Crop Health through Resistance Induction by means of ComCat®

Presented by:
Dr. Riaan Buitendag (PhD, Pr.Sci.Nat, Basos)

AgraForUm SA
natural solutions for the future

TABLE OF CONTENTS

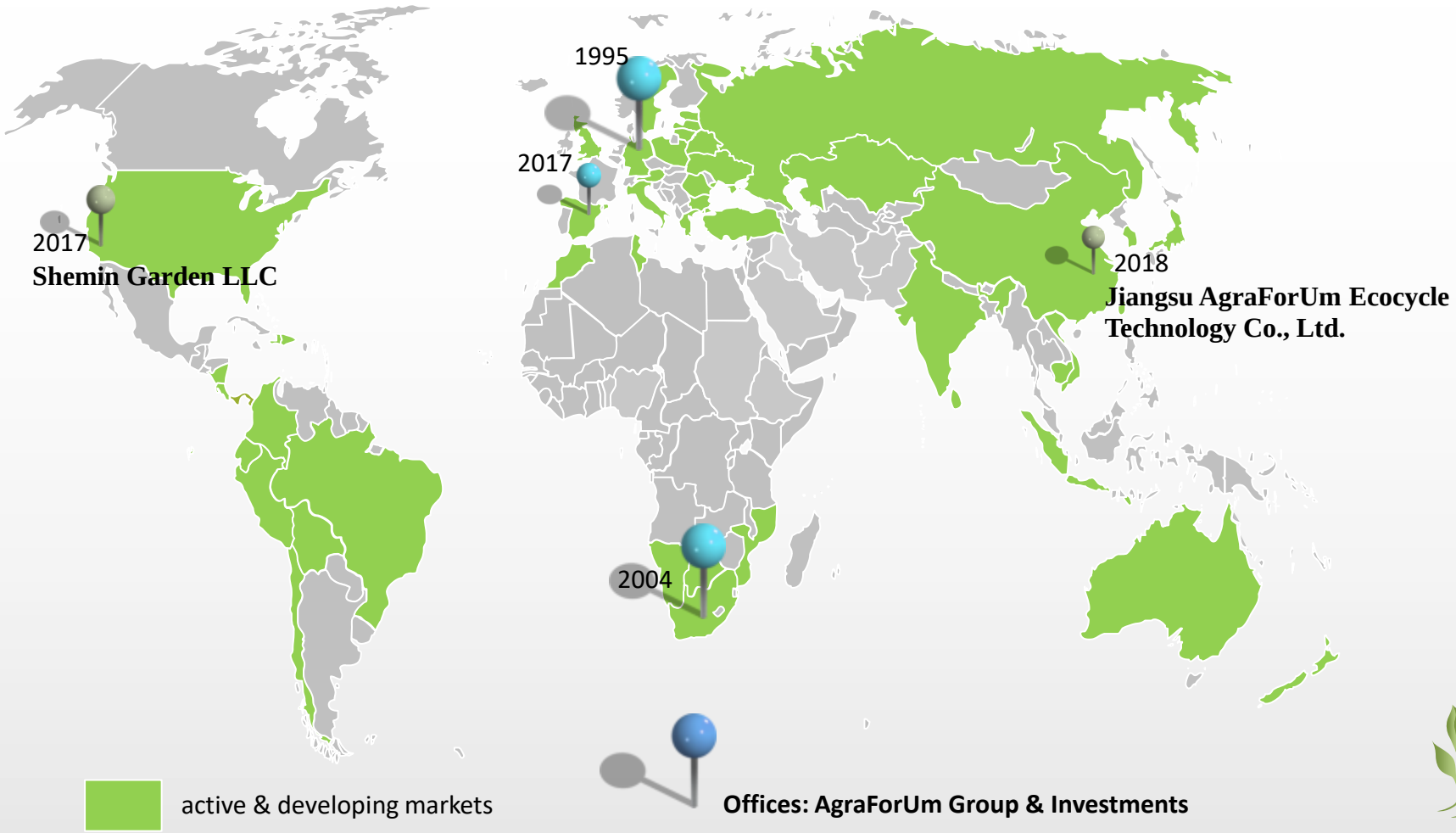

AgraForUm Group
natural solutions for the future

History

- 1995: AgraForUm Germany
- 2004: AgraForUm South Africa (Pty) Ltd. was established mainly for R&D
- 2008: Participation (50%) in BioPher (Pty) Ltd in South Africa for developing the Pheroid Technology in Agriculture
- 2010: Foundation of AgraForUm Produktions GmbH, Bomlitz, Germany
- 2012: Foundation of AgraForUm Vertriebs GmbH, Dohren (present location: Gadebusch, Germany)
- 2016: Shareholding (25%) in Progress Agrar GmbH, Tostedt, Germany
- 2017: Participation (20%) in Shemin Garden LLC Fresno, California
- 2018: Participation (20%) in Jiangsu AgraForUm Ecocycle Technology Co. Ltd. Nantong, China
- 2018: Shareholding (40%) in Lucky Plant GmbH, Bomlitz, Germany

Markets & Offices

AgraForUm Group
natural solutions for the future



INTRODUCTION

What is Resistance Induction of Crops?

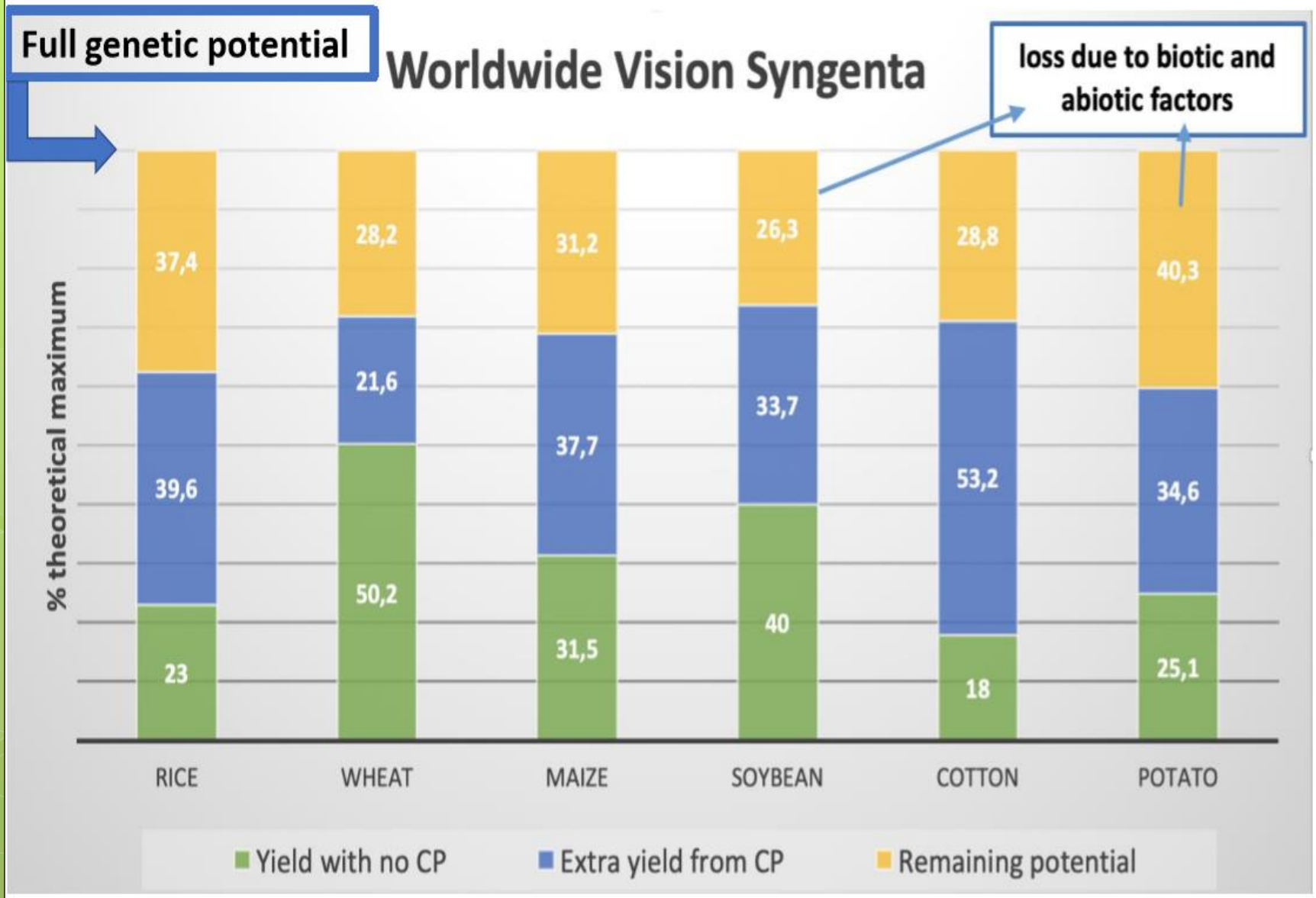
It is defined as an **enhancement** of the plant's own **defensive capacity** against a **broad spectrum of biotic** (pathogens) and **abiotic** (temperature, drought, herbicidal etc.) **stresses** that is ACQUIRED by an appropriate **signalling molecule/activator** (Hammerschmidt and Kuc, 1995).

We need to obtain functional activator/signalling molecules for Optimized Crop Health & Production!!!

Why is Crop Resistance Induction important in Agriculture?

In 1999 Khush reported that world-wide cereal crop **YIELDS are yearly reduced** as a consequence of **Abiotic** stresses (such as **drought** or **abnormal temperatures**), while Huckelhoven and Schweizer indicated in 2011 that **diseases** and **insects (Biotic)** were responsible for **YIELD losses** of up to **25% PER YEAR** over their observation period.

INTRODUCTION



Pius Floris. President of Plant Health Cure BV Netherlands, Fertasa 2019

INTRODUCTION

Can we afford YIELD losses?

The FAO published that by 2050 agricultural production will have to increased by 70% to meet projected food demand.

We need to look for solutions!

MODE OF ACTION

1. Apply Signaling Molecule (Activator)

ComCat®

2. Gene Expression

The memory of the cells carry the architect's plan for the suitable chemical reaction to fight towards stress situation.

4. SAR

The plant is now able to fight against stress factors by means of Systemic Acquired Resistance.

3. Proteins / Physical protection via certain substances

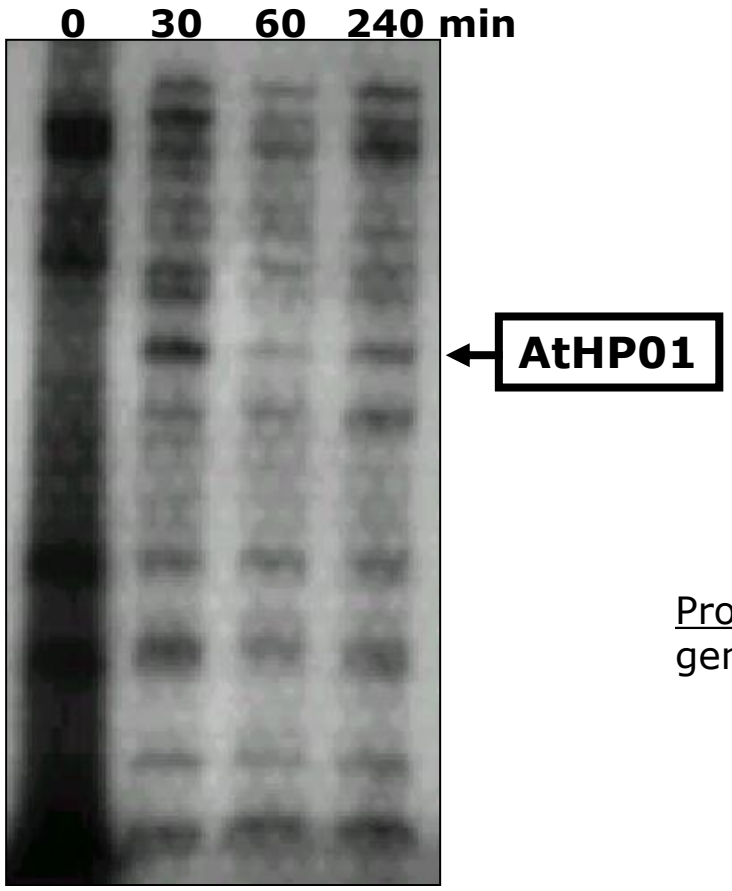
After the first application the plants produce defense **PR-proteins** and lignin/Callose for resistance.

