

Chemical Triggered Defense Mechanisms Against *Phytophthora infestans*

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Dalhousie University
Canada



Plant Genomics
July 14-15, 2016 Brisbane, Australia

International Year of the **POTATO**

2008

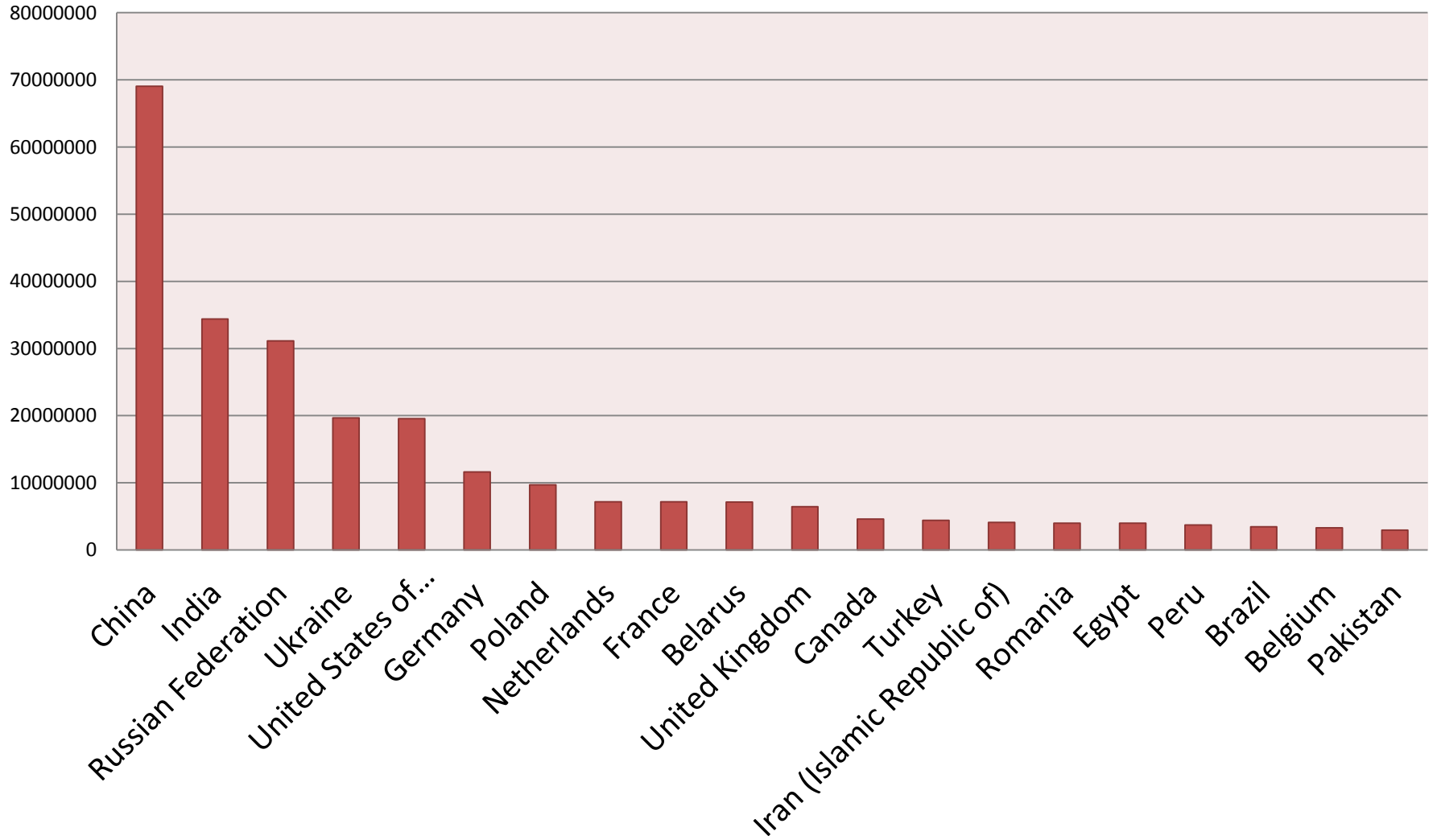


The cultivated potato (*Solanum tuberosum* ssp. *tuberosum*) is one of the four most important food crops in the world after rice, wheat and maize.

Its world production has reached 330 million tones (FAO).

Tubers are underground stems.

Potato Production in Top 20 Countries (tonnes) (FAOSTAT)



Research Programs Overview

Tuber Quality

After-cooking darkening
Enzymatic browning
Maturity

Potato Nutrition

Nutrient mapping
Vitamin C
Chlorogenic acid and
Other antioxidants

Potato Consumer Research Initiative

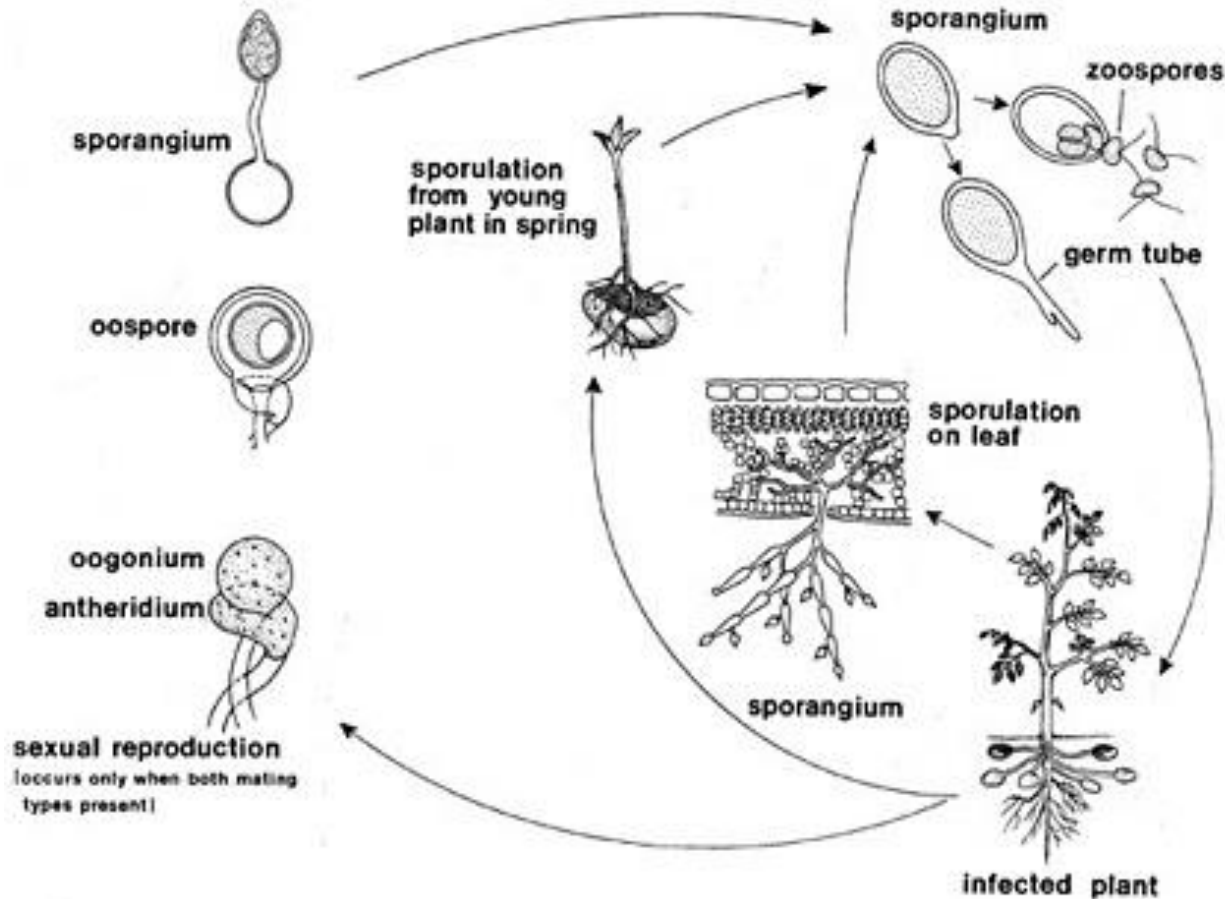
Consumer response
Marketing strategies

Potato Diseases

Late blight

Verticillium wilt

Life cycle of *Phytophthora infestans*, causal agent of late blight of potato and tomato

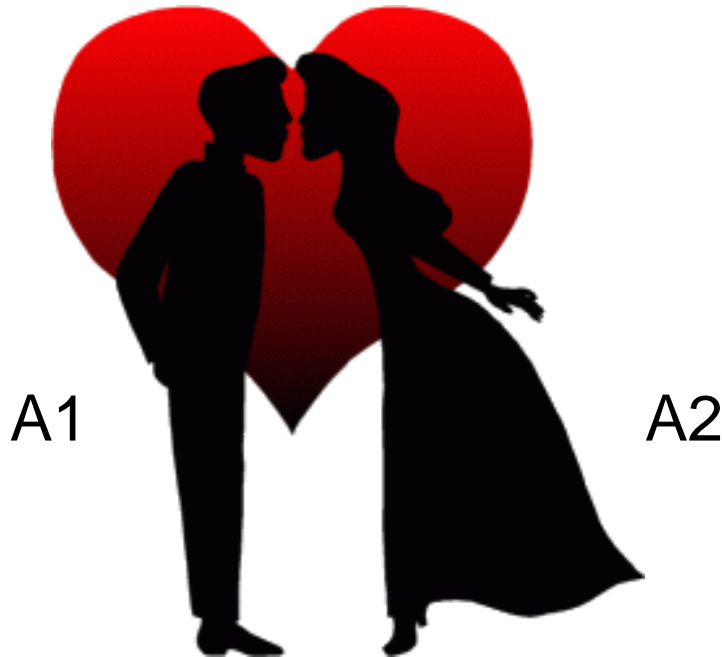


A: zoospores produced within the lemon-shaped sporangia (**B**).



Mating types: A1 and A2

When A1 meets A2.....



