

Formulations to Increase Resistance of Plants to Pathogenic Agents and Environmental Stress

AgNubio

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agNubio

CHILEAN KING CRAB

Lithodes Antartica



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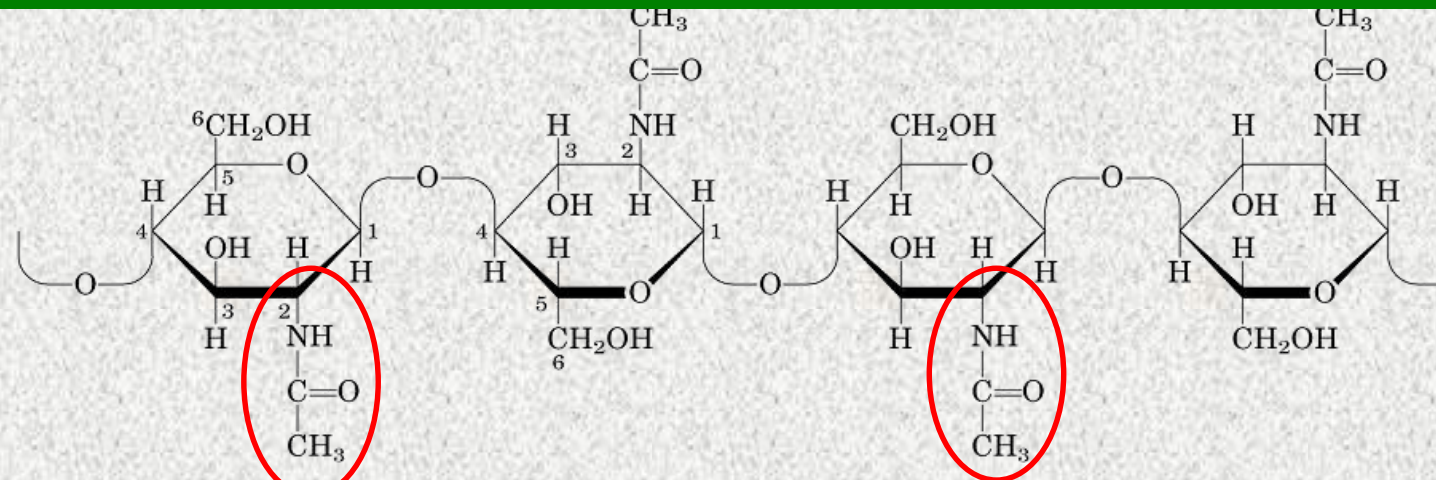
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SOME DEFINITIONS

KING CRAB SHELLS

CHITIN (Natural Polymer - Polysaccharide)

2nd most abundant in nature after cellulose – NON SOLUBLE

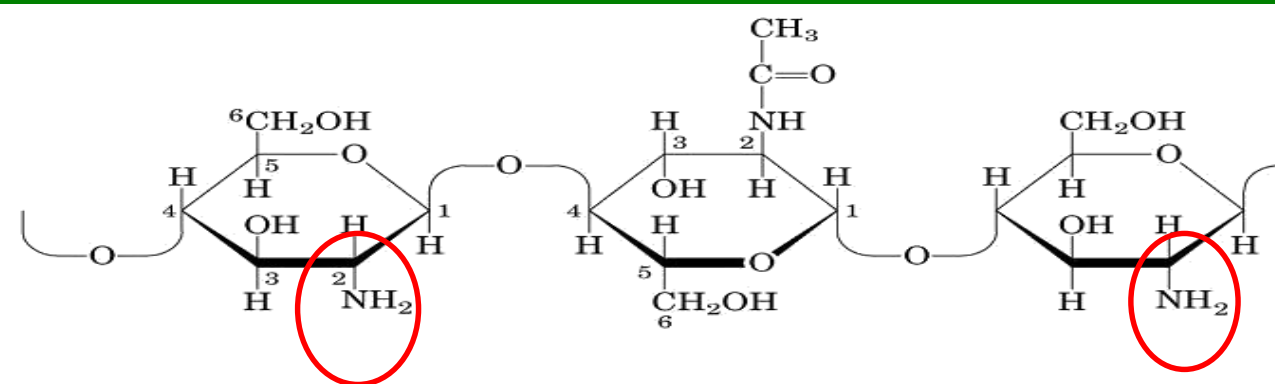


❖ Proteins

❖ Carbonates

❖ Chitin

CHITOSAN : Solubilize Chitin, Chain constituted by 2 types of monomers , glucosamine and N-acetylglucosamine

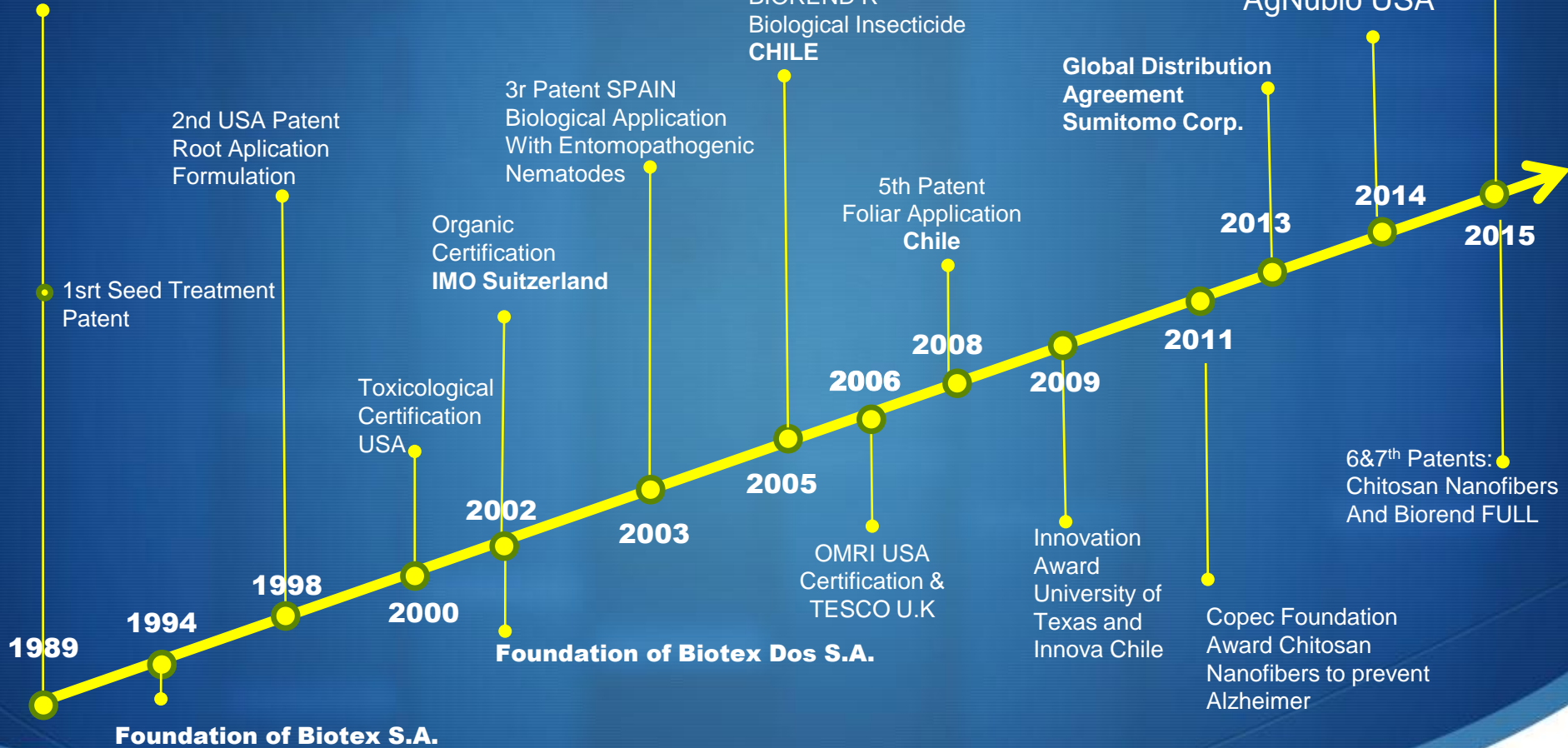


MILESTONES 1989-2015

Foundation of Bioagro

NuPro Technology New Seed Treatment Formulation

Foundation AgNubio USA



Introduction

- **Chitin & Chitosan are very ancient active ingredients used in Agriculture.**
- **Multiple uses for this natural molecule (Agriculture, Medicine, Water Treatments, etc) , It is a polysaccharide extracted from the Animal Kingdom (King Crabs, Crabs, Shrimps, etc).**
- **Its a natural elicitor of Immunological System in plants called Systemic Aquired Resistance (SAR). Chitosan simulates a virtual fungal disease. Also stimulates plant roots to enhance their growth rate.**
- **It works at Secondary Metabolism Level. Triggers signalling through Chitosan receptors in Plant Cell Tissues.**

