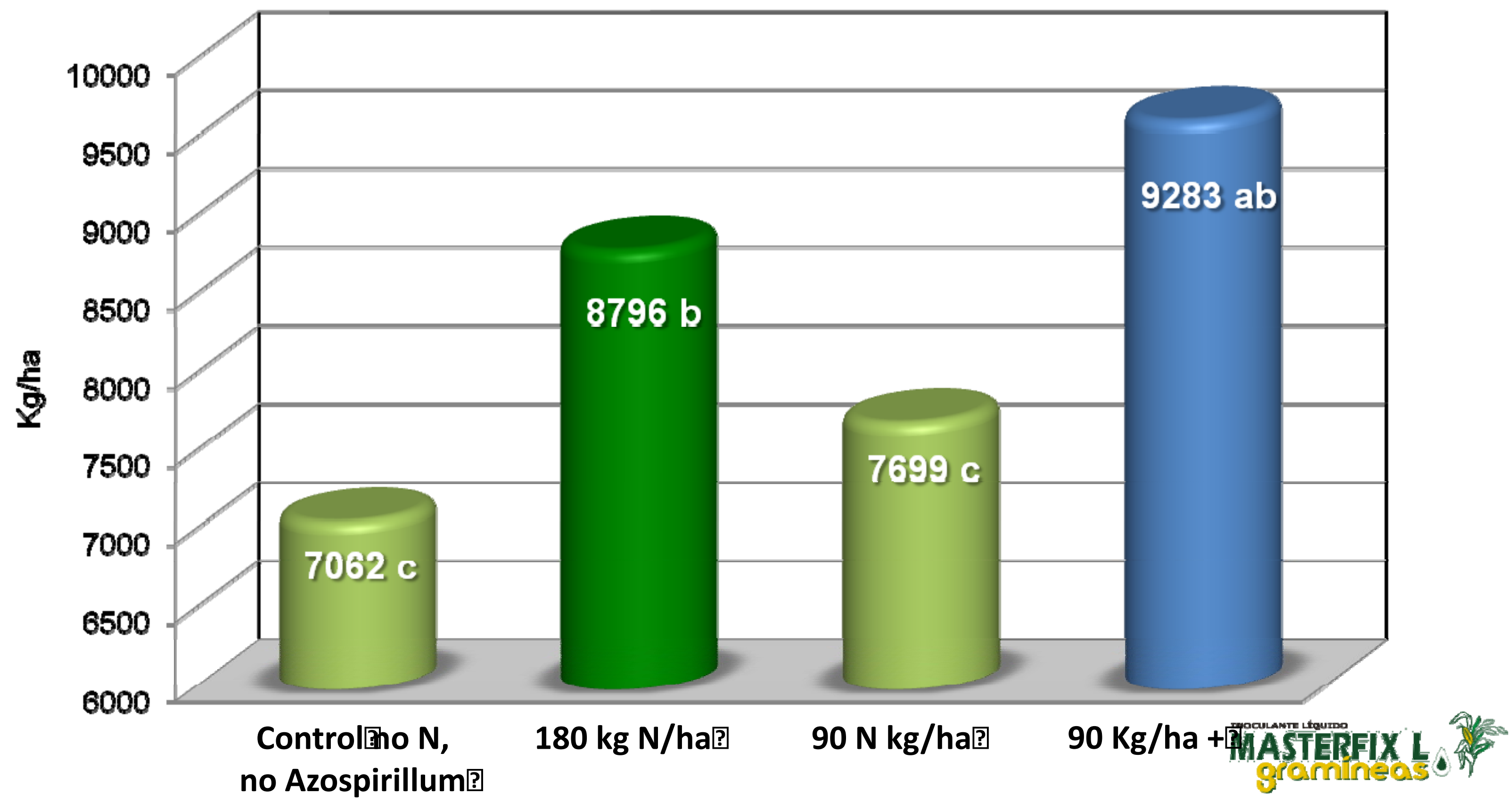
Reinhold-Hurek and Hurek (1998) TIM 6:139



Predicted and demonstrated interactions



Effect of inoculation of maize CD 304 with a commercial inoculant containing *Azospirillum brasilense* AB-V5 and Ab-V6 on grain productivity.



Data provided by Dr. Fabio Pedrosa

Experimental field in Maringá/PR

Maize plants inoculated with *Azospirillum brasilense* AB-V5 e Ab-V6 were more resistant to drought

Non inoculated

MASTERFIX® L gramineas

Nova Santa Rosa, PR, 2009

Data provided by Dr. Fabio Pedrosa

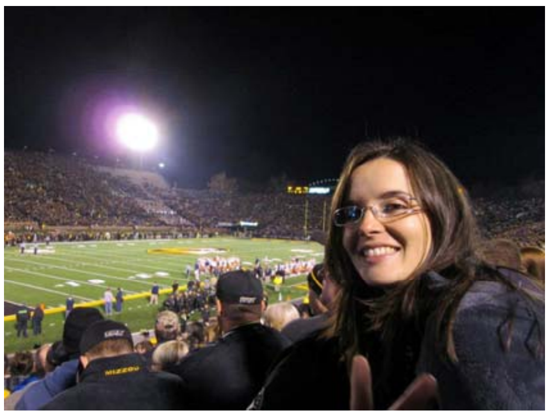
The introduction and encouragement of diazotrophic bacteria and cereal crop interactions

Was my original ranking justified….does this area indeed hold promise?