=



- generation of new strains
- overwintering oospores

DISTRIBUTION OF GENOTYPES OF *P. INFESTANS* IN CANADA

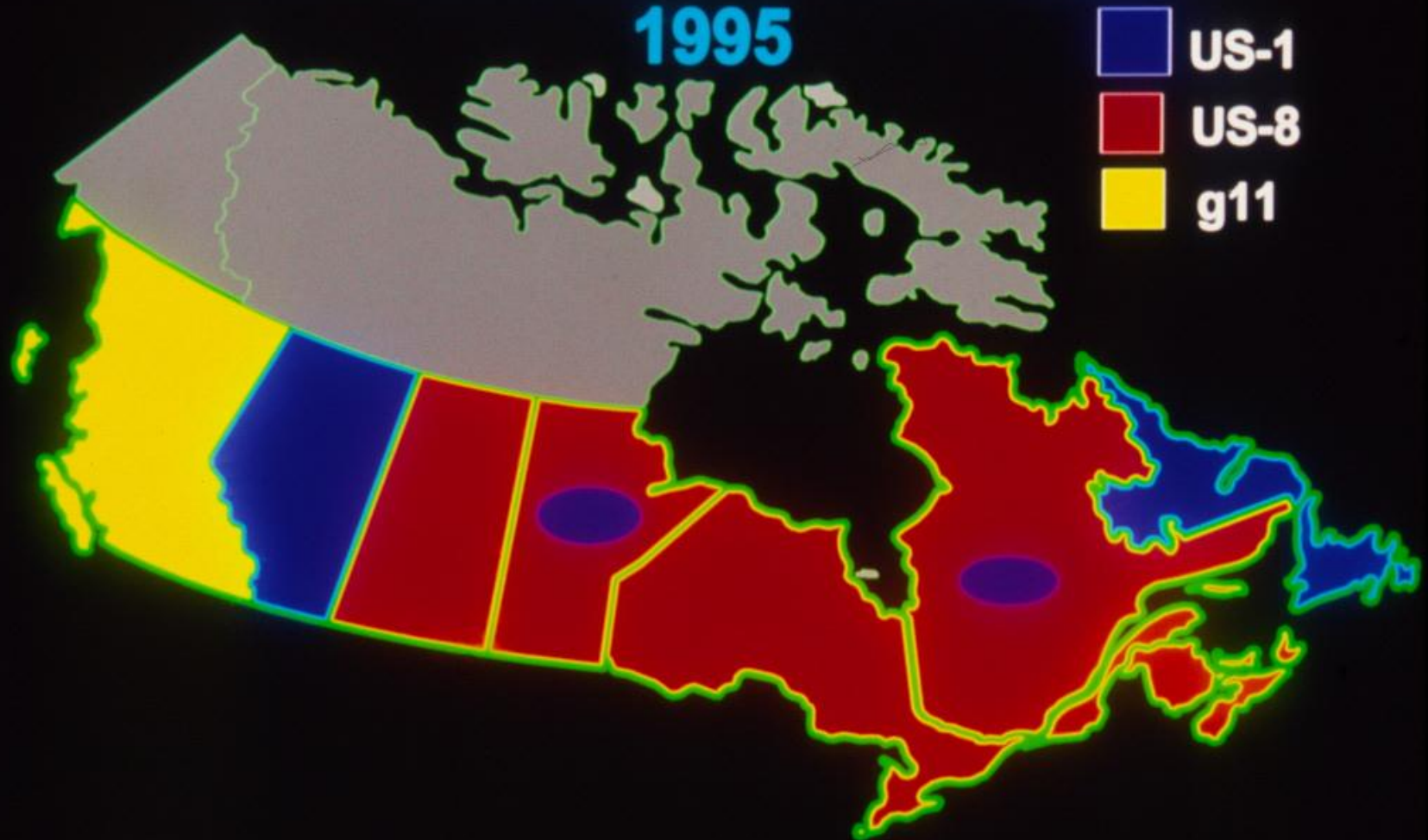
1993



Rick Peters

DISTRIBUTION OF GENOTYPES OF *P. INFESTANS* IN CANADA

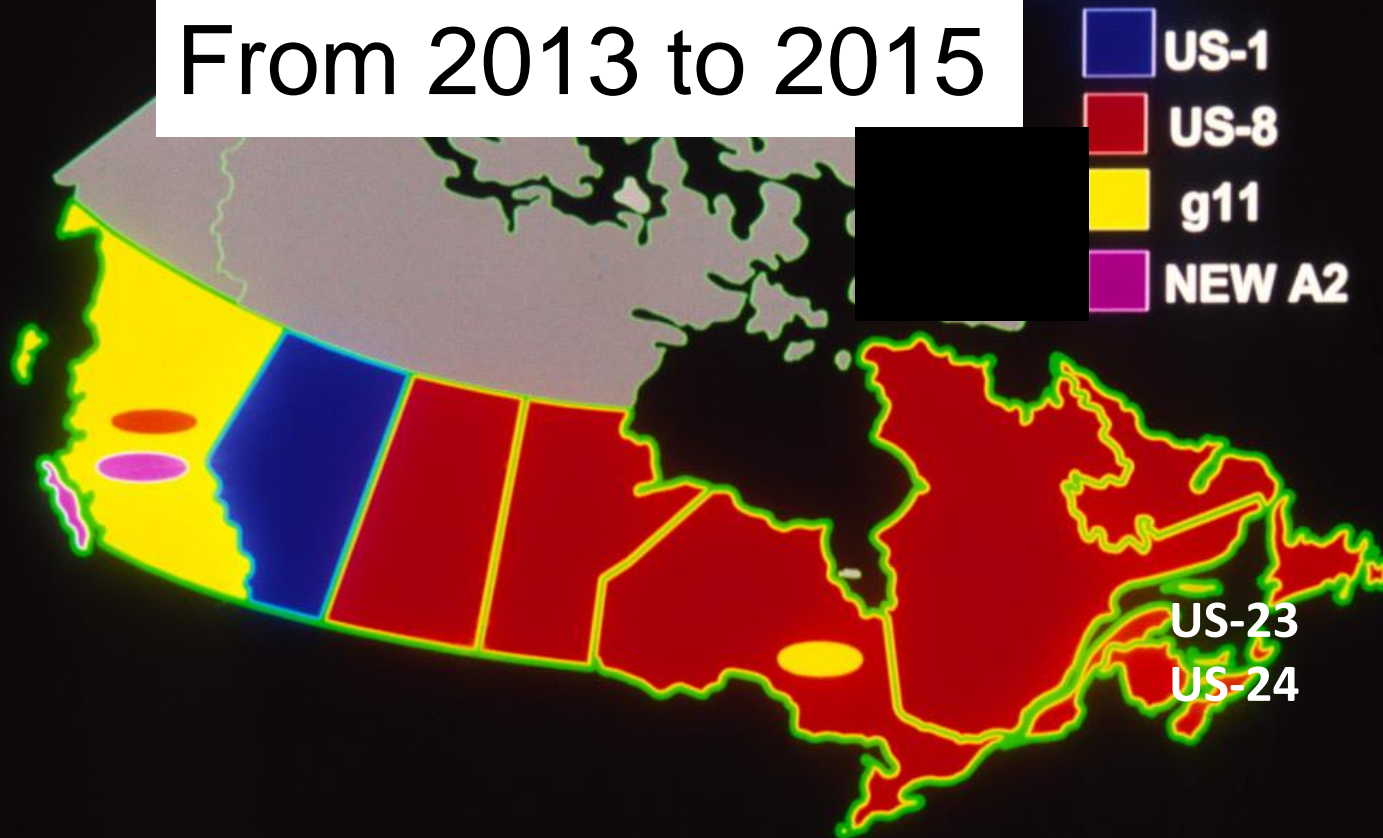
1995



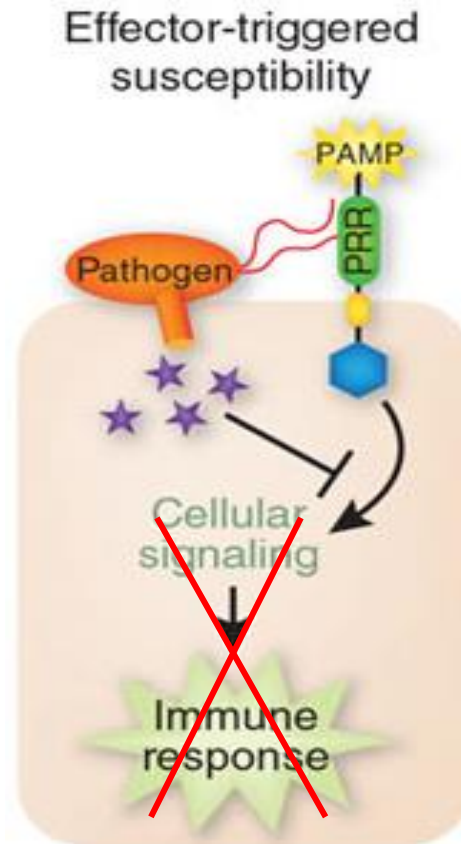
Rick Peters

DISTRIBUTION OF GENOTYPES OF *P. INFESTANS* IN CANADA

From 2013 to 2015

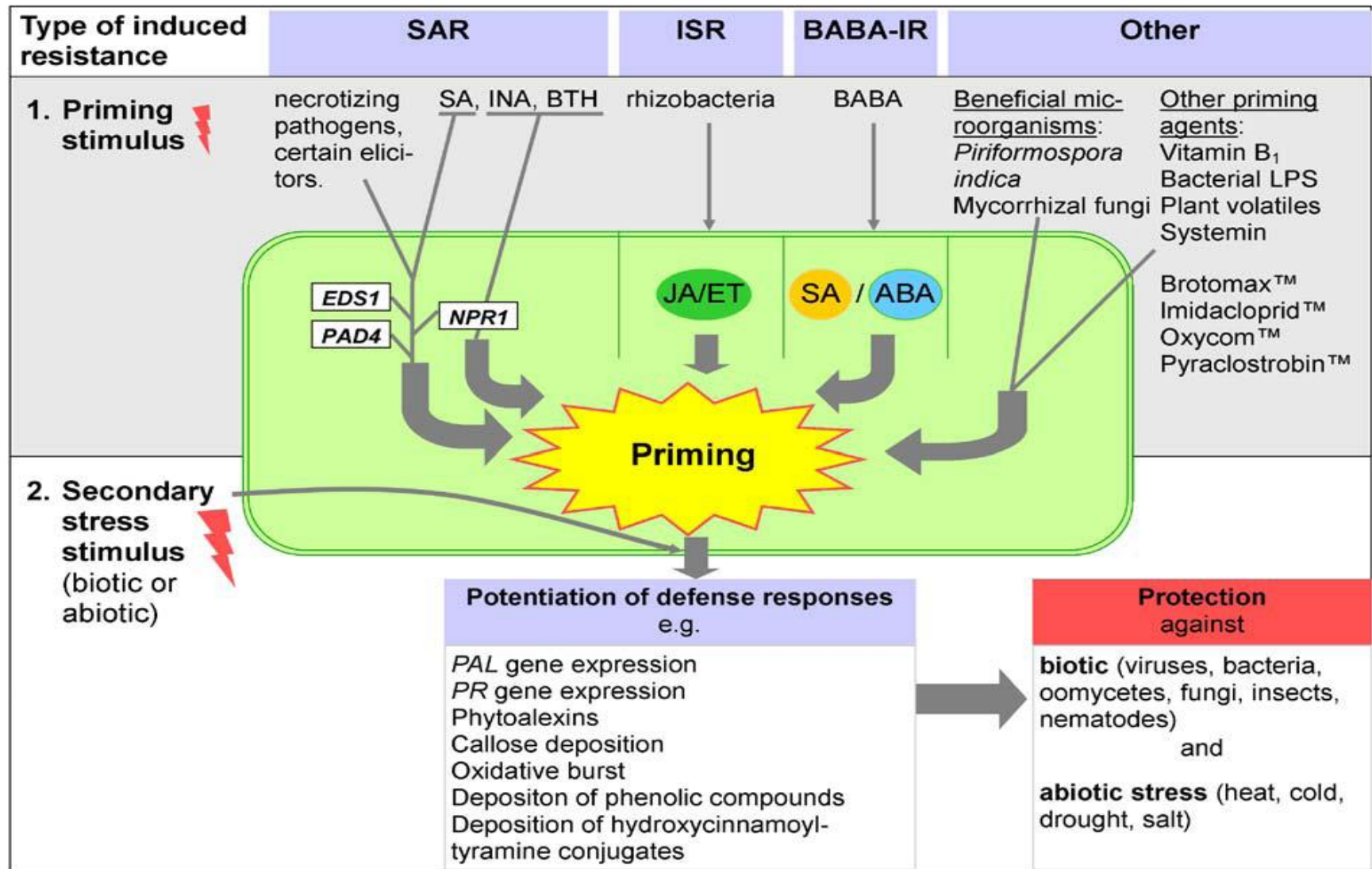


Cultivated potatoes are susceptible to *P. infestans*



(Pieterse *et al.*, Nature Chem. Biol. 2009, 5: 308-316)


Three major defense responses (SAR, ISR, BABA-IR) associated with induced resistance in plants (Goellner and Conrath, 2008)



Priming

 Phosphite as inducing agent

 ConfineTM:

 Phosphite: mono- and di-potassium salts of phosphorous acid (KH_2PO_3 and K_2HPO_3)

ConfineTM

WINFIELDTM
SOLUTIONS

CONFINETM

For the post - harvest treatment of russet – skinned potatoes and potatoes intended for processing for the suppression of late blight and pink rot storage infection.

GROUP	33	FUNGICIDE
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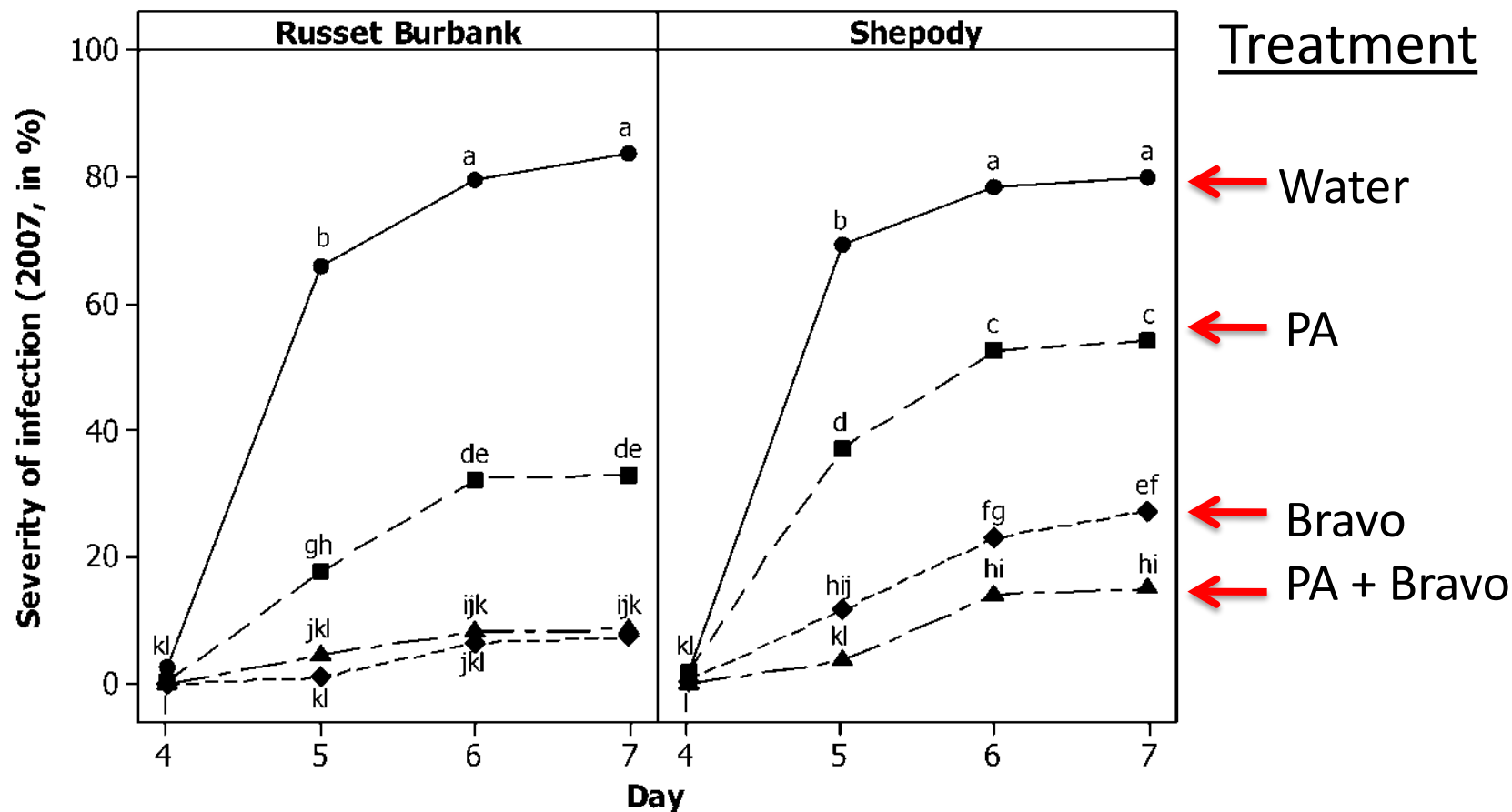
LIQUID
AGRICULTURAL

REGISTRATION NO. 29100 PEST CONTROL PRODUCTS ACT

GUARANTEE: Mono- and di-potassium salts of Phosphorous Acid 45.8%



Disease severity (%) in two cultivars in 2007, 2008, 2009



Field plots before harvest

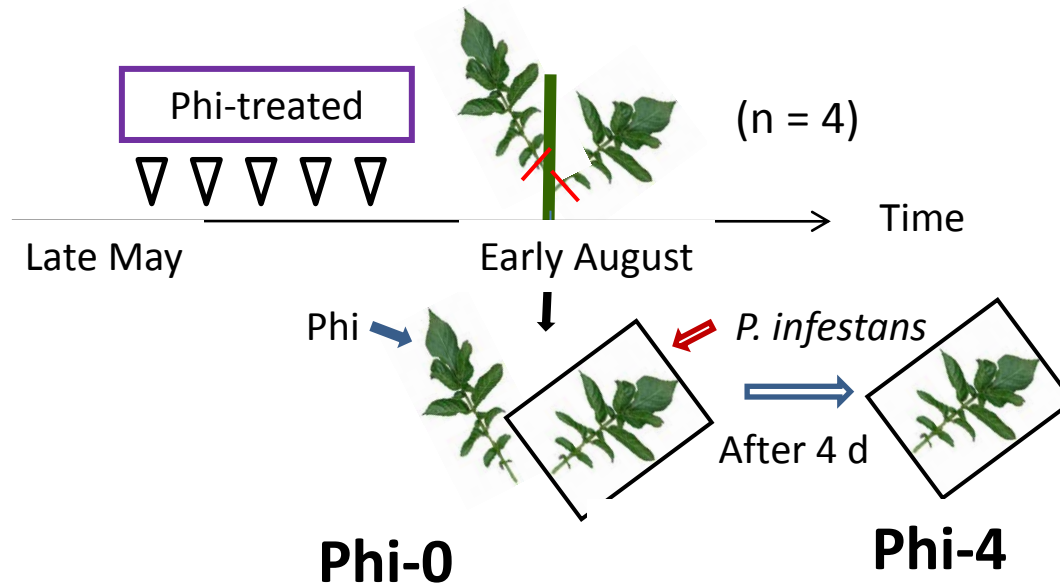
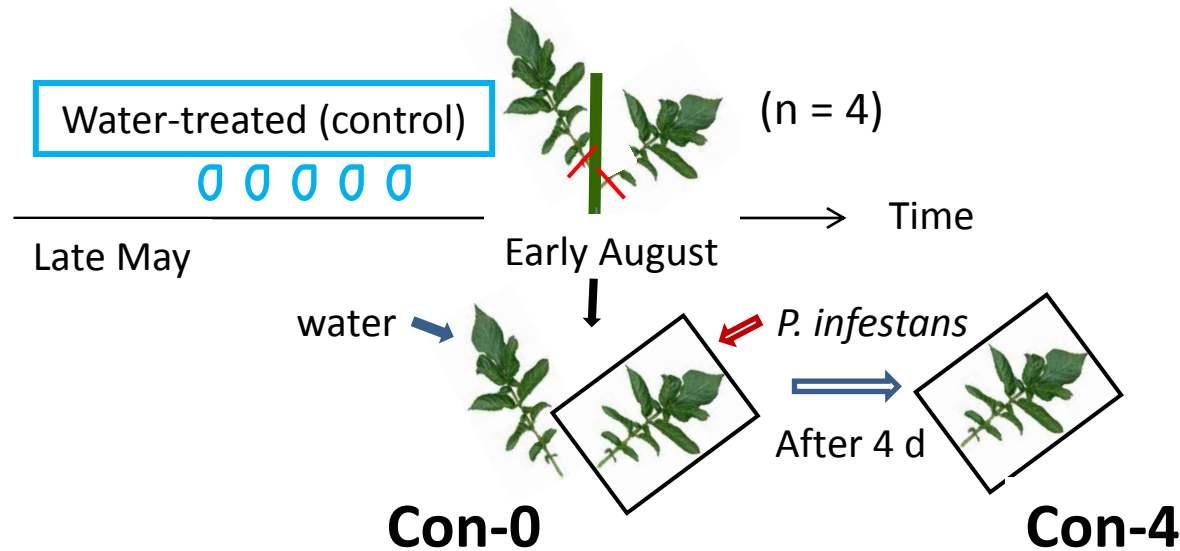


