

# Laminarin Elicits Defense Responses in Grapevine and Induces Protection Against *Botrytis cinerea* and *Plasmopara viticola*

Aziz Aziz,<sup>1</sup> Benoit Poinsot,<sup>2</sup> Xavier Daire,<sup>2</sup> Marielle Adrian<sup>2,3</sup>, Annie Bézier,<sup>1</sup> B. Lambert,<sup>1</sup> Jean-Marie Joubert,<sup>4</sup> and Alain Pugin<sup>2</sup>

<sup>1</sup>Unité de Recherche Vignes et Vins de Champagne, URVVC - UPRES EA 2069, UFR Sciences, Moulin de la Housse, Université de Reims Champagne-Ardenne, BP 1039, F-51687 Reims cedex 2 France; <sup>2</sup>Unité Mixte de Recherche, Plante-Microbe-Environnement, INRA 1088/CNRS 2625/ Université de Bourgogne, 17 rue Sully, BP 96510, 21065 Dijon cedex, France; <sup>3</sup>Institut Jules Guyot, UMR 1088 INRA/Université de Bourgogne, 17 rue Sully, BP 96510, 21065 Dijon cedex, France; <sup>4</sup>Société Goëmar, Avenue du Général Patton, BP 55, 35413 Saint Malo cedex, France

Submitted 21 April 2003. Accepted 30 June 2003.

Grapevine (*Vitis vinifera* L.) is susceptible to many pathogens, such as *Botrytis cinerea*, *Plasmopara viticola*, *Uncinula necator*, and *Eutypa lata*. Phytochemicals are used intensively in vineyards to limit pathogen infections, but the appearance of pesticide-resistant pathogen strains and a desire to protect the environment require that alternative strategies be found. In the present study, the  $\beta$ -1,3-glucan laminarin derived from the brown algae *Laminaria digitata* was shown both to be an efficient elicitor of defense responses in grapevine cells and plants and to effectively reduce *B. cinerea* and *P. viticola* development on infected grapevine plants. Defense reactions elicited by laminarin in grapevine cells include calcium influx, alkalinization of the extracellular medium, an oxidative burst, activation of two mitogen-activated protein kinases, expression of 10 defense-related genes with different kinetics

