

# **Biological Products and Their Role in Soil Fertility**

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## **Biologicals- What are they?**

- **Chemicals that mimic chemical agents already produced by plants or by microorganisms in association with a plant that induce some plant benefit.**

**or**

- **Organisms isolated from the soil that perform a beneficial function in the soil or plant.**

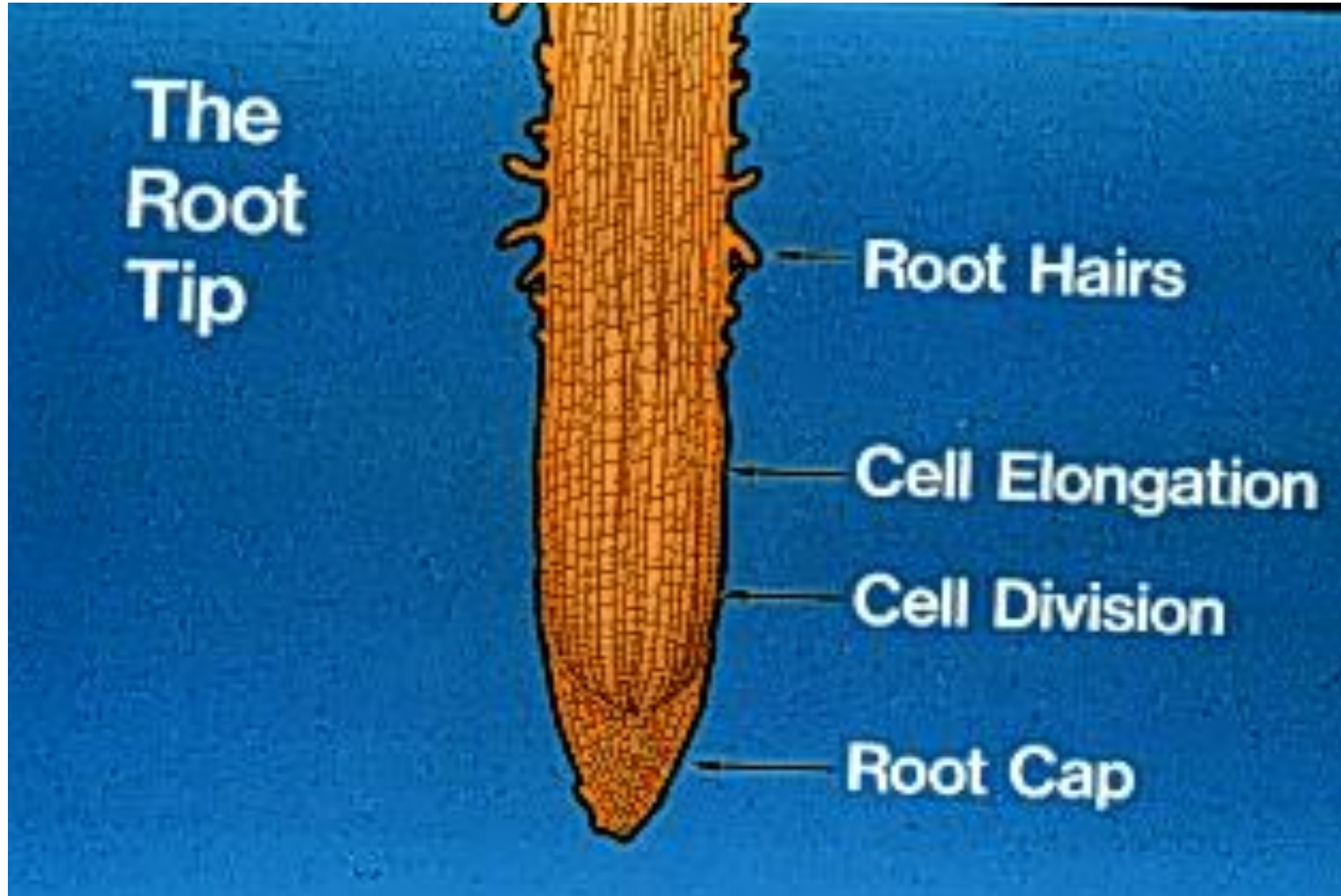
## Signal molecules-

Bacteria with plant associations have what are termed *Quorum Sensing* and *Quorum Quenching* molecules.

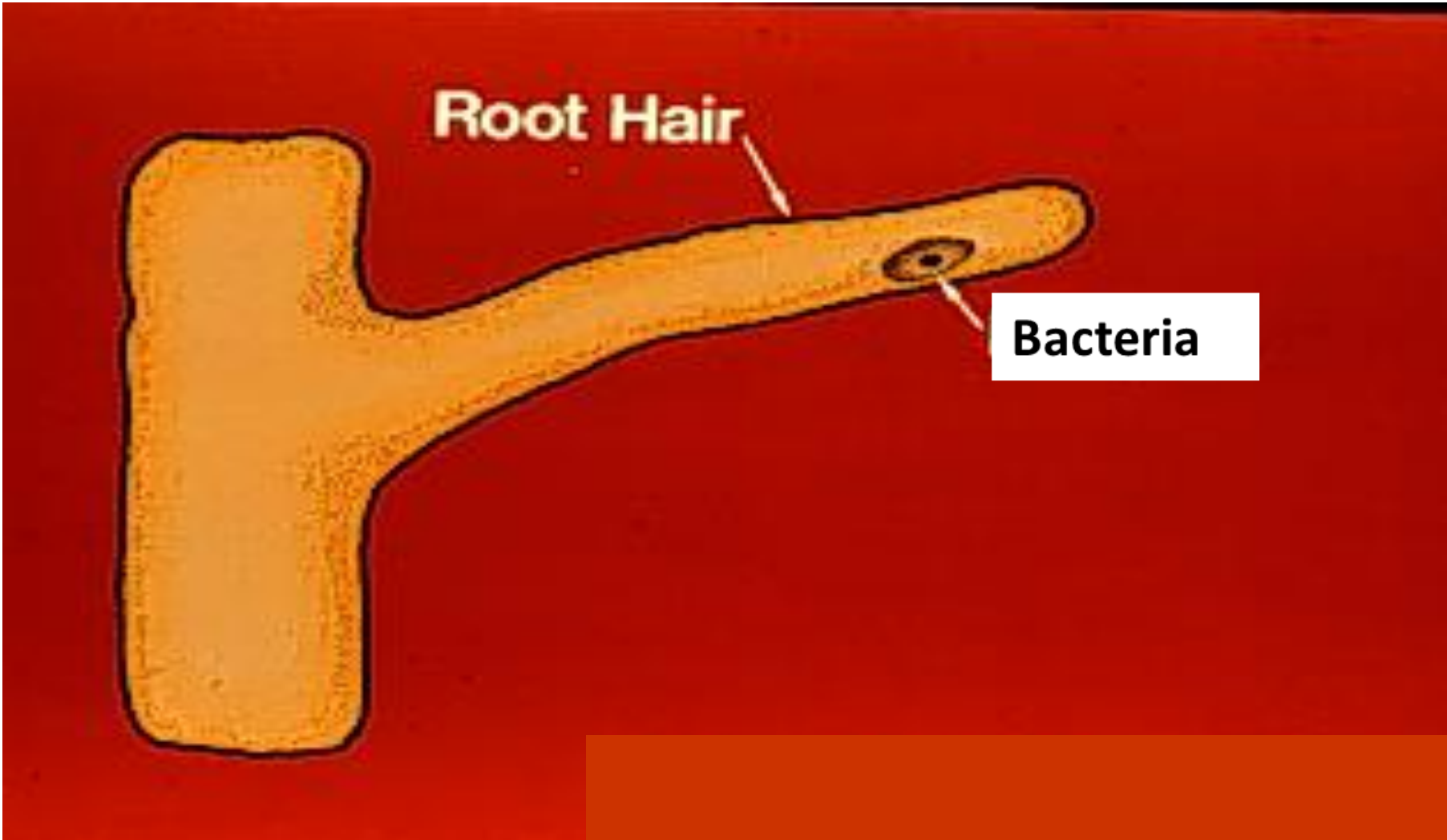
The Quorum Sensing molecules 'communicate' the status of their environment to enable production of proteins and other products that enable a stronger association with the plant.

The Quorum Quenching molecules inhibit production of associations and activity of other neighbors.