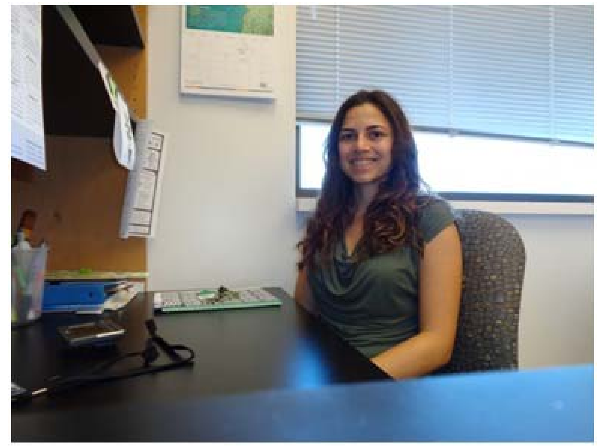
- ✓ **There are clearly well supported reports in the literature, albeit sporadic, of significant levels of nitrogen fixation and incorporation in plants, although only a few in crop plants (e.g., sugarcane)**
- ✓ **However, these reports and, indeed, the entire area is met with some skepticism by the wider scientific community.**
- ✓ **I believe this is largely due to the fact that the field is dominated by phenomenological reports, with few mechanistic studies, and even fewer molecular/ genetic studies….**

Setaria viridis – A model for the study of diazotrophic-plant interaction

Fernanda P. Do Amaral



Vania C. Pankievicz

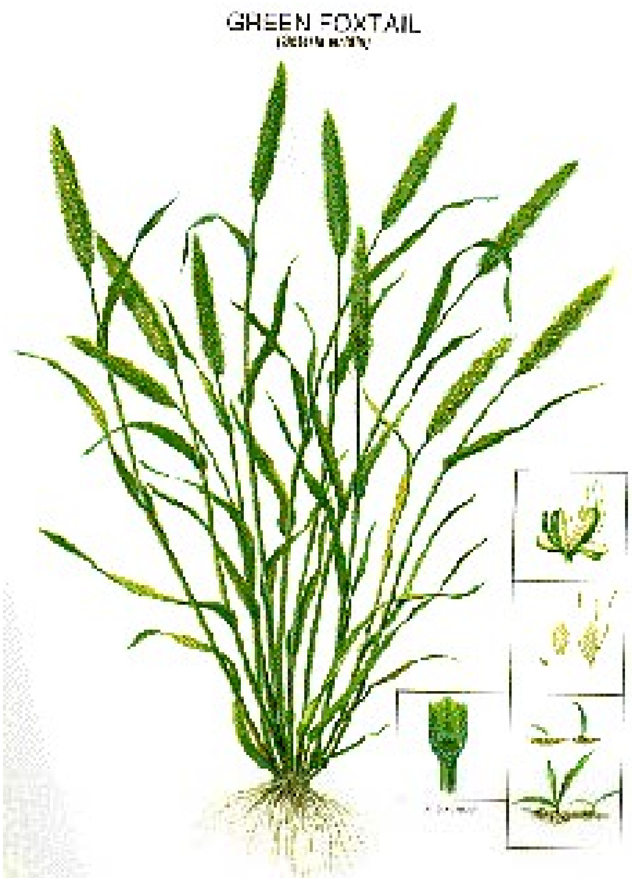


Karina Freire d'Eça Nogueira Santos

Fabio Pedrosa
Emanueal de Souza
Univ. of Curitiba, Brazil

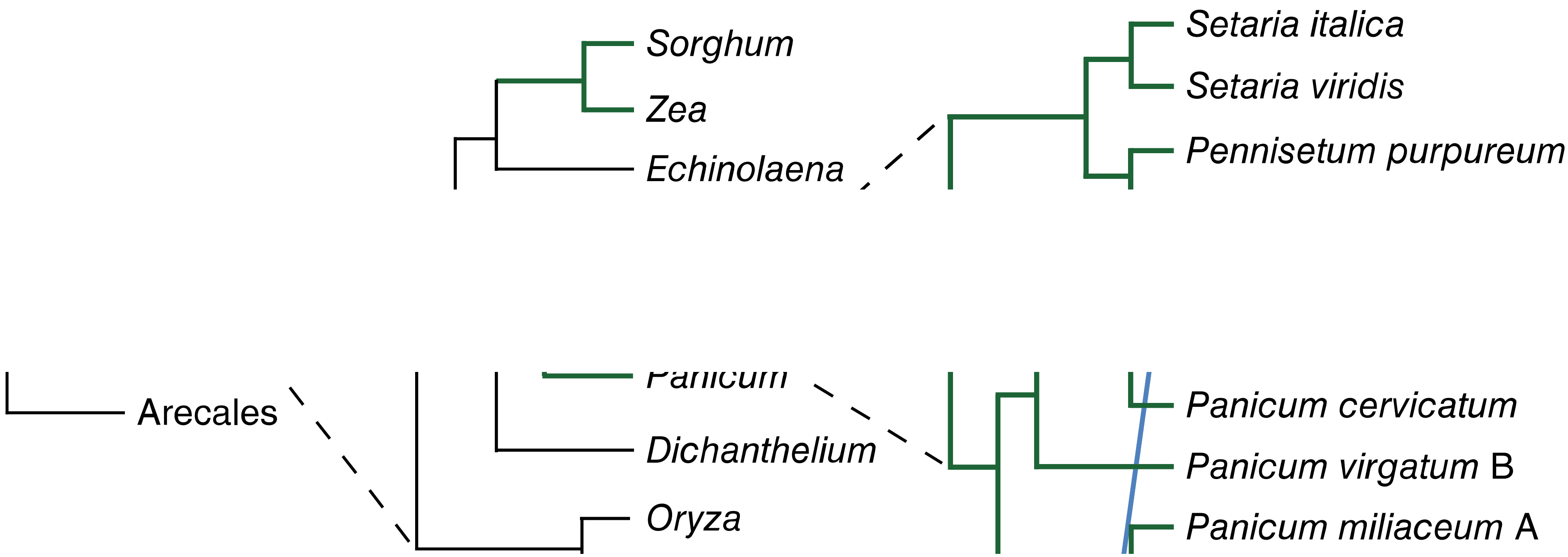
Setaria

S. viridis
Green foxtail



- *S. viridis* is a problematic weed
- Foxtail Millet (*S. italica*) was domesticated from *S. viridis*
- Foxtail Millet is a significant crop and dietary staple in Northern China
- Setaria has been used as a potential model species for understanding basic biological processes
- Setaria is a C4 plant
- *S. viridis is* a small plant, easily grown under greenhouse conditions, with a rapid growth cycle (60 days)
- Setaria has a recently sequenced genome.

Phylogenetic position of *S. italica* and *S. viridis* relative to selected important grass species.

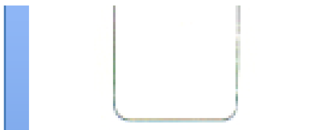


switchgrass

Jeffrey L Bennetzen, Nature Biotech, 2012

System development to grow *S. viridis* to study the bacterial colonization.

48h dark, 30 C
Hoagland + phytagel 1%



Bacterial culture
O.D_{600}=1,0

20''
13000 rpm
(3X)

1ml of washed Bacterial culture per seedling for 30 min.