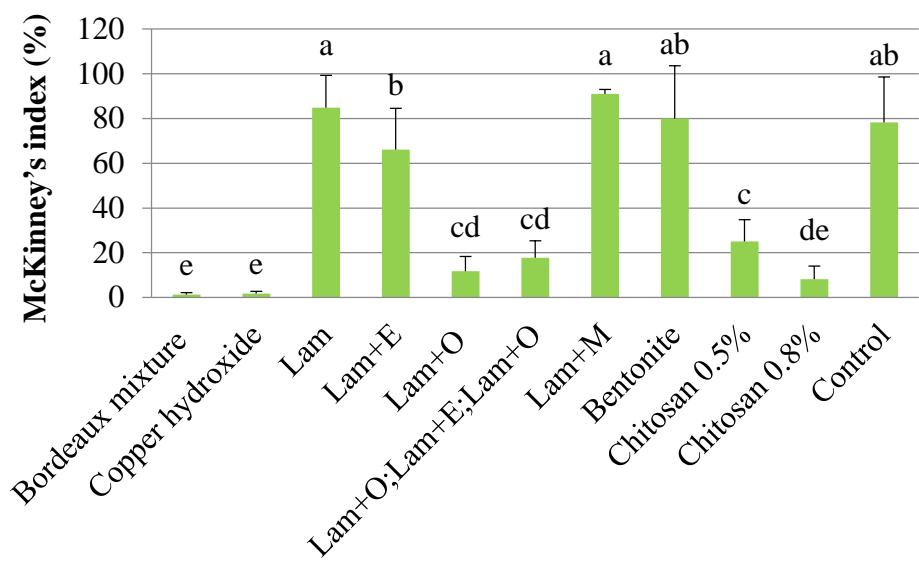
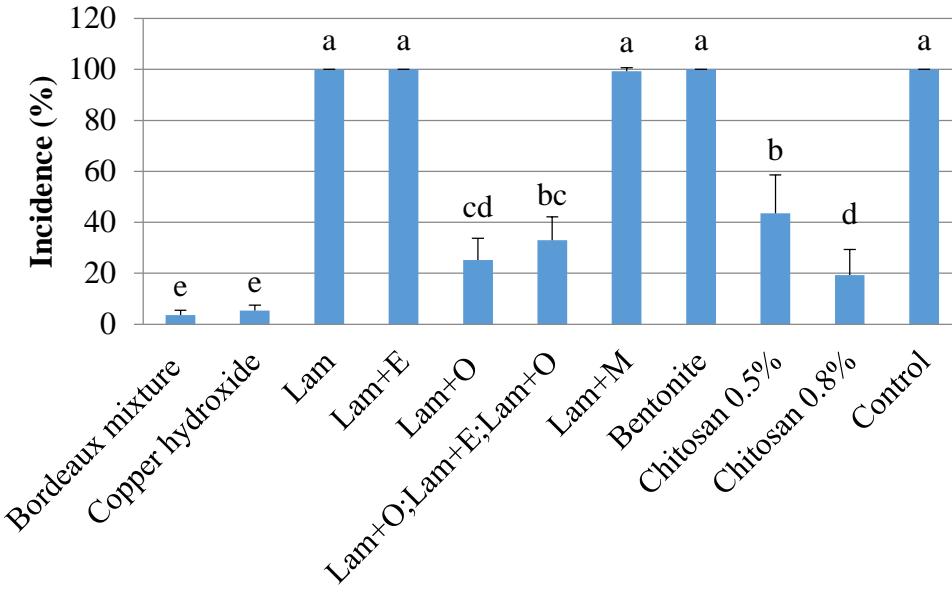
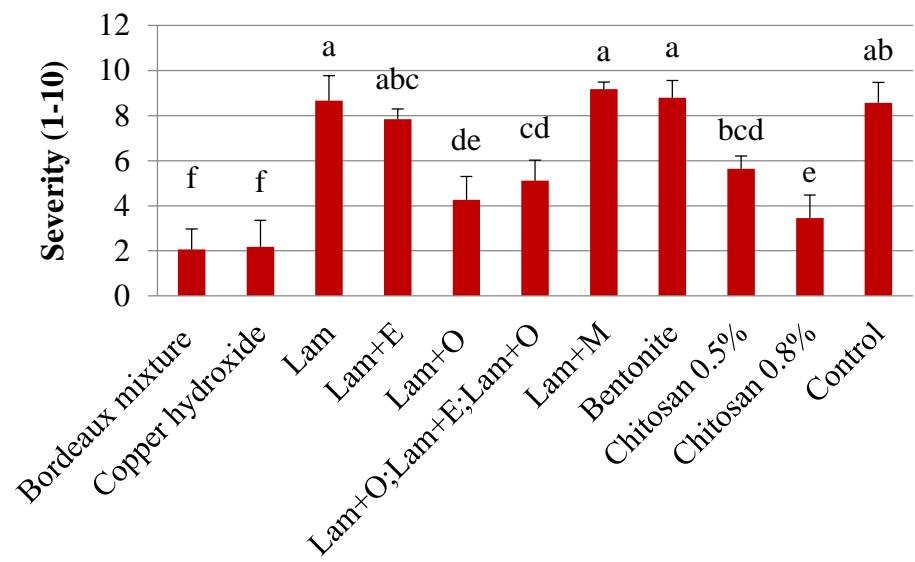
genesis-related (PR) proteins, some of which possess antimicrobial properties (Fritig et al. 1998; Hammerschmidt 1999; Somssich and Hahlbrock 1998; Van Loon and Van Strien 1999). All these defense reactions, which sometimes are associated with a localized cell death known as the hypersensitive reaction (HR), are considered to be important responses for delimiting the pathogen's growth. Moreover, plants have the ability to develop systemic acquired resistance (SAR), which reduces subsequent infection of healthy tissues by a broad range of pathogens. However, if these defense reactions occur too late, the infection process will spread successfully.