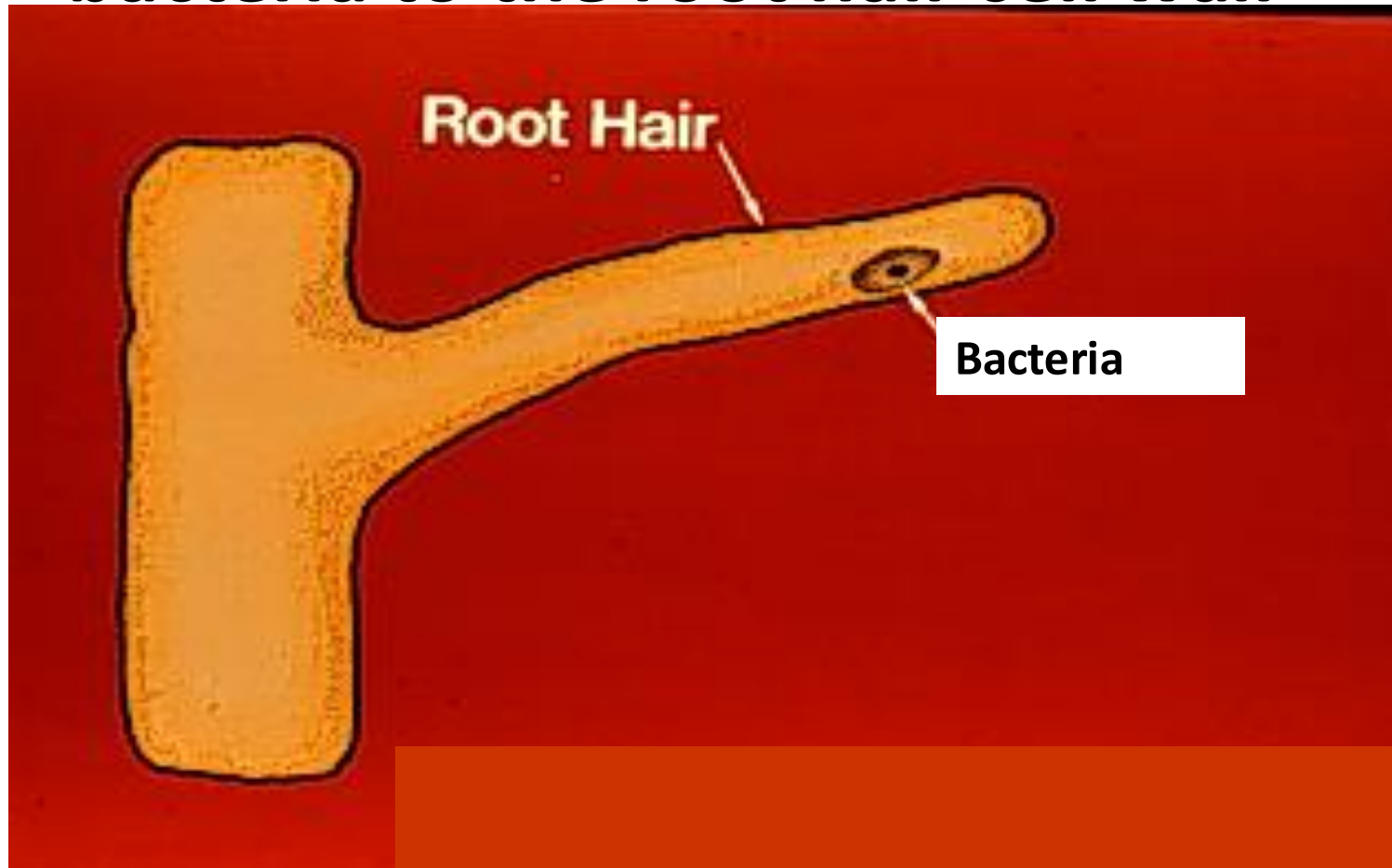
**Roots absorb nutrients and interact with soils primarily through root hairs**



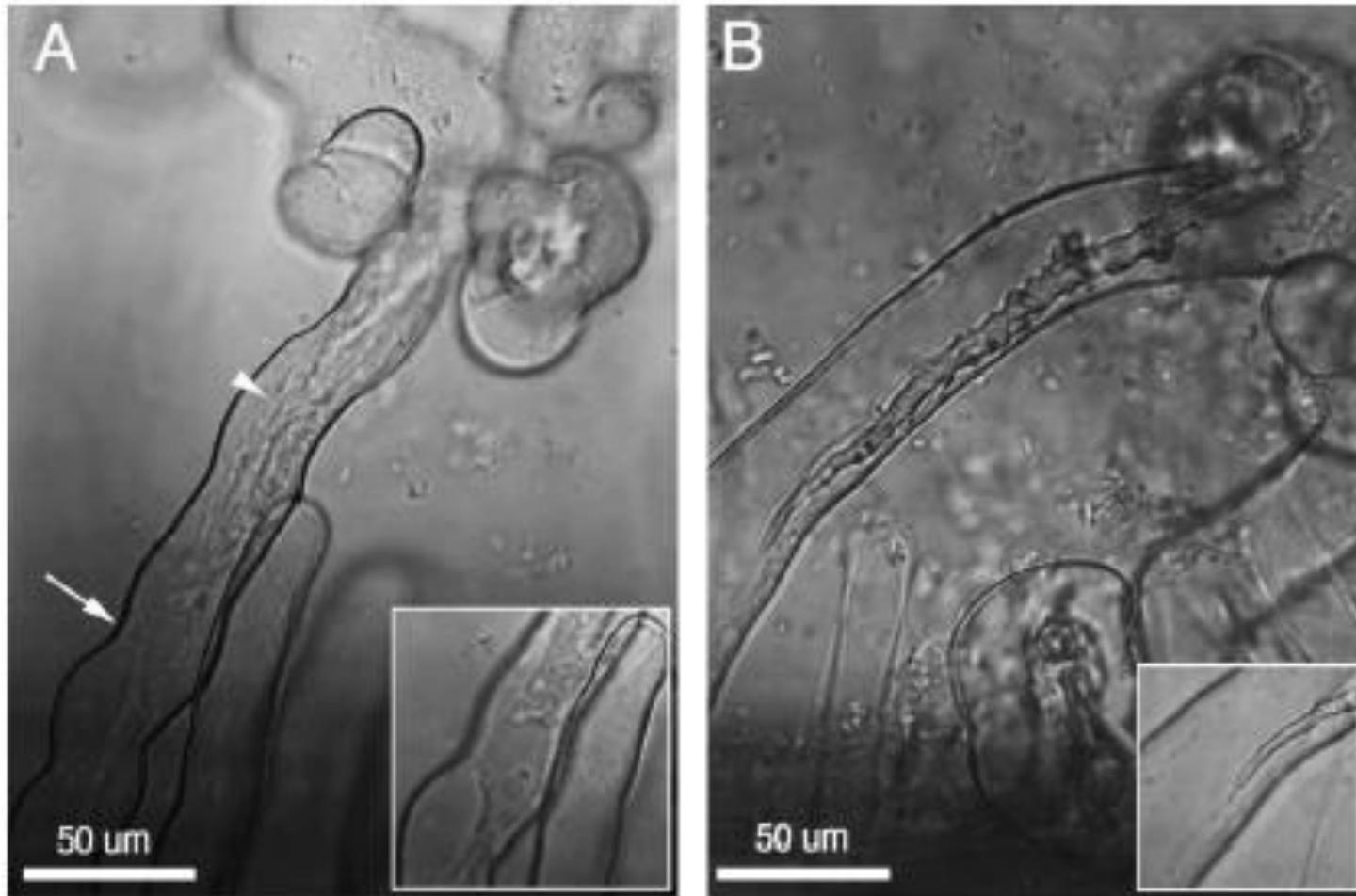
- **Bacteria are attracted to the root through chemical signals from the root hair**