Bacterial mixture
H. seropedicae Ram4 – *dsRed* fusion
A. brasilense FP2 *nifH:gusA*

Turface 3:1 Vermiculite
Greenhouse 16/8h 30 C

The plants were watered with Hoagland nutrient solution twice a week, nitrogen was applied depending on the experiment

Parameters analyzed

- Plant height
- Root weight
- Total root length (WinRhizo Scanner and Software)
- Number of tips of the root (WinRhizo Scanner and Software)
- Shoot weight
- Flag leaf area
- number of seeds
- number of tiller
- Bacterial Recovered
- Bacterial Colonization followed by microscopy

Parameters analyzed during plant development

Germination	Radicle emerged from caryopsis Coleoptile emerged from caryopsis First leaf just at coleoptile tip
Leaf development	First leaf through coleoptile First leaf unfolded 2nd leaf unfolded 3rd leaf unfolded 4th leaf unfolded 5th leaf unfolded 6th leaf unfolded
Tillering	First tiller detectable 2nd tiller detectable 3rd tiller detectable 4th tiller detectable 5th tiller detectable
Main stem elongation	First node at least 1 cm above tillering node Node 2 node 3 node 4 node 5 Flag leaf just visible Flag leaf fully enrolled (ligula just visible)
Booting	Early boot: flag leaf sheath extending Flag leaf sheath opening First awns visible

Heading	Beginning: tip of inflorescence emerged from sheath One-fourth of head emerged and beginning of peduncle elongation Middle of heading: half of inflorescence emerged Three-fourths of head emerged End of heading: inflorescence fully emerged
Flowering	Beginning of flowering: first anthers visible End of flowering: all spikelets have completed flowering but some dehydrated anthers may remain
Development of fruit	Watery: first grains have reached half their final size
Ripening	Early dough Fully ripe: grain hard, difficult to divide with thumbnail
Senescence	Overripe: grain very hard, cannot be dented by thumbnail Grains loosening in daytime Plant dead and collapsing
Harvested	

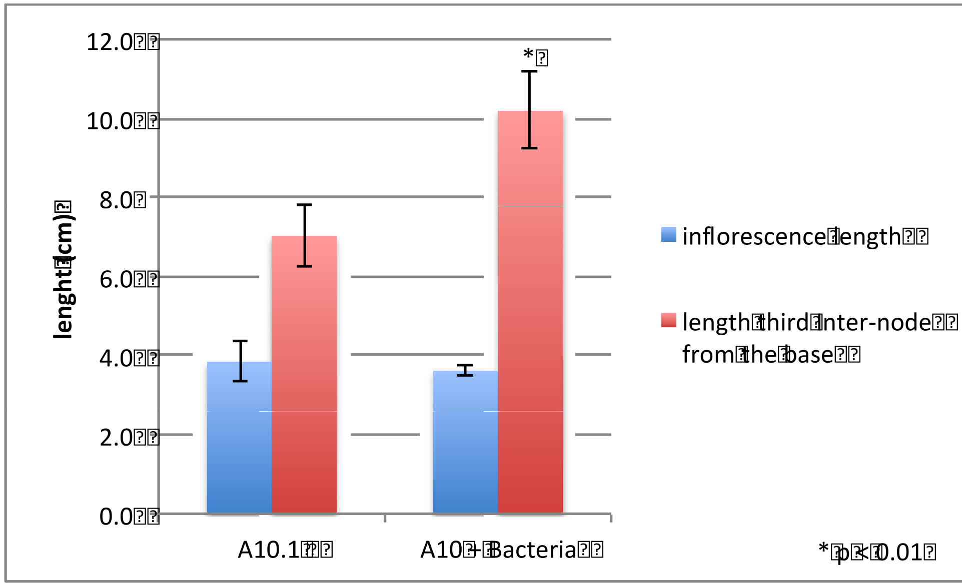
S. viridis Genotypes (~50 available)