SAR Mechanism in Plants

- **Despite the deployment of antifungal defense strategies, fungal diseases occurs in all types of multicellular organisms. In plants, the role of fungal chitin/chitosan as pathogen-associated molecular pattern that activates host defense is well established [1].**

[1] [J. Integr. Plant Biol. 47 \(2005\) 452–456.](#)

SAR MECHANISM

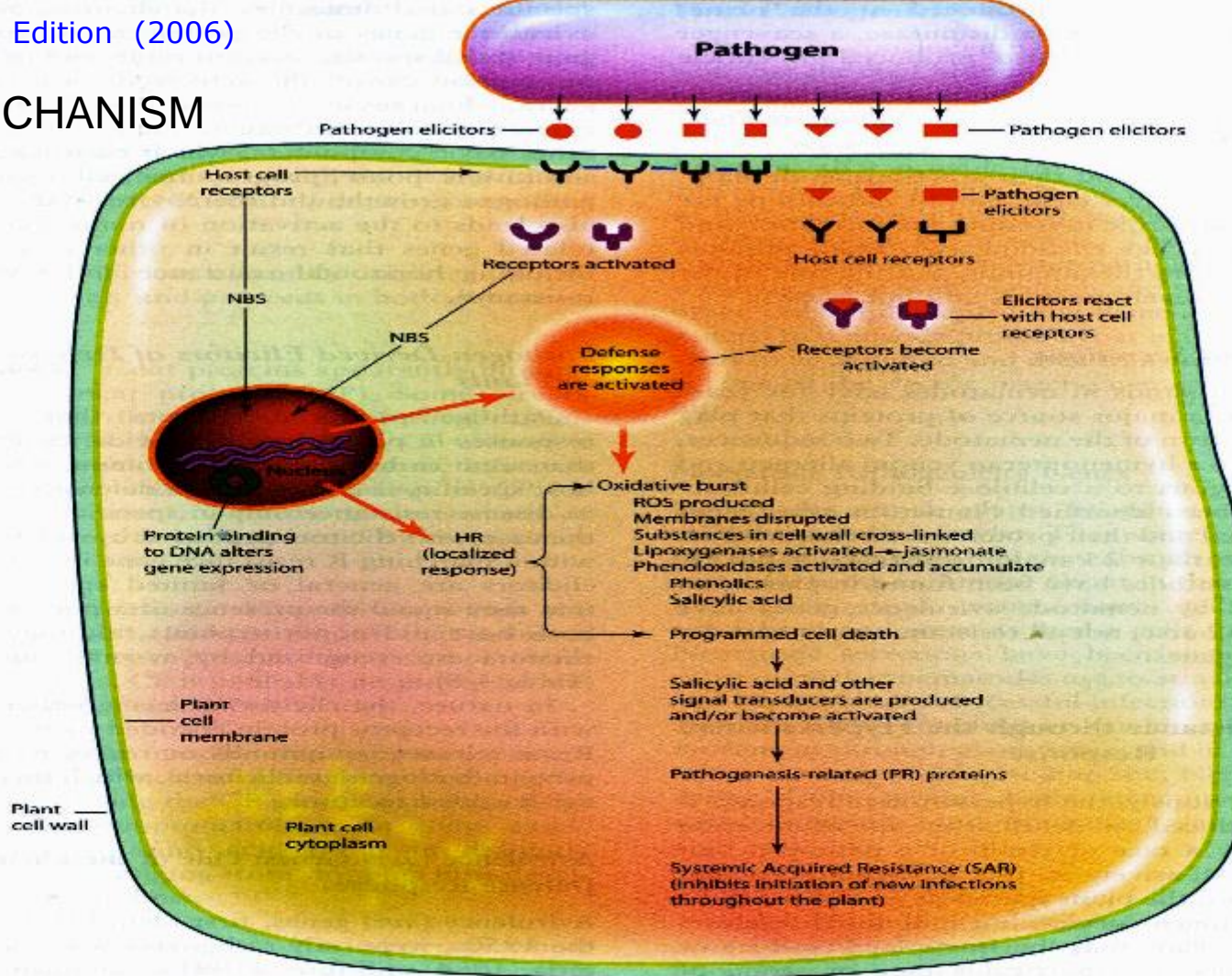
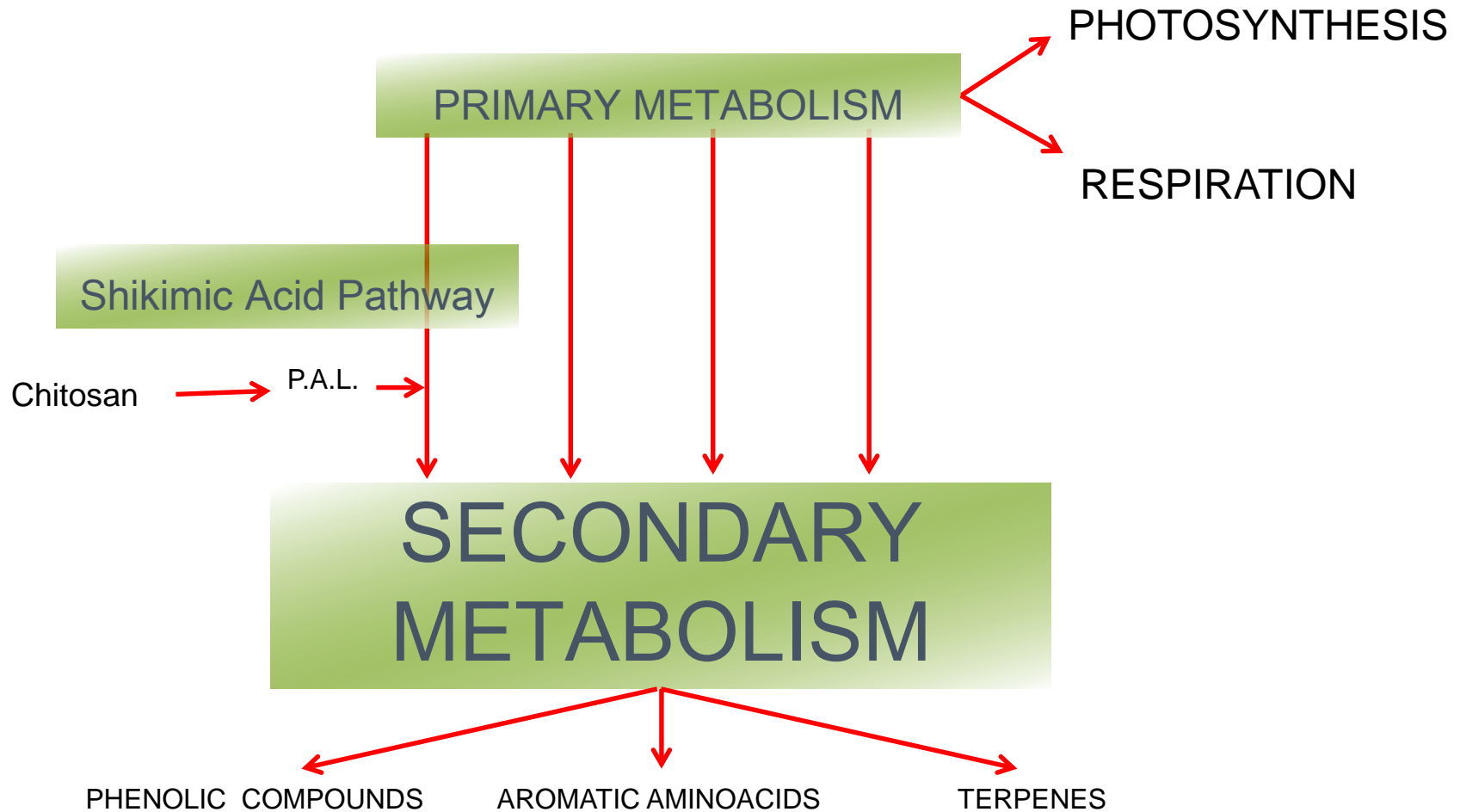