**Once in contact with the root hair,  
a root compound, lectin, binds the  
bacteria to the root hair cell wall**

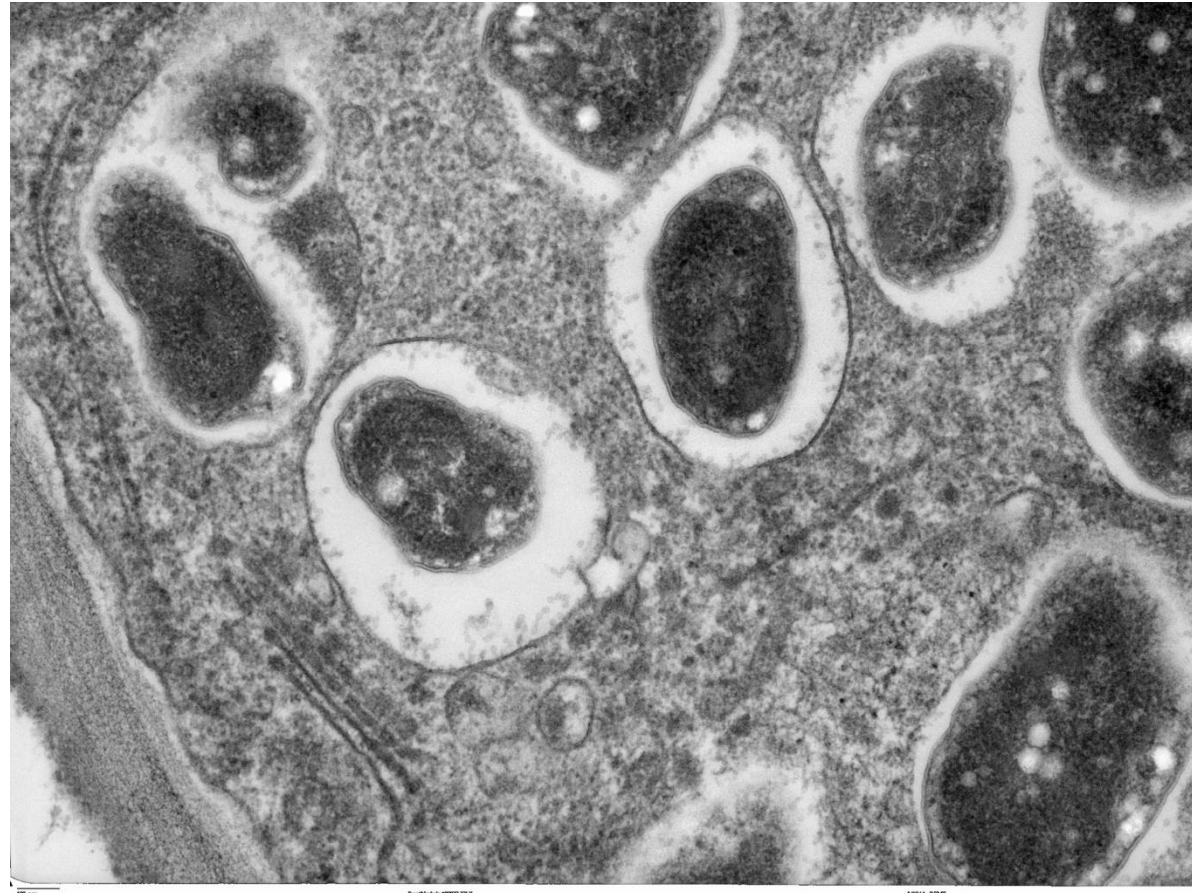


- **The bacteria releases a chemical that causes the root hair to curl and crack, producing a means of internal infection**



**D.J. Gage,  
Microbiology and  
Molecular Biology  
Reviews  
2004:68 280-300**

**Bacteria take over control of some cortex cells, transforming them into nodules, which contain colonies of bacteria called bacteroids, each containing up to 10,000 bacteria.**





- **Bacteroids are bathed in nutrients from the host.**
- **Bacteria take  $N_2$  gas from soil air and transform into  $NH_4^+$  using the enzyme nitrogenase.**



**The legume/bacteria symbiosis process requires the receptiveness of the plant to infection, and continued nutrition of the bacteroids.**

**If the plant has enough N, it is not receptive, nor does it support bacteria nutritional needs.**

**If the conditions are too cold, too hot, too wet, too dry, then then plant protects itself, with nutritional needs of bacteria secondary.**

**Growth hormones are also signal molecules.**

**Cytokinens, Gibberillins, Auxins, all signal responses in the plants. All of our crops have the ability to produce these molecules, and deconstruct them if they are not needed.**

**Timing of application of any of these molecules is critical to their beneficial use. Thus a low frequency of reports of their beneficial use.**

# **Organism which may enhance plant nutrient availability**

- **Phosphorus?**
- **Nitrogen?**

## Phosphorus enhancers?

***Penicillium bilaii*, a soil fungus isolated that can solubilize P from occluded P (P in calcareous soil).**

**Karamanos et al. (2011) examined 47 field trials over 3 Canadian Provinces with P fertilizer and the use of a commercial *P. bilaii* product.**

**Wheat yield increased with P fertilizer in 33 trials, and yield increased with *P. bilaii* in 5 trials, but none of these increases could be attributed to P enhancement.**

**Latest in the soil fertility enhancement product offerings are the asymbiotic N-fixing bacteria.**

***Point # 1- Asymbiotic N-fixing organisms are a natural part of soils***

**Organisms, usually a species of bacteria, that have the ability to fix atmospheric N ( $N_2$ ), transforming it into  $NH_3$ , which is immediately attached to a 'carbon-skeleton', safening it.**

**The fixation requires energy, which when conducted in soil comes from organic matter.**

## Asymbiotic N-fixing organisms

**Evidence for asymbiotic N-fixing organisms finds that these organisms were active 1.5 billion years ago- some of the oldest organisms found in the fossil record.**

**(Boyd & Peters, 2013, Frontiers in Microbiology)**

**Compared with about 59 million years ago for symbiotic N-fixers (Sprent and James 2007, Plant Physiology)**



## Asymbiotic N-fixing organisms

**N-fixation is an energy-expensive process.**

**The enzyme that serves as ‘fixation facilitator’ in bacteria is *nitrogenase*.**

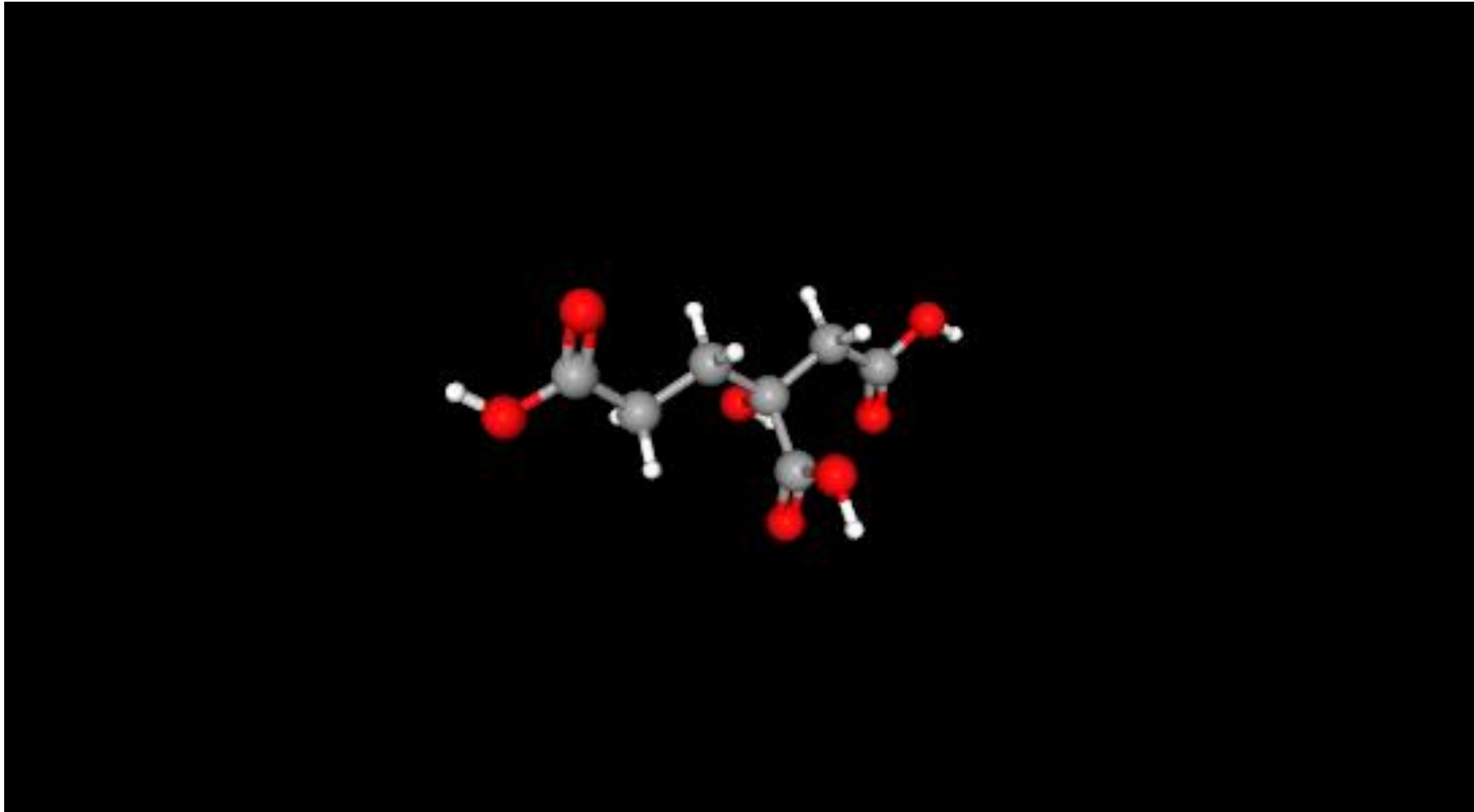
**To convert 1 N<sub>2</sub> to 1 NH<sub>3</sub> requires 16 ATP molecules (produced during photosynthesis) and 8 electrons.**