ComCat® and Induced Resistance against Abiotic stresses

- Drought
- Cold

Gene Induction in *Arabidopsis thaliana*

Cloning of *At-HP01*



[VISSER, B., 2004]

PERFORMED RT-PCR

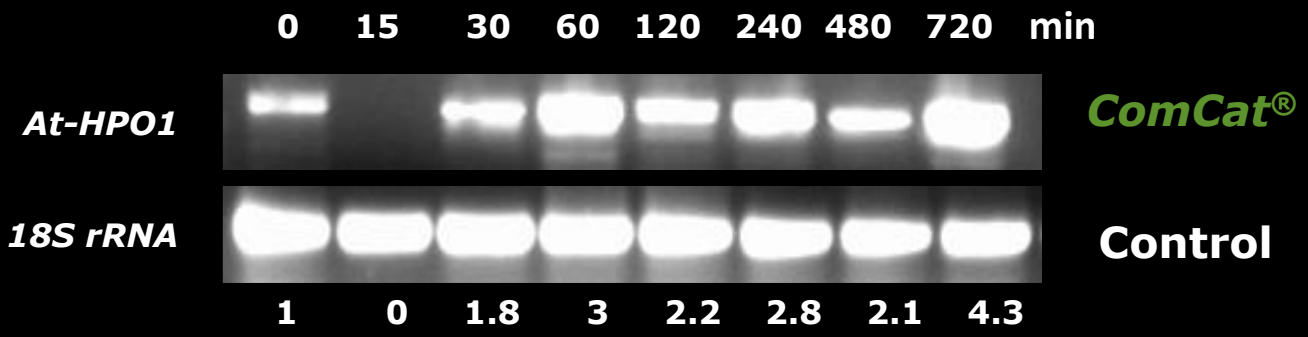
Identified a gene in ***A. THALIANA*** that was expressed within 30 min after spraying with **ComCat®**.

Promoter analysis of AtHP01 indicated that gene At-HPO1 is ALSO induced upon:

DROUGHT STRESS

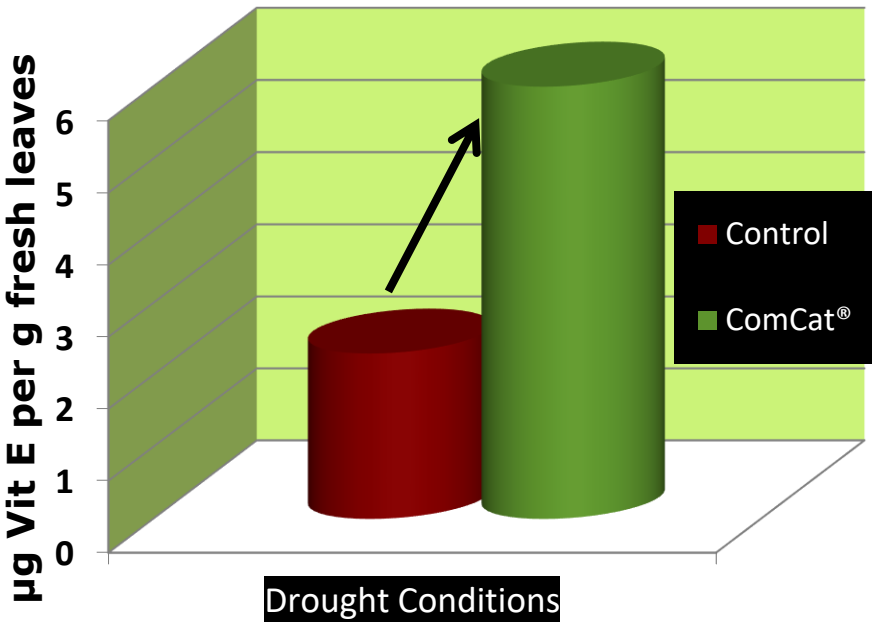
Activation of ***AT-HP01 GENE*** **EXPRESSION** compared to an internal **18S r-RNA CONTROL**

(Visser, 2004)



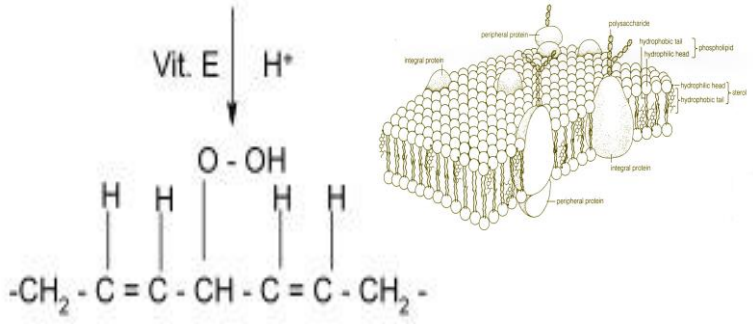
Hypothesis on Vitamin E and Cellmembrane Protection

Creeping Bent grass Trial (2001) USA (Pretorius, 2001)



Vitamin E is an antioxidant!!!
(Hofius & Sonnewald, 2003; Munne-Bosch & Alegre, 2002; Porfirova *et al.*, 2002)

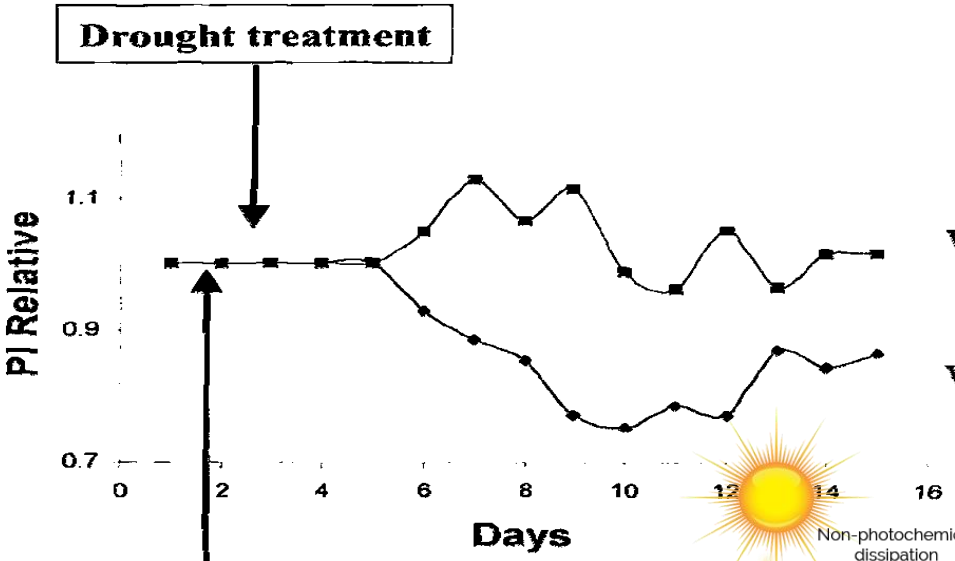
[Vit. E prevents this chain reaction of free radical formation by supplying an H-atom to the lipid free radical and forming a non-harmful HYDROPEROXIDE]



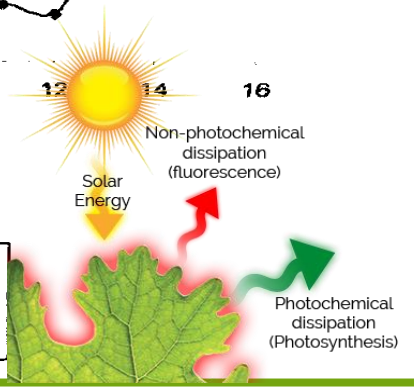
CHARACTERIZATION OF EIGHT VARIETIES OF PEA (PISUM SATIVUM) BY THE JIP-TEST (Chlorophyll Fluorescence)

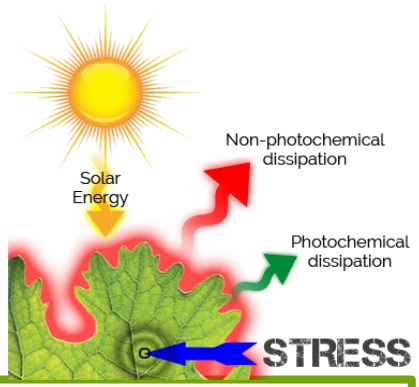
A. Gonzales [1], L. Ayerbe[1], J. Sanches[1], F. Reverchon[2], R. Maldonado-Rodriguez[2] and R. J. Strasser[2]

Relative Performance Index of Var 1 under Mild drought stress with and without (blue) Comcat



Average of days before drought normalized for each condition (ComCat/control)





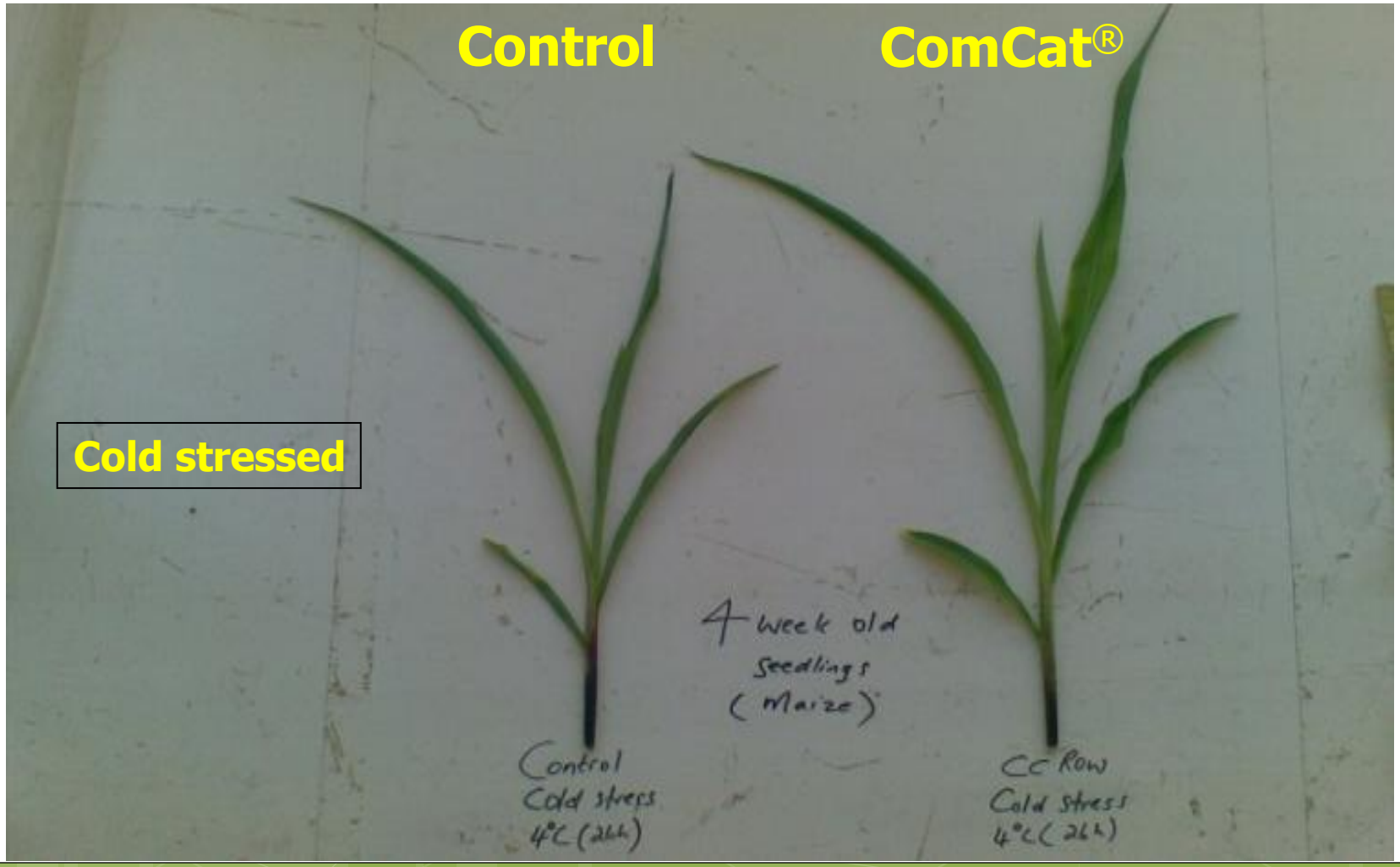
Comcat helps to prevent the decrease of the Performance Index under mild drought stress conditions.