Various types of elicitors have been characterized, including carbohydrate polymers, lipids, (glyco)peptides, and (glyco)proteins. These products are secreted by microorganisms or derived from the cell walls of fungi, bacteria, or host

# Formulations, application rates and number of treatments

Active ingredient (%)	Commercial product	Company	Application rate (g or ml/ha)	Number of applications	Year trial
Bordeaux mixture (20)	Poltiglia Disperss	Cerexagri Italia S.r.l. (IT)	5000	11	2012; 2013
Copper hydroxide (19.2)	Funguran	Certis Europe (IT)	2800	11	2012; 2013
Laminarin	Frontiere	BioAtlantis (IRL)	1000	11	2012; 2013
Laminarin + microbial extract of <i>Saccharomyces</i> spp. (10), carbossilamine (10)	Frontiere + Oomisine	BioAtlantis (IRL); Kalosgate (IT)	1000 + 2000	11	2012; 2013
Laminarin + copper hydroxide/copper oxide (33)	Frontiere + Coptrel	BioAtlantis (IRL); Kalosgate (IT)	1000 + 500	11	2012; 2013
Laminarin + copper hydroxide/copper oxide (33);	Frontiere + Coptrel;		1000 + 500	1	
Laminarin + microbial extract of <i>Saccharomyces</i> spp. (10), carbossilamine (10);	Frontiere + Oomisine;	BioAtlantis (IRL); Kalosgate (IT)	1000 + 500	7	2012; 2013
Laminarin + copper hydroxide/copper oxide (33)	Frontiere + Coptrel		1000 + 500	3	
Laminarin + microorganisms ( <i>Glomus</i> spp., <i>Bacillus subtilis</i> , <i>Streptomyces</i> spp., <i>Trichoderma</i> spp., <i>Pichia pastoris</i> )	Frontiere + Micosat TAB fogliare	Kalosgate (I); CCS Aosta S.r.l. (IT)	1000 + 2000	11	2012; 2013
Bentonite, lithothamne, potassic stone meal	Bentotamnio	Cerrus (IT)	14167	11	2012
Vermicompost extract (3.5)	Humixa polivalente	Farmtech (SI)	6000	11	2013
Chitosan (99.9)	Chito Plant	ChiPro GmbH (D)	5000	11	2012; 2013
Chitosan (99.9)	Chito Plant	ChiPro GmbH (D)	8000	11	2012; 2013
Control	Untreated	-	-	-	

