

YEA!<sup>®</sup> Elicitor Response Comparison to Chitin / Chitosan  
in Mung Bean and Adzuki Bean Germination Experiments

by

James C. Linden and Richard J. Stoner  
Colorado State University and AgriHouse, Inc.  
October 21, 2008

The purpose of this abstract is to focus on differences between AgriHouse's YEA!<sup>®</sup> and chitin / chitosan elicitation effects using mung beans and adzuki beans, which we have recently published<sup>1</sup>.

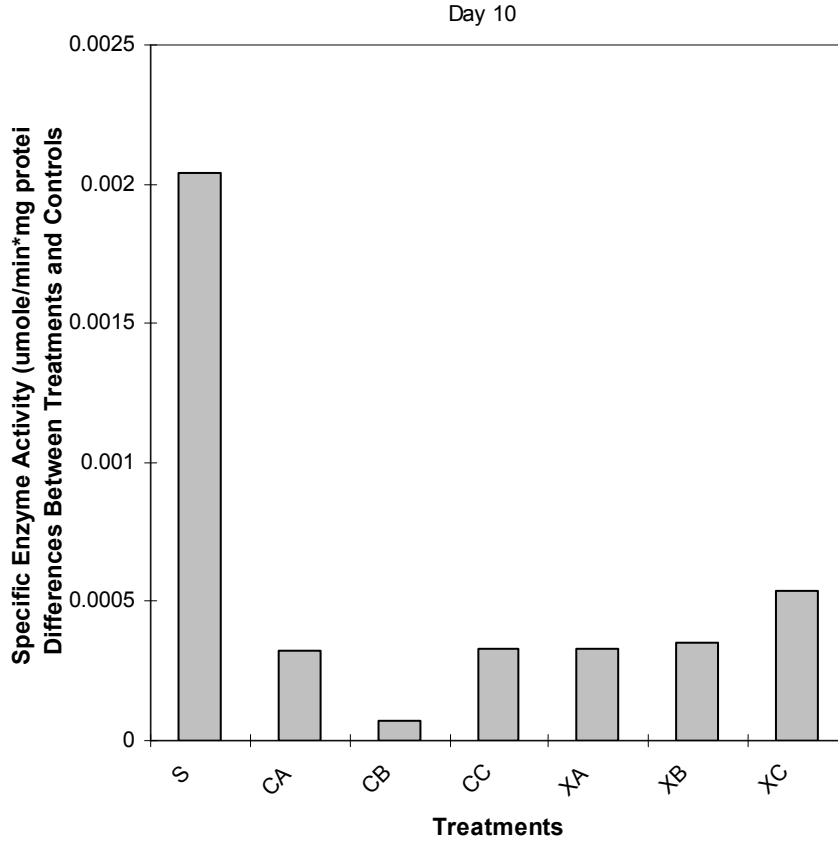
YEA!<sup>®</sup> is derived from chitin / chitosan by a proprietary process. The YEA!<sup>®</sup> derivative is 100% water soluble, whereas chitin / chitosan is not water soluble. Chitin / chitosan occurs naturally in a range from 100% chitin to 100% chitosan as a mixed polymer<sup>2</sup>; an NMR analysis of YEA!<sup>®</sup> revealed characteristics of 20% chitin and 80% chitosan<sup>3</sup>. We show below that the performance of YEA!<sup>®</sup> outperforms chitin / chitosan as an elicitor.

One of the classical responses to elicitation of plants is induction of certain enzyme activities. Enzyme activity measurements relate to the level of a given enzyme protein in the plant tissue. The enzyme,  $\beta$ -1,3-glucanase, was assayed using laminarin (a soluble  $\beta$ -1,3-glucan) as substrate. Crude homogenates of the seedlings from treated seeds yielded the data in Figure 1. Increased  $\beta$ -1,3-glucanase activity compared to controls (without seed treatment) was obtained in the YEA!<sup>®</sup> treatments ten days following germination. Elicitation of mung bean seeds that were treated with YEA!<sup>®</sup> were compared to those treated with two types of elicitors. First, various concentrations of purified colloidal chitin / chitosan were used. The dose response to chitin / chitosan concentrations of 9, 0.9 and 0.09 mg/seed followed no regular pattern. A nearly equivalent concentration of YEA!<sup>®</sup> (1 mg/seed) to the 0.9 mg/seed treatment with chitin / chitosan elicited five times as much  $\beta$ -1,3-glucanase enzyme activity. Secondly, lower concentrations of the chitin oligosaccharide containing six glycan moieties, N-acetylchitohexaose were studied. The importance of the chitin oligosaccharide is that short chains of chitin have been found optimal in elicitation of many types of plants. The dose response relationship to the oligosaccharide concentrations of 0.5, 0.05 and 0.005 mg/seed is negative; i.e. higher doses resulted in lower specific enzyme activities. Comparisons similar to those with chitin / chitosan could be made between the performance of 1 mg/seed YEA!<sup>®</sup> and lower concentrations of the more optimal oligosaccharide.