Sorghum: Growth under drought conditions, Wilber Ellis, USA

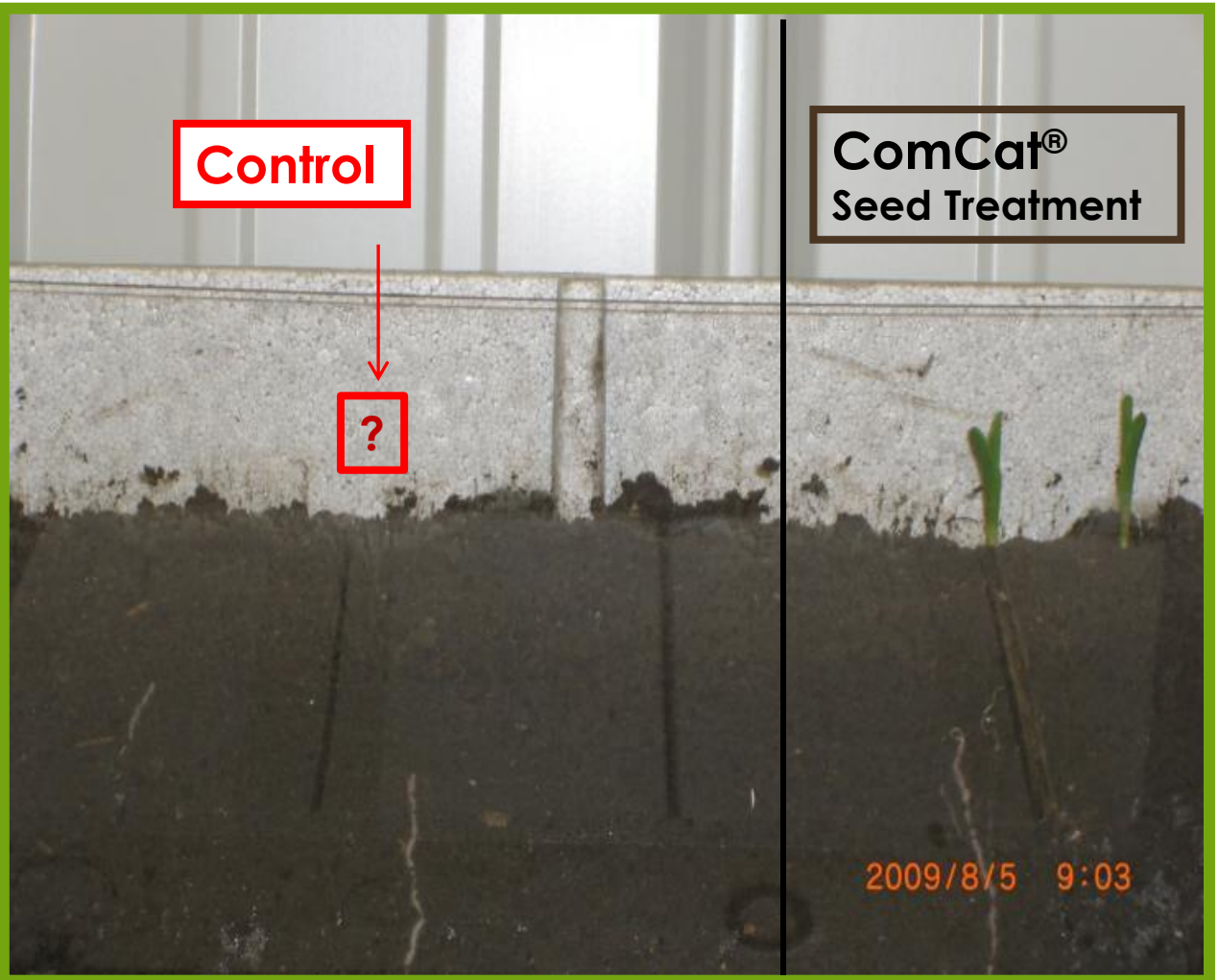
Sorghum ComCat® seed treatment [Drought tolerance], Wilber Ellis, USA

COLD STRESS

Maize seed treatment trial with ComCat® and cold stress
University of the Free State
4°C for 24h



Sorghum: Cold stress Wilber Ellis, 2009, USA

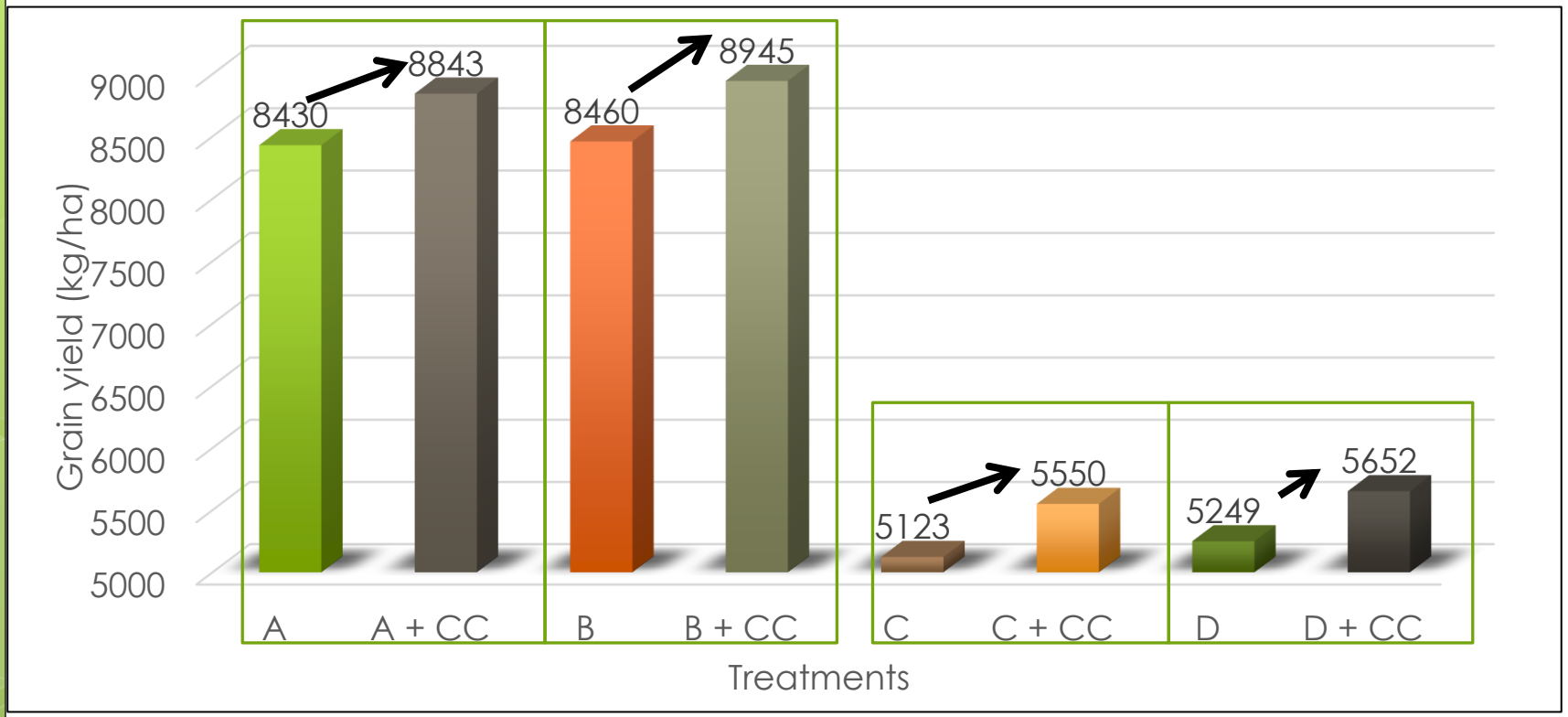


Literature references & Temperature stresses - He and others 1991, Katsumi 1991, Kamuro and Takatsuto 1991

HERBICIDE STRESS & ComCat®

- Crop Yield & **Maize**
- Chlorophyll Fluorescence & **Maize**
- Crop growth & **Wheat**
- **Herbicide efficacy** & ComCat®
- **Herbicide residues** in soil & ComCat®

Initial trials: Herbicide stress and Maize South Africa

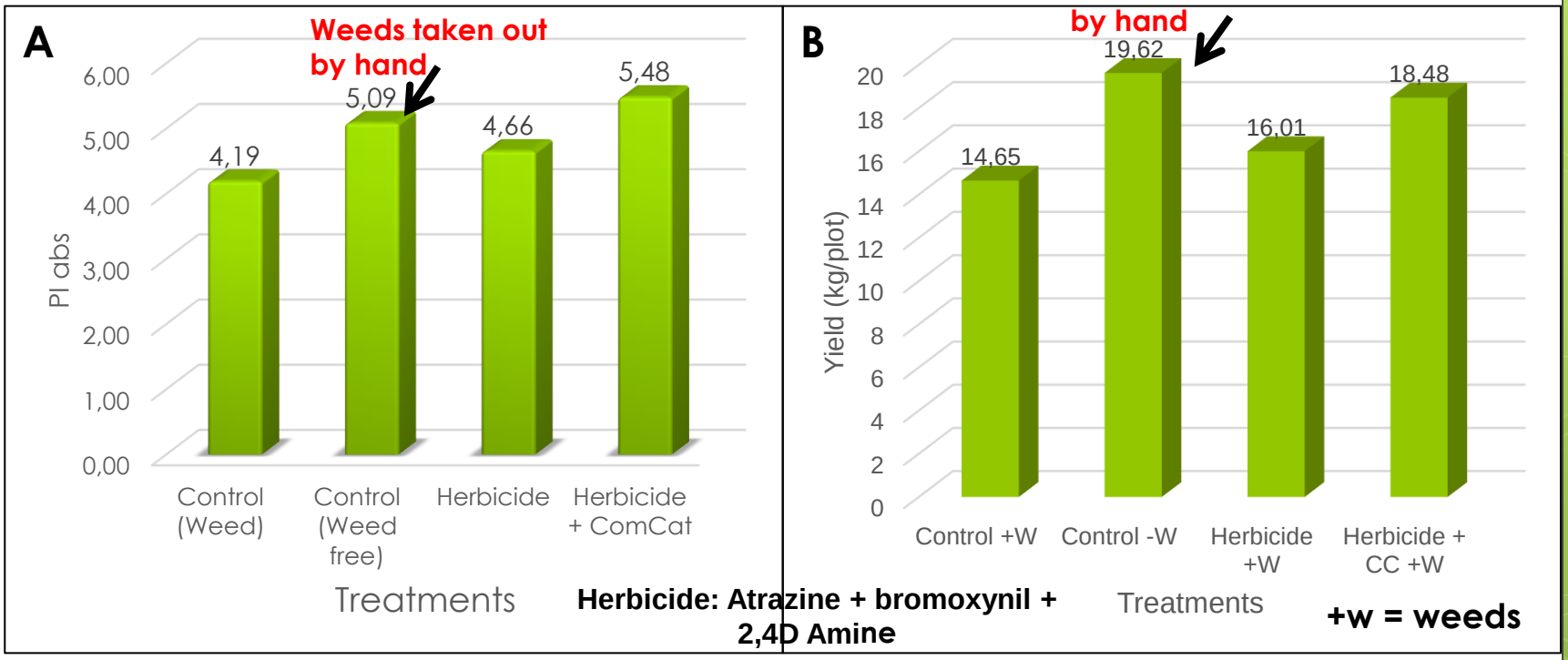


CC = ComCat®.