FIGURE 4-13 Basic events in an incompatible host-pathogen interaction: Elicitors from pathogen interact with plant cell receptors. Signal transductions activate hypersensitive (host defense) responses that lead to programmed cell death and systemic acquired resistance.

resistant host corresponds to each avirulence gene in the pathogen. In some cases, however, because two independent resistance (R) genes may correspond to a single *avr* gene, there apparently are genes-for-gene interactions as well. Some *avr* genes, when transferred artificially to other pathovars, are active in the new pathovars, making the recipient pathogen unable to infect their previously susceptible hosts and, instead,

causing the hypersensitive response in these plants. In some host-pathogen systems, *avr* genes determine not only which cultivars of a species the pathogen can attack, but also which plant species it can attack. For example, an *avr* gene (*avrBs1*) in the tomato-infecting group of strains of the bacterium *Xanthomonas campestris* pv. *vesicatoria*, the pathogen of bacterial spot in tomato and pepper, enables the bacterium to induce

DIAGRAM OF SECONDARY METABOLISM



Secondary Metabolism

”Shikimic Acid pathway gets activated through **Phenilalanine Amonnia Liase enzyme activity (P.A.L.).**“

“By this pathway more than 85% of phenolic compounds of a plant are synthesized. This pathway also promotes Triptophan synthesis.”

“PAL activity raises up because some external factors such as Fungal Disease Infection. Chitosan, as an elicitor, represents a fungal infection.”

How much do Chitosan affects PAL activity?

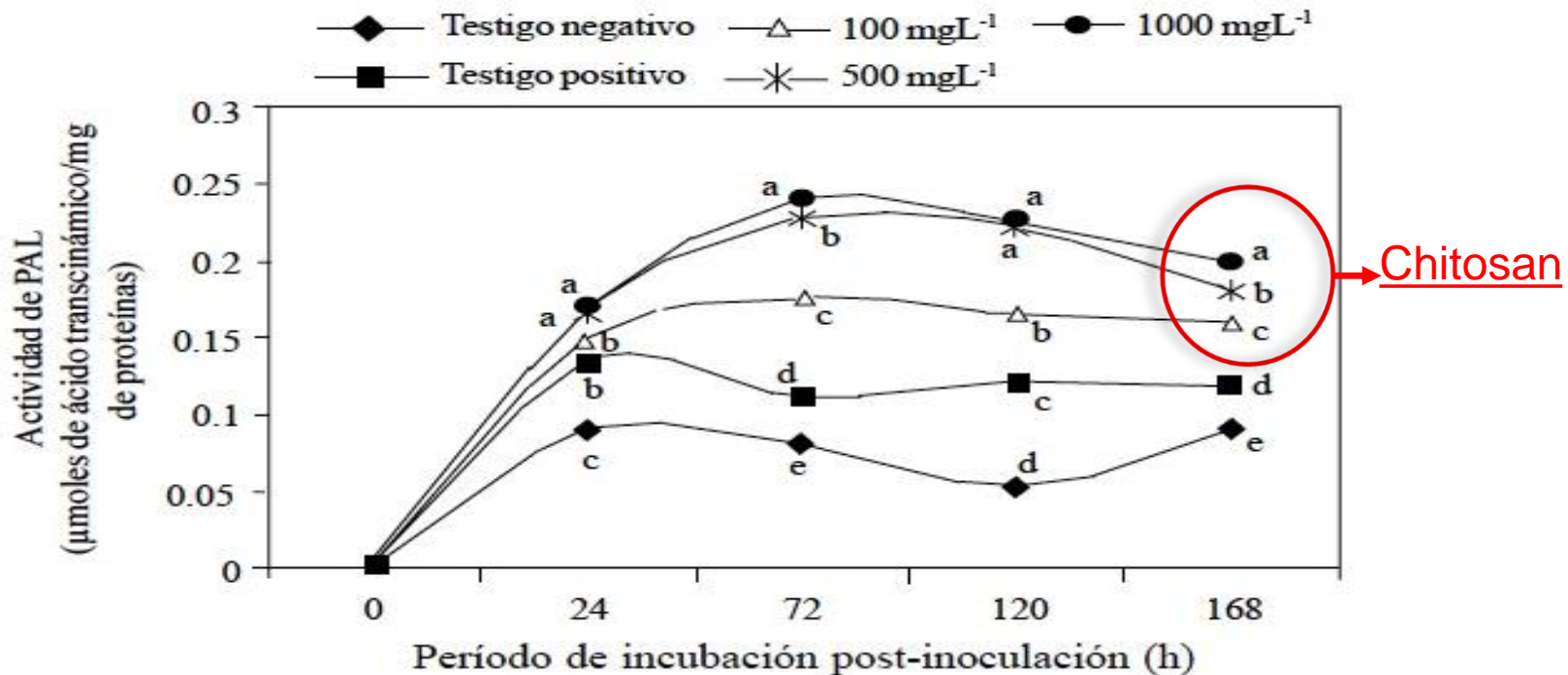


Fig. 1. Cinética enzimática de la fenilalanina amonio liasa (PAL) en plántulas de arroz (*Oryza sativa*) obtenidas de semillas tratadas con diferentes concentraciones de quitosana e infectadas con *Pyricularia grisea*.

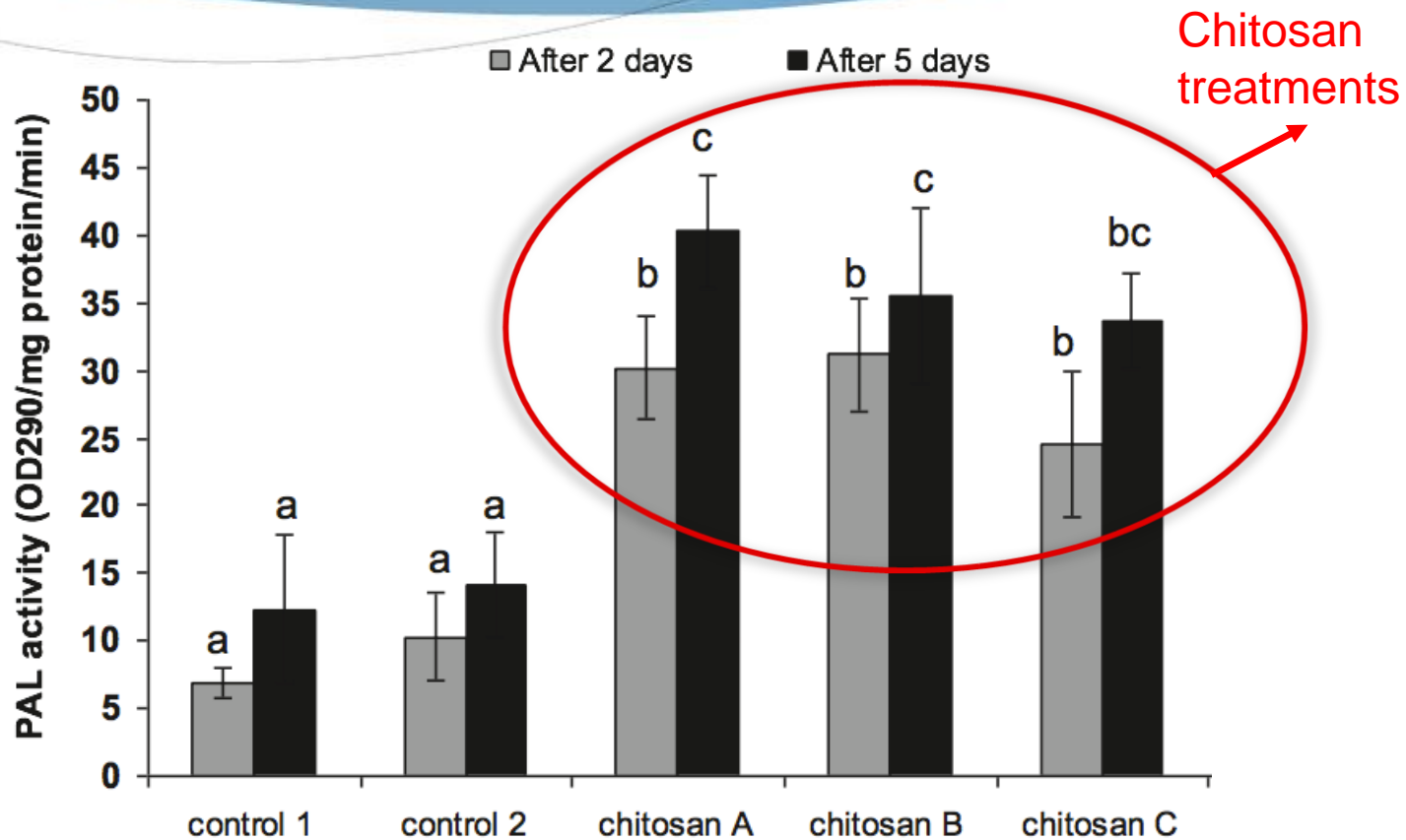
Effect of Different Chitosans with different Degrees of Polymerization (DP) on PAL Activity