I. Comparative Proteomic Analysis





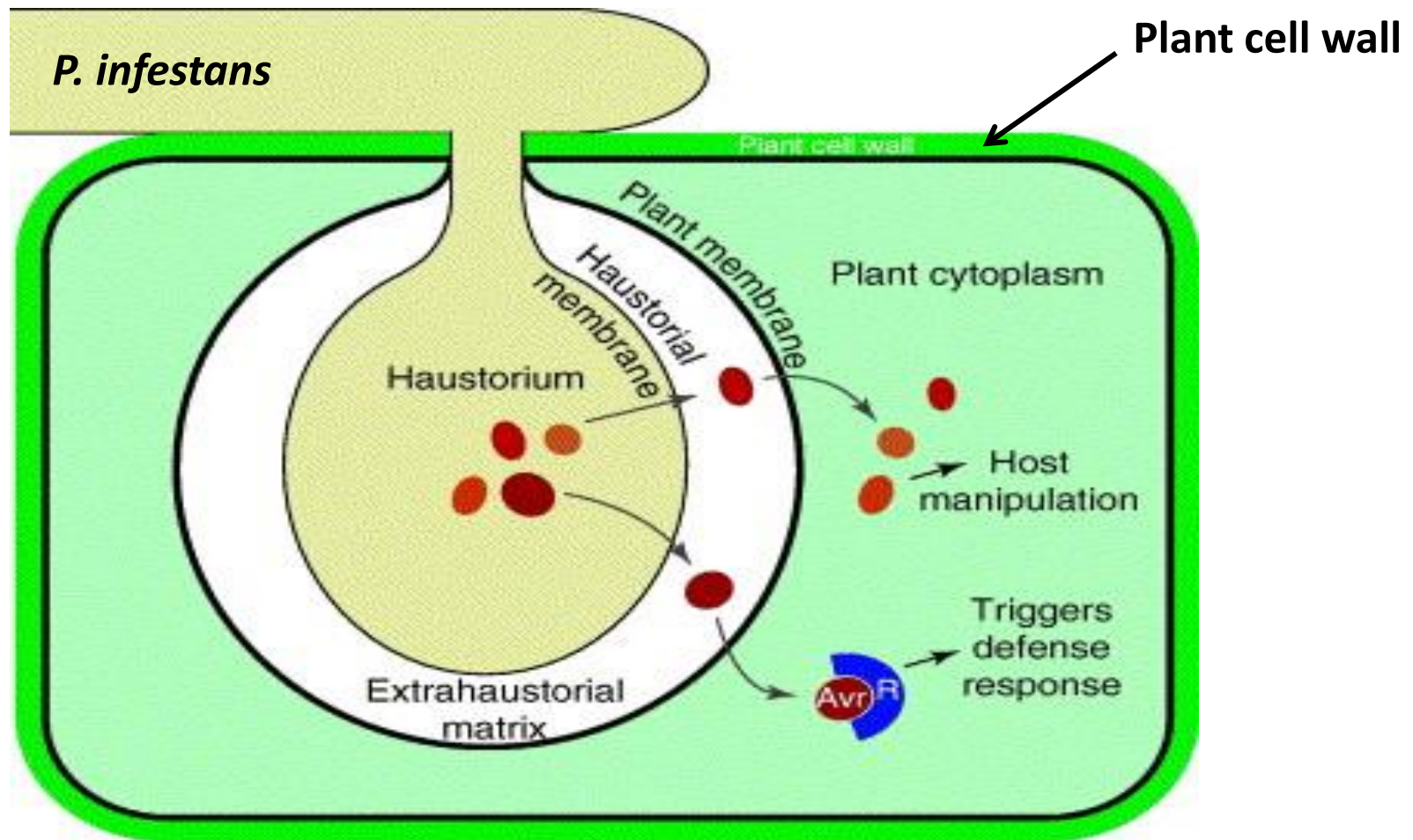
Field trials



4 leaves/rep
X 3 reps
X 4 treatments

Phi - 5.8 L product (Confine)/250 L water/ha – 5 times

Identification of cellular proteins induced by Phi



Protein Profiling in Potato (*Solanum tuberosum* L.) Leaf Tissues by Differential Centrifugation

Sanghyun Lim,^{†,‡} Kenneth Chisholm,[§] Robert H. Coffin,^{||} Rick D. Peters,[⊥] Khalil I. Al-Mughrabi,[#] Gefu Wang-Pruski,^{*,†} and Devanand M. Pinto[§]

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[‡]Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada

[§]National Research Council Institute for Marine Biosciences, Halifax, Nova Scotia, Canada

^{||}Cavendish Farms

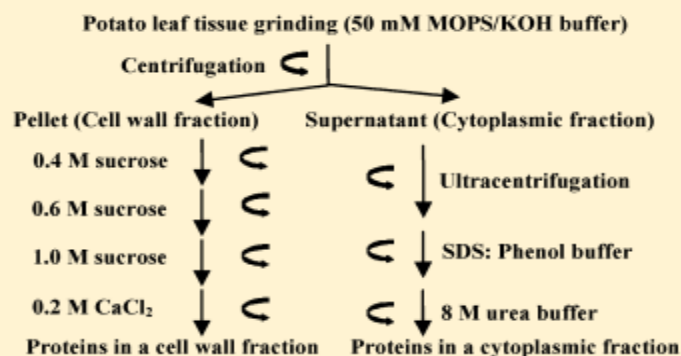
[⊥]Agriculture and Agri-Food Canada

[#]New Brunswick Department of Agriculture and Aquaculture

S Supporting Information

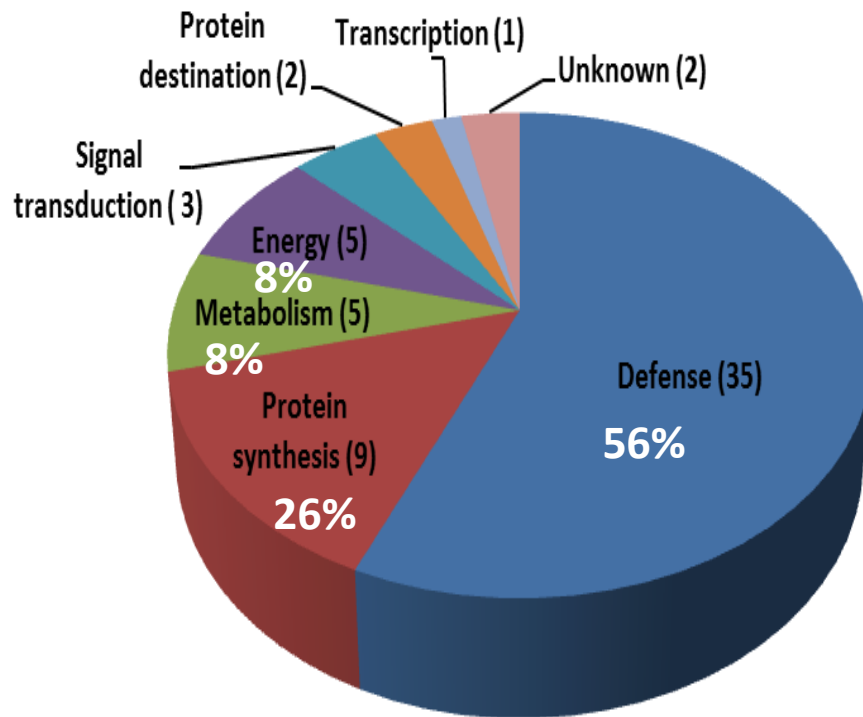
ABSTRACT: Foliar diseases, such as late blight, result in serious threats to potato production. As such, potato leaf tissue becomes an important substrate to study biological processes, such as plant defense responses to infection. Nonetheless, the potato leaf proteome remains poorly characterized. Here, we report protein profiling of potato leaf tissues using a modified differential centrifugation approach to separate the leaf tissues into cell wall and cytoplasmic fractions. This method helps to increase the number of identified proteins, including targeted putative cell wall proteins. The method allowed for the identification of 1484 nonredundant potato leaf proteins, of which 364 and 447 were reproducibly identified proteins in the cell wall and cytoplasmic fractions, respectively. Reproducibly identified proteins corresponded to over 70% of proteins identified in each replicate. A diverse range of proteins was identified based on their theoretical pI values, molecular masses, functional classification, and biological processes. Such a protein extraction method is effective for the establishment of a highly qualified proteome profile.

KEYWORDS: potato leaf, protein profiling, differential centrifugation, reproducibility, cell wall and cytoplasmic fractions

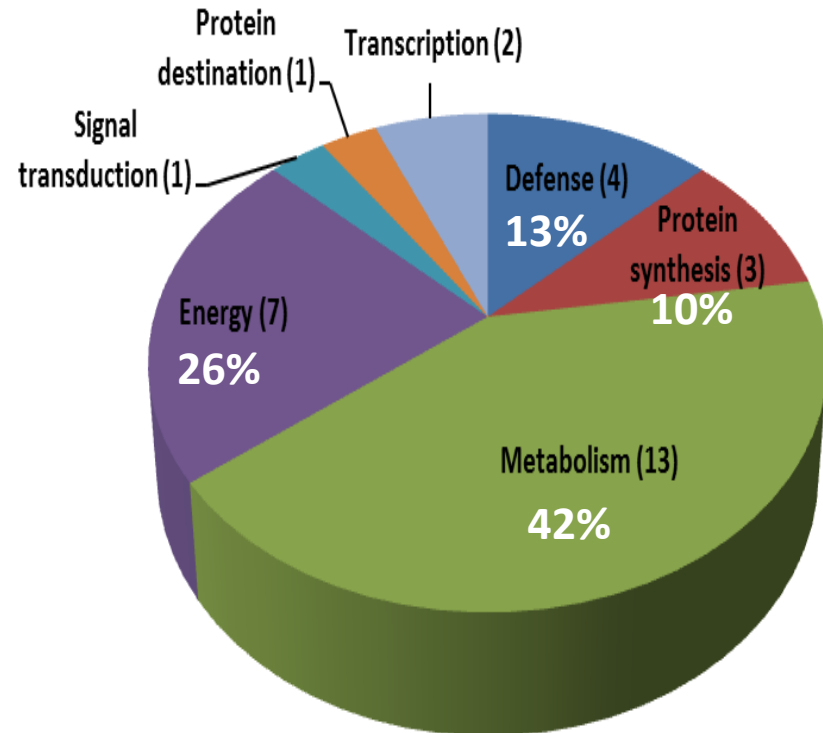


Classification of 93 up- or down-regulated proteins (93 of 1172 potato leaf proteins, $p < 0.05$)

62 up-regulated proteins



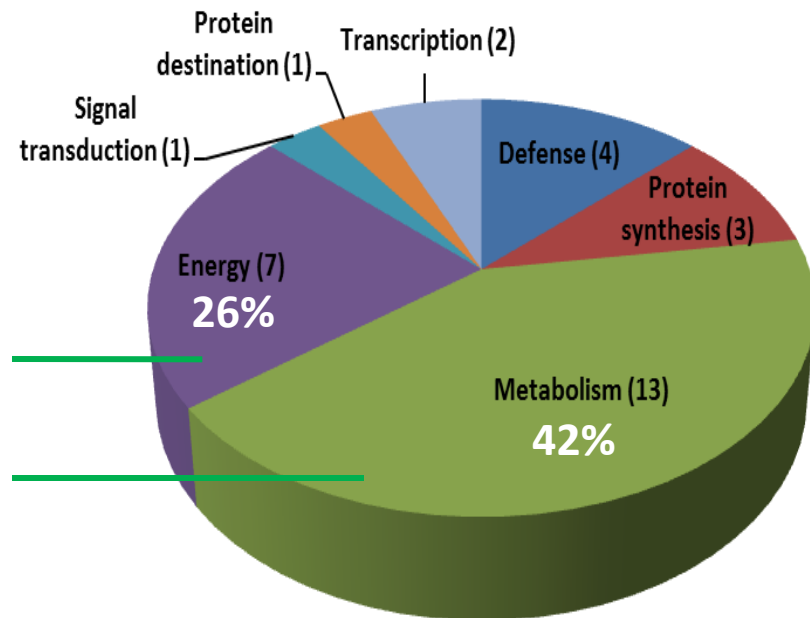
31 down-regulated proteins



Classification of down-regulated proteins

(The ratio of Phi-0/Con-0 <0.75 fold change)