A/C/D: Acetochlor (HRAC K3 - growth inhibitor) + Atrazine +Terbuthylazine (HRAC C1- PSII inhibitor).
B: Glyphosate: HRAC G9 (EPSP synthase inhibitor).

Reduction of herbicide stress on the maize plant by ComCat®

Bloemfontein, RSA, 2017



Fv/Fm is a parameter widely used to indicate the maximum quantum efficiency of Photosystem II. **Plabs** shows how efficient is the Fv/Fm system functioning.

Acetochlor (HRAC K3 - growth inhibitor) + Atrazine +Terbuthylazine (HRAC C1- PSII inhibitor).

Bromoxynil (HRAC C3 – PSII), 2,4 D (HRAC 0, Growth regulator)

Effect of ComCat ® to support the growth of wheat plants when herbicides were applied 2018

Dr. Rasine Zhang
Plum Agrochemical Consulting & Service Co., Ltd., China

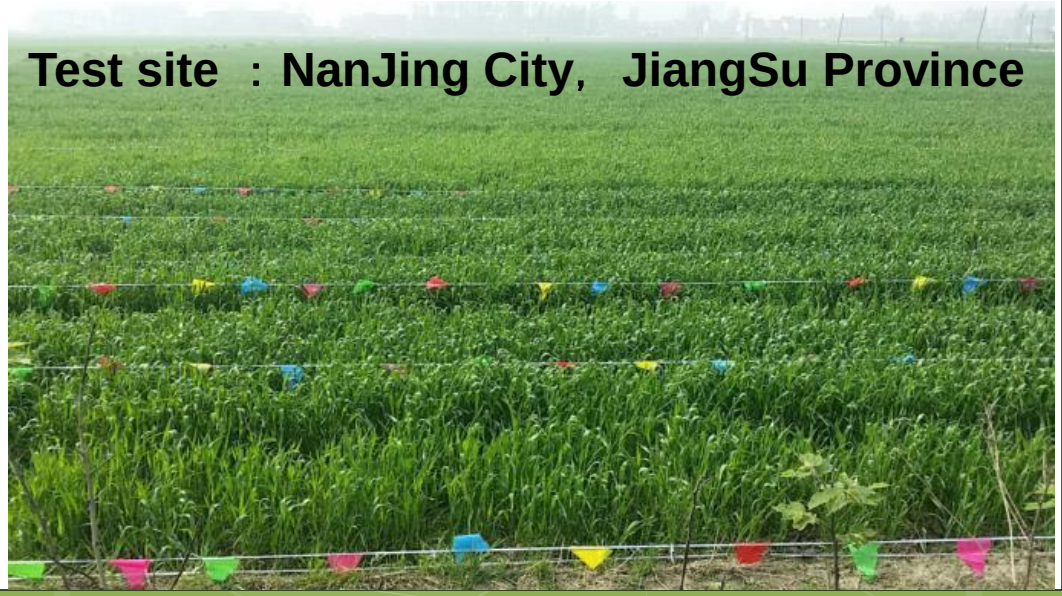
Treatment Date：**10th March. 2018**

Survey time ：**18d after treatment**

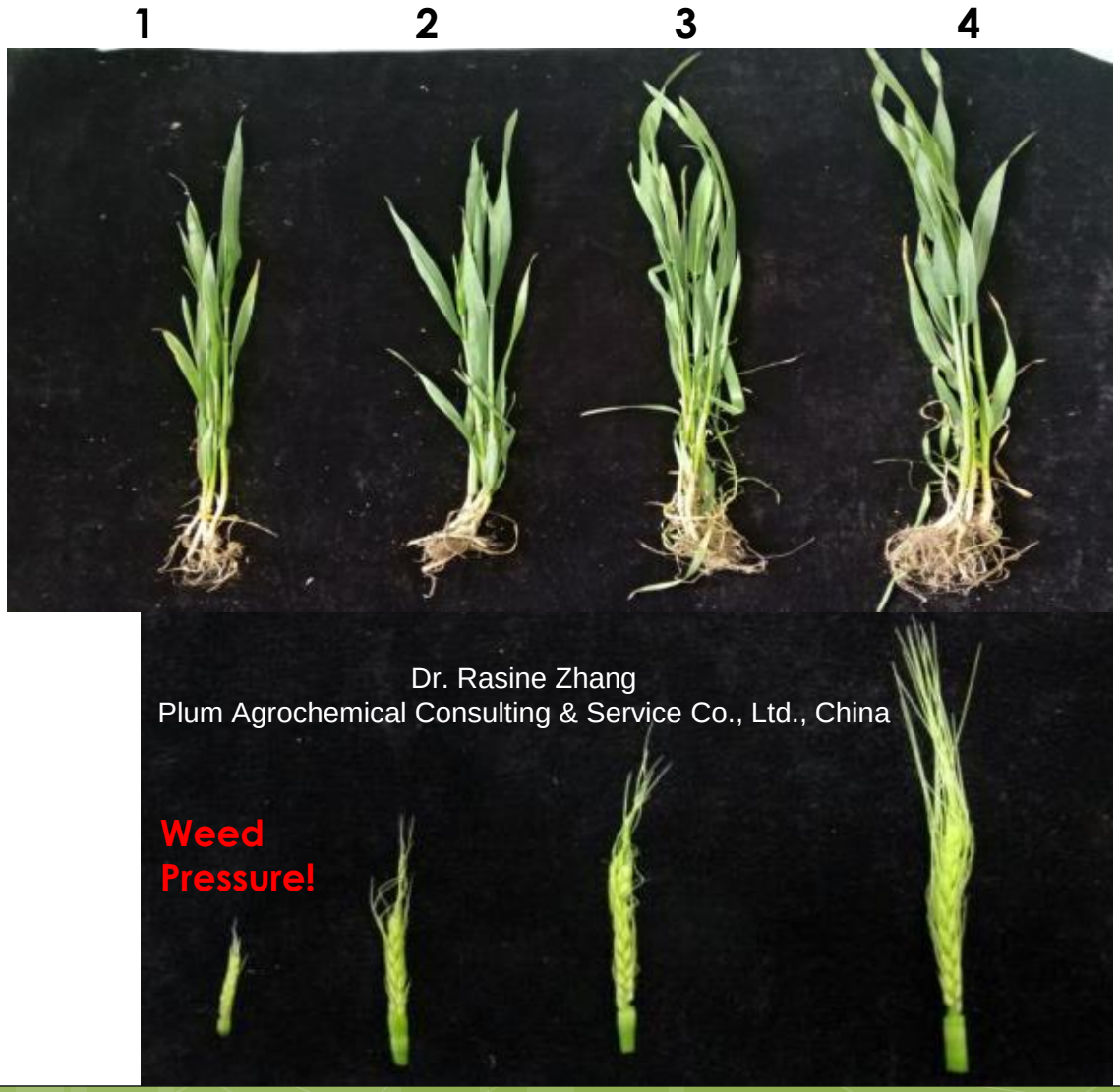
Wheat variety: Ning Mai 5

Sowing date ：**14th Nov, 2017**

Field weeds ：amur foxtail, Alopecurus japonicus
Density of weeds ：**2578/m²**



Results-Wheat-Pyroxsulam-Isoproturon-ComCat ®



18d after treatment

Treatments:

1. Control
2. 7.5% Pyroxsulam 20ml/mu+ 50% Isoproturon 250g/mu
3. 7.5% Pyroxsulam 20ml/mu+ 50% Isoproturon 250g/mu+ AnnGro
4. 7.5% Pyroxsulam 20ml/mu+ 50% Isoproturon 250g/mu + ComCat

More studies on herbicide stress and ComCat® planned!

ALS inhibitor (HRAC B2)

Results-Wheat-Pyroxsulam-Isoproturon-ComCat ®

Quantification of data 18d after treatment application

Treatment	Dosage （g or ml/mu）	Plant height（cm）	Stem diameter（mm）
Control	——	38 b	2.765 c
7.5% Pyroxsulam + 50% Isoproturon	20mL/mu+250g/mu	46.5 b	3.4625 bc
7.5% Pyroxsulam + 50% Isoproturon +AnnGro	20mL/mu+250g/mu	48.25 b	4.0625 b
7.5% Pyroxsulam + 50% Isoproturon + ComCat	20mL/mu+250g/mu 100 g /ha	54.5 a	5.4525 a

Weed Pressure!

Dr. Rasine Zhang
Plum Agrochemical Consulting & Service Co., Ltd., China

Herbicide efficacy and ComCat®?

Locality:	**"In De Middel",Schoemanskloof, Mpumalanga Province, South Africa**									
Co-operator:	Stone Creek Research Facility									
GPS:	S 25^0 23' 52.50 E 30^0 37' 23.55									
Climatic Zone:	CWa – Temperate climate. Dry winters, hot summer									
Crop:	Maize				Variety:	PAN3P502R				
Plant Date:	15th Jan 2016				Water regime:	Dry Land				
Soil:	Clay:	37%	Silt:	15%	Sand:	58%		3.9%	pH:	6

Product usage	Active ingredient
ComCat	
Cantron 480 SC	Mesotrione 480g/ℓ SC
Terbusien Super 600 SC	Atrazine/terbuthylazine 300/300g/ℓ SC
Villa 51 (adjuvant)	Isotridecanol 918 g/ℓ SL - NIS

Common Name	Scientific name	Abbrev	% Ground Cover
GRASSES/SEDGES			
Goose grass	*Eleusine coracana*	ELECO	10%
BROADLEAF			
Field bindweed	*Convolovulus arvensis*	CONAR	20%
Mexican Richardia	*Richardia braziliensis*	RCHBR	15%
Cocklebur	*Xanthium strumarium*	XANST	10%