Three different kinds of chitosan were named as chitosan A, chitosan B and chitosan C. Its molecular weight is as follows:

Kinds of Elicitor	Molecular Weight (kDa)
Chitosan A	1.129
Chitosan B	521
Chitosan C	607

Ref.: Postharvest Biology and Technology 84 (2013) 51–60

Effect of Chitosan on PAL Activity In Figure

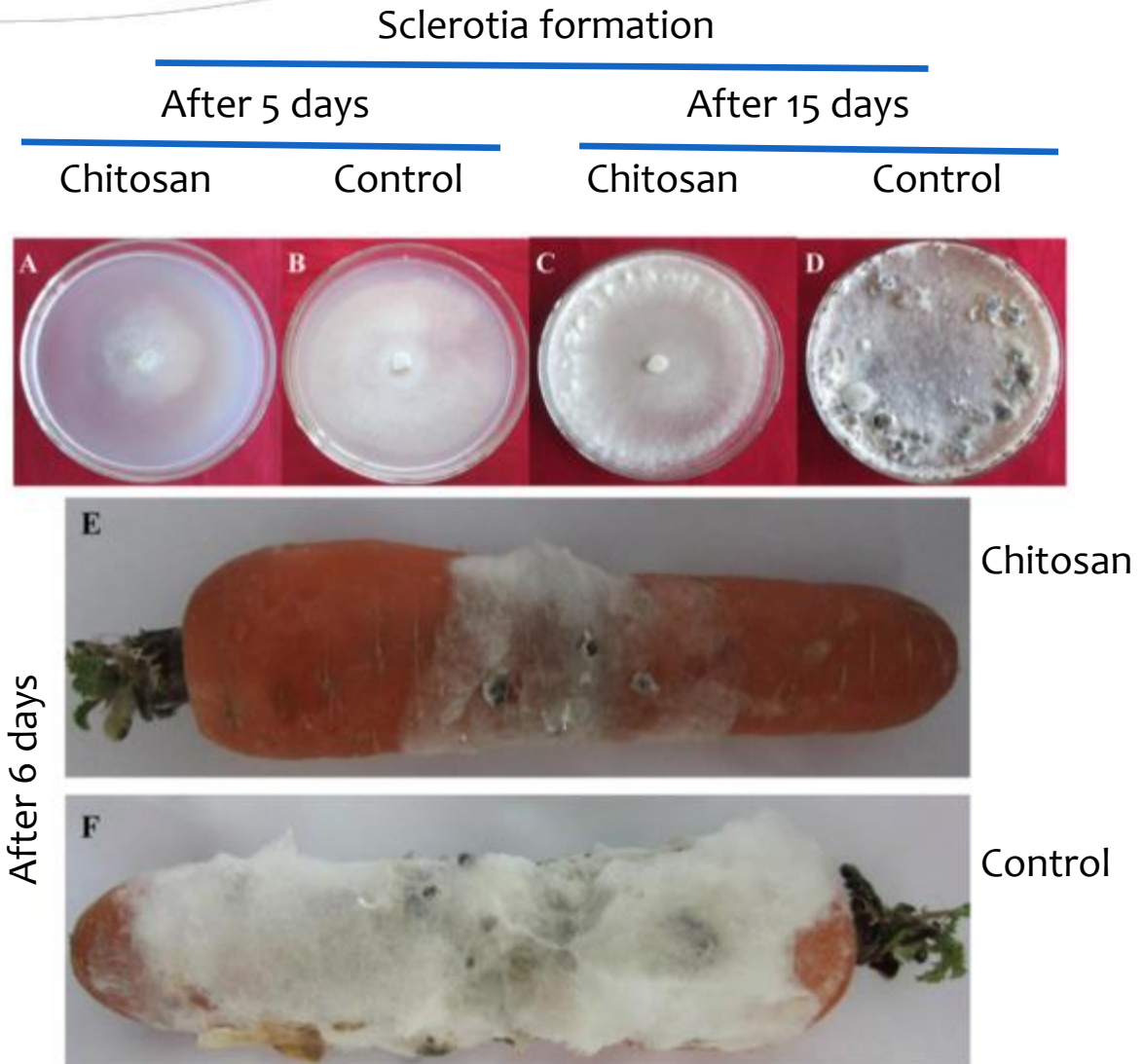


Ref.: Postharvest Biology
and Technology 84
(2013) 51–60

Changes of phenylalanine ammonia lyase (PAL) activity in carrot roots treated with **three chemically different chitosans** (5g/L), 600 mg/L) and inoculated with isolates of *S. sclerotiorum*. Columns having a common letter do not differ significantly ($P > 0.05$) according to Fishers' LSD test.

Effect of Chitosan on PAL Activity and its role in SAR

As an example, Inhibitory effect of **chitosan B** against mycelial growth, sclerotia formation and pathogenicity of *Sclerotinia sclerotiorum*.