31 down-regulated proteins in Phi-treated sample

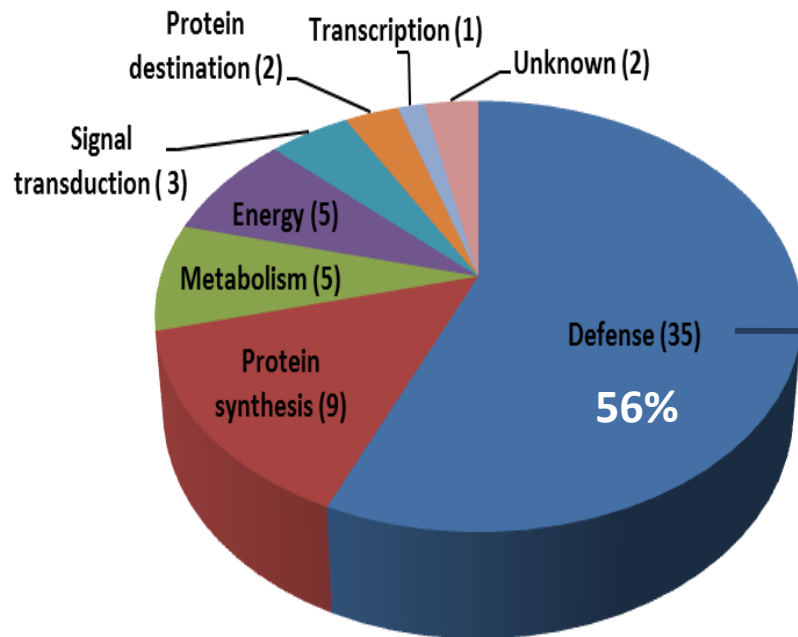


Glycolysis
Photosynthesis
Starch & sucrose metabolism
Amino acid metabolism

Classification of up-regulated proteins

(The ratio of Phi-0/Con-0 >1.4 fold change)

62 up-regulated proteins in Phi-treated sample



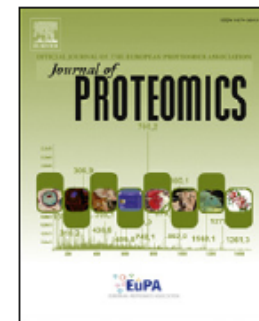
Antifungal activity
SA (salicylic acid)
ROS (reactive oxygen species)
HR (hypersensitive response)



Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jprot



Proteomics analysis suggests broad functional changes in potato leaves triggered by phosphites and a complex indirect mode of action against *Phytophthora infestans*☆

Sanghyun Lim^{a,b,1}, Tudor Borza^a, Rick D. Peters^c, Robert H. Coffin^d, Khalil I. Al-Mughrabi^e, Devanand M. Pinto^f, Gefu Wang-Pruski^{a,*}

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^bDepartment of Biology, Dalhousie University, Halifax NS B3H 4R2, Canada

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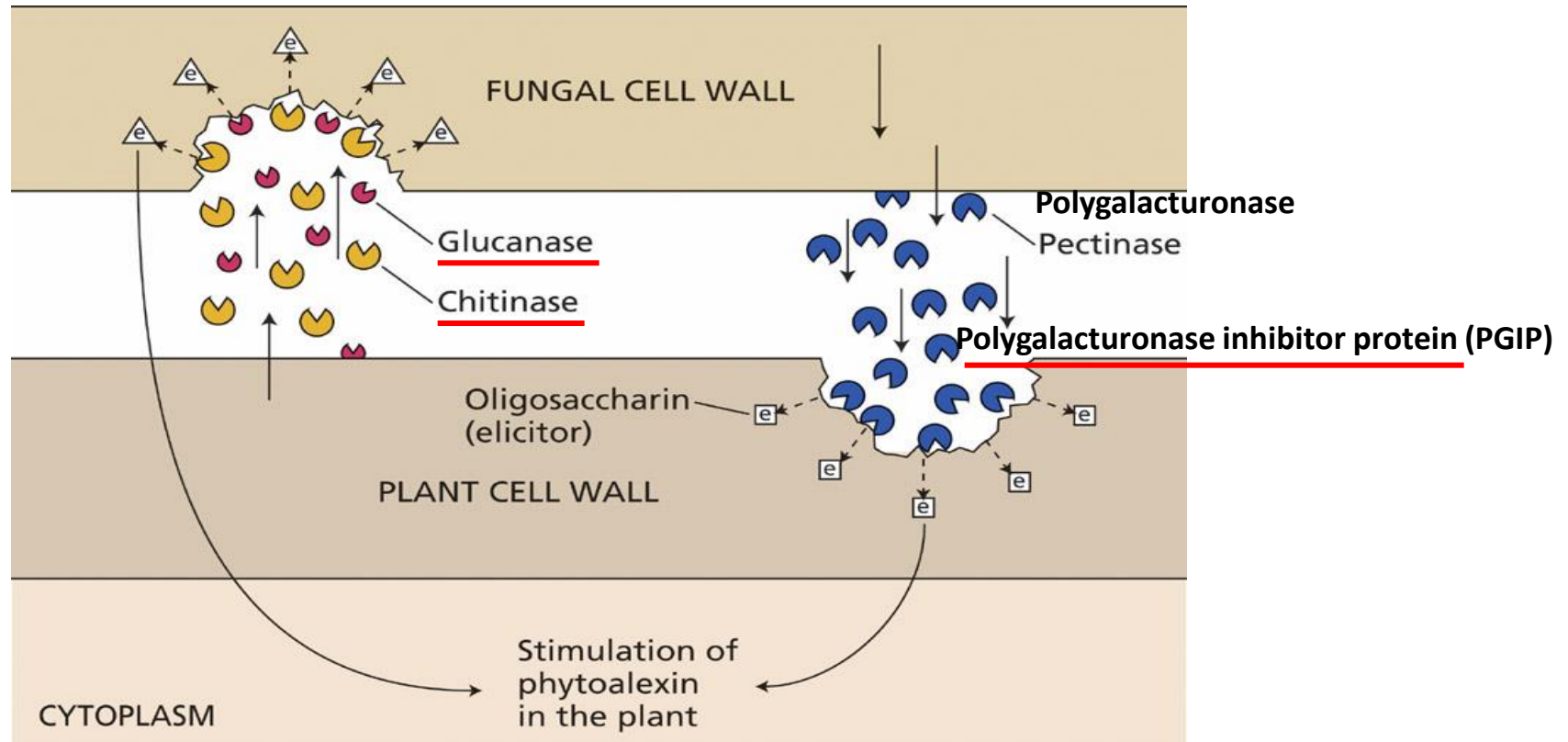
^dCavendish Farms, Kensington PE C0B 1M0, Canada

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^fNational Research Council Institute for Marine Biosciences, Halifax NS B3H 3Z1, Canada

Proteins related to antifungal activity

 : up-regulated proteins in this study



(Taiz and Zeiger, Plant Physiology 5th ed., 2010, Chap. 15)

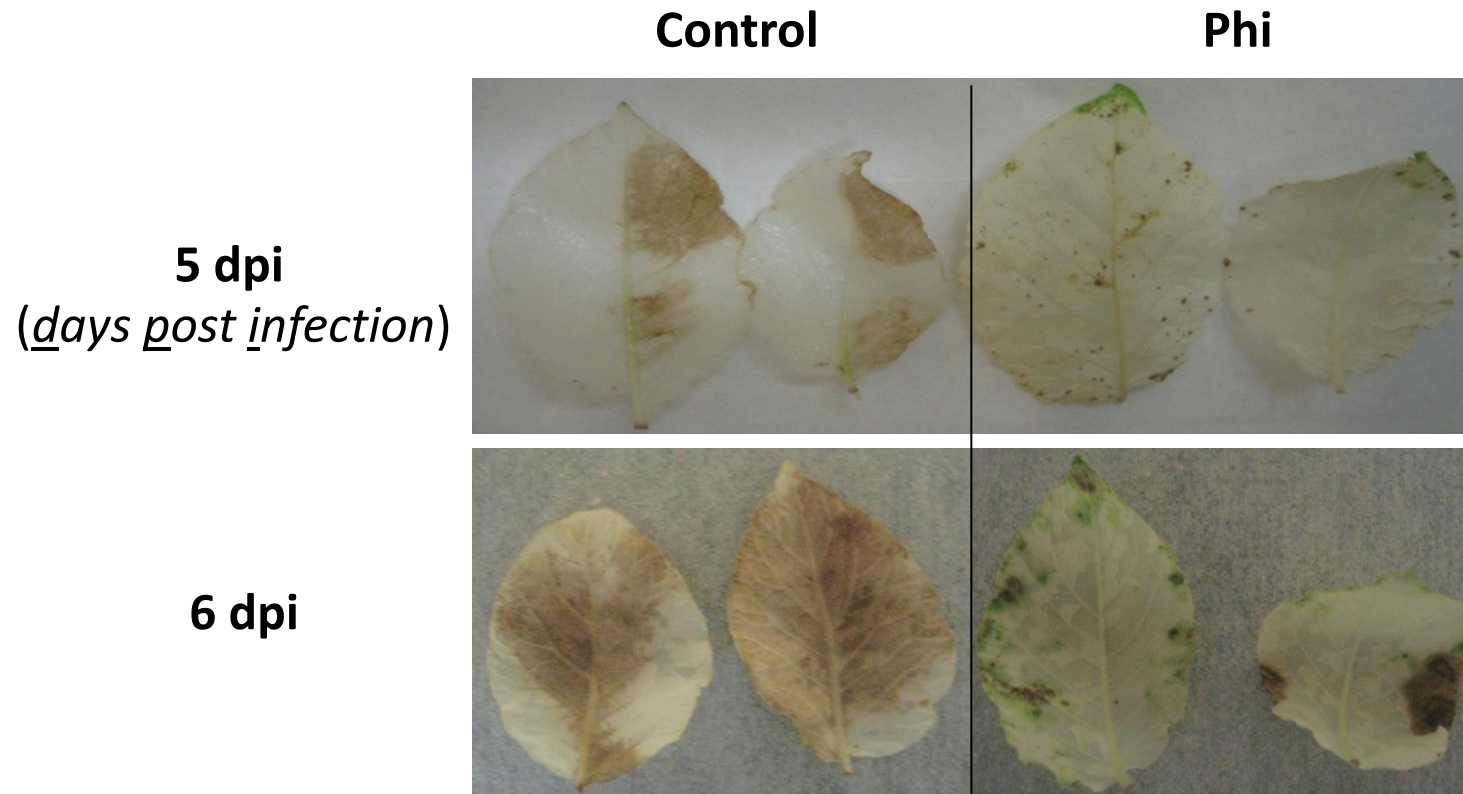
Glucanases: **TC173865**
TC163195

Chitinases: **TC168318**
CK263954
TC168794

PGIP: **TC172573**

The observation of H₂O₂ accumulation

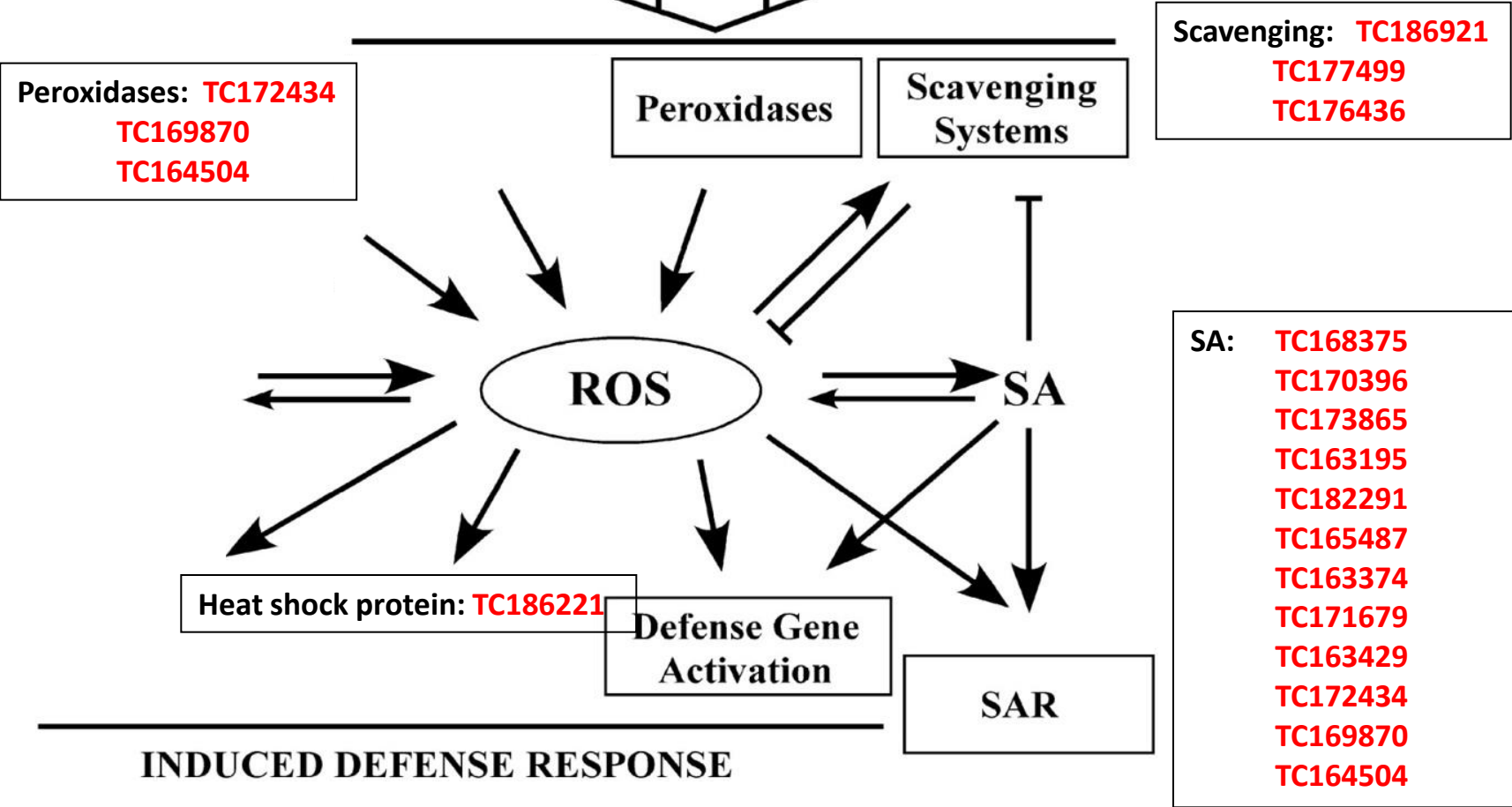
- H₂O₂ accumulation (ROS) is localized at the infection sites



Proteins related to ROS generation and functions

(In this study)

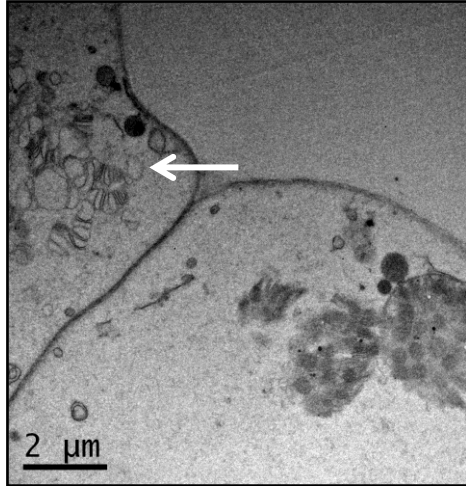
Phi treatment



Observation of ultrastructural changes related HR in cells of infected Phi-treated leaves by TEM