**Energy limits N fixation.**

**(Smercina et al., 2019, Applied Environmental Microbiology)**

**For comparison, production of 1 peptide bond in protein synthesis requires only 5 ATP (still considered ‘high energy requirement’)**

# Nitrogenase enzyme

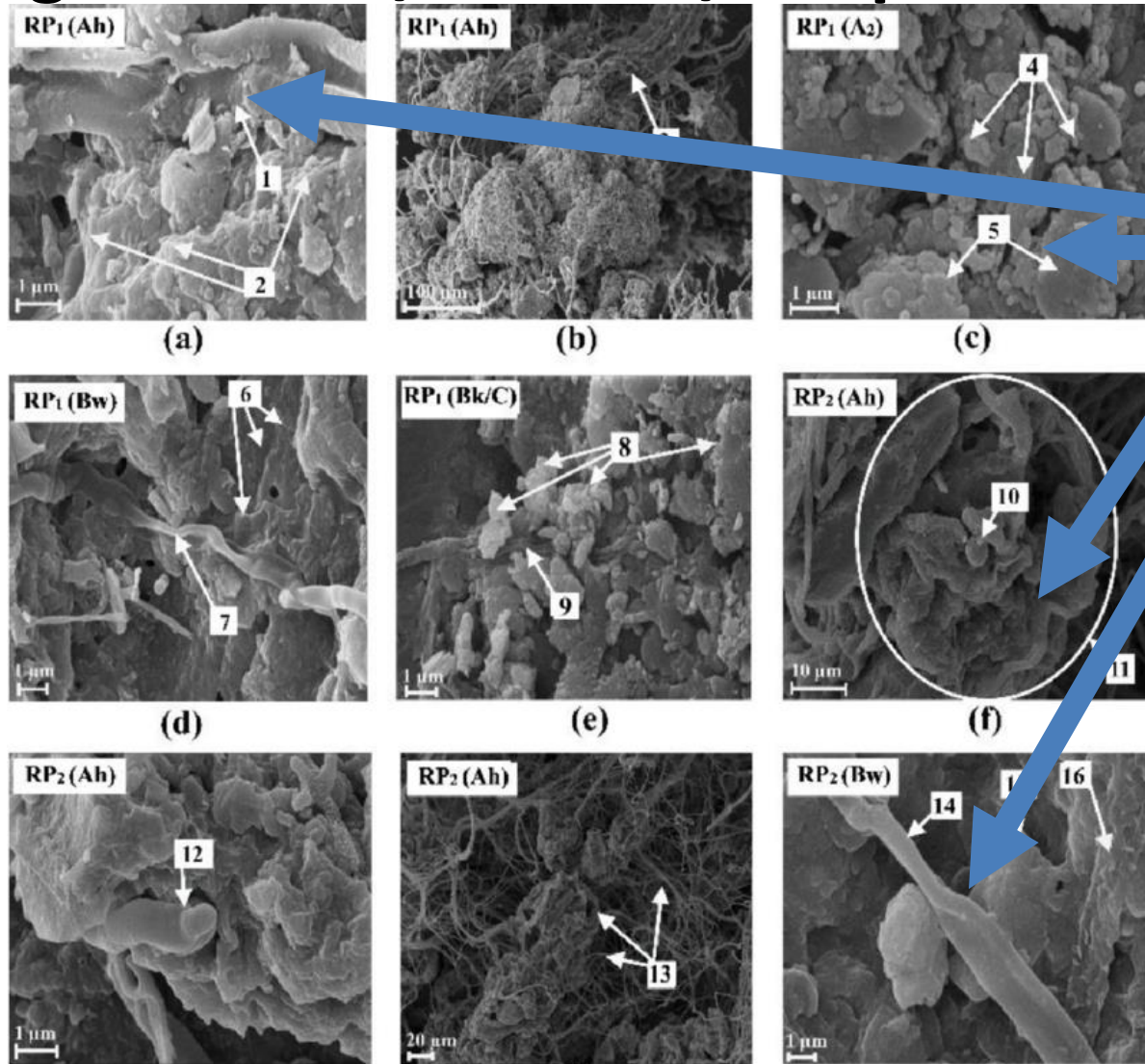


## ***Substrates to meet Asymbiotic bacteria energy requirement***

**Energy sources for asymbiotic N-fixing organisms close to plant roots are the root exudates that surround many roots, and also includes components of soil organic matter and residue decay intermediary compounds in the bulk soil.**

**N-fixing organisms that exist inside the plant tissue have to receive substantial energy from the plant within which they reside.**

# Images of root/exudate/soil particle interfaces



**Root Exudates/  
biofilms**

RP 1 images (SEM) of fine root (1), root exudates/biofilms (2), fine roots (3), clay mineral grains (4), clay mineral aggregates (5), root exudates/biofilms (6), organic filament (7), fine clay aggregates (8), fine root (9), and RP 2 images (SEM) of aggregates with abundant root remnants (10), pollen (11), root tap protruding through clay aggregate coated by exudates (12), dominant network of fine roots (13), and organic filament, primary mineral, clay minerals coating organic filament (14, 15, 16).  
From Razzaghi et al., 2017.

## Diversity of Asymbiotic bacteria types-

A Chinese/Tibetan paper, using genetic screening, found Asym-bacteria from 6 Phyla, 13 Classes and 43 genera (Li et al, 2021, Front. Ecol. Evol. 13 August. Vol 9)

Genera include species from

*Azotobacter*, *Ocrhobactrum*, *Sphingomonas*, *Opitutus*, *Clostridium*, *Pseudomonas* and 'a host of others'.

There are many species, some more efficient than others, Their activity is linked to substrate and soil condition.

**Asymbiotic N-fixing bacteria are in most soils.**

**Their activity increases when tillage decreases. *Food & Housing***

**Lamb, Doran and Peterson, 1987**

**Nonsymbiotic dinitrogen fixation in no-till and conventional tillage SSSAJ 51:356-361**

**Recorded greater activity with no-till, but concluded that it was not great enough to contribute to any N credit. They considered the values from incubation to be values that might be experienced in the field. But the disturbance of soil probably killed billions of N-fixing critters, so values are index.**

**In North Dakota, there is an N credit for 6 or more years of continuous no-till, one-pass shallow tillage, shank strip-till, amounting to 40-50 pounds N per acre.**