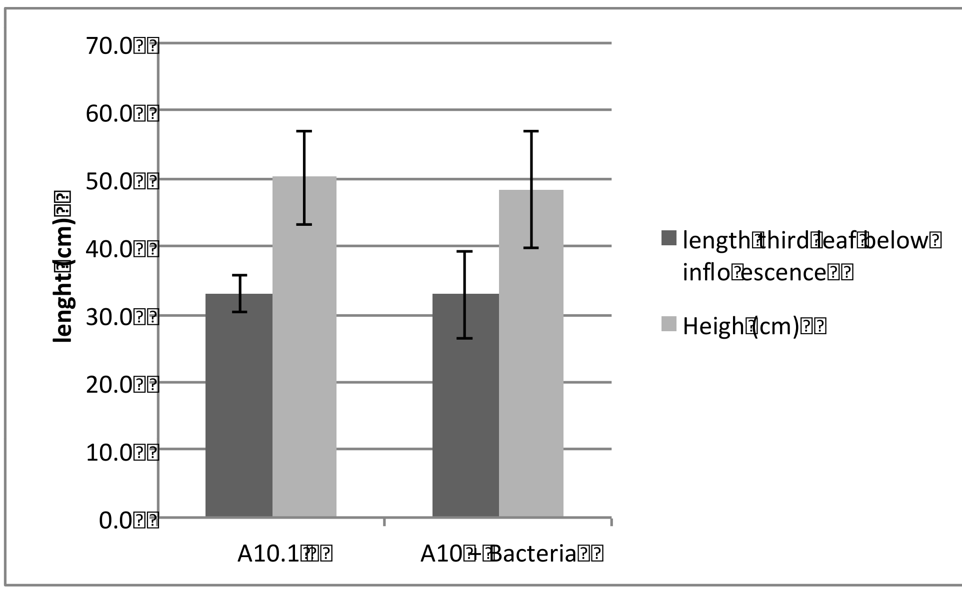
Exp #	NAME	Genera	Species	#seeds	Germination Rate	GA added	Visual Plant Growth promotion response	Bacteria inoculated-RAM4	Bacteria inoculated FP2-7	Result	Day of analyses (40 DAI)	Next step
1	**Thompson**	Setaria	viridis	100	34.8%	yes	na	1.00E+06	1.00E+05	only one plant left	na	harvest seeds
2	**Estep ME035**	Setaria	viridis	50	40.0%	yes		3.80E+06	8.80E+07	7 plants left	46 DAI	Experiment replica
2	**S. viridis A10-1**	Setaria	viridis	>1000	90.0%	yes	++	3.80E+06	8.80E+07	sufficient to make statisti	40 DAI	follow colonization
3	**Estep ME015**	Setaria	viridis	14	42.8%	yes		4.20E+07	7.30E+06	only one plant left	na	harvest seeds
3	**Estep ME025V**	Setaria	viridis	16	50.0%	yes		4.20E+07	7.30E+06	only one plant left	na	harvest seeds
3	**Estep ME026**	Setaria	viridis	50	29.2%	yes		4.20E+07	7.30E+06	4 plants left	40 DAI	Experiment replica
3	**Estep ME032V**	Setaria	viridis	50	40.8%	yes		4.20E+07	7.30E+06	4 plants left	40 DAI	Plant to get seeds
3	**Estep ME034V**	Setaria	viridis	50	48.0%	yes		4.20E+07	7.30E+06	6 plants left	40 DAI	Experiment replica
4	**Vela 88**	Setaria	viridis	94	29.7%	yes	growing	3.90E+06	1.00E+05		12/5/12	measurements
4	**Ahart**	Setaria	viridis	54	37.0%	yes	growing	3.90E+06	1.00E+05		12/5/12	measurements
5	**Roché 10106**	Setaria	viridis	110	28.2%	yes	growing	1.60E+08	1.30E+06		12/6/12	measurements
6	**Vela 86**	Setaria	viridis	21	42.8%	yes	growing	1.03E+08	8.00E+05		12/13/12	measurements
6	**KELLOGG 1186**	Setaria	viridis	87	43.9%	yes	growing	1.03E+08	8.00E+05		12/13/12	measurements
6	**KELLOGG 1237**	Setaria	iridis sma	89	12.4%	yes	growing	1.03E+08	8.00E+05		12/13/12	measurements
7	**Estep ME028V**	Setaria	viridis	131	58.0%	no	growing	1.70E+08	1.00E+05		12/22/12	measurements
7	**Waselkov Vandali**	Setaria	viridis	41	48.7%	no	growing	1.70E+08	1.00E+05		12/22/12	measurements
7	**Waselkov Rock Fal**	Setaria	viridis	72	27.7%	no	growing	1.70E+08	1.00E+05		12/22/12	measurements
7	**Waselkov Momen**	Setaria	viridis	23	21.7%	no	growing	1.70E+08	1.00E+05		12/22/12	measurements
	Estep ME017	Setaria	viridis	--	--	--	--	--	--			
	Estep ME019	Setaria	viridis	--	--	--	--	--	--			
	Estep ME043	Setaria	viridis	--	--	--	--	--	--			
	Estep ME044	Setaria	viridis	--	--	--	--	--	--			
	Estep ME046	Setaria	viridis	--	--	--	--	--	--			
	Estep ME51V	Setaria	viridis	--	--	--	--	--	--			
	PENAGOS P8	Setaria	viridis	--	--	--	--	--	--			

Of the first 30 genotypes screened, only 3 showed a significant growth response to bacterial inoculation…hence, we conclude that plant genotype is a crucial factor

Effects of inoculation of *S. viridis* A10-1 with *H. seropedicae* and *A. brasilense*

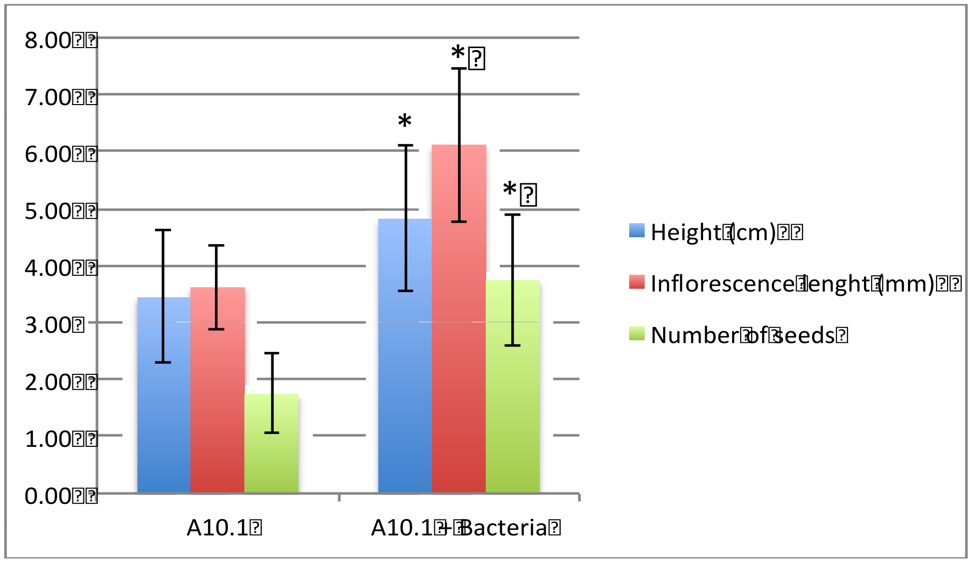
Soil
Promix 1:1 Sunshine
No nitrogen added