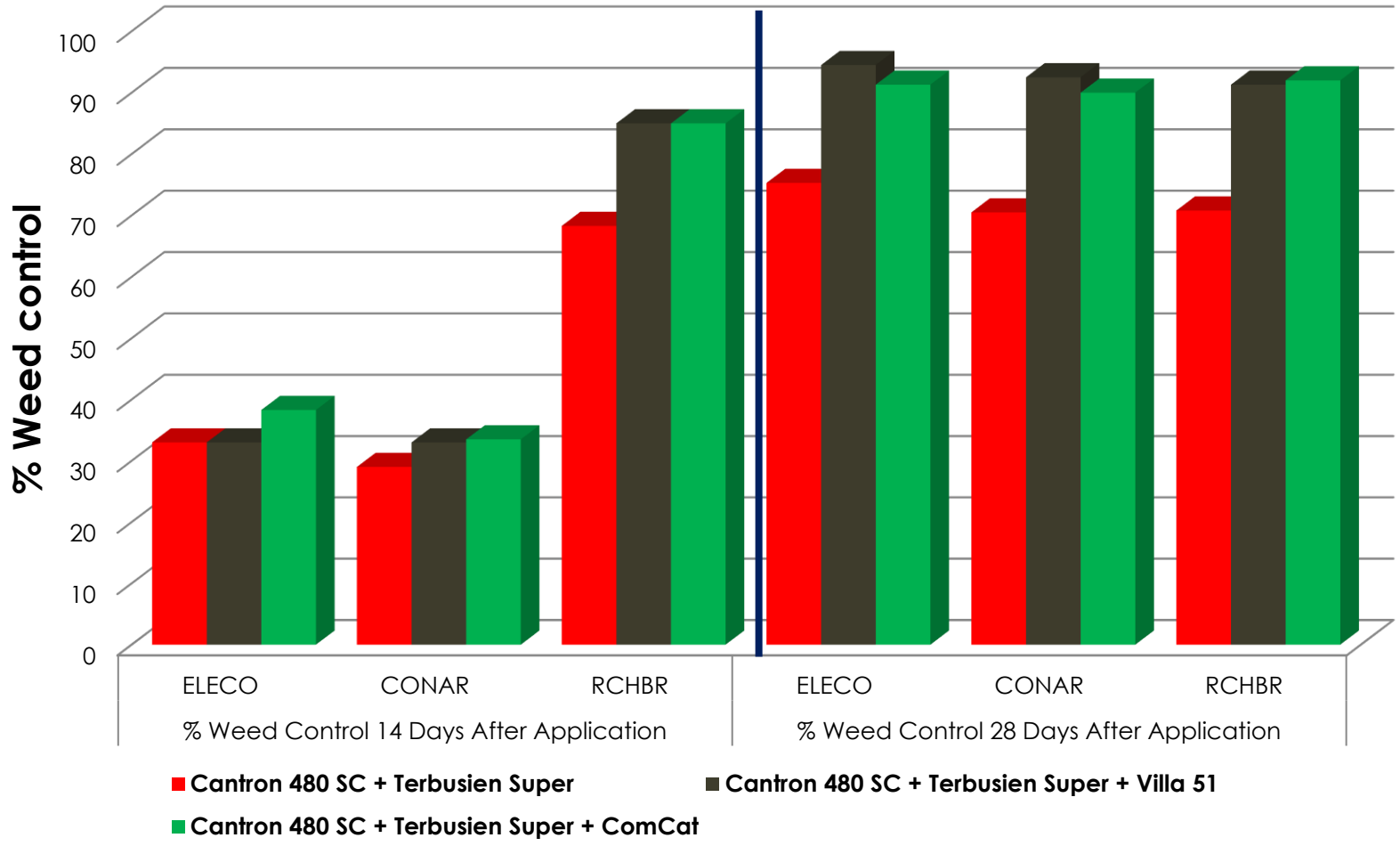
Independent study – Brink Enterprises

Post emergence treatments applied as a broadcast application over the rows at the BBCH 14 and BBCH17 growth stages of maize with a spray volume of 200 ℓ/ha.

Mesotrione – HRAC F2 (HPPD inhibitor), atrazine + terbuthylazine (HRAC C1- PSII inhibitor)

HERBICIDE STRESS

Comparative Influence of ComCat® on *CANTRON 480 SC* + *TERBUSIEN SUPER 600* SC treatments with regards to HERBICIDAL EFFICACY in maize



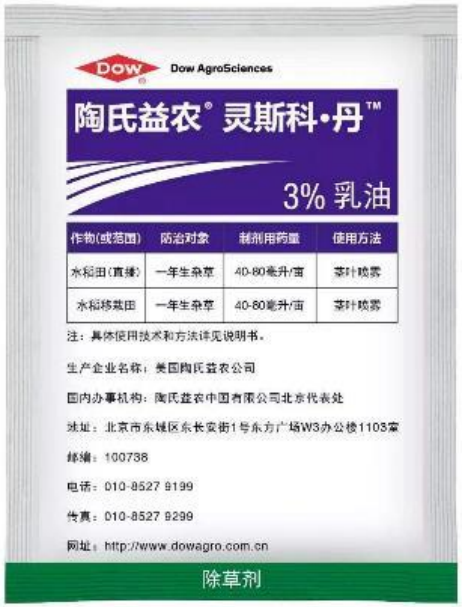
植物生长调节剂碧护®与除草剂混用对杂草和作物的影响

Effects of herbicide tank-mixed with Vitacat® on weeds and crops

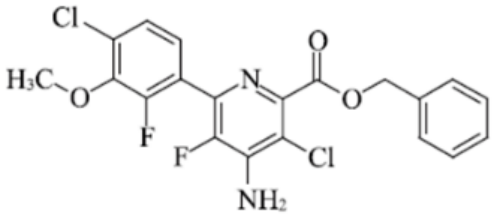
Weeds Research Laboratory of
Nanjing Agricultural University
2019

研究背景/Research Background

Rinskor （3% florpyrauxifen-benzyl EC）——Hormone herbicide



is an aryl pyridine carbamate and synthetic auxin herbicide developed by Dow AgriSciences Company. **Group 4**



The herbicide is mainly used in paddy fields to control grasses, broad-leaved weeds, sedges and aquatic weeds. Affect cell wall plasticity and nucleic acid metabolism. Synthetic auxins also affect protein synthesis, cell division and growth, and stimulate ethylene evolution

Materials： Barnyard grass （*Echinochloa crusgalli* var. *mitis*）and Rice（*Oryza sativa*） minghui63

表1 供试药剂的种类、剂型和品牌

Table 1 Type, formulation and brand of test herbicides

供试药剂 Chemical	剂型 formulation	商品名 Trade name	生产单位 Manufacturer
Florpyrauxifen-benzyl	3%（EC）	Rinskor	Dow AgroSciences
Secondary metabolites	0.136%（WP）	VitaCat®	AgraForUm

The symptoms of barnyard grass and rice were observed and recorded on the 1st, 3rd, 5th and 7th day after treatment, and the damaged comprehensive index of barnyard grass and rice were calculated. The leaves of barnyard grass and rice were taken for the determination of physiological indexes.

Rinskor is an aryl pyridine carbamate and synthetic auxin herbicide

INVESTIGATION OF PHYTOTOXICITY

表3 氯氟吡啶酯杂草和作物药害症状判断药害级别标准

Table 3 Scoring criteria of Florpyrauxifen-benzyl injury level on weeds and crops

HIS	Injury symptoms on treated plants
0	No symptom
1	Plant growth inhibited slightly. Entire plant wilted, all leaves drooped slightly, but no other symptom occurred. Yellow leaves appear on a small part of the leaves. Less than 20% of leaves had necrotic spots
2	Entire plant wilted including the young leaves. The discolored stem and old leaf petiole wilted and the degree of whitening of the stem is more serious. 20-40% of leaves were dead.
3	Young leaves curled and etiolated. Entire plant etiolated. Roots decomposed and separated to stem.40-60% of leaves were dead.
4	The leaves of the plants are completely brown and fall off, the roots and stems are deformed, the stems are separated with the roots. Plants tissue discomposed. Entire plant etiolated more seriously. 60-80% of leaves were dead.
5	The whole plant withered completely. The leaves of the plants are completely brown and fall off, and the roots and stem completely separated and decomposed. More than 80% of the leaves die, or the entire plant dies.

表4 3%氯氟吡啶酯乳油药后不同天数的下的无芒稗药害综合指数表

Table 4 Composite injury index of *Echinochloa crusgalli*. var. *mitis* treated by 3% Florpyrauxifen-benzyl EC and 3% Florpyrauxifen-benzyl EC + VitaCat at different days after application

处理 Experimental treatments	剂量 Dose	1D	3D	5D	7D	9D
对照 Control Check		0%	0%	0%	0%	0%
碧护® VitaCat	45 g/ha	0%	0%	0%	0%	0%
3%氯氟吡啶酯乳油 3% Florpyrauxifen-benzyl EC	1200mL/ha	25%	45%	57%	65%	83%
3%氯氟吡啶酯乳油+碧护® 3% Florpyrauxifen-benzyl EC + VitaCat	1200 mL/ha +45 g/ha	28%	49%	60%	83%*	97%*

备注：氯氟吡啶酯组与氯氟吡啶酯+碧护组差异显著用*表示

Remarks: The difference between the chlorofluoropyridyl ester group and the chlorofluoropyryl ester + Bi protection group is significant. * indicates。

Investigation on the safety of rice

Comprehensive index of rice **Injury symptoms**

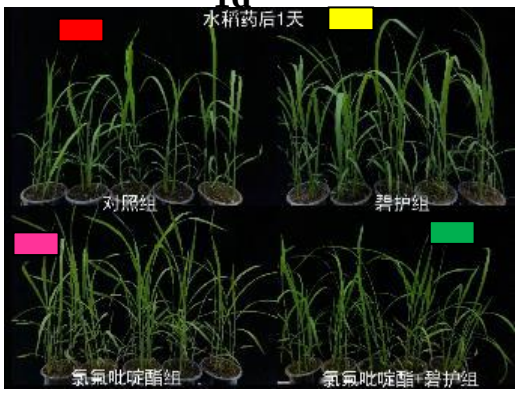
处理 Experimental treatments	剂量 Dose	1d	3d	5d	7d
对照 Control Check		0%	0%	0%	0%
碧护® VitaCat	45 g/ha	0%	0%	0%	0%
3%氯氟吡啶酯乳油 3% Florpyrauxifen-benzyl EC	1200mL/ha	0%	0%	0%	0%
3%氯氟吡啶酯乳油+碧护® 3% Florpyrauxifen-benzyl EC + VitaCat	1200 mL/ ha +45 g/ha	0%	0%	0%	0%

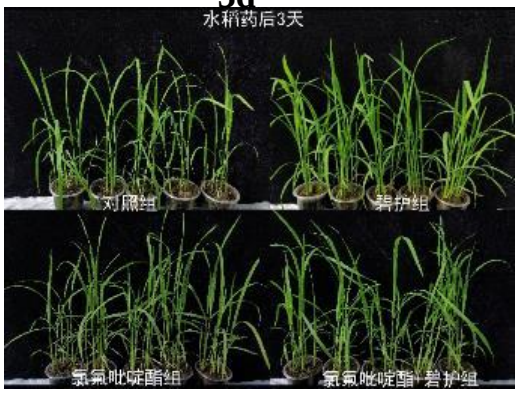
Control **VitaCat**

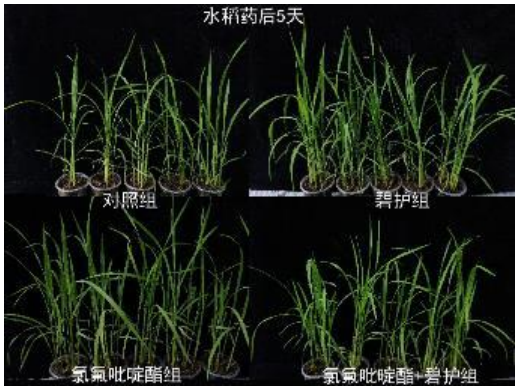
3% Florpyrauxifen-benzyl EC

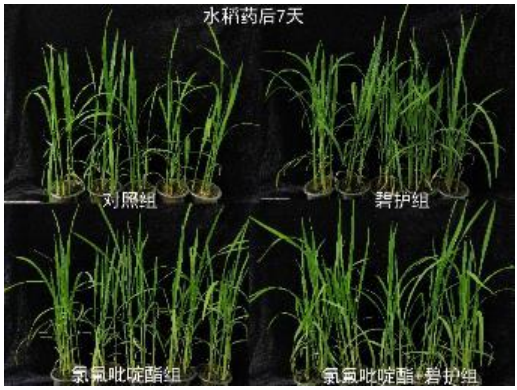
3% Florpyrauxifen-benzyl EC + VitaCat

1d **3d**









5d **7d**

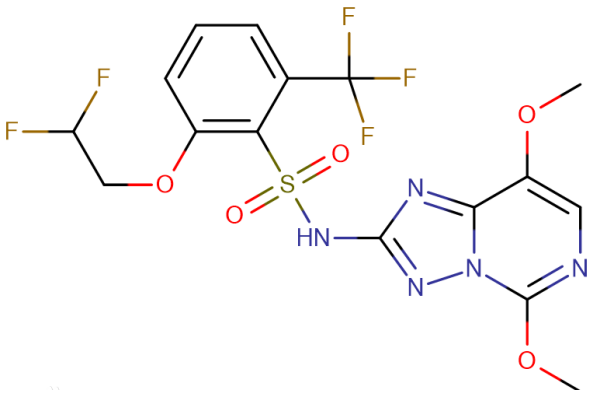
Yield characteristics of rice

处理Treatments	株高 height（cm）	剑叶面积 flag leaf area（cm^2）	分蘖数 tiller number	穗长 panicle length（cm）	单穗饱粒数 number of full grains per panicle	结实率 seed setting rate（%）	千粒重 thousand grain weight（g）	单株产量 grain weight per plant（g）	理论产量 theoretical yield（kg/mu）
Control	127.87±1.46b	58.52±2.2b	8.89±0.38c	23.32±1.08 b	110.33±18.03b	82.69±5.19b	30.74±0.95b	45.09±0.32a	1002.94±40.86b
VitaCat	147.13±1.35a	67.97±2.74a	12.22±0.77a	28.27±1.47 a	128.56±8.18a	87.15±1.76a	33.39±1.68a	48.2±1.81a	1185.53±40.23a
3% Florpyrauxifen-benzyl EC	127.37±3.94b	57.9±6.57b	9.33±1.53bc	22.41±2.18 b	97.67±7.23b	77.14±2.44b	28.13±1.67c	44.53±3.6a	973.98±100.2b
3% Florpyrauxifen-benzyl EC + VitaCat	131.63±4.71b	65.74±4.44ab	11.22±1.35ab	24.9±1.45b	115.78±7.37a	84.27±1.84a	32.02±0.6ab	49.64±3.97a	1184.08±83.1a

Note: Significance of difference between different lowercase letters at 0.05 level .