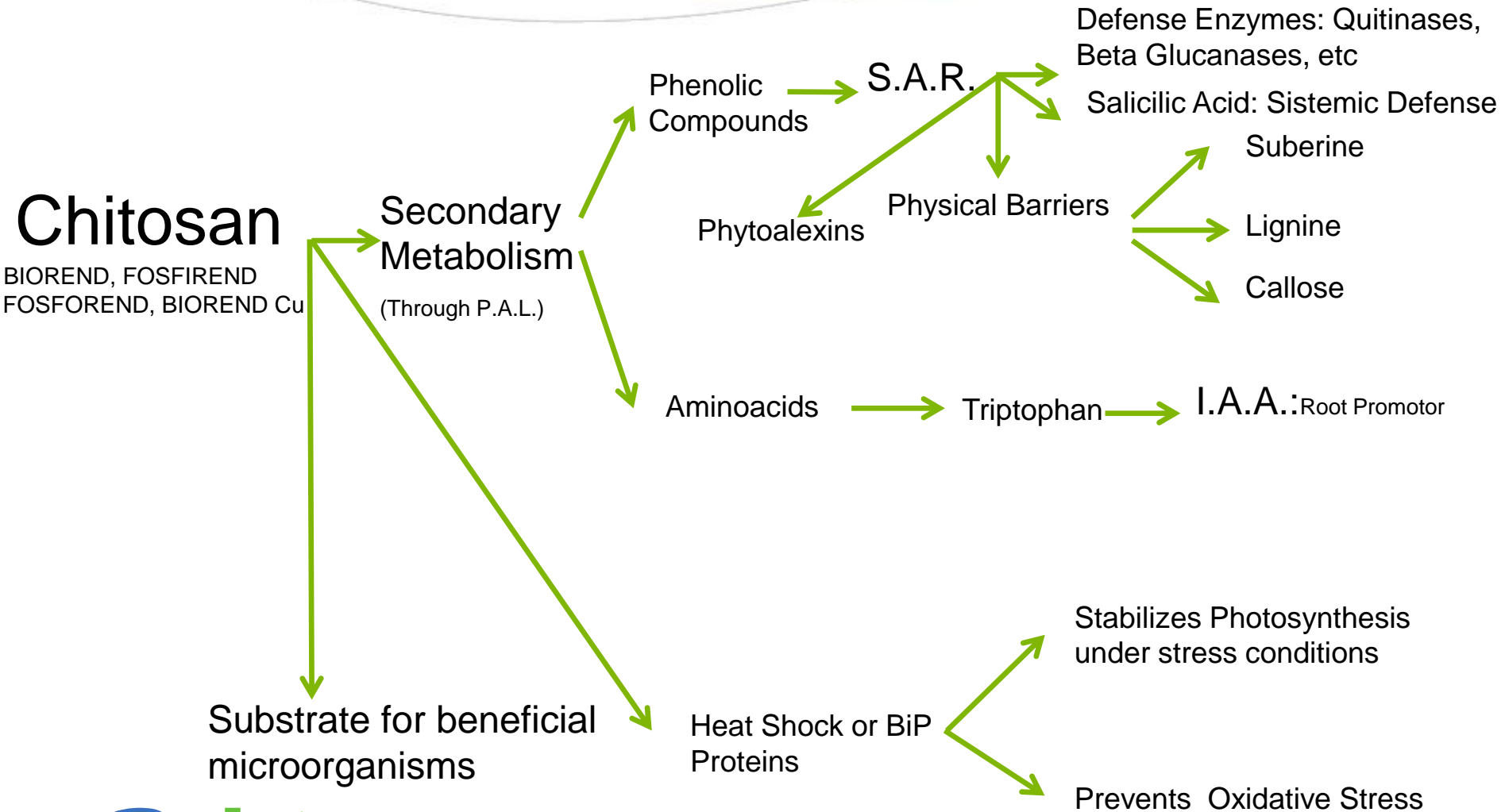


SUMMARY

HOW DOES CHITOSAN WORK AT THE END?



CHITOSAN'S MODE OF ACTION



Chitosan
BIOREND, FOSFIREND
FOSFOREND, BIOREND Cu

BIOAGRO S.A.

Technology Evolution with Chitosan in Seed Treatment

agNubio

CHILE



**EVOLUTION OF CHITOSAN
FORMULATIONS DOSES
IN SEED TREATMENT
(Liters per 100 Kg of Seed)**

	1990	2000	2015
Soybean	1,5	0,6	0,13
Wheat	2,0	1,0	0,18
Corn	4,0	2,0	0,36

BRASIL: IHARABRAS TRIALS 2012

Treatments	Doses (ml/50 Kg of seed)
Untreated	-
BIOREND Plus	300
BIOREND Plus	600
BIOREND Plus	1000
FERTAMIN CoMo	150

Seed Treatment Results With BIOREND Chitosan formulation (Experiments made in Petri Plates)

Treatments	Doses (ml/50 Kg of seed)	Germination (%)	Seedling Average Height (cm)
Untreated	**	50,00	11,38
BIOREND Plus	300	60,00	13,71
BIOREND Plus	600	60,00	14,65
BIOREND Plus	1000	53,33	11,00
FERTAMIN CoMo	150	56,67	11,83

Treatments	Doses (ml/50 Kg of seed)	Green Matter Weight (g)	Dry Matter Weight (g)	% (Dry/Green Matter)
Untreated	**	10,09	2,173	21,536
BIOREND Plus	300	13,84	2,372	17,139
BIOREND Plus	600	14,33	2,410	16,818
BIOREND Plus	1000	14,31	2,497	17,449
FERTAMIN CoMo	150	13,02	2,577	19,793

New Technology

NuPro
By AgNubio

New Pentasacharide, made out of King Crab Chitosan 500 to 800 KDa Degree of polimerization.

Unique manufacturing process & formulation for SEED TREATMENT

DOSAGE : 1.5 a 6,5 cc of Nupro per 100 Kg of seed

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Question

Why small DP chitosan?

Pentasaccharide of Chitosan

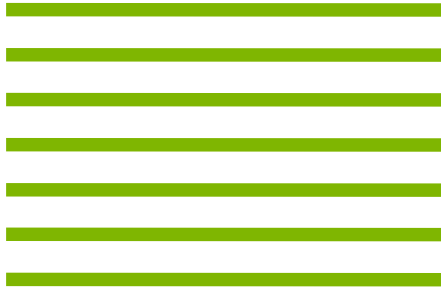
Different Types of Chitosan regarding DP

Type of Chitosan	Degree of Polimerization (DP) in Kilo Daltons (KDa)
High DP Chitosan	500 to 1000
Medium DP Chitosan	100 to 500
Low DP Chitosan	10 to 100

King Crab's Chitosan molecular weight has a DP between 500 to 800 kDa.

WHAT DO WE DO???

500 to 800 KD



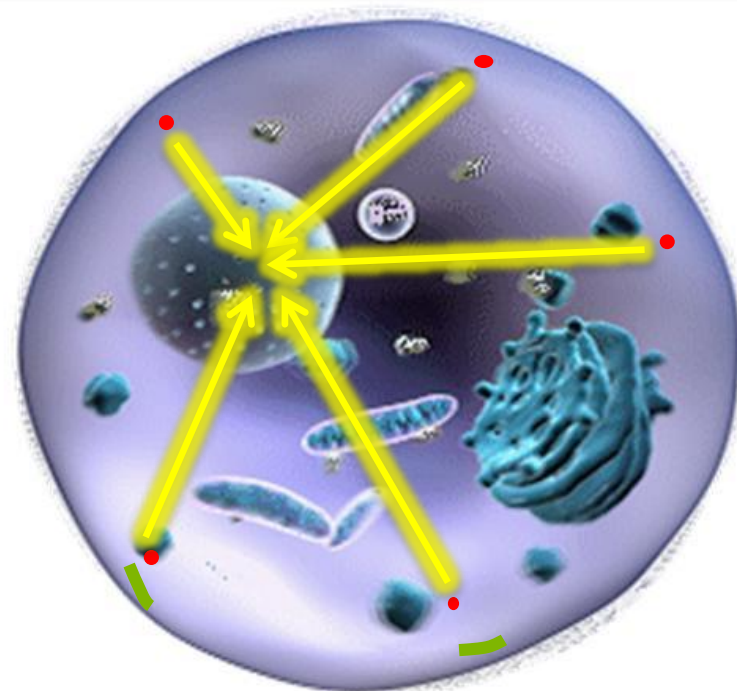
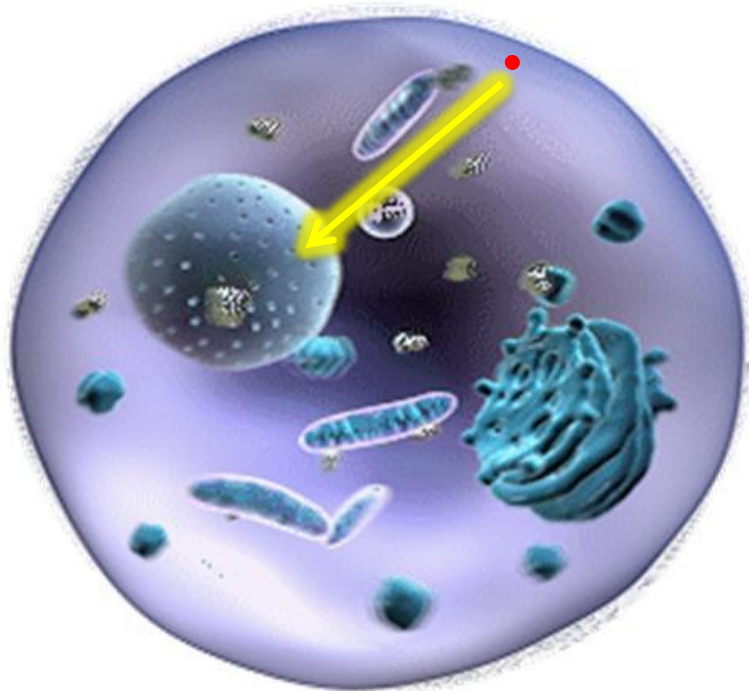
1 Kd

$$MW_1 > MW_2$$

500 to 700 KD

Chitosan Molecules

1 KD



● Chitosan Receptors

→ Signaling to DNA

Some Mathematics

1 Molecule of NuPro's Chitosan = 1 Pentasaccharide = 1 KDa

1 Chitosan Molecule of 700 KDa = 700 NuPro's Molecules

So 1 ml of NuPro is effective as 700 ml of a 700 KDa Chitosan solution ?