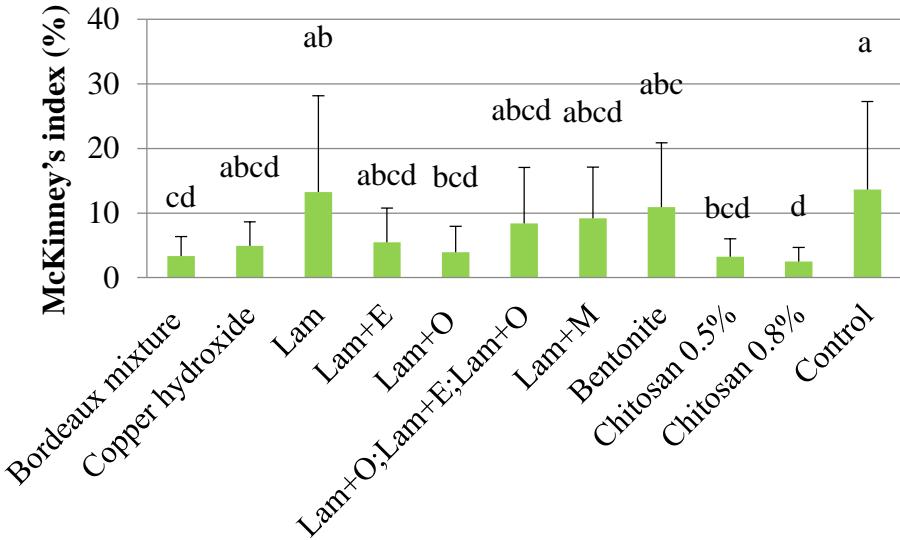
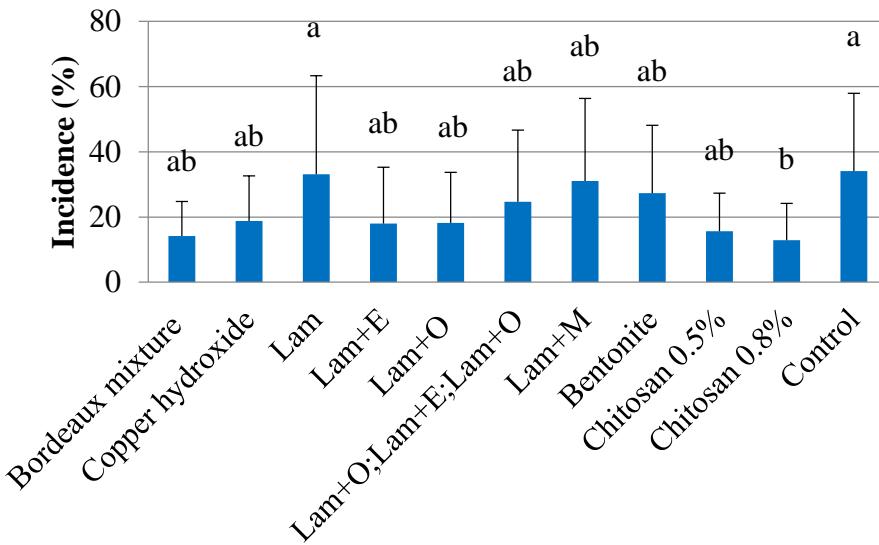
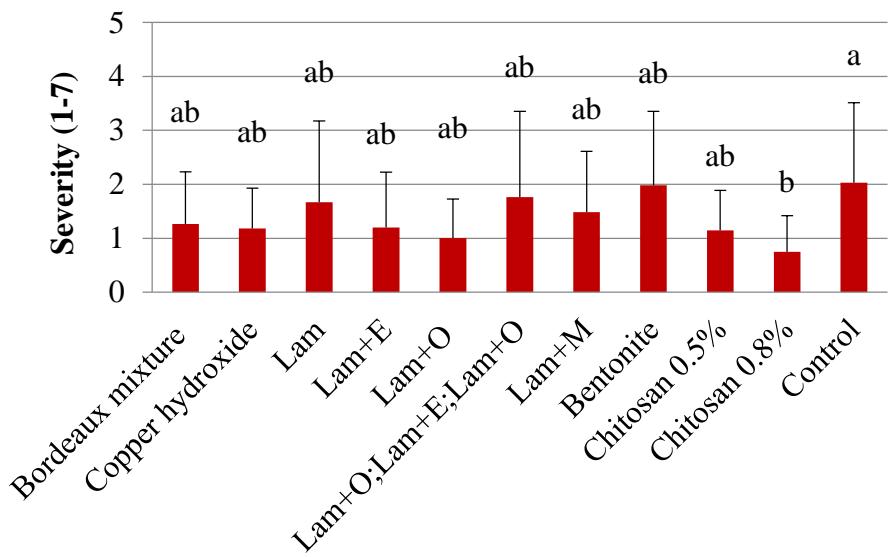
# Results – September 24, 2012



Values followed by different letter(s) in the same column are significantly different according to Tukey's HSD ( $P<0.05$ ).

**Lam** = laminarin, **O** = copper hydroxide/copper oxide, **E** = microbial extract of *Saccharomyces* spp., **M** = microorganisms (*Glomus* spp., *Bacillus subtilis*, *Streptomyces* spp., *Trichoderma* spp., *Pichia pastoris*)