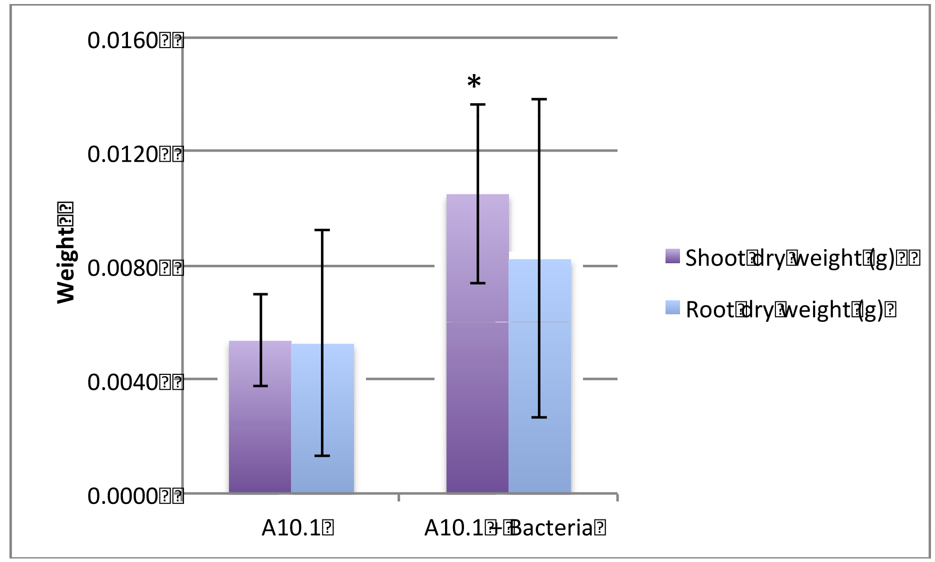


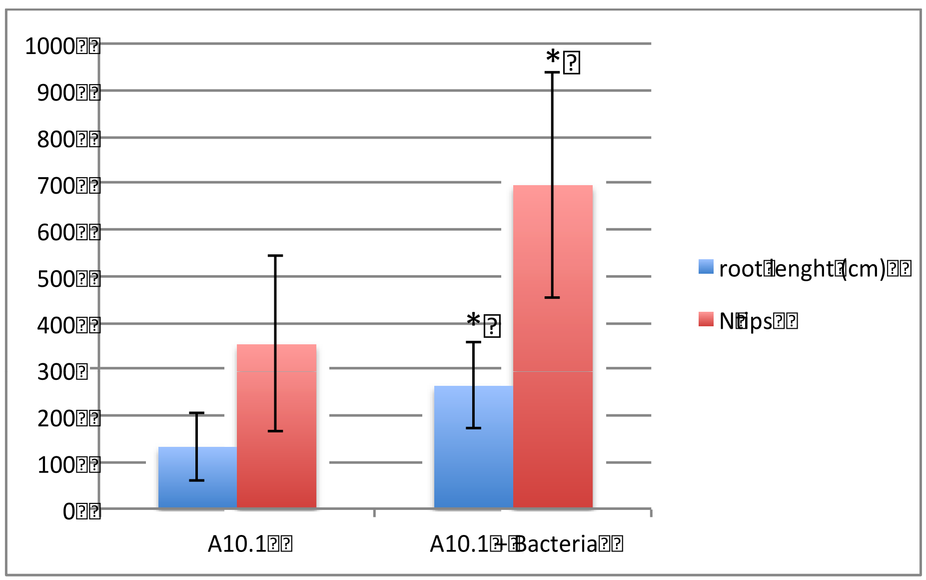


Exp 2: Soil
Turface 3:1 Vermiculite
No nitrogen

Effects of inoculation of *S. viridis* A10-1 with *H. seropedicae* and *A. brasilense*