What's Next for Chitosan Formulations

Mixtures of Chitosans with different Degrees of Polymerization and Different Degrees of Deacetylation.

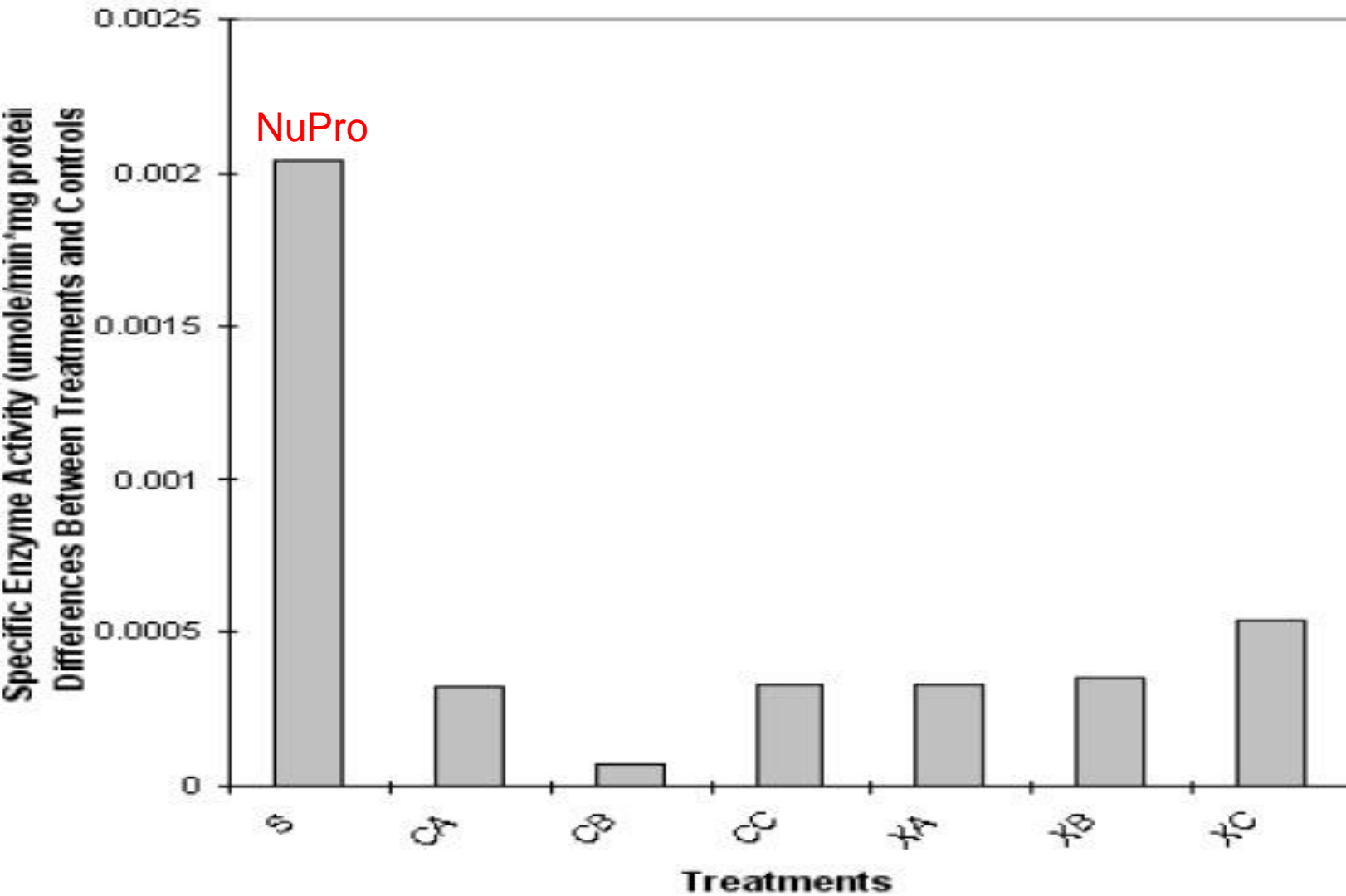
From Pentasacharides to 800 KDa.

Proportion will depend on target, Quickness vs Residual Effect.

NuPro Field Data up to this point

Beta Glucanase Activity Comparing Pentasaccharide Chitosan (NuPro) with other Chitosans

Colorado State University, 2008
Day 10



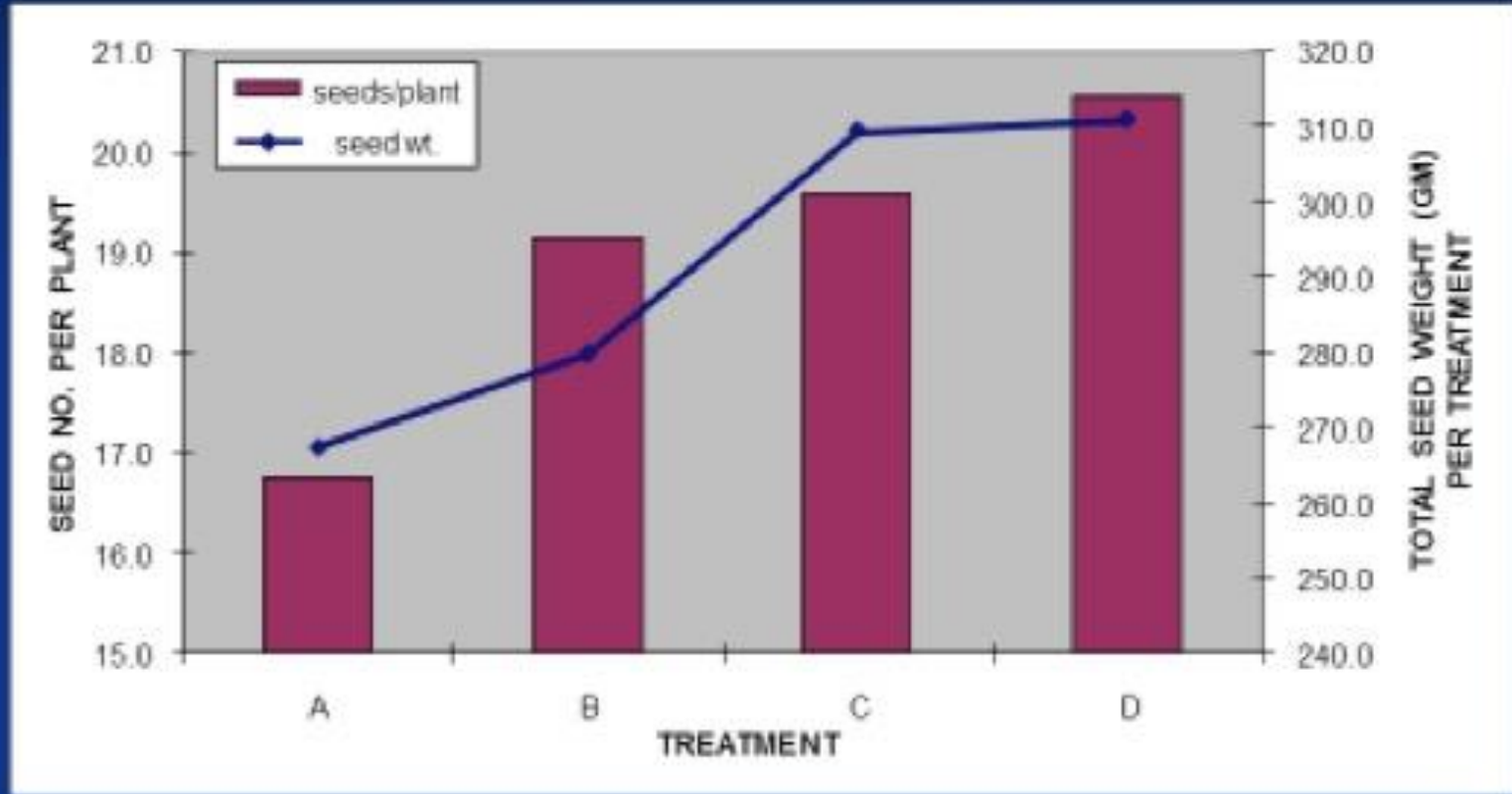
Seed Treatment with Pentasaccharide Chitosan (NuPro)