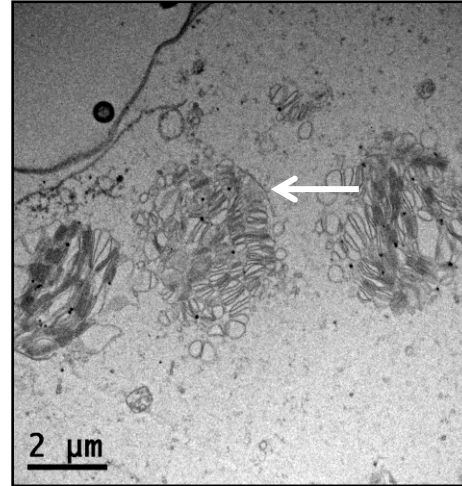
(TEM, Transmission Electron Microscopy)

(c)



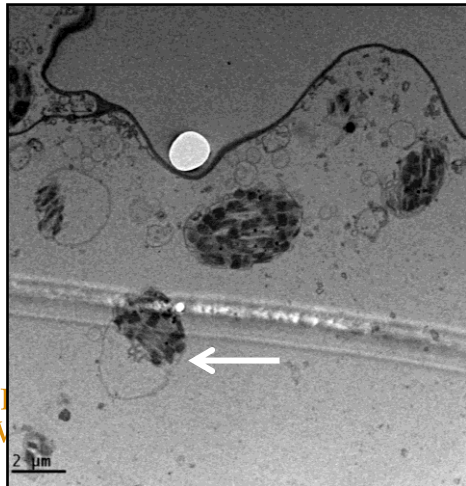
Con 5 dpi
sample

(e)



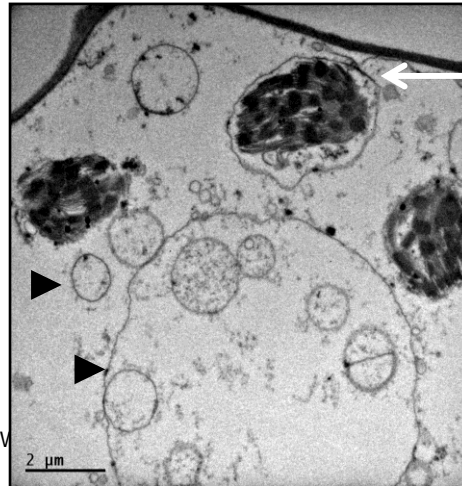
disrupted chloroplasts

(d)



Phi 5 dpi
sample

(f)



remain intact chloroplasts
enveloped chloroplasts

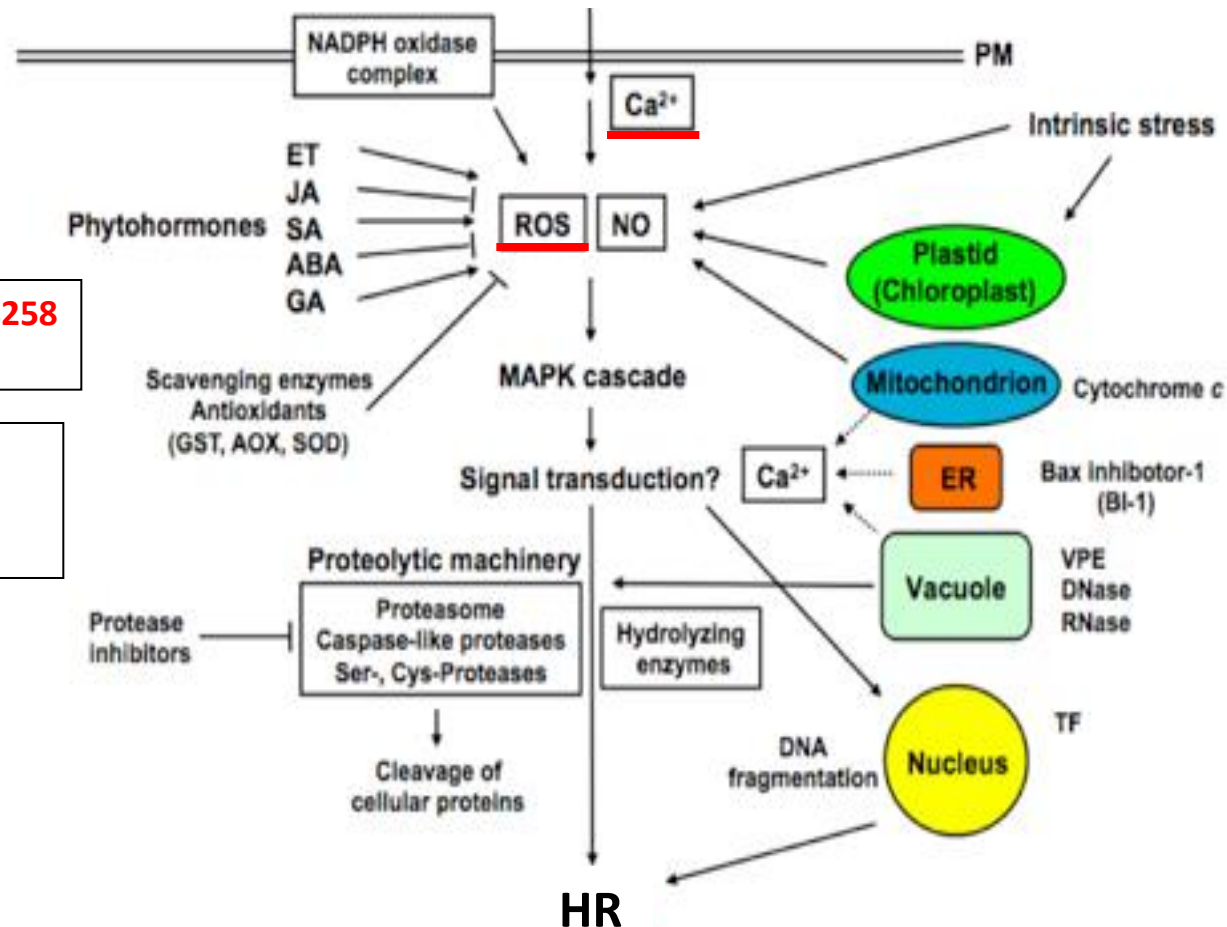
vacuolization

Proteins related to hypersensitive response in potato leaves

(In this study)

— : up-regulated proteins

Phi treatment



Calmodulins: **TC168258**
TC166307

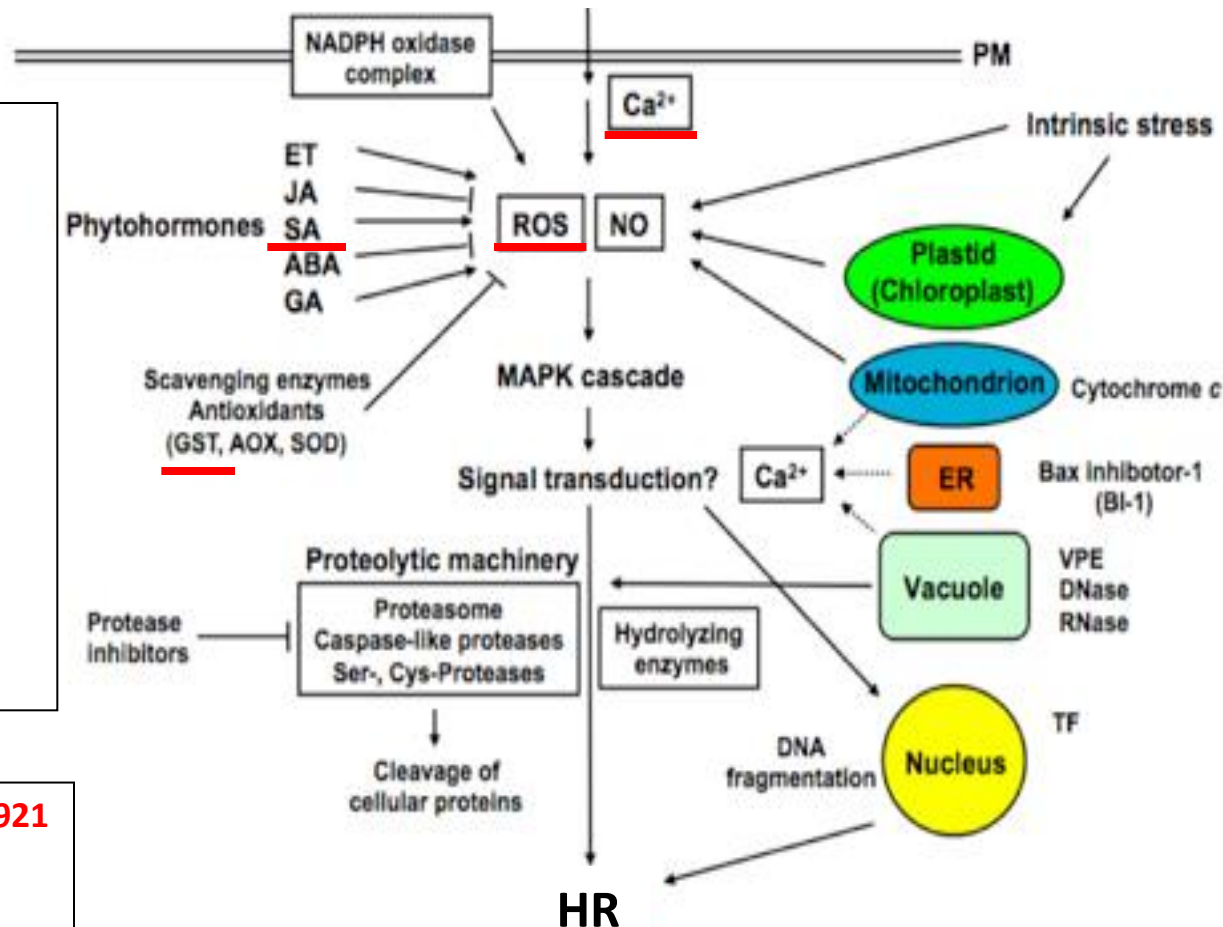
ROS: **TC172434**
TC169870
TC164504

Proteins related to hypersensitive response in potato leaves

(In this study)

— : up-regulated proteins

Phi treatment



SA: TC168375
TC170396
TC173865
TC163195
TC182291
TC165487
TC163374
TC171679
TC163429
TC172434
TC169870
TC164504
TC163769

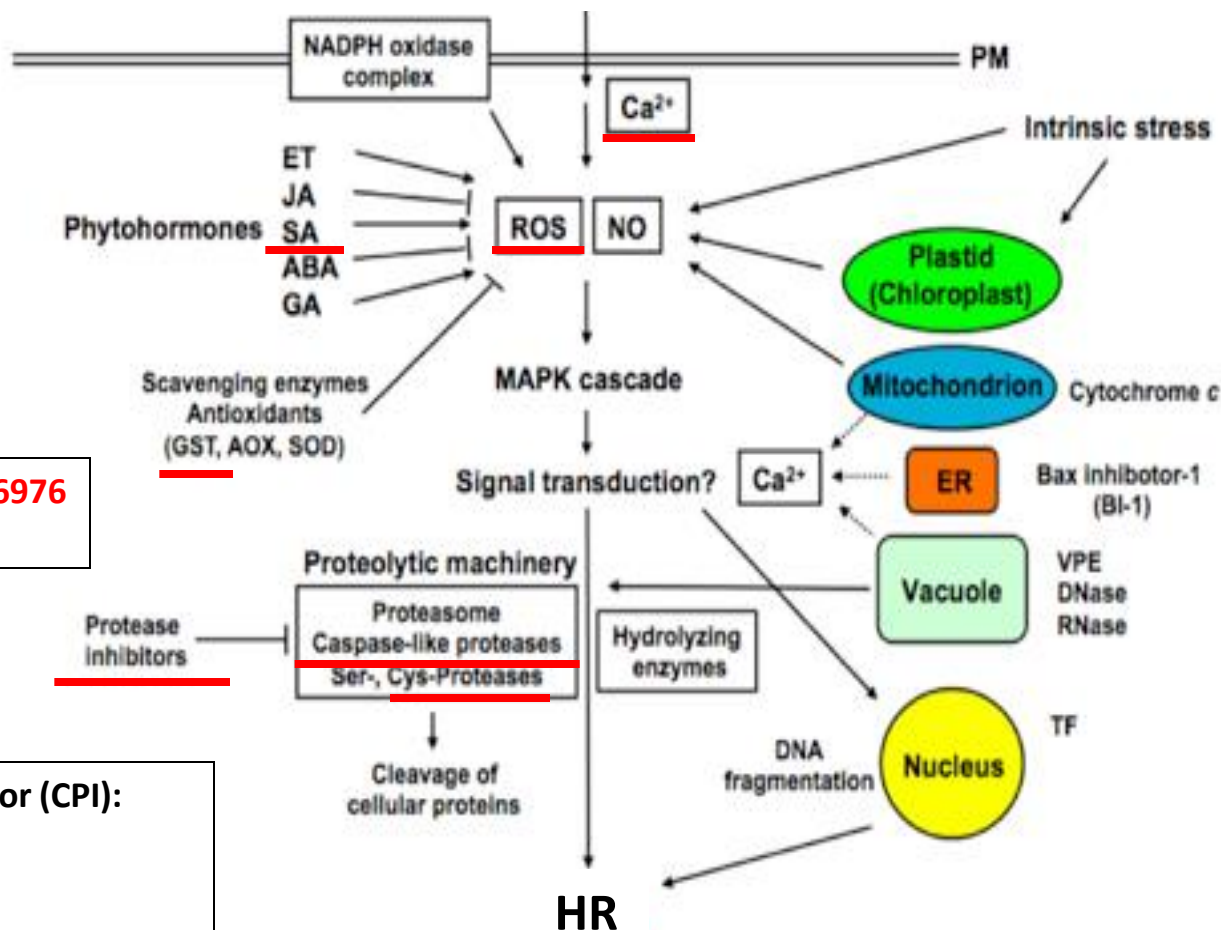
Scavenging: TC186921
TC177499
TC176436

Proteins related to hypersensitive response in potato leaves

(In this study)