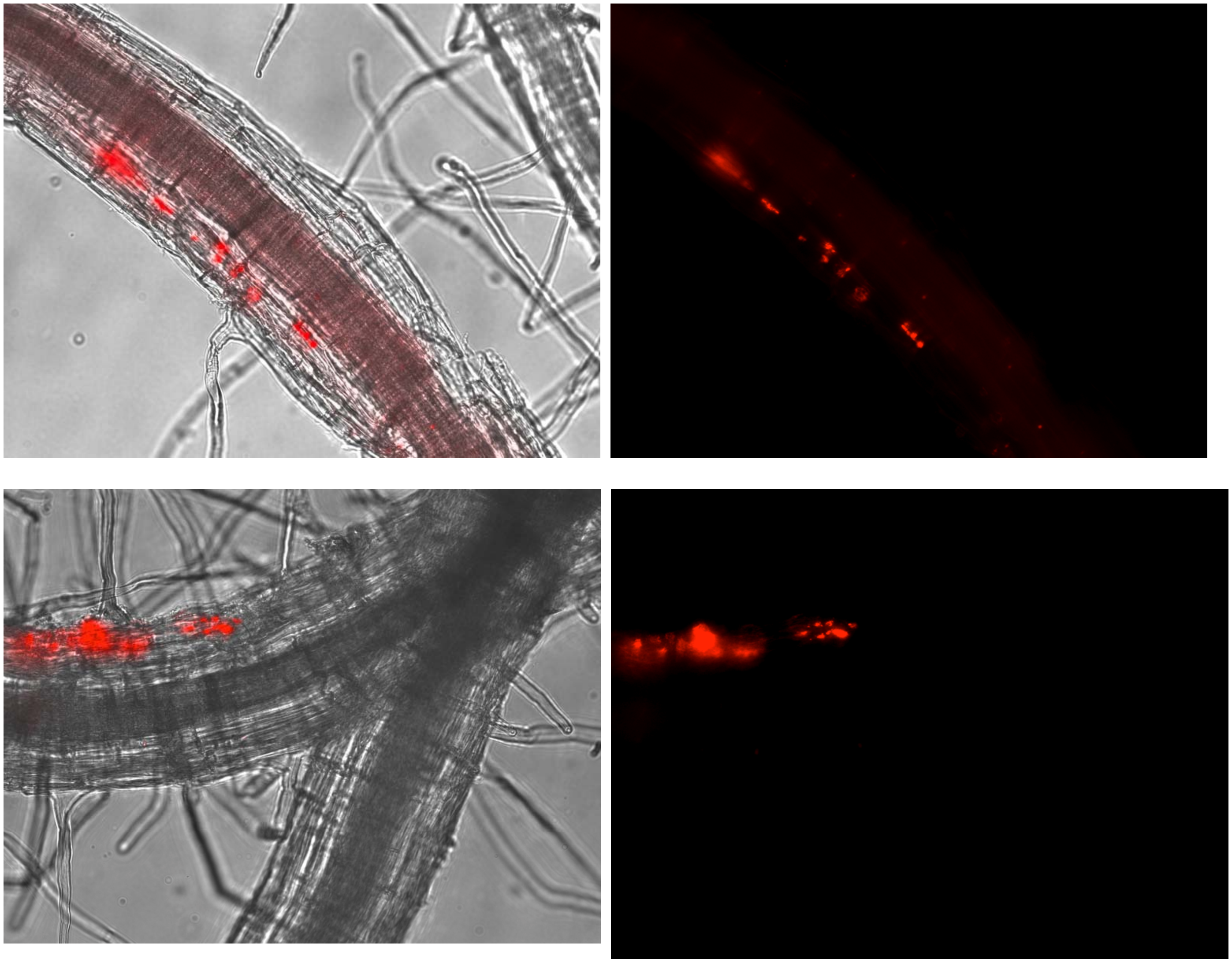




*p < 0.05

Exp 2: Soil Turface 3:1 Vermiculite
No nitrogen

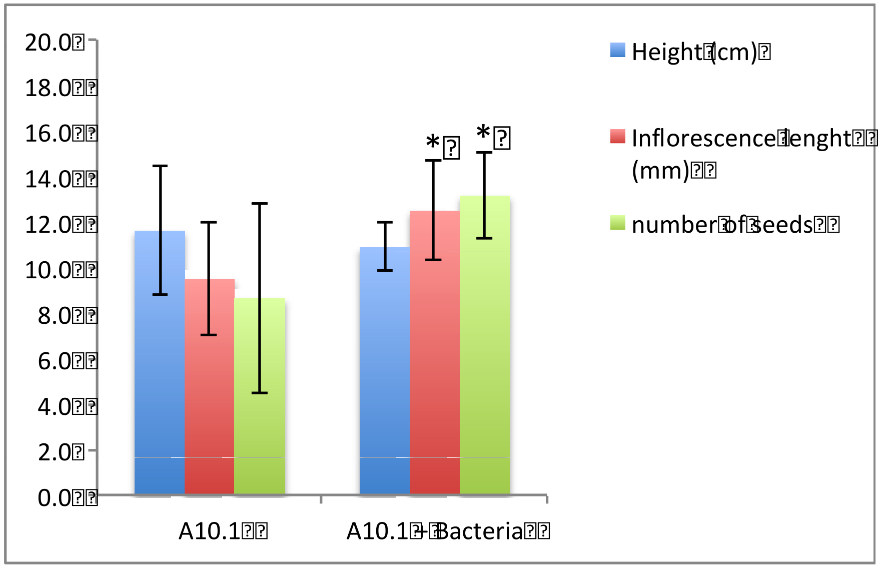
Effects of inoculation of *S. viridis* A10-1 with *H. seropedicae* and *A. brasilense*

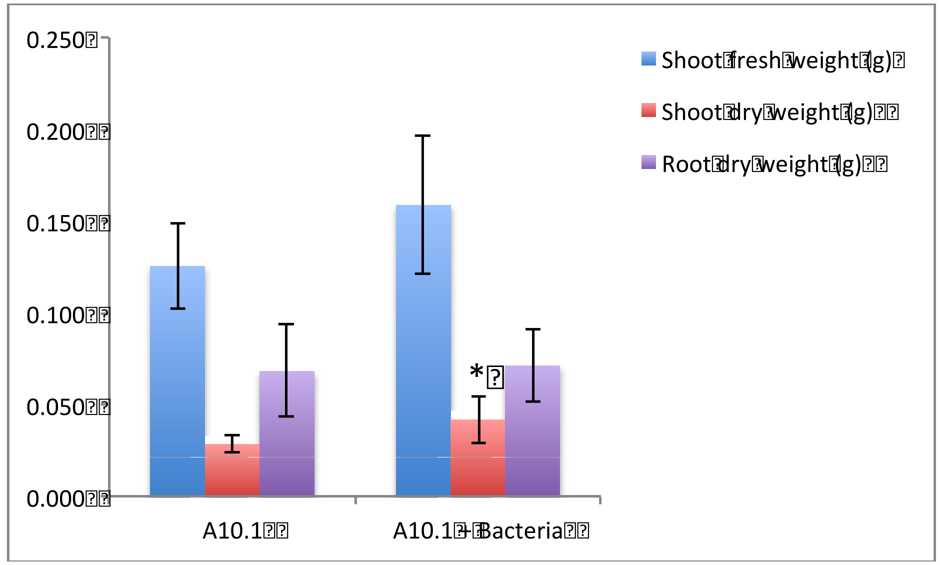


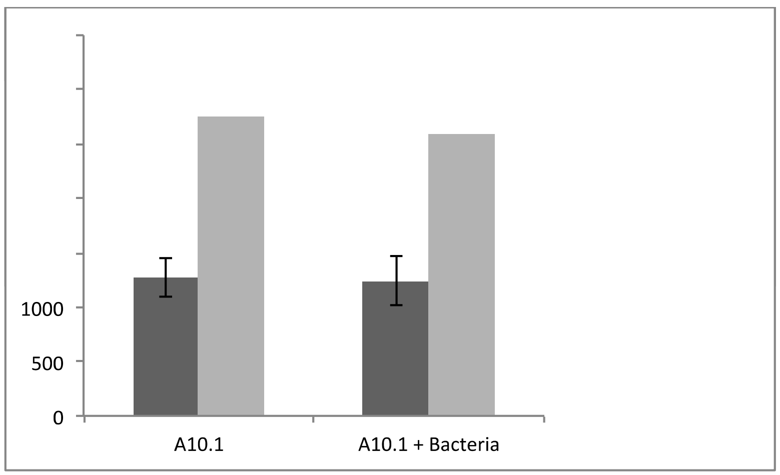
Roots colonized after 40 Days of inoculation (D.A.I)

Effects of inoculation of *S. viridis* A10-1 with *H. seropedicae* and *A. brasilense* – **Low N**

Exp 2: Soil
Turface 3:1 Vermiculite
0.5mM of nitrogen





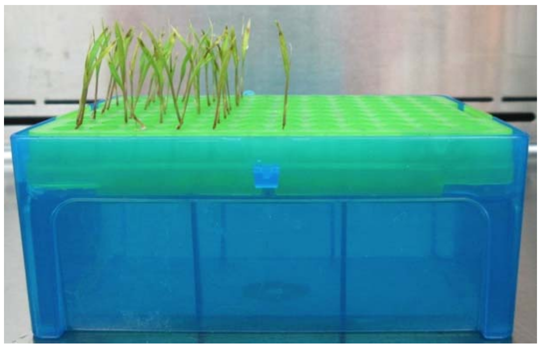


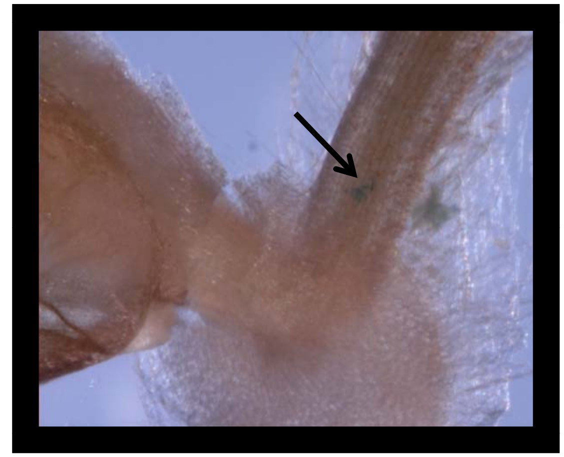
* $p < 0.05$

NifH-Gus-Staining could be observed on *S. viridis* A10.1 growing under sterile conditions.