	Yield Difference	Treatment Difference
2004 Field Tests		(% increase over Control)
Soybean	5.4 (bu/A)	10
2005 Field Tests		
Soybean	6.6 (bu/A)	12
Wheat	2.5 (bu/A)	5
Corn	7.0 (bu/A)	4
Peanut	645 (lbs/A)	39

Figure 10: Greenhouse results on soybean 2005

2005 Greenhouse Soybean Study

CSU Plant Growth Facilities Manager, J. Matsuura



A: Control

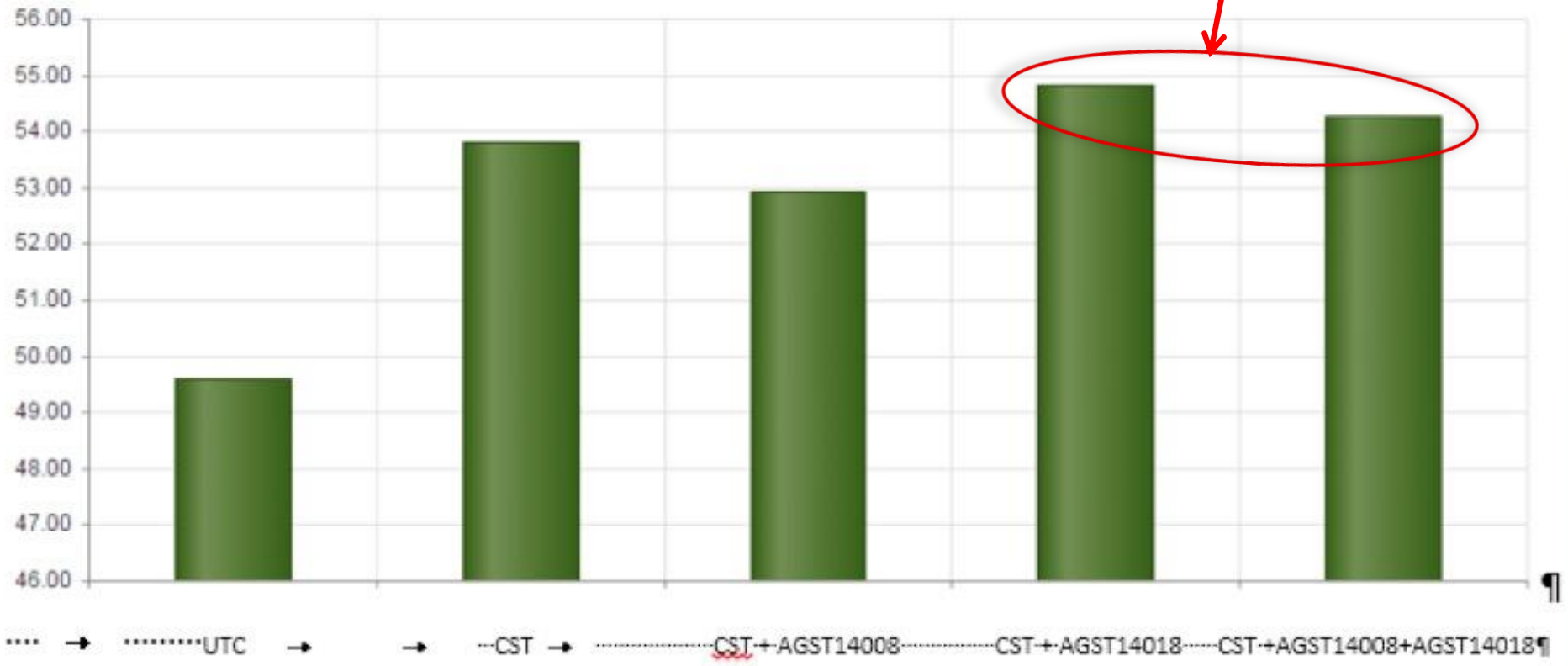
B: Irrigation with NuPro

C: Seed Treatment with NuPro

D: Double Seed Treatment with NuPro

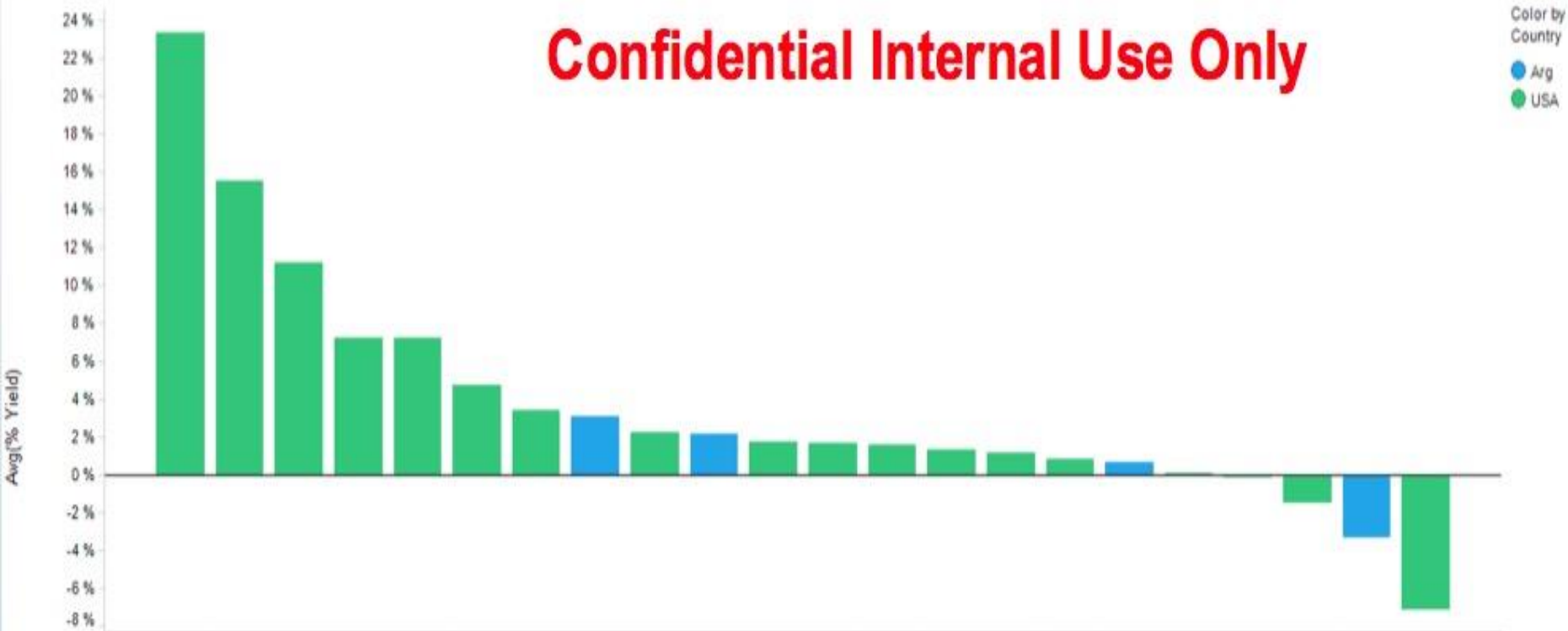
Yield Results in Soybean comparing Control, Standard and two treatments with NuPro (Avg of 6 locations in USA, 2014).