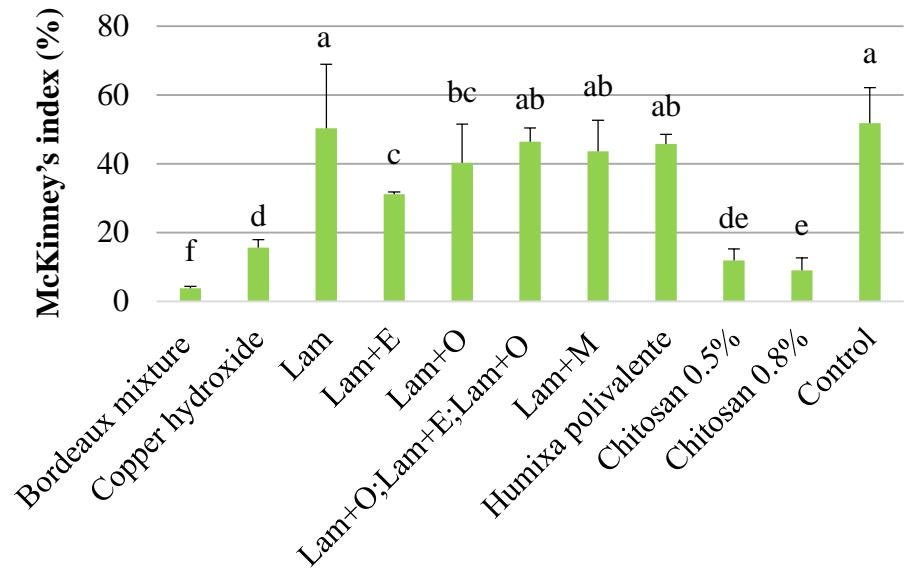
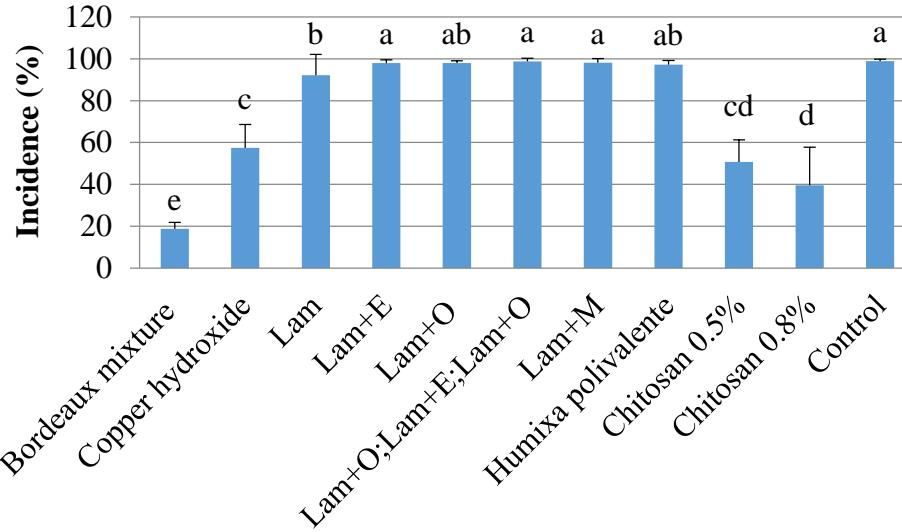
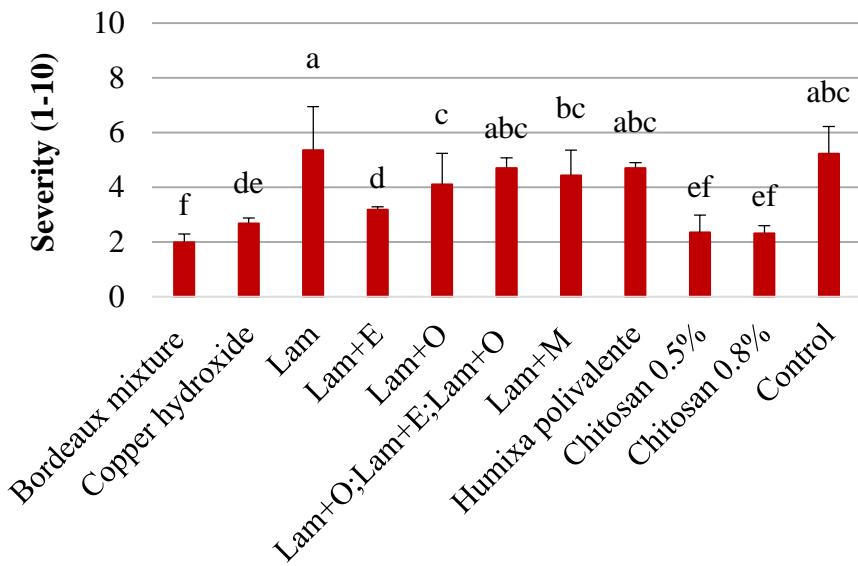
# Results – July 23, 2012



Values followed by different letter(s) in the same column are significantly different according to Tukey's HSD ( $P<0.05$ ).