Effects on yield characteristics of rice treated with 3% Florpyrauxifen-benzyl EC （1200 mL/ha）± VitaCat （45 g/ha）

Penoxsulam——**Strong ALS inhibitor (Group 2, HRAC B2)**



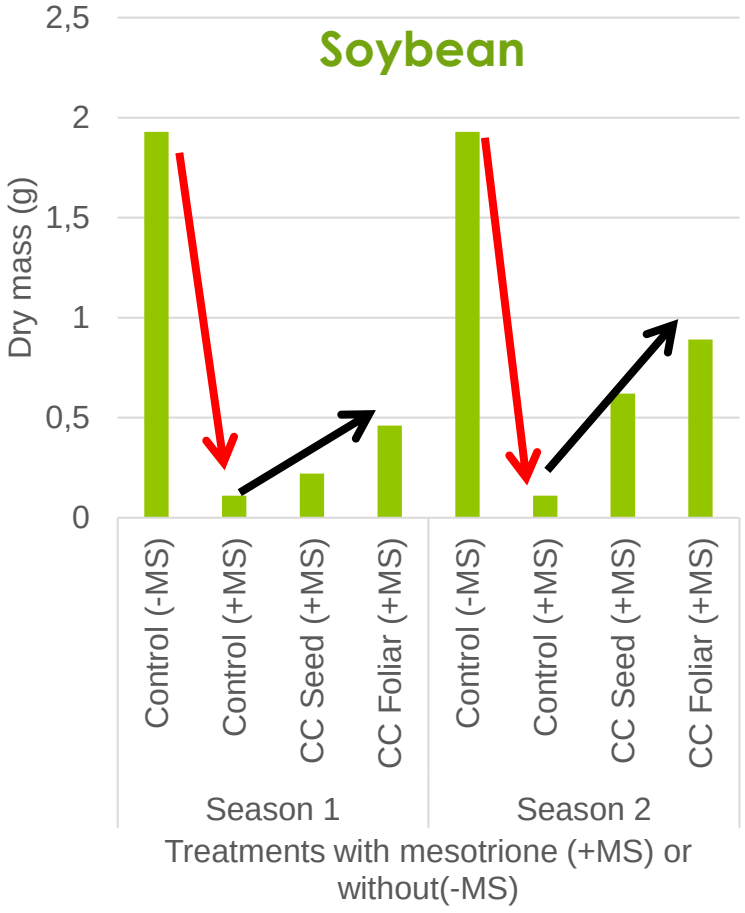
Structural formula

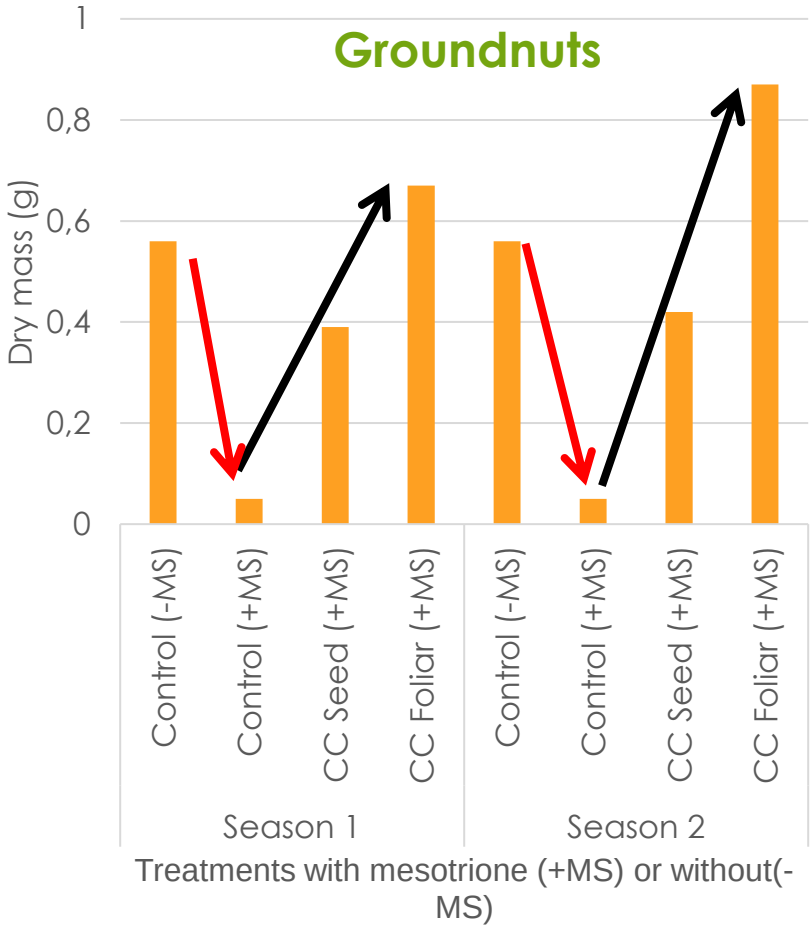
25g/L Penoxsulam OD, from Dow AgroSciences. It works by inhibiting acetyllactate synthetase (ALS). ALS is a catalytic enzyme involved in the biosynthesis of various amino acids.

Composite injury index of Barnyard grass

Treatments	Time（d）			
	1d	3d	5d	7d
Control	0	0	0	0
VitaCat	0	0	0	0
25g/L penoxsulam OD	1.6 a	7.2 a	47.6 a	59.2 b
VitaCat+25g/L penoxsulam OD	2.4 a	8.8 a	49.2 a	70.8 a

Alleviation of Mesotrione residue phytotoxicity on legumes using ComCat® University Free State, RSA