**Part of this credit probably comes from the increased microbial biomass under no-till that protects N from loss.**

**But a part of the credit, perhaps 25-33% may come from greater asymbiotic activity in long-term no-till.**

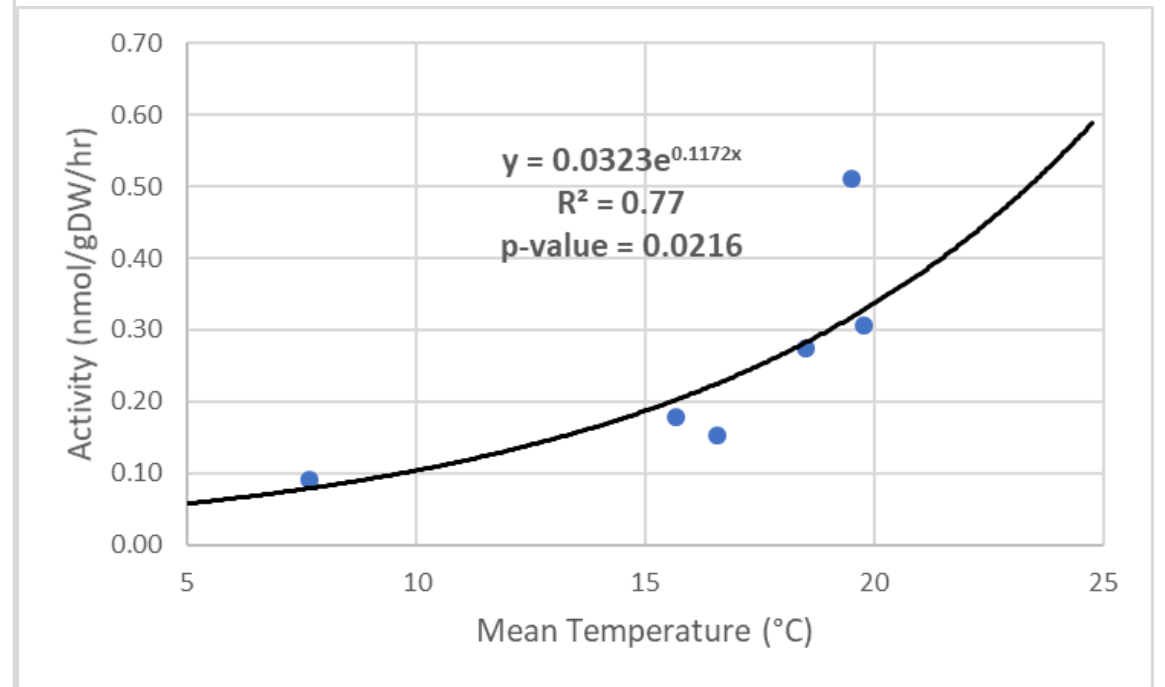
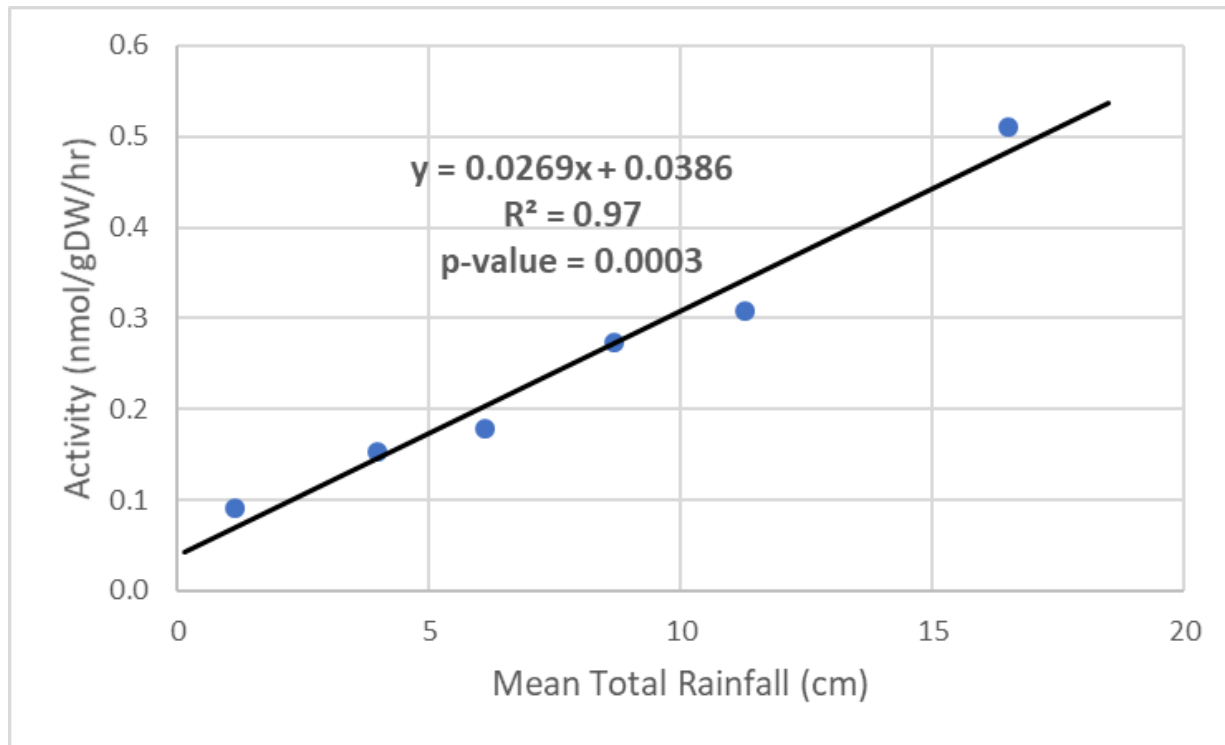
**Franzen et al. 2019, SSSAJ**

**I took paired no-till/conventional till across state.**

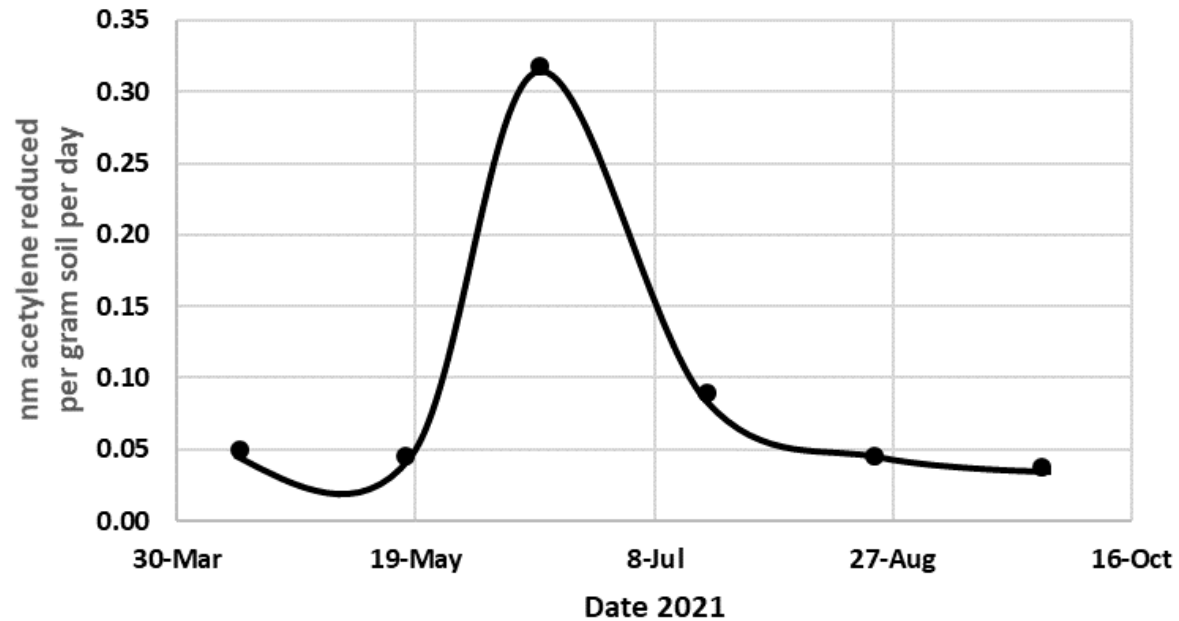
**In all 11 of 11 sites where conventional tillage microorganism activity was compared across the fence with long-term no-till soil, the no-till soil activity was much greater.**



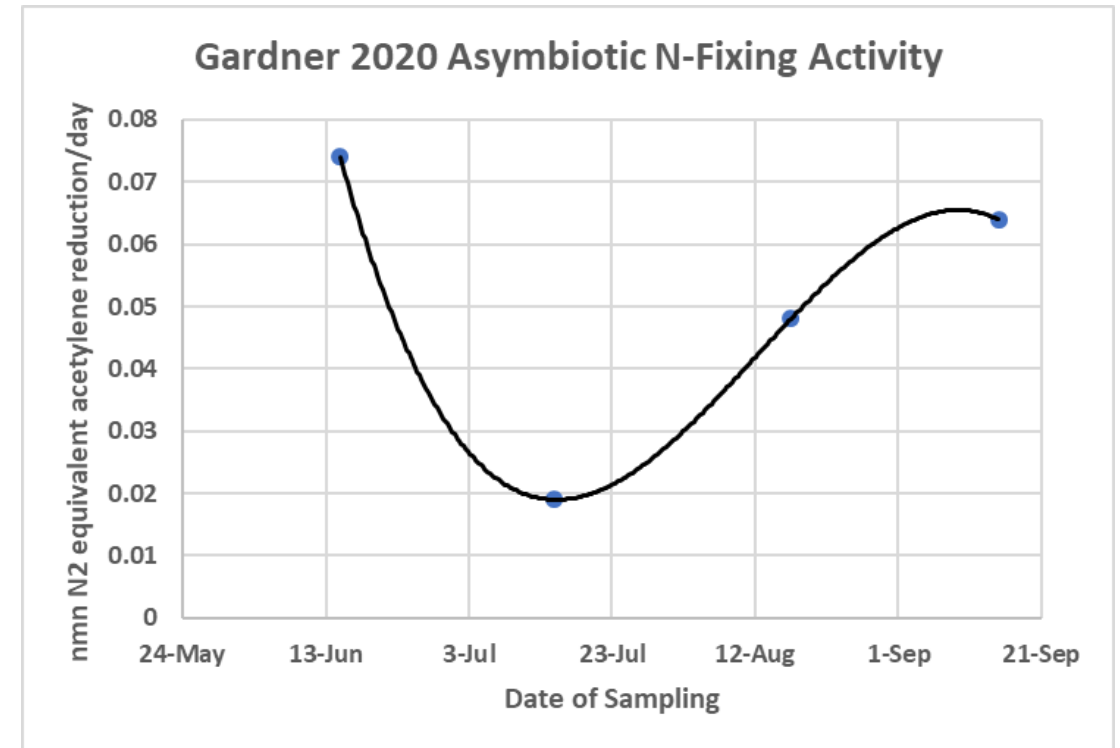
**From 2019 to 2021, 6 sites in eastern North Dakota were sampled each month for asymbiotic N fixing activity. Change in activity was related to rainfall within 30 days before sampling and mean air temperature.**



# The relationship of N-fixing activity to soil condition made a large impact on the trend of activity over a season



Jamestown, 2021. Period from late May to early July moist, then very dry.