**Lam** = laminarin, **O** = copper hydroxide/copper oxide, **E** = microbial extract of *Saccharomyces* spp., **M** = microorganisms (*Glomus* spp., *Bacillus subtilis*, *Streptomyces* spp., *Trichoderma* spp., *Pichia pastoris*)

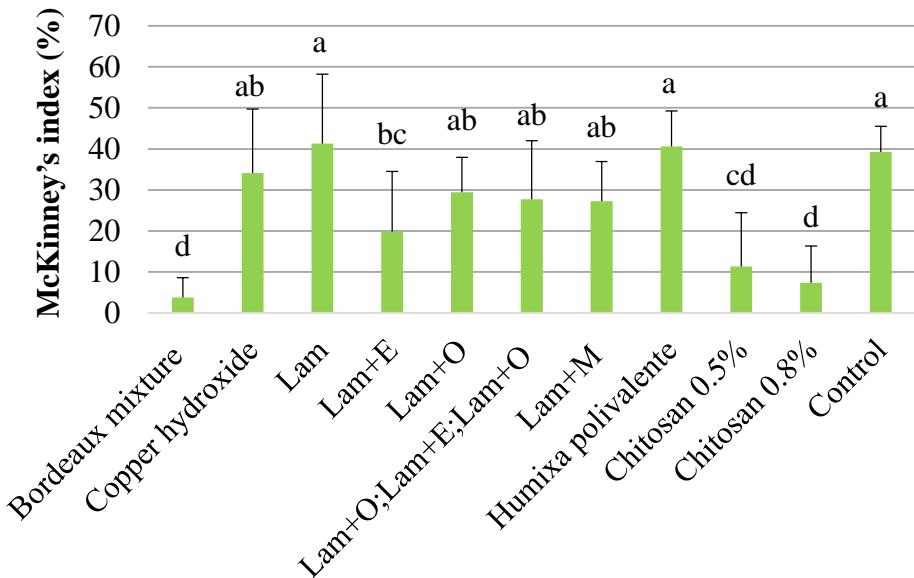
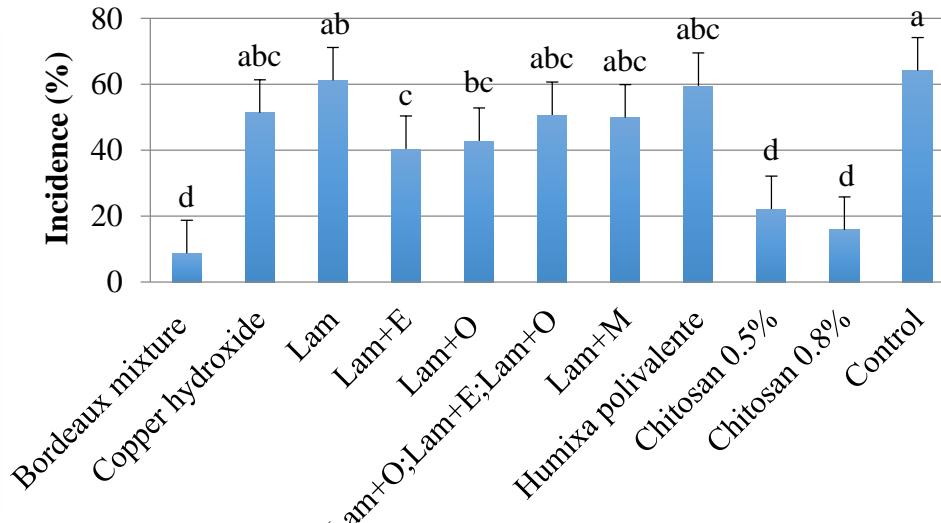
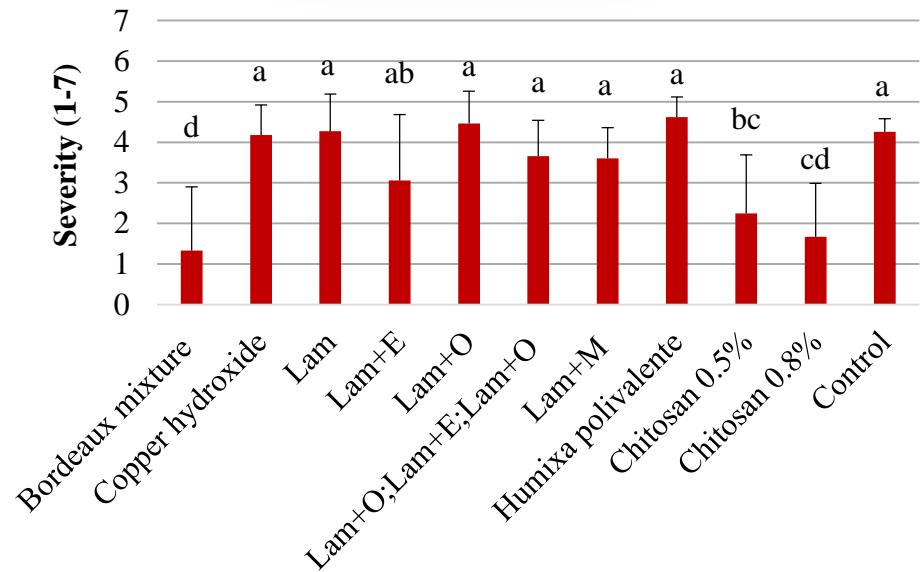
# Results - July 31, 2013



Values followed by different letter(s) in the same column are significantly different according to Tukey's HSD ( $P<0.05$ ).