— : up-regulated proteins

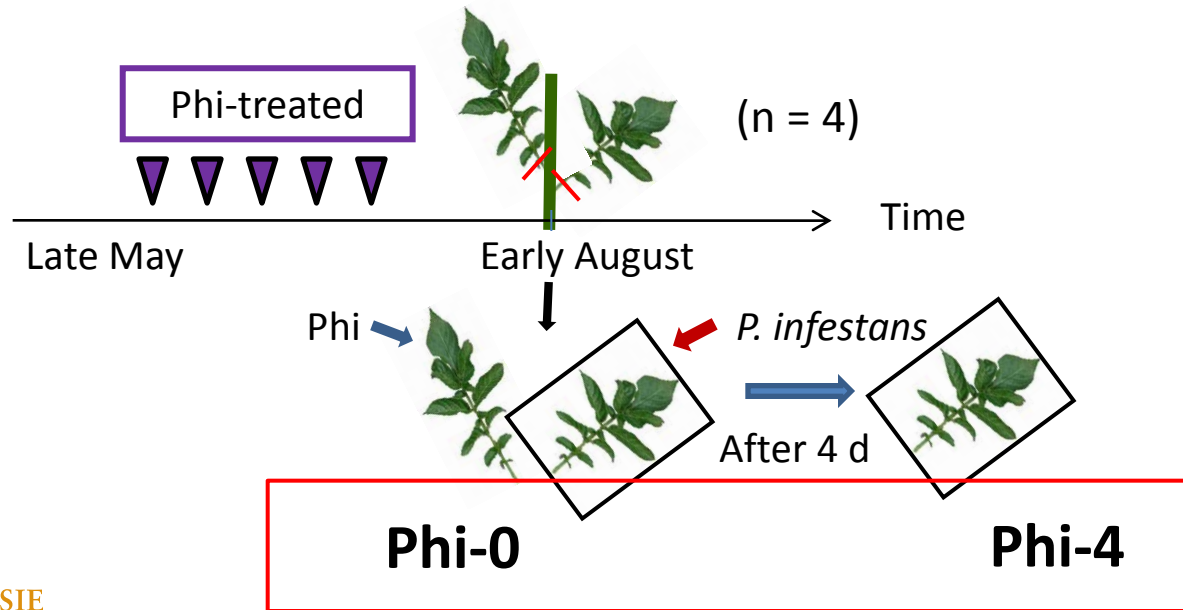
Phi treatment



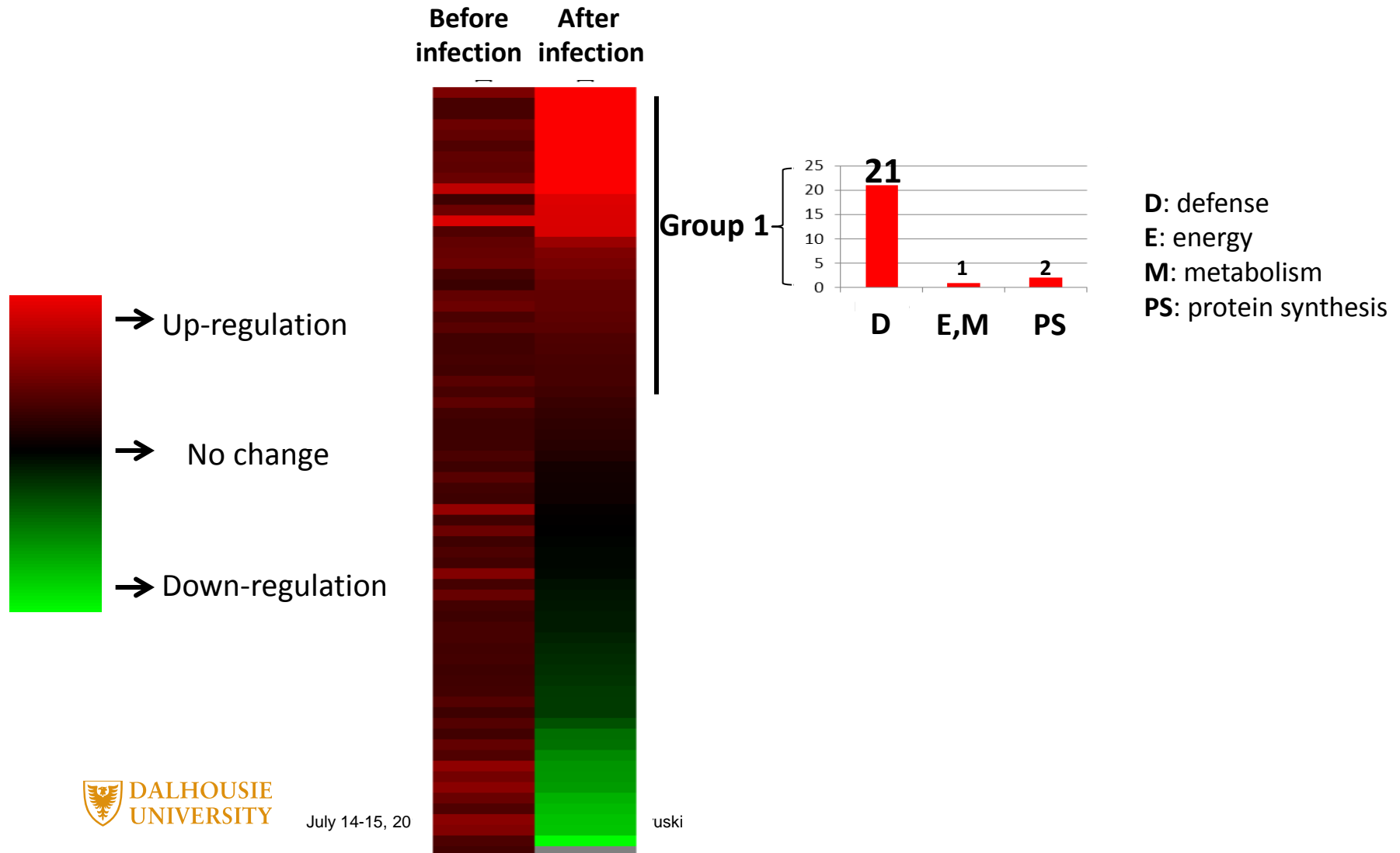
Cys-protease: **TC176976**
TC172593

Cys-protease inhibitor (CPI):
TC181645
TC166886
TC166762

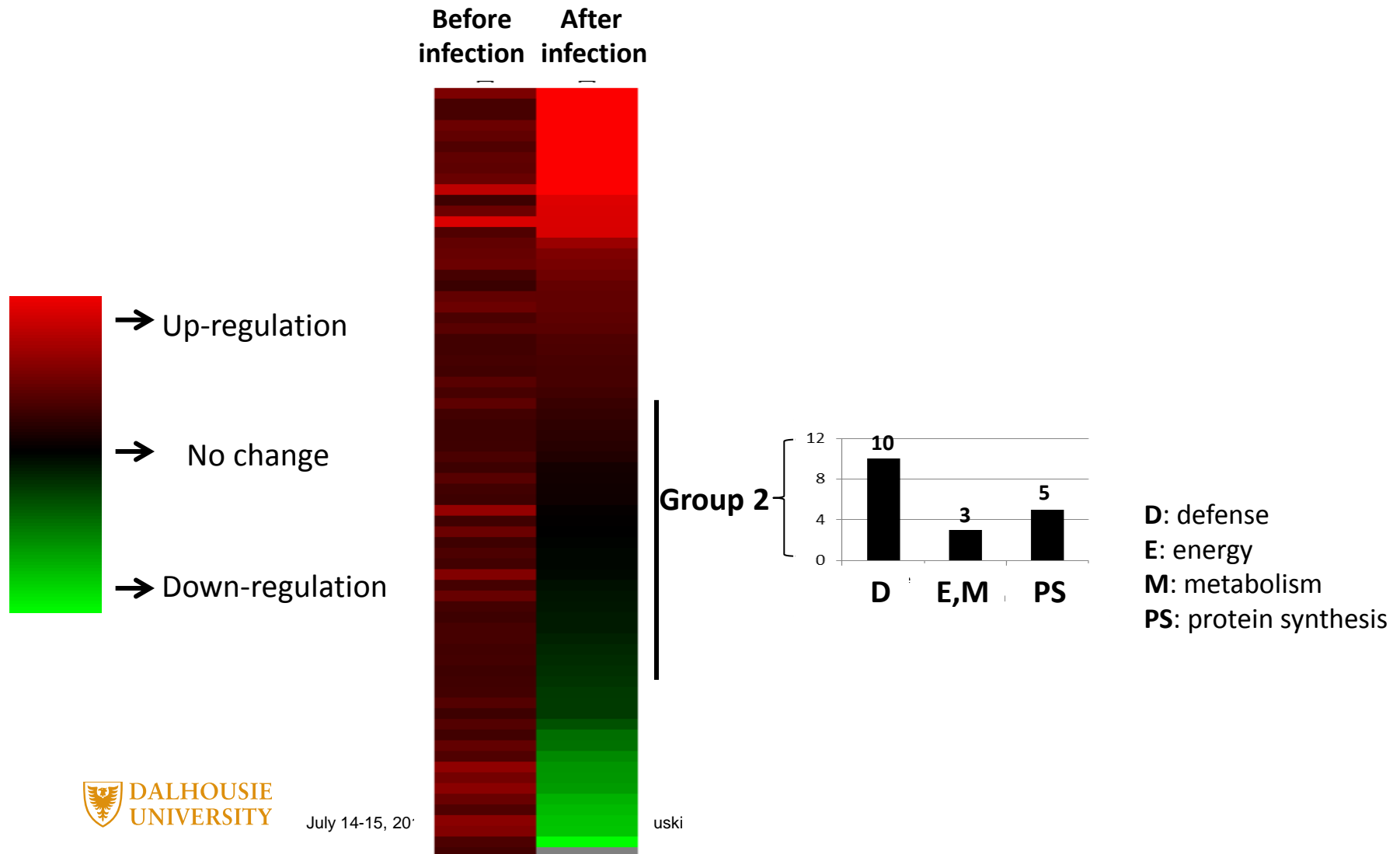
After infection with *P. infestans*, how does the abundance of the proteins change?



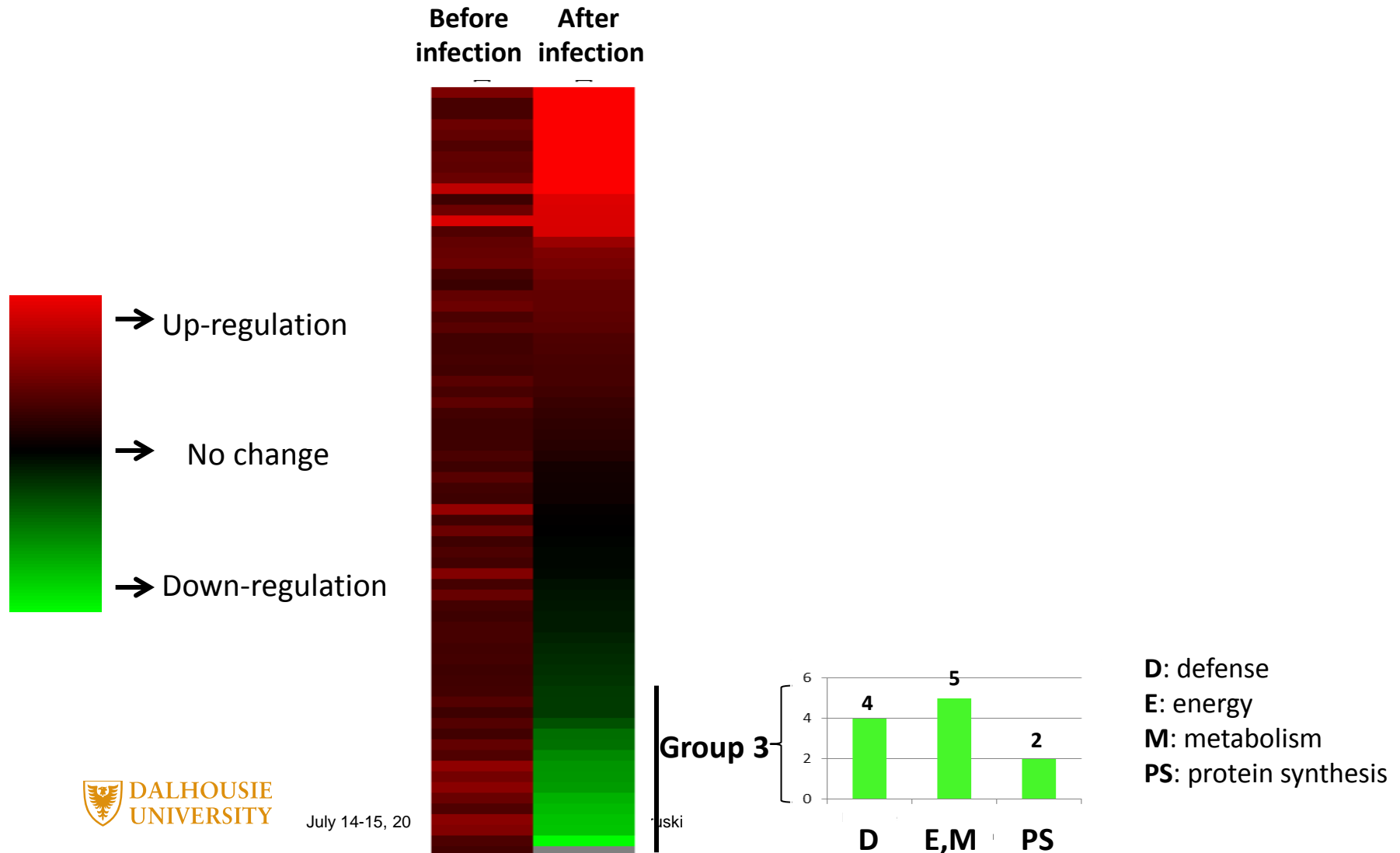
Changes in abundance of 62 up-regulated proteins in Phi-treated plants after infection



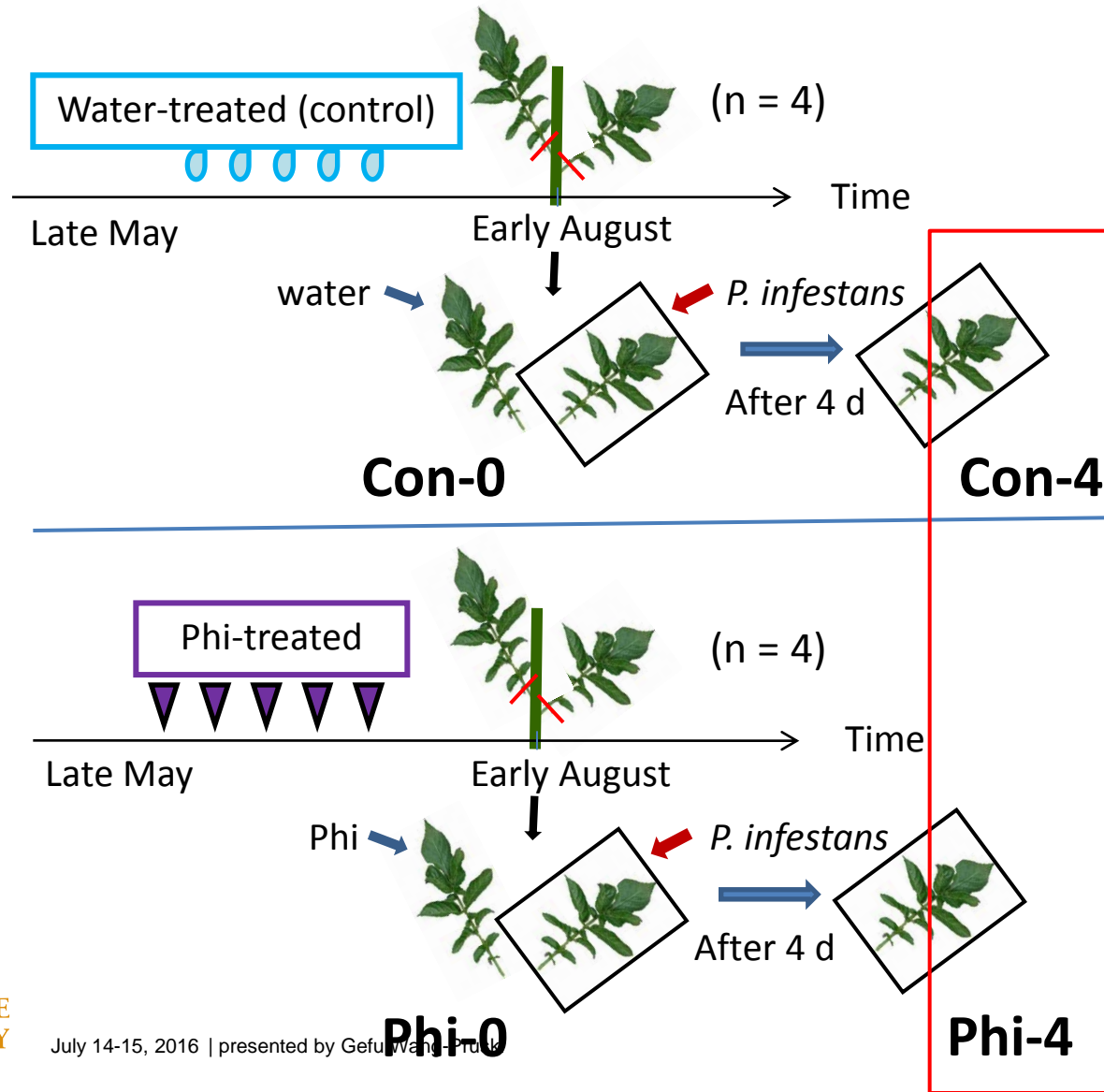
Changes in abundance of 62 up-regulated proteins in Phi-treated plants after infection