Tip Box

11 D.A.I



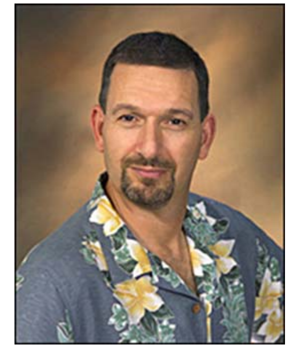


Test Tube

15 D.A.I

Setaria viridis: A Model Grass to Explore Bacterial Plant Growth Promotion and Associative N_2 Fixation.

Collaboration with
Dr. Richard Ferrieri
Brookhaven National
Laboratory

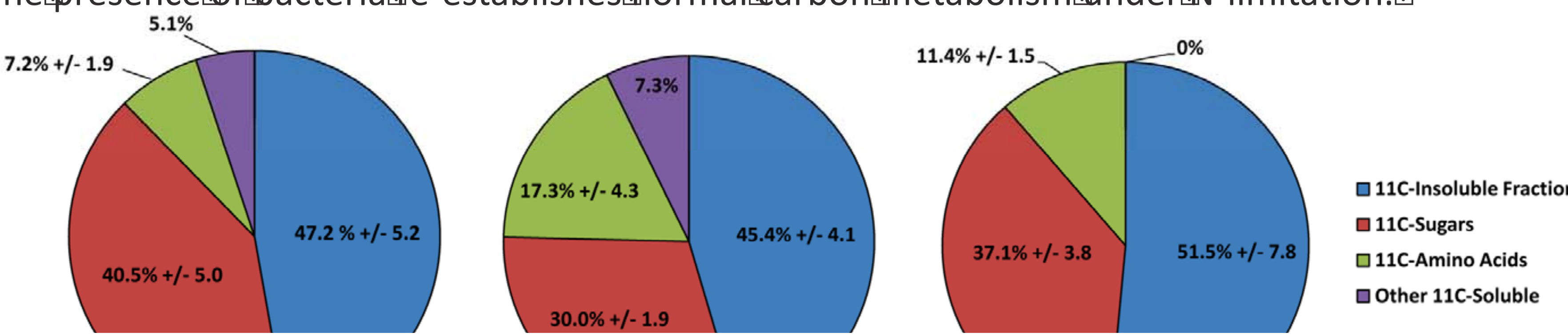


Fernanda P. Do Amaral

Objective: To provide mechanistic insight underpinning host plant growth promotion.

Approach: Metabolic partitioning of new carbon into key pools was quantified using $^{11}CO_2$ administered to plants grown under normal nitrogen and nitrogen limitation. Azospirillum *brasilense* and Herbaspirillum *seropedicae* N_2-fixing bacterial strains were introduced under N-limitation.

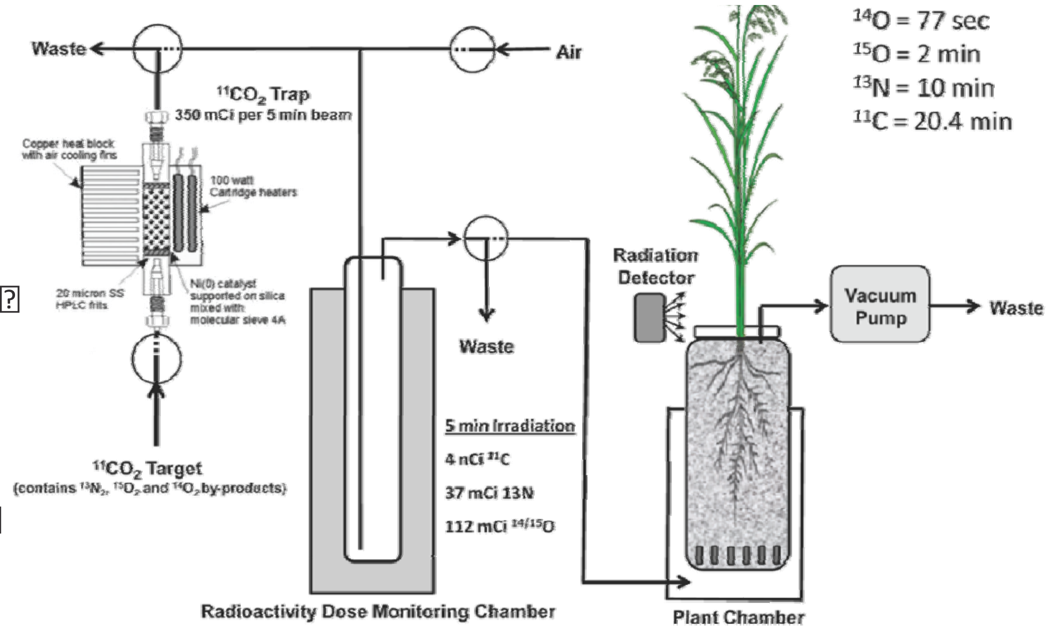
Results/Impact: N-limitation causes stress to the plant resulting in changes in carbon metabolism. The presence of bacteria re-establishes normal carbon metabolism under N-limitation.

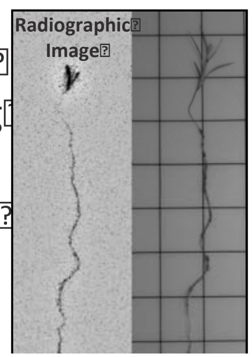


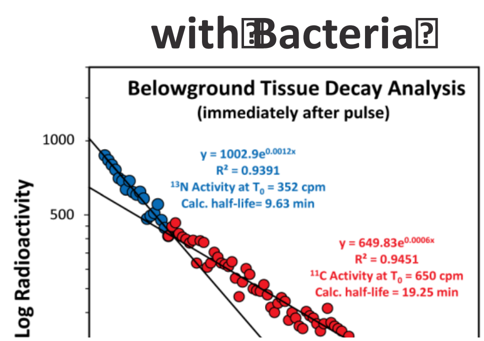
rhizobacteria in non-leguminous grass systems has only inferred that host plants acquire biological nitrogen based on growth characteristics, but without direct evidence of this. Our objective was to provide this evidence leveraging the power for measuring minute amounts of fixed radioactive ^{13}NN.