Gardner, 2020, Period for July sampling, soil was saturated in a high clay soil.

***Summary of what we know about native  
asymbiotic N-fixing organism activity-***

**Greater in long-term no-till. Their activity increases when their 'homes' are not destroyed and there is sufficient food to support their N-fixation.**

**Moist soil and warm conditions favor N-fixation.  
Dry soil conditions, saturated soil conditions,  
and cold soil temperatures inhibit their activity.**

# Regional studies on commercial asymbiotic N-fixation products

## Products tested-

**Envita, Azotic North America**    *Gluconacetobacter diazotrophicus*

**Utrisha, Corteva Agriscience**    *Methylobacterium symbioticum*

**ProveN, PivotBio**    *Klebsiella variicola*

**ProveN 40, PivotBio**    *Kosakonia sacchari & Klebsiella variicola*

# Summary of results from 10 states.

**No** means no difference between same N rate with or without additive  
**Yes** means a yield increase present at least 1 N rate

State	Envita IF†	Utrisha	ProveN	ProveN 40 IF	ProveN 40 ST
	Number of site years included in evaluations				
ND	4 No	4 No	-----	-----	-----
MN	1 No	-----	3 No/1 Yes	-----	-----
IL	2 No	-----	4 No	5 No	2 No
IN	1 No	-----	-----	-----	-----
MO	2 No / 1 Yes	3 No	2 No	1 No	-----
KS	-----	-----	1 No	-----	-----
MI	1 No	1 No	-----	1 No	-----
KY	-----	2 No	-----	-----	-----
NE	-----	-----	5 No	6 No	-----
OH		1 No			
<b>Total</b>	11 No/1 Yes	11 No	15 No/1 Yes	13 No	2 No

**Total corn experiments 54.**

**52 no benefit to yield over N rate alone.**

**2 benefits with N rate benefits 12-20 lbs N/a**

***Growers should be skeptical about new products***

***Point # 2-***

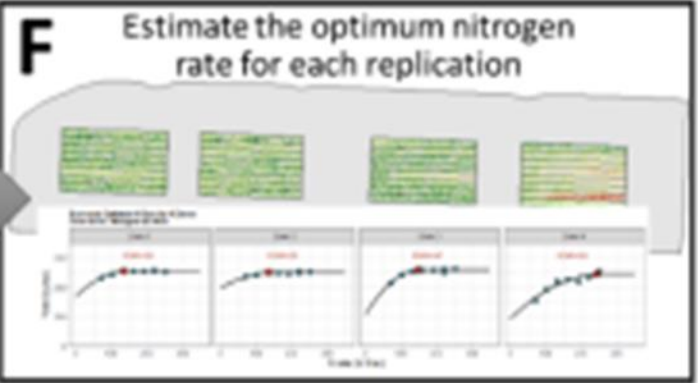
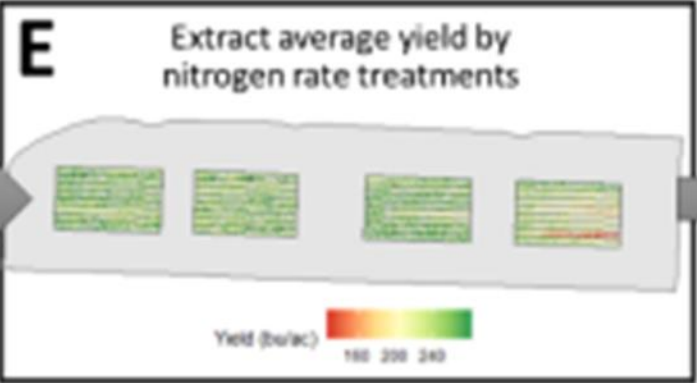
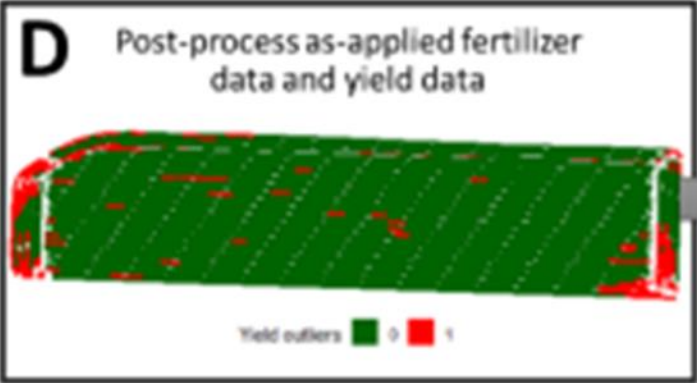
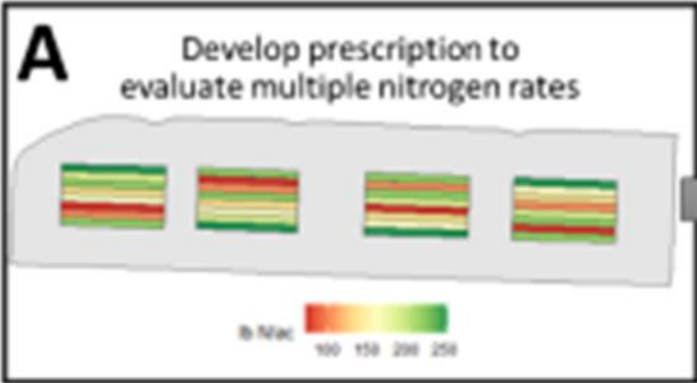
**Try new products and ideas on replicated strips on the farm.**