Yield in Bu/acre



Yield % advantage in Soybean comparing NuPro vs Base in Different locations in different countries

% Yield per Loc_trt



EPA LABEL



NuPro™ ST Seed Treatment Agricultural Use

NuPro™ is a trademark of Ag NuBio™, Inc.

When used as directed on this label, NuPro™ ST can boost seed germination and vitality, stimulate emergence and sprouting, enhance plant vigor, promote root growth, promote foliar growth, increase crops yields, improve crop vigor and quality, suppress diseases and pathogens, activate plant resistances to environment stress, drought, and disease pressures, including the suppression of parasitic nematodes.

SYSTEMIC INDUCED RESISTANCE AND INNATE IMMUNITY PROTECTION

NuPro ST Protects grasses, grains, cereals, legumes, sugar beets and vegetables against fungus, mosaic virus, stem wilt, damp-off, early blight, late blight and mold.

For use on, corn, canola, soybeans, rice, sugar beets, vegetables, herbs, sunflowers, wheat, grains, cereals, legumes, and flowers.

ACTIVE INGREDIENT:

Chitosan (poly-D-glucosamine)*	0.25%
Other Ingredient	99.75%
Total	100.00%

Product contains .021 pounds (0.01 kg) of chitosan/gallon

KEEP OUT OF REACH OF CHILDREN

CAUTION

HAZARDS, TO HUMANS AND DOMESTIC ANIMALS

CAUTION: Wear the appropriate Personal Protective Equipment (PPE)

SEE SIDE PANEL FOR PRECAUTIONARY STATEMENTS

Manufactured for: Ag NuBio, Inc. 11125 N. Ambassador Dr., Ste. 120 Kansas City, MO 64153	EPA Establishment No.: 91950-CHL-001 EPA Registration No.: 91439-1-91664
Net Contents	5 gallons

FIRST AID	
If in eyes	<ul style="list-style-type: none"> Hold eye open and rinse slowly and gently with water for 15-20 minutes Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye Call a poison control center or doctor for treatment advice
If on skin or clothing	<ul style="list-style-type: none"> Take off contaminated clothing Rinse skin immediately with plenty of water for 15-20 minutes Call a poison control center or doctor for treatment advice
If inhaled	<ul style="list-style-type: none"> Move person to fresh air If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth to mouth if possible Call a poison control center or doctor for further treatment advice
Have the product container or label with you when calling a poison control center or doctor, or going for treatment. You may also contact 1-800-222-1222 for emergency medical treatment information.	

PRECAUTIONARY STATEMENTS HAZARDS, TO HUMANS AND DOMESTIC ANIMALS

CAUTION: Causes moderate eye irritation. Avoid contact with eyes. Wash thoroughly with soap and water after handling.

ENVIRONMENTAL HAZARDS

Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when cleaning equipment or disposing of equipment wash waters.

For 5 gallon product: Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans or public waters unless in accordance with the requirements of National Pollutant Discharge Elimination System (NPDES) permit and the permitting authority has been notified in writing prior to discharge. Do not discharge effluent containing this product into sewer without previously notifying the sewage treatment plant authority. For guidance, contact your State Water board or regional Office of the EPA.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

Applicators and other handlers must wear: Long-sleeved shirt, long pants, waterproof gloves, protective eyewear, and shoes plus socks. Follow manufacturer's instructions for cleaning/maintaining PPE. If no such instructions for washables, use detergent and hot water. Keep and wash PPE separately from other laundry.

DIRECTIONS FOR USE

It is a violation of federal laws to use this product in a manner inconsistent with its labeling.

To prevent contamination of the dilute solution of NuPro™ ST by possible pesticide or other chemical residues in the spraying equipment, use dedicated equipment, or be sure that the equipment is thoroughly clean before use.

FOR AGRICULTURAL USE

Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handler may be in the area during application. For any requirements specific to your State or Tribe, consult the agency responsible for pesticide regulation.

AGRICULTURAL USE REQUIREMENTS

Use this product only in accordance with its labeling and with the Worker Protection Standard, 40 CFR Part 170. This standard contains requirements for the protection of agricultural workers on farms, forests, nurseries and greenhouses, and handlers of agricultural pesticides. It contains requirements for training, decontamination, notification and emergency assistance. It also contains specific instructions and exceptions pertaining to the statements on this label about personal protective equipment (PPE) and restricted-entry intervals (REI). The requirements in this box only apply to uses of this product that are covered by the Worker Protection Standard.

Do not allow worker entry into treated areas until sprays have dried.

PPE requirements for early entry to treated areas (that is permitted under the Worker Protection Standard and that involves contact with anything that has been treated, such as plants, soil, or water) is:

- Coveralls
- Waterproof gloves
- Protective eyewear
- Shoes plus socks

GENERAL INSTRUCTIONS

Shake before using. Follow specific crop instructions for dilution rates and application usage. Do not apply within three days of harvesting. For best results use pH neutral (pH 6.9 to 7.0) mix water.

Crops	Mixing and Application Instructions
Seed Treatment for True Seed Crops	<ul style="list-style-type: none"> • Mix 30 ml into 1.5 gallon • Application treats: approximately 70 acres • Apply enough product to soak seeds • Apply 80 ml of product per approximately 50 to 100 lbs. seed/acre • Apply directly to seeds. Do not rinse. Allow to dry and/or plant soaked seeds.

STORAGE AND DISPOSAL

Do not contaminate water, food or feed by storage or disposal.

Pesticide Storage: Store in original container in a cool dry place, away from sunlight and out of the reach of children and pets. Do not reuse container.

Pesticide Disposal: Wastes resulting from use of this product may be disposed of on site or at an approved waste disposal facility.

Container Handling: Non-refillable container. Do not reuse or refill this container. Offer for recycling, if available. Triple rinse container (or equivalent) promptly after emptying. (For product less than 5 gallons) Triple rinse as follows: Empty the remaining contents into application equipment or a mix tank and drain for 10 seconds after the flow begins to drip. Fill the container ¼ full with water and recap. Shake for 10 seconds. Pour rinate into application equipment or a mix tank or store rinate for later use or disposal. Drain for 10 seconds after flow begins to drip. Repeat this procedure two more times. (For product greater than 5 gallons) Triple rinse as follows: Empty the remaining contents into application equipment or a mix tank. Fill the container ¼ full with water. Replace and tighten closures. Tip container on its side and roll it back and forth, ensuring at least one complete revolution, for 30 seconds. Stand the container on its end and tip it back and forth several times. Turn the container over on its other end and tip it back and forth several times. Empty the rinate into application equipment or a mix tank or store rinate for later use or disposal. Repeat this procedure two more times. An empty container retains vapor and product residue. Observe all labeled safeguards until container is cleaned, reconditioned or destroyed. Completely empty contents into formulation equipment. Then offer for recycling or reconditioning or dispose of container in a manner approved by state and local authorities.

WARRANTY

Ag NuBio warrants that this product conforms to the chemical description on the label and is reasonably fit for the purposes set forth in the Complete Directions for Use label booklet ("Directions") when used in accordance with these Directions under the conditions described therein. TO THE EXTENT CONSISTENT WITH APPLICABLE LAW, NO OTHER EXPRESS WARRANTY OR IMPLIED WARRANTY OF FITNESS FOR PARTICULAR PURPOSE OR MERCHANTABILITY IS MADE. This warranty is also subject to the conditions and limitations stated herein.

Manufactured for:
Ag NuBio, Inc.
11125 N. Ambassador Dr., Ste. 120
Kansas City, MO 64153

Batch code:

Thank you very
much and Merry
Christmas!

agNubio