ComCat® and Induced Resistance against Biotic stresses

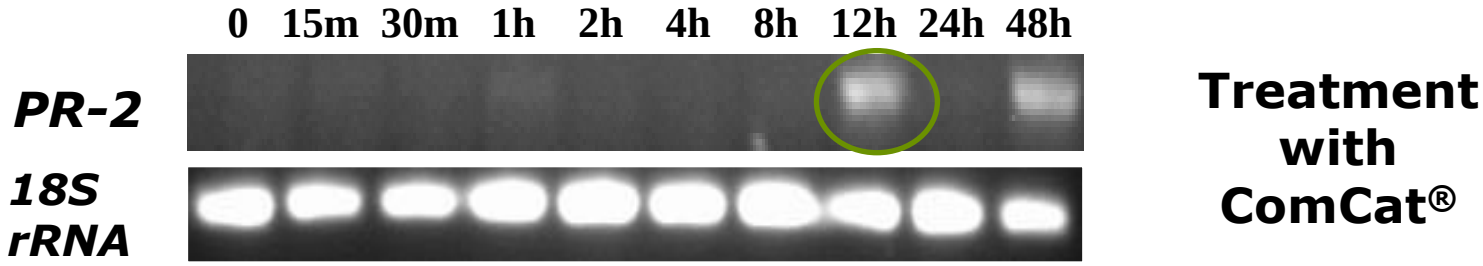
- Fungal infections

Fungal Infection

Expression of **PR-2 GENE (β-1,3-GLUCANASE)**

in

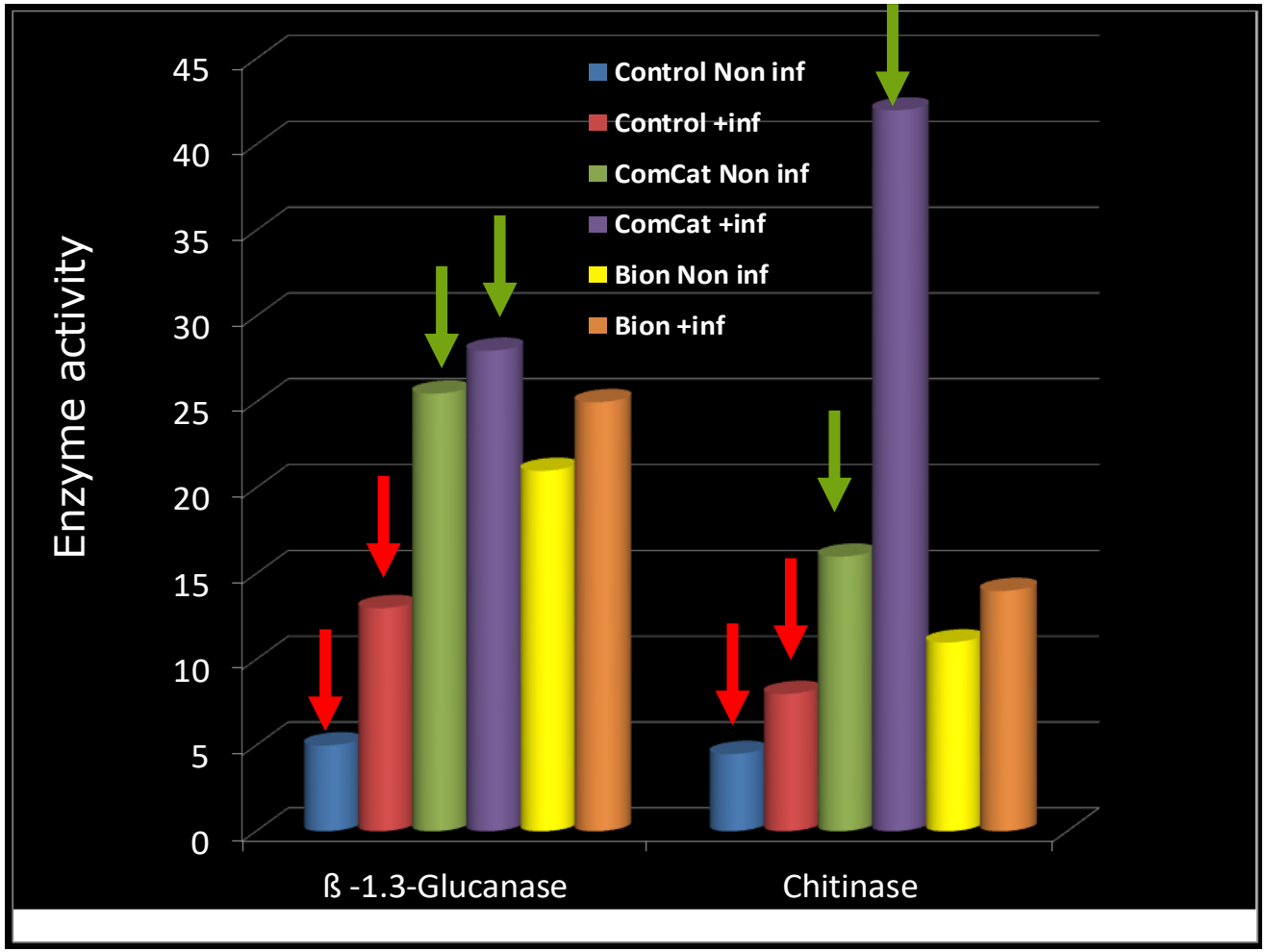
***A. thaliana* by ComCat®**

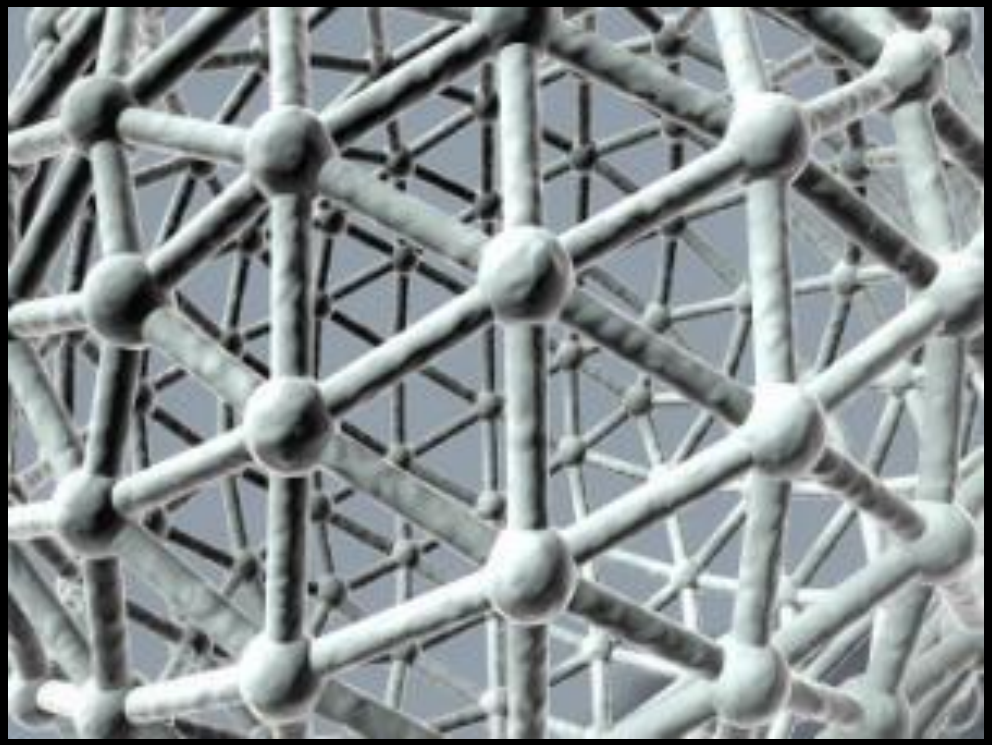


NOTE

PR-2 GENE known to **INDUCE β-1,3-GLUCANASE** (PR-protein) and **RESISTANCE TO** biotic stress **SUCH AS** fungal infection

PR-PROTEIN INDUCTION IN SUNFLOWER, UFS





ComCat®

BIOCHEMICAL MECHANISMS

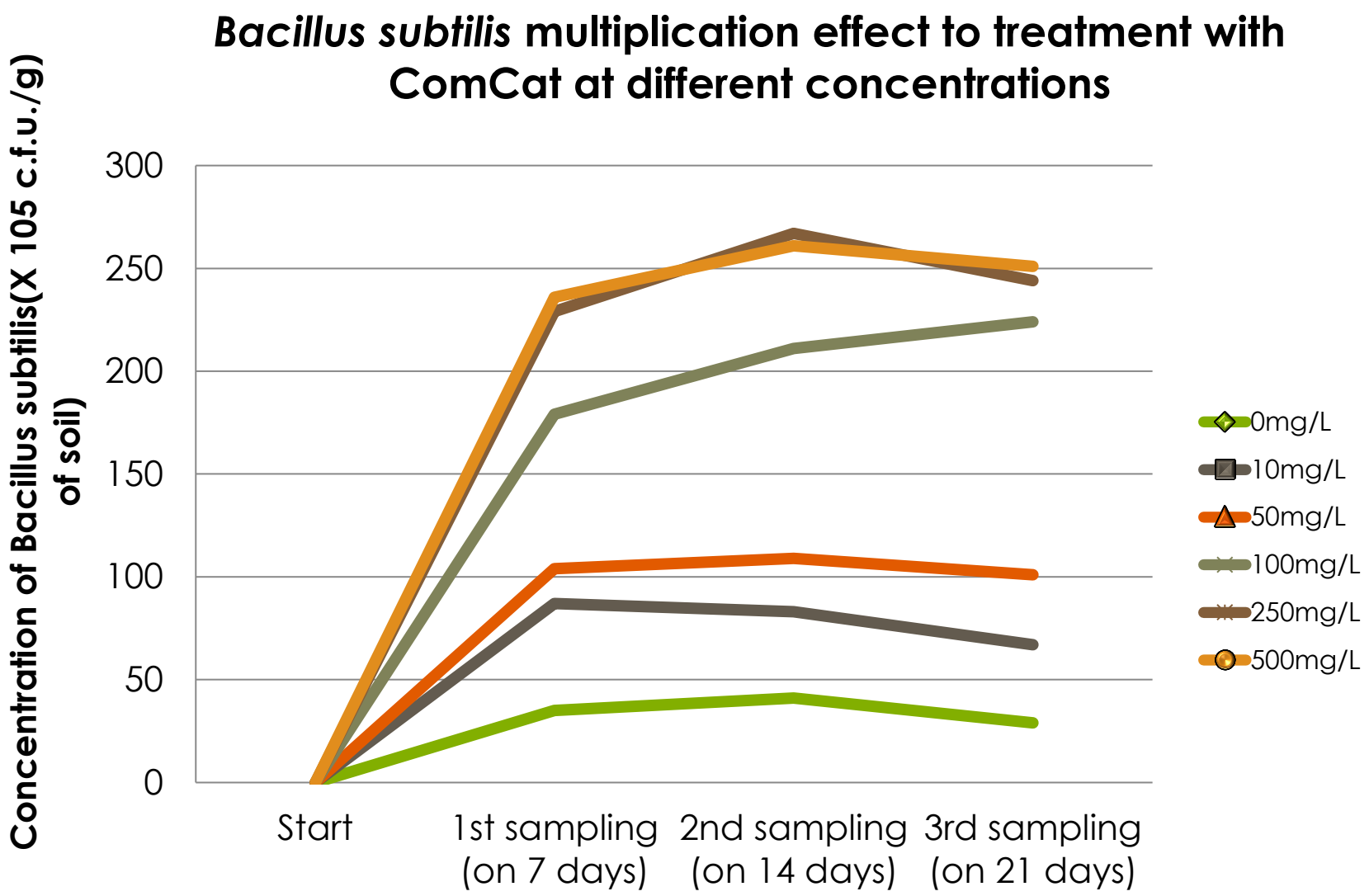
Information not presented, available on request

Initial trial on *B. subtilis* multiplication effect by ComCat® under laboratory conditions

Test institute: Research Institute of Korea Bio Co.LTD

Test procedure:

- Soil was sterilized via dry oven at 160°C for 3h.
- Preparation of aqueous ComCat® (5% a.i.) solutions: 0 mg/L, 10 mg/L, 50 mg/L, 100 mg/L, 250 mg/L, 500 mg/L.
- Addition of each solution to soil.
- Inoculation of *Bacillus subtilis* to soil (final concentration of *B. subtilis* was 2.1 X 10*5/g wet soil).
- Incubated in growth chamber (humidity 95%, temperature 27-30°C)
- Soil sampling and count of *B. subtilis* was done at 7 days intervals.


Bacillus subtilis multiplication effect to treatment with ComCat at different concentrations
Concentration of Bacillus subtilis(X 105 c.f.u./g) of soil)
300
250
200
150
100
50
0
Start
1st sampling (on 7 days)
2nd sampling (on 14 days)
3rd sampling (on 21 days)
0mg/L
10mg/L
50mg/L
100mg/L
250mg/L
500mg/L

UNIVERSIDADE FEDERAL DE PELOTAS
FACULDADE DE AGRONOMIA ELISEU MACIEL
PROGRAMA DE PÓS GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE SEMENTES

Compliments to:

Andermatt do Brasil Soluções Biológicas Ltda.

Responsáveis:
Prof. Dr. Antonio Carlos Souza Albuquerque Barros
Dr. Cassyo de Araujo Rufino;
Msc. Otávio Correa;
Msc. Ewerton Gewehr