**Refer to L. Thompson, 2022 from**

***Proceedings of the North Central Extension-Industry Soil  
Fertility Conference***

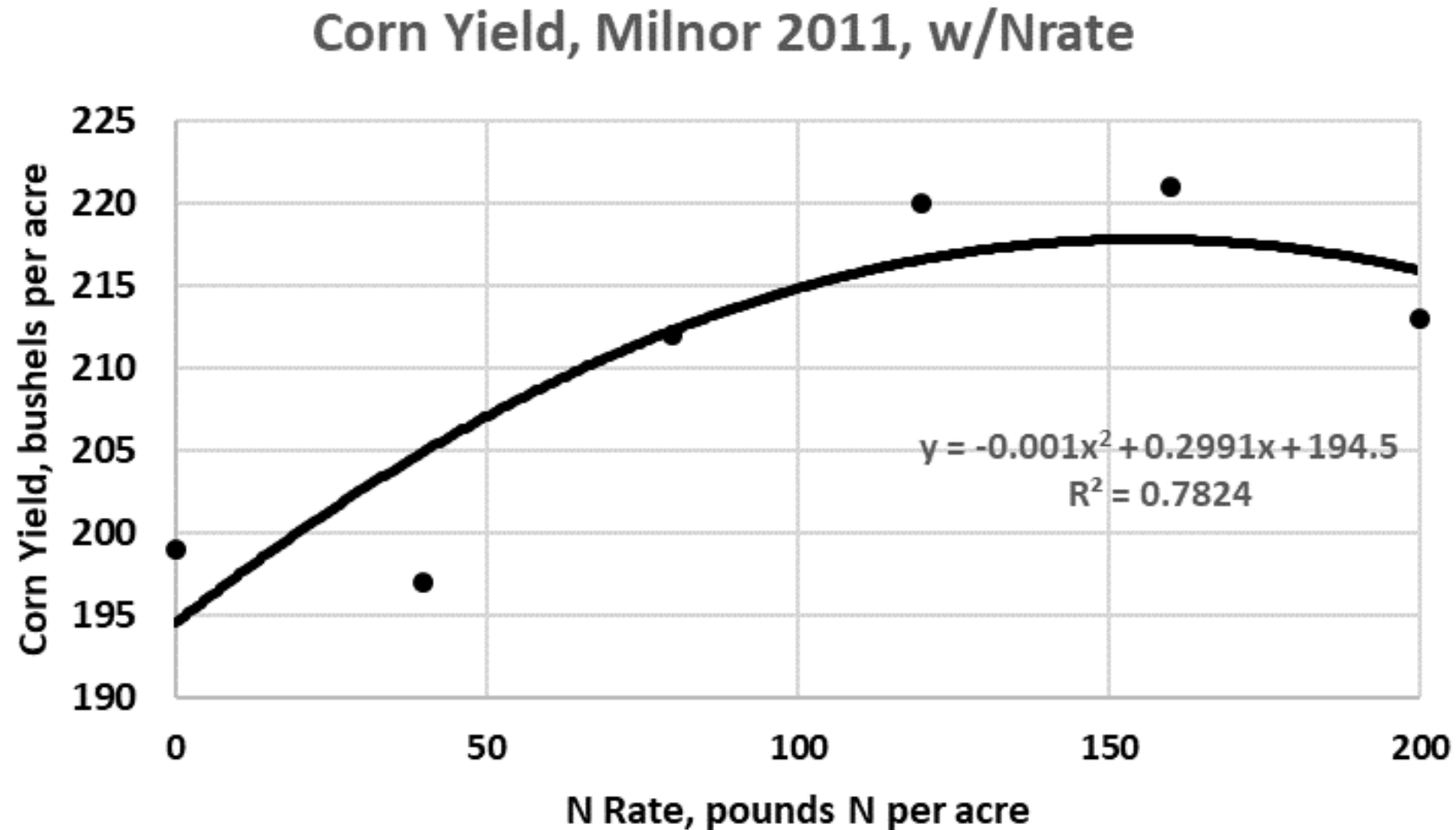
**for ideas regarding on-farm testing and data analysis.**

# Examples from Thompson Paper:

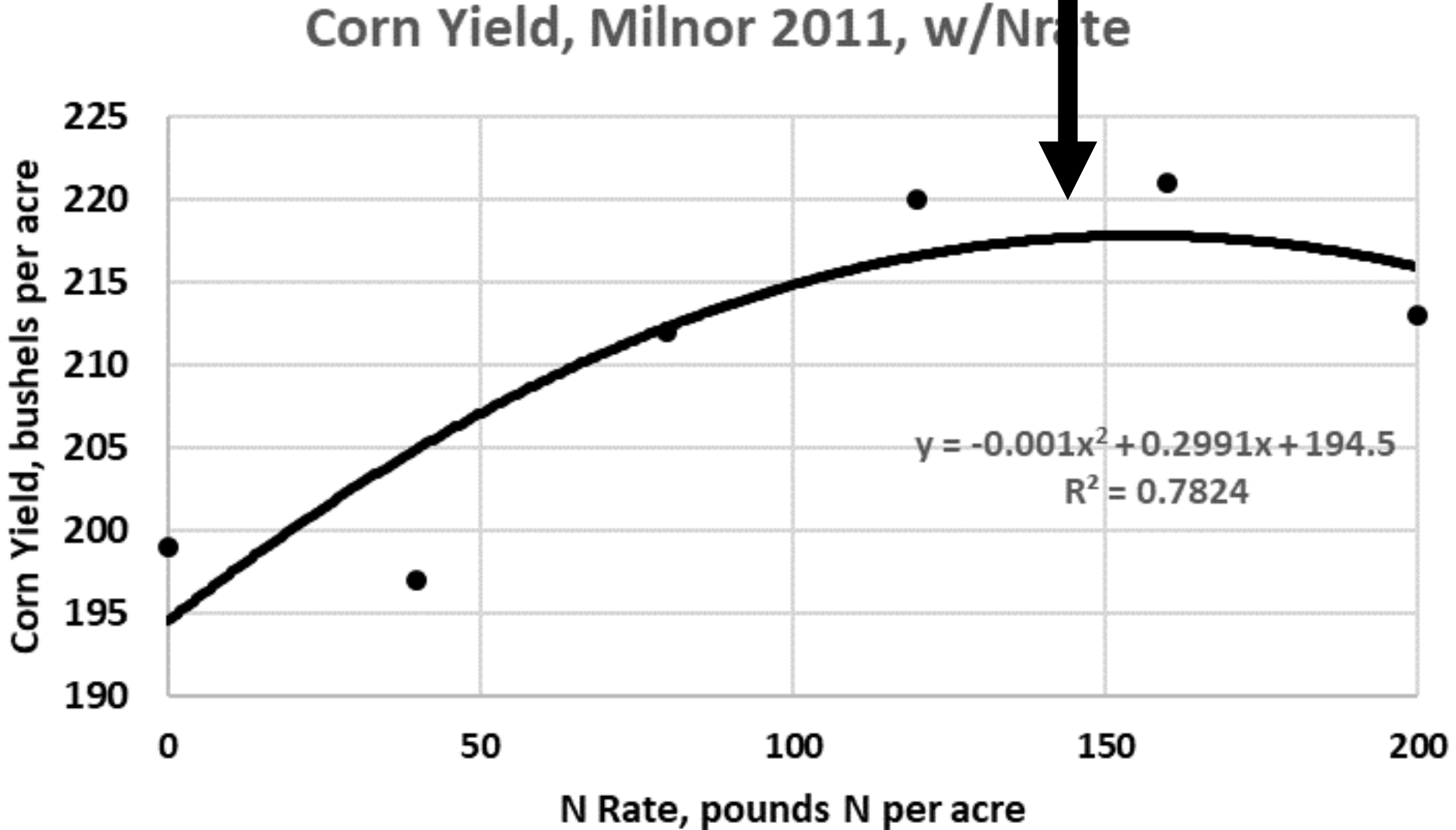




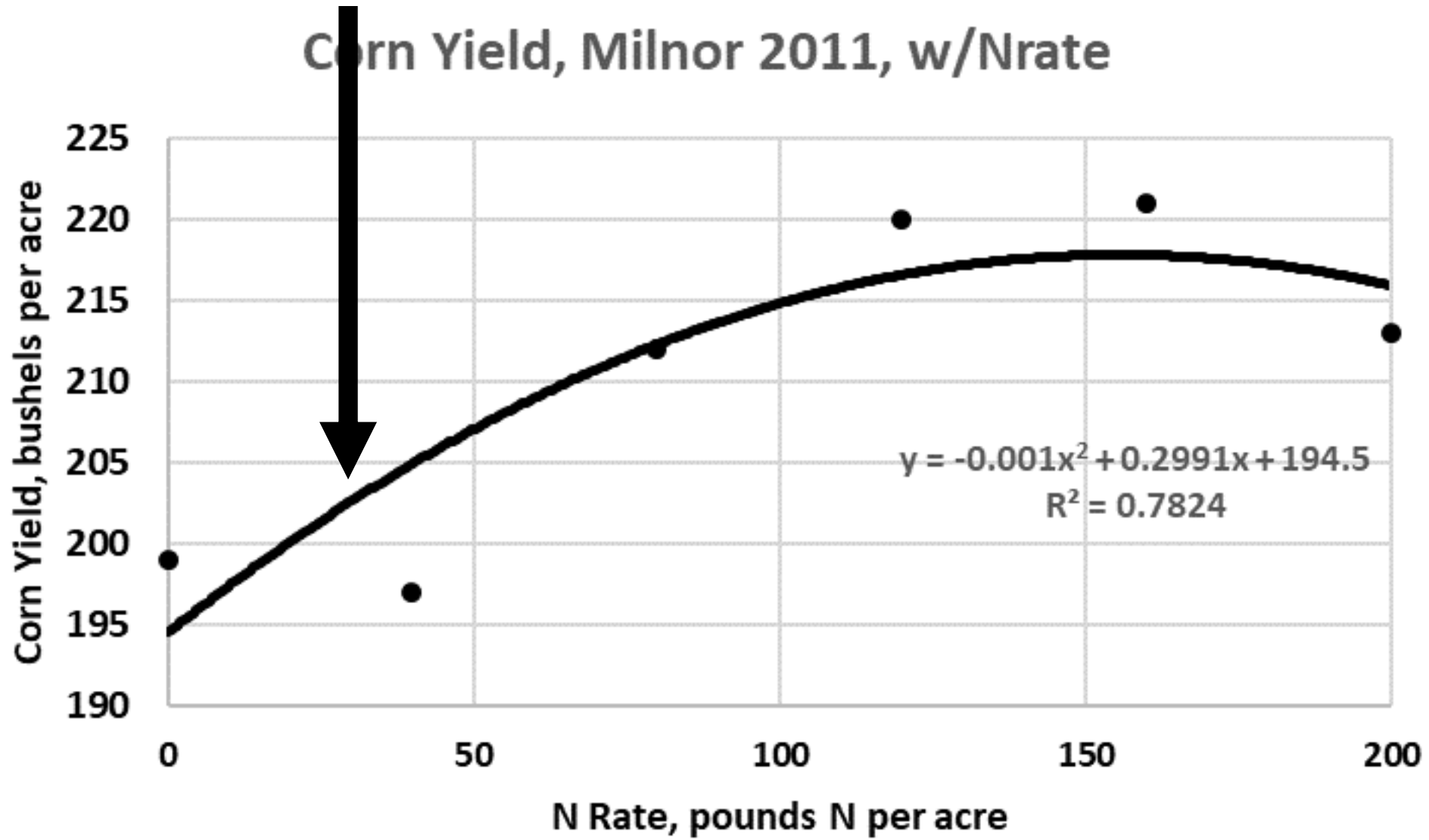
# How to set up an N-rate experiment, or an experiment with a different N product or biological N provider-