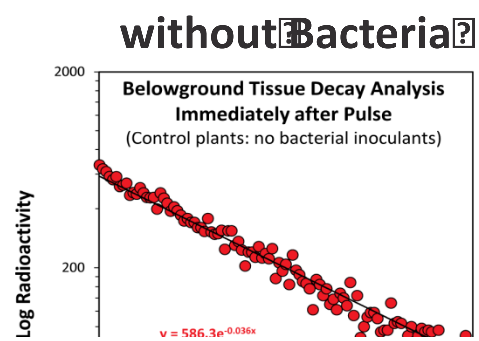
Approach: A remotely operated ^{13}NN pulsing station was recently installed at the BNL Plant Radiotracer Facility that taps ^{13}NN as a by-product from the $^{11}CO_2$ cyclotron production target and re-directs the ^{13}NN tracer through the soil column.

Results/Impact: Based on ^{13}N data we calculated a cumulative N_2-fixing rate of 125±36 nmoles per day. Approximately 30% of that nitrogen is acquired by the host and moved to aerial tissues. We estimate this

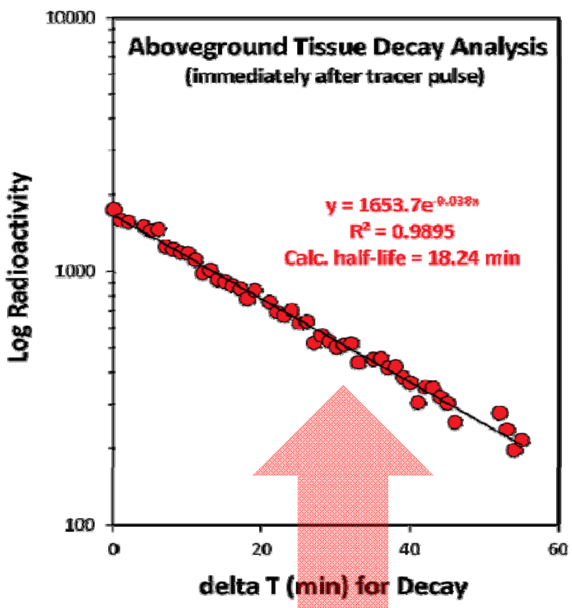


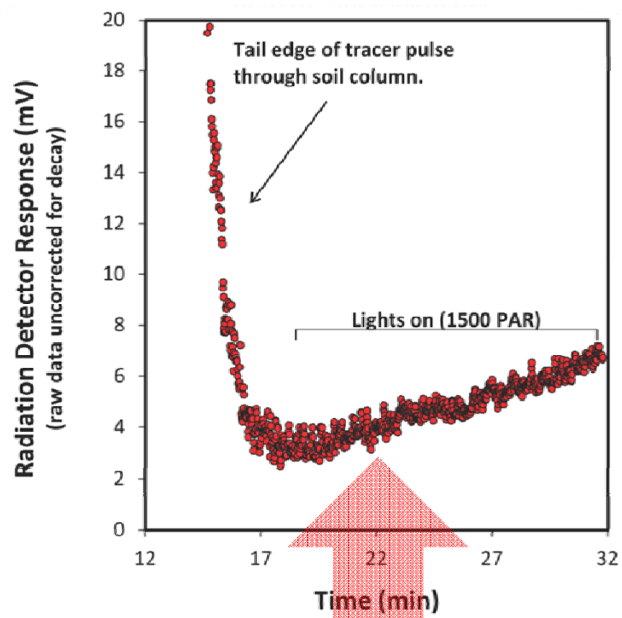


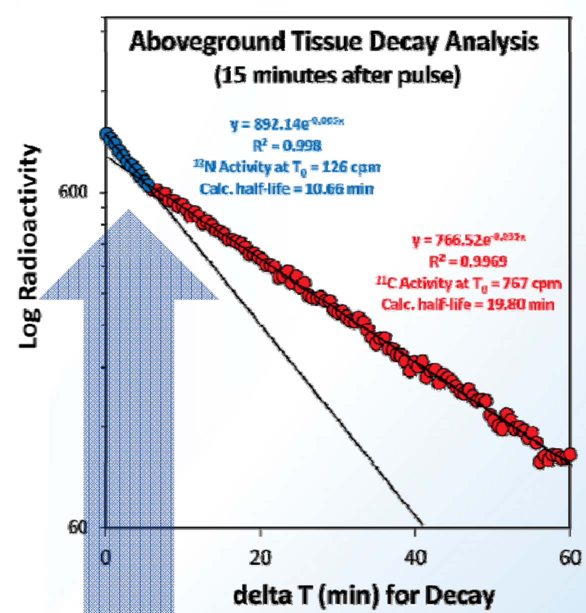




Biological Transport of Fixed ^{13}N to Aboveground *Setaria* Shoots Demonstrated.







Only a ^{11}C signature is seen in shoots immediately after pulse.

Biological transport of radiotracer from roots-to-shoots is evident under high light.

A ^{13}N signature can be seen in shoots after biological transport.

BROOKHAVEN NATIONAL LABORATORY

CONCLUSIONS

- **We seem to be in an era of increasing interest and appreciation for biological nitrogen fixation**
- **The ongoing strong record of research advances in the area of biological nitrogen fixation provide optimism for the notion that this research can be translated for practical benefit**
- **Changes in the agricultural industry have created a more receptive environment for biological products**
- **However, challenges remain and agricultural research continues to be undervalued.**

- **Our research suggests that it is an inability to couple Nod factor recognition to symbiotic developmental pathways that is the missing link in non-legumes, not an inability to recognize the NF.**
- **We believe that non-symbiotic, associative nitrogen fixation continues to hold significant promise and research in this area will be stimulated by the adoption of Setaria as a model system**

Many people to thank…

My lab:

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National Center for Soybean Biotechnology NCSB

MSMC

NCSRP North Central Soybean Research Program

Brookhaven National Laboratory

Pacific Northwest National Laboratory

EMSL

Thanks for listening…