**Lam** = laminarin, **O** = copper hydroxide/copper oxide, **E** = microbial extract of *Saccharomyces* spp., **M** = microorganisms (*Glomus* spp., *Bacillus subtilis*, *Streptomyces* spp., *Trichoderma* spp., *Pichia pastoris*)

# Results – July 11, 2013



Values followed by different letter(s) in the same column are significantly different according to Tukey's HSD ( $P<0.05$ ).

**Lam** = laminarin, **O** = copper hydroxide/copper oxide, **E** = microbial extract of *Saccharomyces* spp., **M** = microorganisms (*Glomus* spp., *Bacillus subtilis*, *Streptomyces* spp., *Trichoderma* spp., *Pichia pastoris*)

# Late downy mildew symptoms on plots treated with different compounds on September 24, 2012



**laminarin**

**0.5% chitosan**

## II

(*Non-legislative acts*)

## REGULATIONS

### COMMISSION IMPLEMENTING REGULATION (EU) No 563/2014

of 23 May 2014

approving the basic substance chitosan hydrochloride in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending Commission Implementing Regulation (EU) No 540/2011

(Text with EEA relevance)

ELICITOR	TARGET PATHOGEN				
	Chromista	Fungi	Bacteria	Viruses	Phytoplasma
Acibenzolar-S-Methyl or Benzothiadiazole (BTH)	X	X	X	X	X
$\beta$ -aminobutyric acid (BABA)	X	X		X	
Cerevisane	X	X			
Chitosan	X	X	X	X	X
Glutathion + oligosaccharines	X				X
Isonicotinic acid (INA)	X	X	X		
Jasmonic acid (JA, MeJA)		X			
Laminarin	X	X			
Phosetyl-Al	X	X	X		X
Potassium phosphyte	X	X			
Prohexadione-Ca			X		X
Protein hydrolysates	X	X			
Salicylic acid (SA)		X		X	X
Yeast extracts	X	X			

REGISTERED FOR USE AS PPP Registered and not registered products on the market

# Conclusions

The elicitation of host resistance is in agreement with current trends in plant protection (see Directive 2009/128/EC on sustainable use of pesticides) because of reduced chemical inputs in agriculture and lower risk of residues on commodities

Induced resistance is advisable also for organic agriculture, where disease prevention is the rule and use of direct means to control pathogens and pests is allowed only in particular conditions

# Conclusions

Induced resistance is usually broad spectrum and can be long lasting, although rarely has complete effectiveness (20-85%)

Resistance inducers are usually safer than chemical pesticides, and can contribute, alone or by integration with other means, to manage plant diseases in a sustainable way

**Marche Polytechnic University**

*Erica Feliziani*

*Lucia Landi*

*Sergio Murolo*

*Valeria Mancini*

*Andrea Servili*

**University of Bari**

*Antonio Ippolito*

*Franco Faretra*

*Vito Savino*

**SFR Marche Region**

*Sandro Nardi*



**USDA Parlier, CA, USA**

*Joe Smilanick*

**California Table Grape Commission**

*Franka Mlikota Gabler*

**Volcani Center, Israel**

*Samir Drobly*

*Amnon Lichter*

**Tswane University, South Africa**

*Dharini Sivakumar*

**National Polytechnic Institute, Mexico**

*Silvia Bautista-Baños*

**Agriculture and Agri-Food Canada**

*Deena Errampalli*

**Thanks for your attention**

*For further information: [g.romanazzi@univpm.it](mailto:g.romanazzi@univpm.it) or  
[www.researchgate.net/profile/Gianfranco\\_Romanazzi/publications](http://www.researchgate.net/profile/Gianfranco_Romanazzi/publications)*

**Vinelink Meeting, Paris – 8 April, 2016**