*Would you insert your product treatment here?*



*Or here?*



**Trials that tell you nothing, or less-**

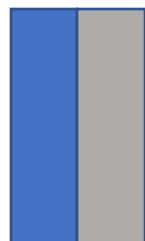
**Compare field 1 to field 2.**

**This is not a test.**

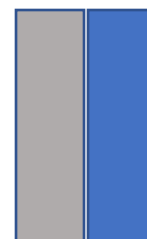
**Compare one-half of a field the other.**

**Either of these has as much value as flipping a coin,  
noting heads, and then expecting heads every time.**

**A real trial  
that will  
provide  
real  
direction.**



**With and without,  
or rate 1 vs rate 2  
3 reps. Blocked trial  
within one field**



<https://cropwatch.unl.edu/farmstat>



Institute of Agriculture and Natural Resources

**CROPWATCH**



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Weather (GDD & ET)  
Info & Resources

Crops

Management

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# FarmStat

FarmStat is a statistical analysis tool that provides quick, accurate, and straightforward analysis for data from agricultural experiments. While FarmStat is provided at no cost, we do require you to register with your name and email address.

First Name Required

David

Last Name Required

Franzen

E-Mail Required

david.franzen@ndsu.edu

# FarmStat Beta Welcome

Thank You

The screenshot displays the FarmStat software interface. At the top, there is a navigation bar with the 'EXTENSION' logo and the title 'FarmStat Complete Guide'. Below this, there are tabs for 'Yield' and 'Net Return'. A red box highlights the 'SHOW DETAILS' button and the text 'Anova table displays most important information -'. Below this, an Anova table is shown with the following data:

Source	D.F.	F-Value	P-Value
Treatments	4		<0.001
Blocks	3		0.883
Error	12		
Total	19		

Below the Anova table, there is a summary table with the following data:

F-Critical	Correction Factor	Standard Deviation	Coefficient of Variation
2.48	1.147219370045e+6	18.665	0.078

At the bottom of the screenshot, there is a 'Watch on YouTube' button and a 'Show all' button.

[Download FarmStat User Guide](#)

[Download CRD Excel Template](#)

[Download RCBD Excel Template](#)

**Farmers have GPS**

**Farmers have GPS yield monitors**

**Farmers have everything they need to  
replicate treatments and test product effectiveness.**



### ***Point 3-***

**There should be a quick method of analysis developed to determine whether the organism is alive and functioning in the container, the field or the plant.**

## ***Point 4-***

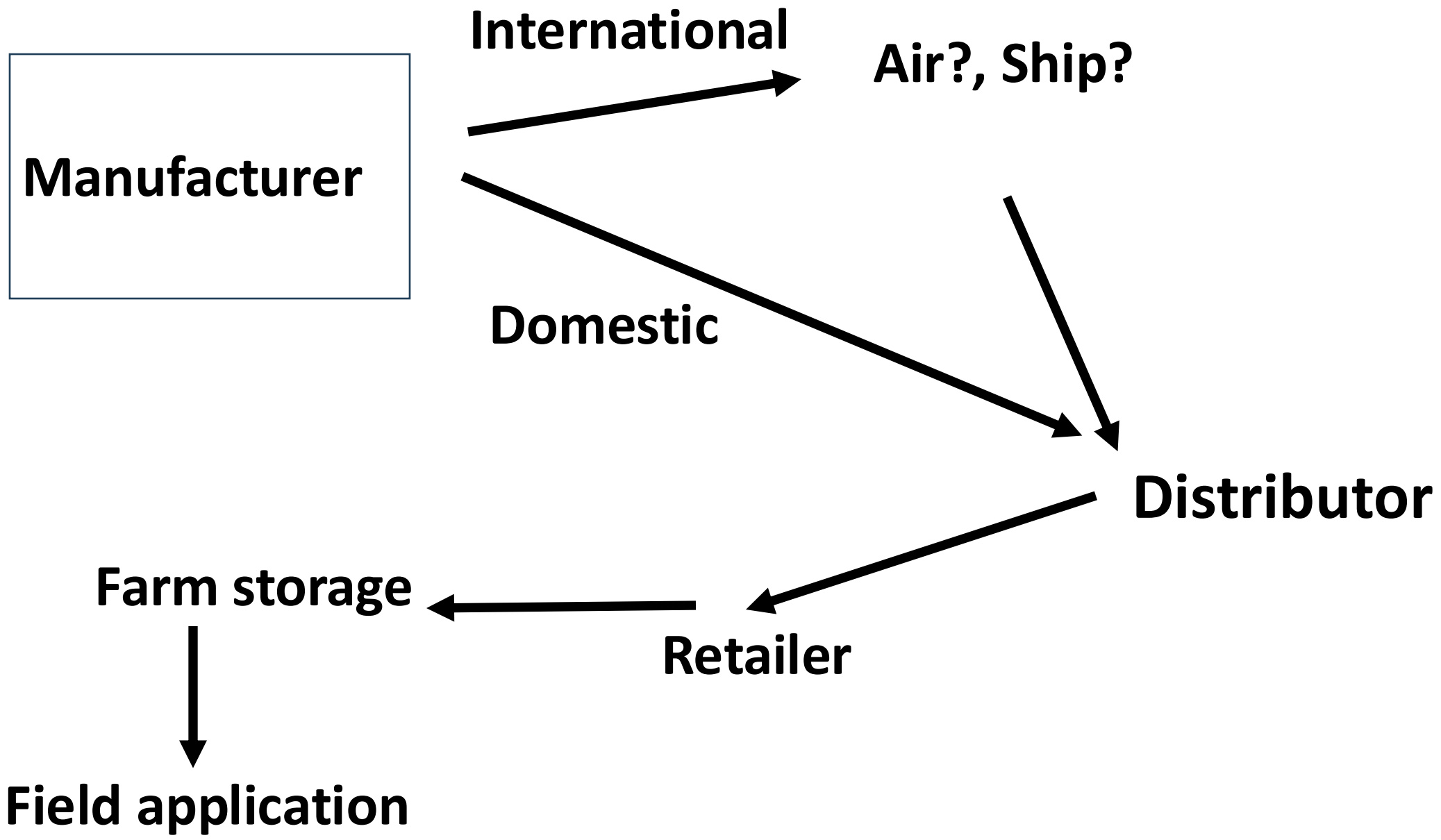
**Organisms need to be kept alive through transportation and storage intervals between manufacturer, shipper, warehouses, distributor, dealer and on the farm awaiting application.**

### **Storage and Disposal**

**Storage Conditions:** Keep the product in a well-ventilated place. Store at room temperature, do not subject to temperatures below 39 °F (4 °C).

Keep the product hermetically closed.

**STORAGE AND HANDLING: DO NOT FREEZE.** This product contains live non-pathogenic organisms. Store between 39°F to 46°F. Store in well ventilated buildings, away from foodstuffs and animal feed. Keep out of reach of children. Keep out of direct sunlight. DO NOT open product container until ready for use.



***Point 5-***

**Organisms should be able to compete and ‘win the war’ with native microorganisms in order to survive and perform its function.**

***Point 6-***

**Organisms should be adapted to variable moisture, variable soil pH and variable soil salts in order to perform its function.**

## Contact information:

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