ComCat®

Pelotas, RS
2015

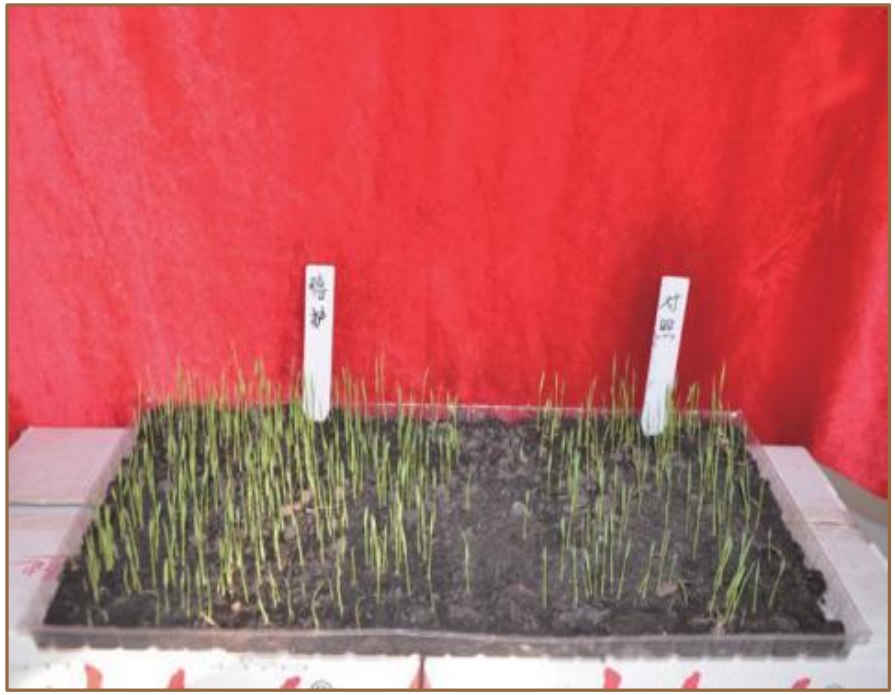
ComCat® & Soybean Rhizobium activity

UNIVERSIDADE FEDERAL DE PELOTAS, BRAZIL

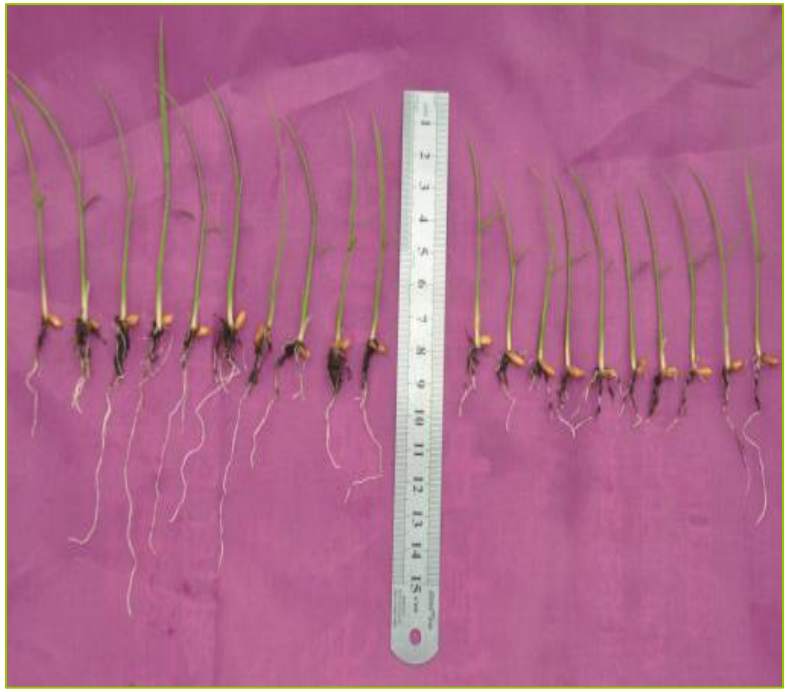
Inoculants and ComCat Synergy					
Treatments	**Leaf Area (cm²/pot)**	**Foliar dry mass (g/pot)**	**Root dry mass (g/pot)***	**Total Chlorophyll**	**Nodules dry mass (g/pot)***
Control negative	716,791 b	3,6813 b	0,5888 b	34,6 c	0,0888 b
Control inoculated	849,024 b	3,5453 b	0,8953 b	36,7 ab	0,2454 b
Control positive	608,573 b	2,7241 b	0,4581 b	35,8 bc	0,1078 b
Primax +SilCoat (5kg/ha)	894,794 b	4,0506 b	0,7691 b	32,6 bc	0,3076 b
Primax +SilCoat (2,5kg/ha)	829,782 b	3,9499 b	0,6474 b	35,8 bc	0,2465 b
ComCat (50mg/kg seeds)	1351,894 a	6,3569 a	2,1352 a	36,7 ab	0,6536 a
ComCat (25mg/kg seeds)	899,946 b	4,2824 b	0,7825 b	36,9 ab	0,2024 b
ComCat (10mg/kg seeds)	742,667 b	4,2323 b	0,5993 b	37,6 a	0,1243 b
Primax+SilCoat (2,5kg/ha) + ComCat(10mg/kg seeds)	875,903 b	3,9285 b	0,6766 b	36,3 abc	0,1257 b
C.V.(%)	22,8	24,7	17,1	9,77	31,5
Duncan test 5%					
*Transformed data "arcsen((x/100)^1/2)"					

MORE SCIENTIFIC STUDIES PLANNED: COMCAT & SOIL MICROBIAL ACTIVITY

ComCat® & Seed treatment on Rice



ComCat® Control



ComCat® Control

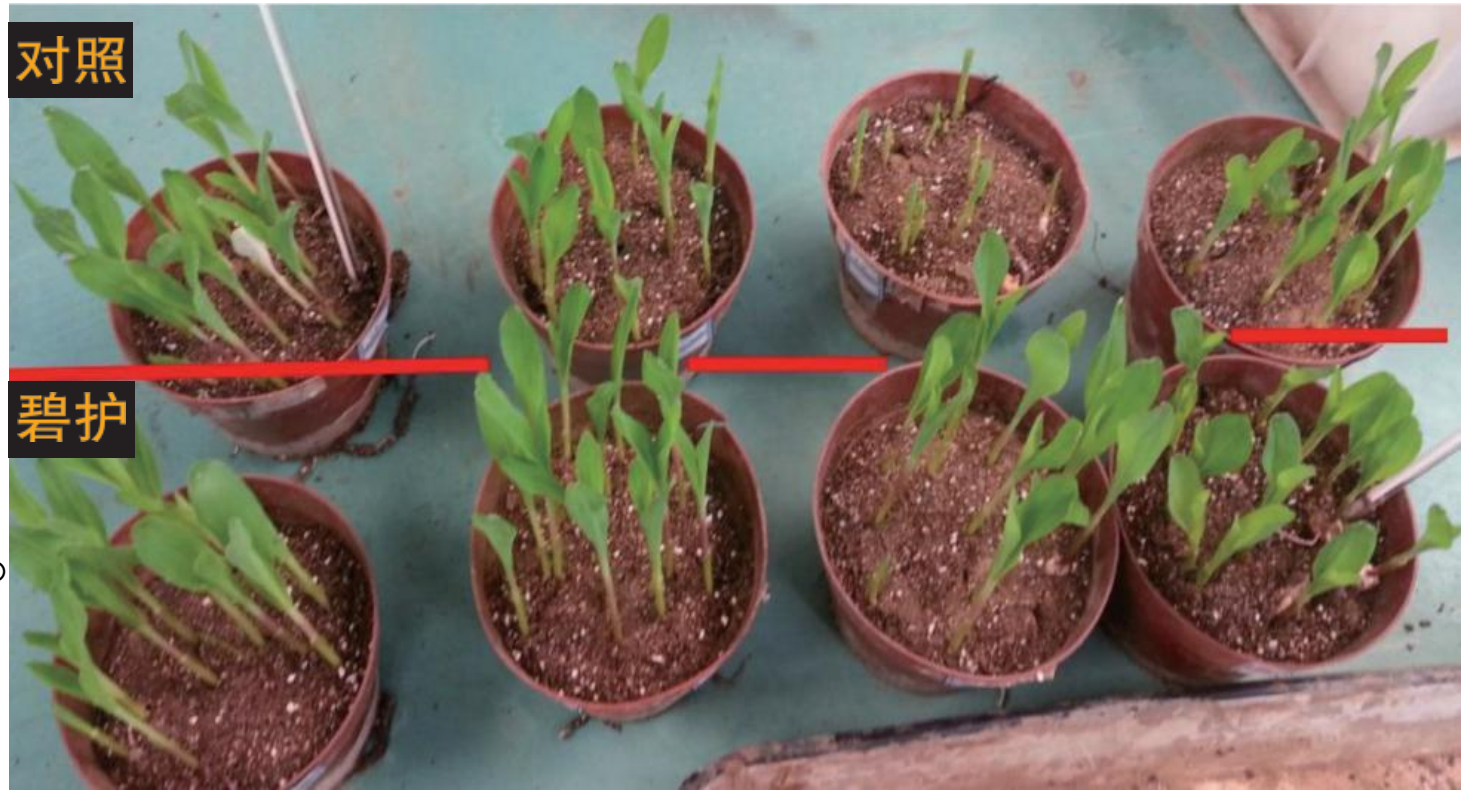
Dr. Rasine Zhang
Plum Agrochemical Consulting & Service Co., Ltd., China

ComCat® seed treatment on rice, Vietnam

ComCat® & Seed treatment on corn

Control

ComCat®



tebuconazole 1x tebuconazole 4x tebuconazole 2x

Tebuconazole is a triazole fungicide

Dr. Rasine Zhang
Plum Agrochemical Consulting & Service Co., Ltd., China

ComCat® & Seed treatment on wheat

Control ComCat®

ComCat® Control

Dr. Rasine Zhang
Plum Agrochemical Consulting & Service Co., Ltd., China

CROP YIELD

INCREASED CROP YIELDS NOTED

BY *ComCat®*

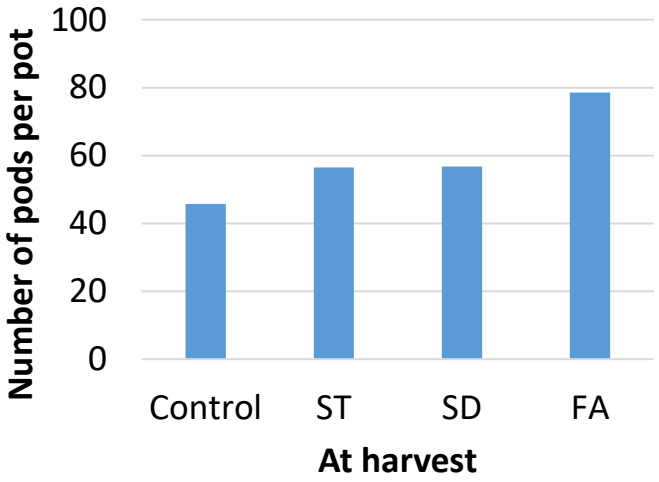
OVER SEVERAL SEASONS

Trial data not presented, available on request

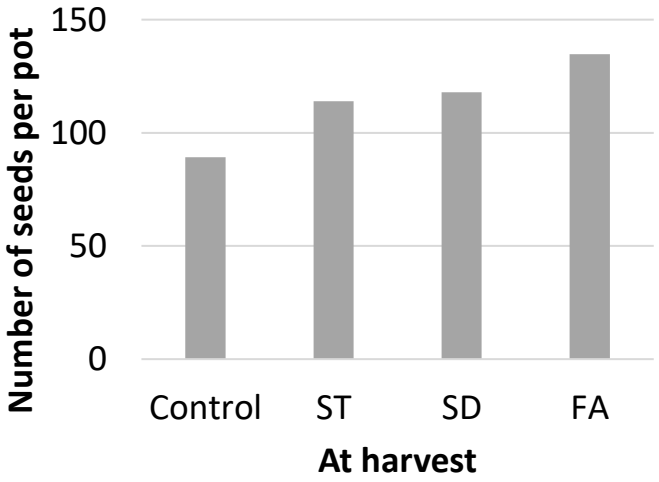
Different application methods of *ComCat®* and the effect on yield parameters of soybean and groundnuts

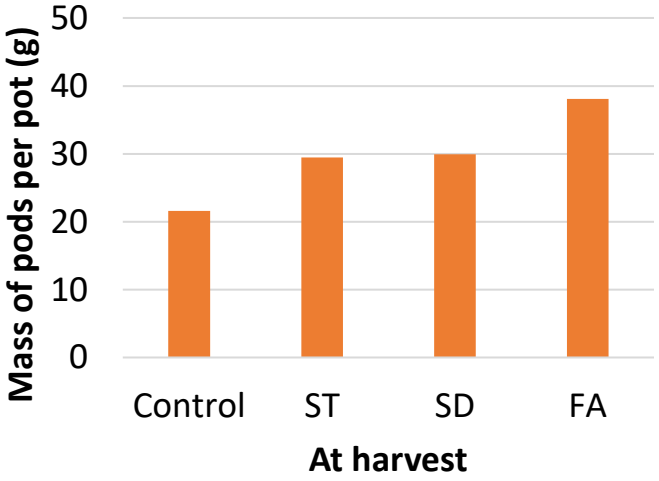
Dr. E van der Watt
MM Mota
University of the Free State

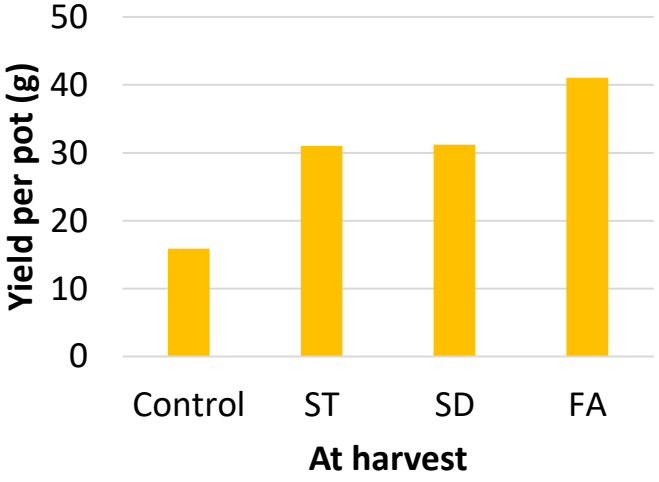
Effect of different ComCat® application methods on yield components of soybean – UFS 2018



ST = Seed treatment
SD = Soil drench
FA = Foliar application







The effect of ComCat® applied as a seed treatment, soil drench and foliar application on groundnut yield components (UFS, 2018)

Treatment	Number of pods per pot	Mass of pods per pot (g)	Number of seeds per pot	Mass of seeds per pot (g) (yield per pot)
Control	47.50 c	22.62 c	72.75 c	18.35 c
Seed treatment	55.75 b	27.84 b	115.75 b	24.53 b
Soil drench	56.75 b	28.21 b	119.00 b	24.76 b
Foliar application	65.25 a	31.32 a	128.00 a	27.38 a
LSD_T (5%)	8.09	2.21	7.25	2.11

CONCLUSION

ComCat®

IS WORKING IN YOUR CROPS

WHEN YOU ARE SLEEPING!

THANK YOU

Thank you for your attention