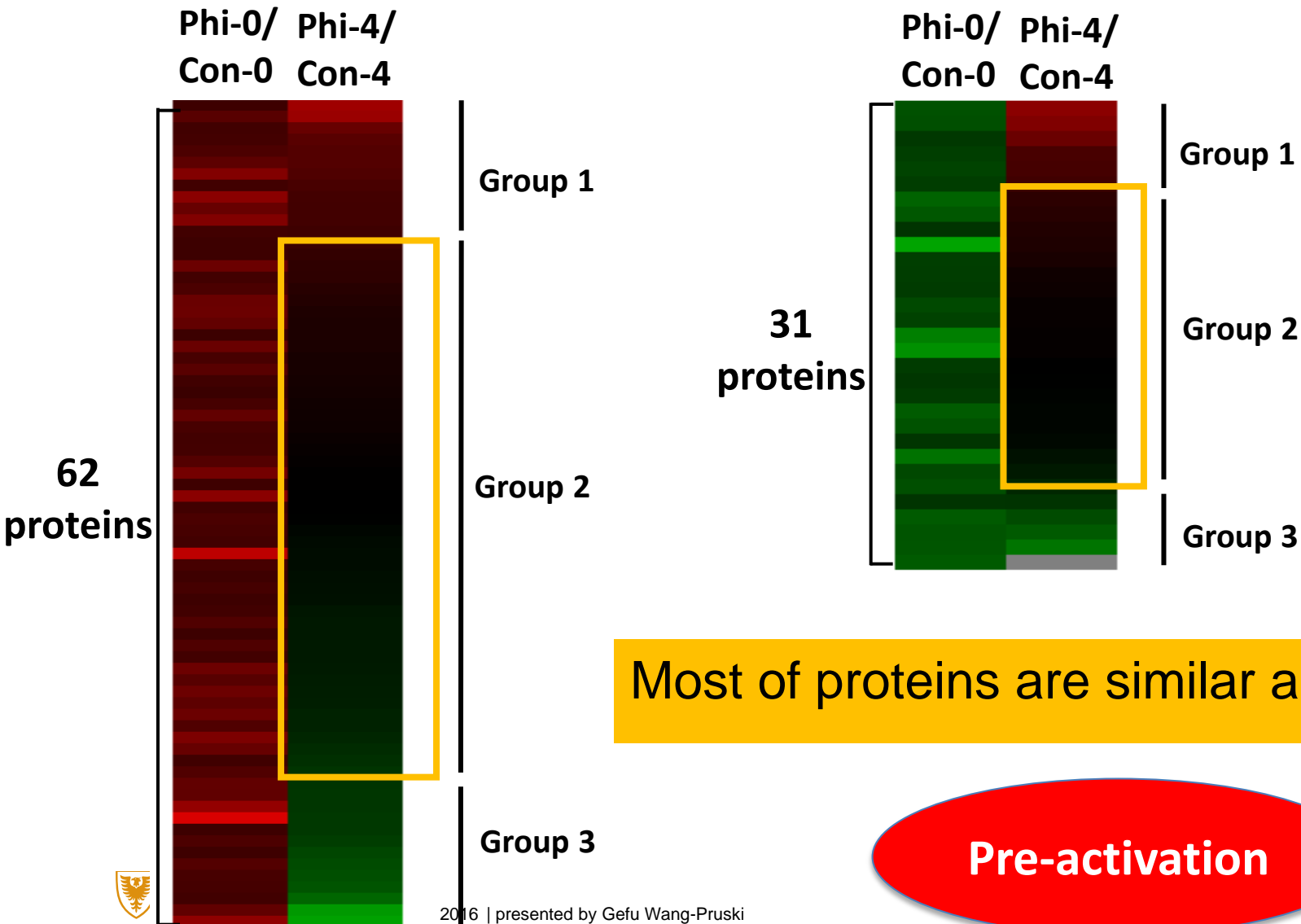
Changes in abundance of 62 up-regulated proteins in Phi-treated plants after infection



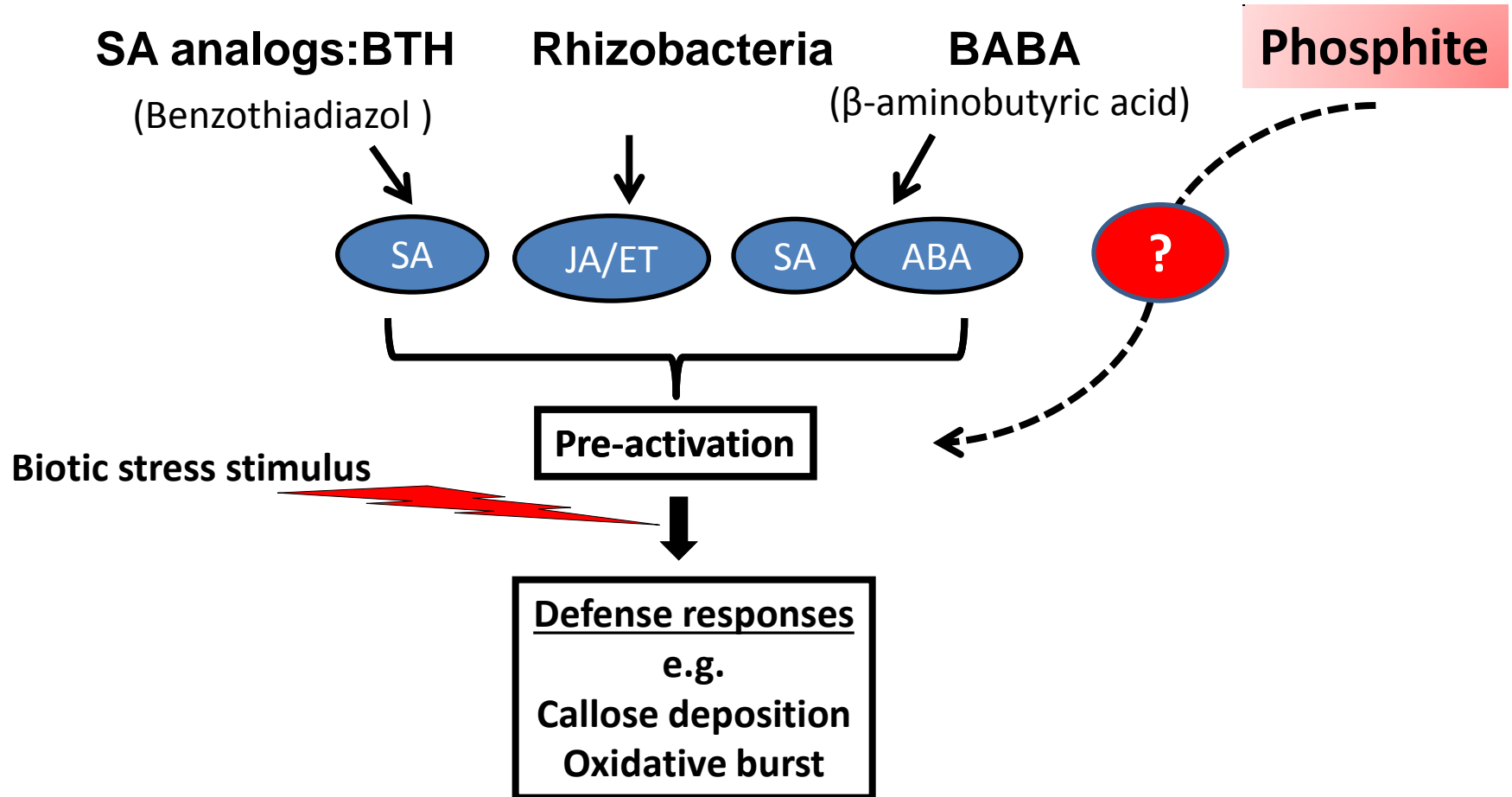
After 4 dpi, how does differential abundance of the proteins change in infected control and Phi-treated samples?



Differential abundance of the proteins in Phi-treated and control plants at 4 dpi

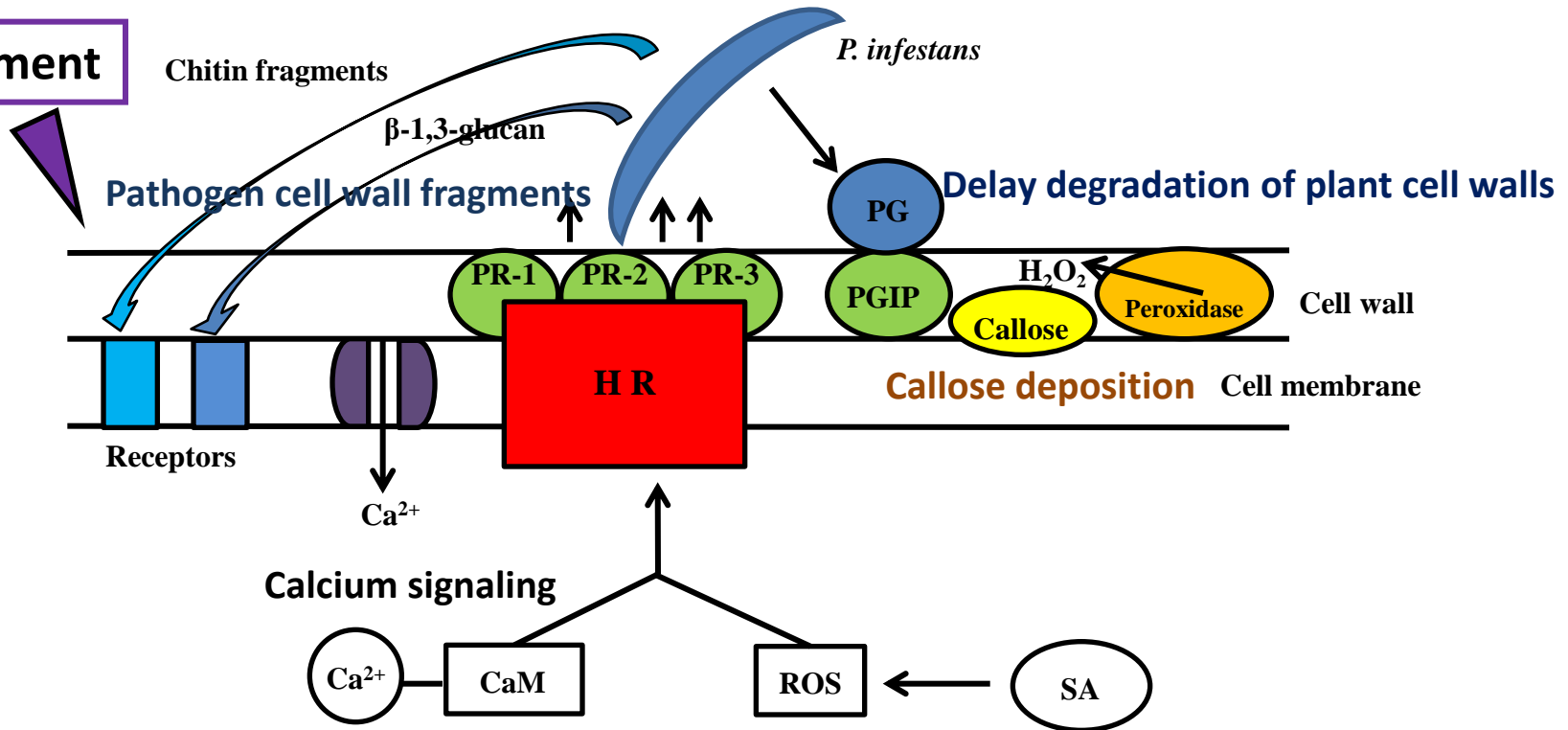


Induced resistance



Model for Phi-induced resistance after infection

Phi treatment



II. Transcriptomic Profiling by Next Generation Sequencing (NGS)

A total of **181,054,327** sequence reads were generated by NGS.

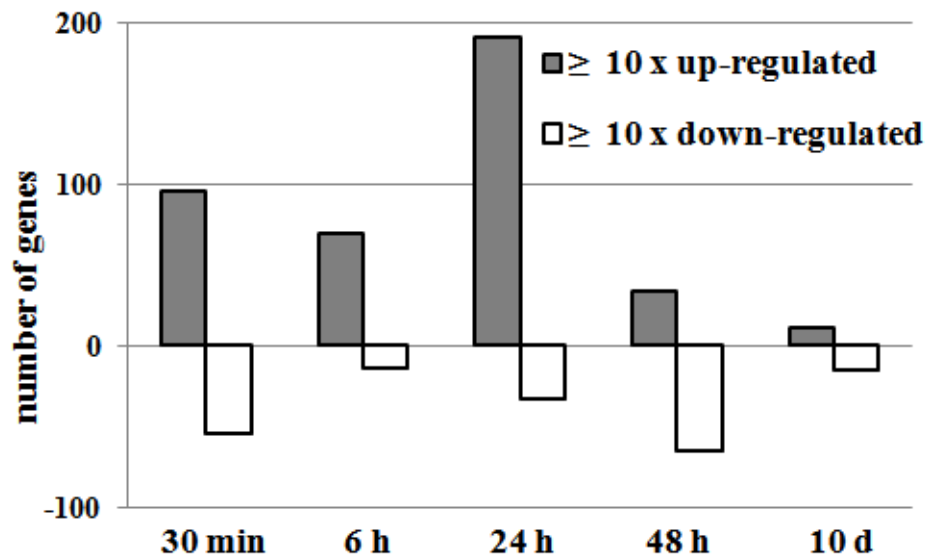
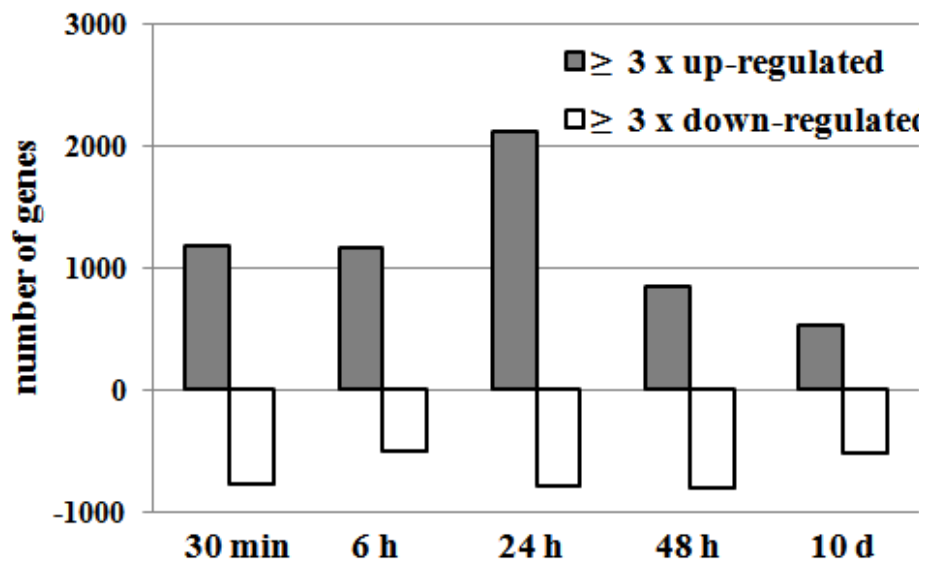
NGS - distribution of reads/library

Sample/cDNA library	Number of reads per sample
Confine treated 30 min	22,774,574
Control 0 h	18,398,449
Confine-treated 6 h	15,400,067
Control 6 h	19,911,440
Confine-treated 24 h	20,680,751
Control 24 h	22,220,249
Confine-treated 48 h	16,586,847
Control 48 h	12,288,124
Confine-treated 10 days	15,221,166
Control 10 days	17,572,660
Total	181,054,327

NGS - # of genes up- and down- regulated by phosphites

Differential gene expression

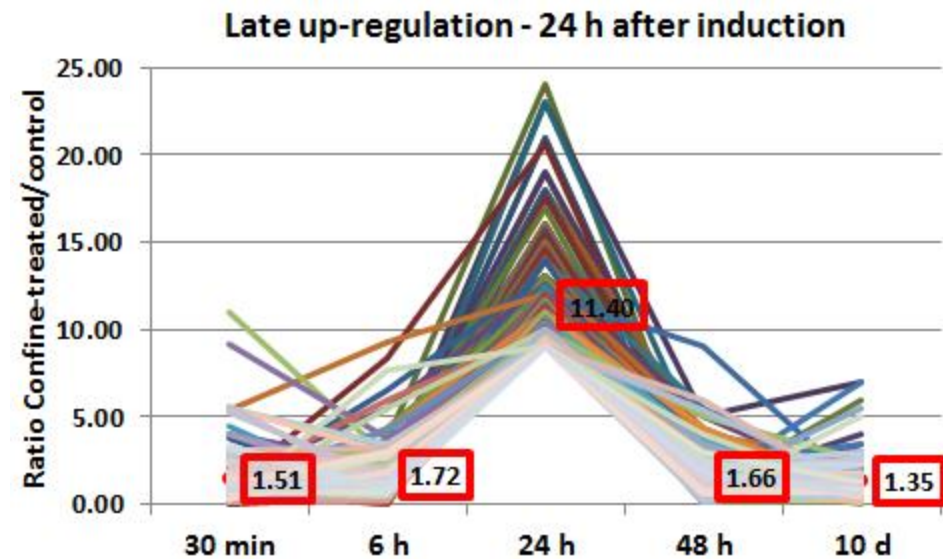
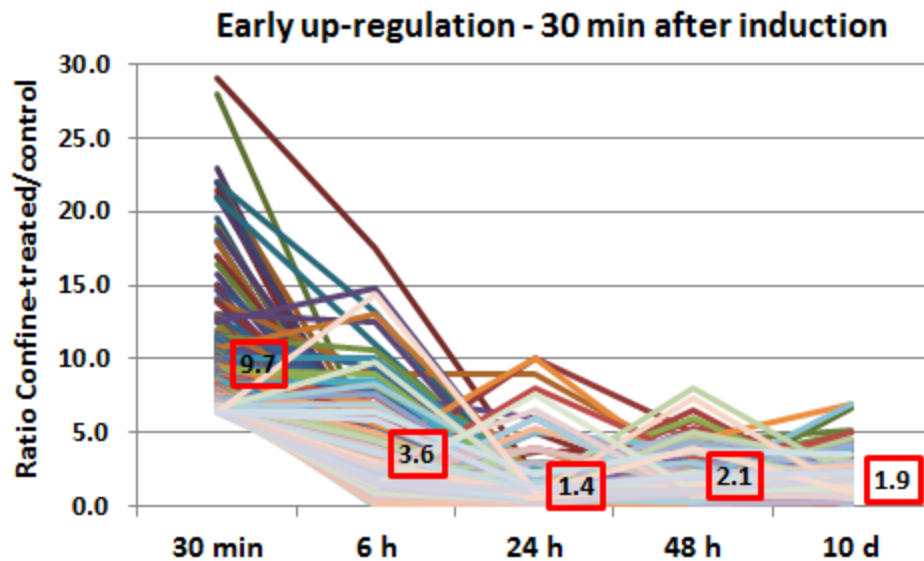
- Pairwise Confine-treated vs control analysis
- Across time points (global) Confine-treated vs control analysis



Expression patterns triggered by phosphites

Differential gene expression

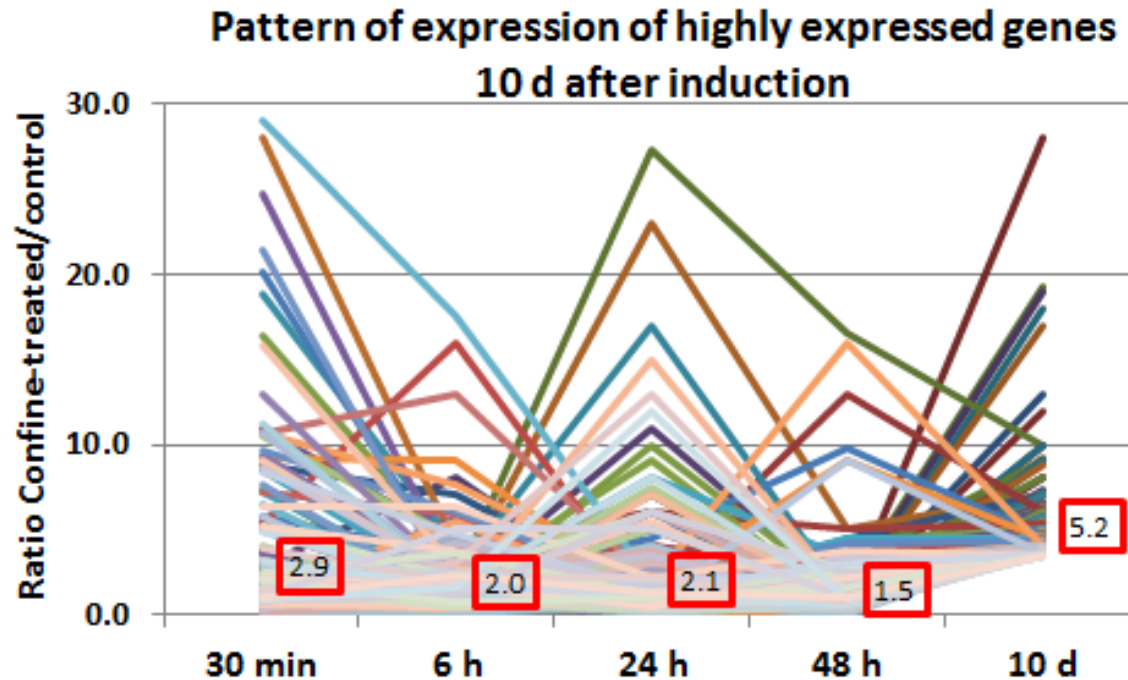
- early response - minutes to 24 h
- late response - ≥ 24 h



Expression patterns triggered by phosphites

Differential gene expression

- 10 days after induction – no clear pattern



Differential gene expression validation

qPCR vs NGS

Group 1: Energy production (phosphate transfer)

Group 2: Starch and sucrose metabolism

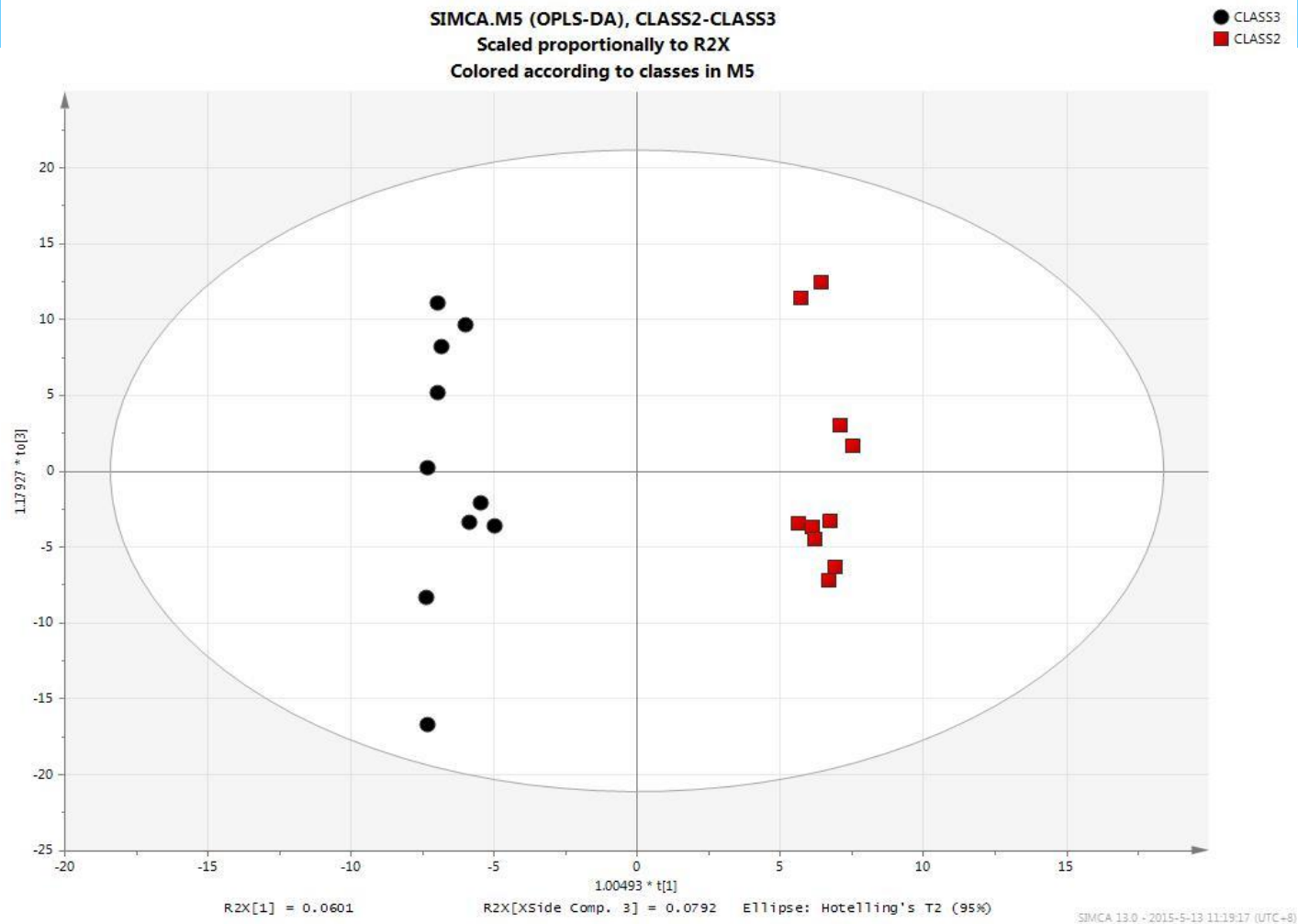
Group 3: Plant defense mechanisms, including the salicylate, jasmonic and ethylene signaling pathways.

How many genes are activated?

A wide array of functions are activated: signalling pathways, genes involved in defense mechanisms, carbohydrate metabolisms and energy production.

III. Metabolic profiling in phosphite treated potato leaves

- GC/TOFMS analysis was performed using an Agilent 7890 gas chromatograph system coupled with a Pegasus HT time-of-flight mass spectrometer.
- A total of 752 peaks were characterized after filtration by interquartile range denoising method, and the LECO/Fiehn Metabolomics Library was used to identify the compounds.
- Principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA) and orthogonal projections to latent structures-discriminant analysis(OPLS).



Score plot of OPLS model obtained from Class 2 (Control group) and Class 3 (Phosphite treated group)

Metabolic pathway (<http://www.genome.jp/kegg>)

Pathways potentially associated with phosphite treatment:

1. biosynthesis of secondary metabolites
2. ABC transporters
3. glutathione metabolism
4. tyrosine metabolism
5. 2-oxocarboxylic acid metabolism
6. ascorbate and aldarate metabolism
7. fructose and mannose metabolism
8. biosynthesis of amino acids
9. galactose metabolism
10. arginine and proline metabolism
11. biosynthesis of antibiotics

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Late Blight:

- R. Coffin and Z. Ganga (Cavendish Farms)
- R. Peters and H. Platt (Agriculture and Agri-Food Canada)
- K. Al-Mughrabi (New Brunswick Department of Agriculture,
Aquaculture and Fisheries)

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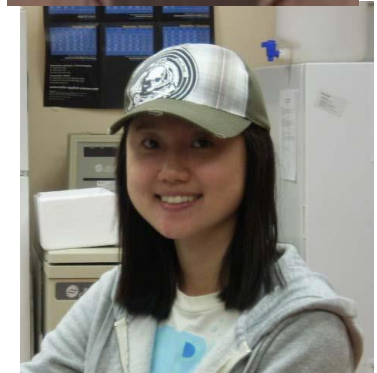
Gary Simpson

Patrick Murphy

Yuanyuan Wu

Dr. Fanming Kong

Dr. Andrew Schofield



NOVA SCOTIA CANADA

Thank you!



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