A dose response for YEA!<sup>®</sup> in induction of elevated  $\beta$ -1,3- glucanase activity in adzuki beans is demonstrated by data in Figure 2. Induction of this enzymatic activity increases with quantity of YEA!<sup>®</sup> applied to the seeds. Comparison of elicitation between treatments with 0, 0.5, 1.0 and 2.0 mg/seed and controls in specific enzyme activity was evaluated in both hypocotyl (root) and epicotyl (shoot) tissues. The specific enzyme activities in both tissues increased with dosage 21 days after germination. The differences become significant in root tissue using 2.0 mg/seed with twice the level of activity, compared to controls.

**Figure 1**

**COMPARISONS OF GERMINATING MUNG BEANS  
ELICITATION FOLLOWING SEED TREATMENT WITH  
YEA!<sup>®</sup> AND CHITIN / CHITOSAN**



<u>Name</u>	<u>Treatments:</u>	<u>Rate:</u>
S:	<b>YEA!<sup>®</sup></b>	1 mg/seed;
CA:	Chitin / Chitosan	9.06mg/seed;
CB:	Chitin / Chitosan	0.906mg/seed;
CC:	Chitin / Chitosan	0.0906mg/seed;
XA:	N-acetylchitohexose	0.5mg/seed;
XB:	N-acetylchitohexose	0.05mg/seed;
XC:	N-acetylchitohexose	0.005mg/seed.

Figure 1. Differences between controls and seed treatments with YEA!<sup>®</sup> chitin/ chitosan and N-acetylchitohexose oligosaccharide on specific activities of  $\beta$ -1,3- glucanase in homogenates of mung bean seedlings ten days after germination in test tubes.

**Figure 2**

COMPARISONS OF GERMINATING ADZUKI BEANS  
ELICITATION FOLLOWING SEED TREATMENT WITH  
VARIOUS CONCENTRATIONS OF YEA!<sup>®</sup>

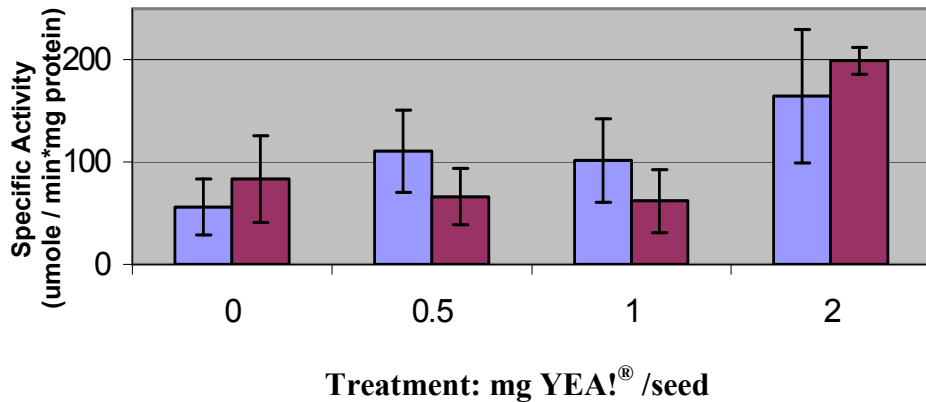


Figure 2. Specific activities of  $\beta$ -1,3- glucanase in homogenates of adzuki bean shoot tissue (blue) and root tissue (red) twenty-one days after germination in test tubes of water controls and those of treatments in the presence of various concentrations of YEA!<sup>®</sup> as follows:

<u>Treatments:</u>	<u>Rate:</u>
YEA! <sup>®</sup>	0 mg /seed
YEA! <sup>®</sup>	0.5 mg /seed
YEA! <sup>®</sup>	1.0 mg /seed
YEA! <sup>®</sup>	2.0 mg /seed

Conclusion

The ability of the proprietary elicitor to elicit plant growth characteristics by means of signal transduction processes makes YEA!<sup>®</sup> that differs from other types of elicitors and treatments, including chitin and chitosan. As a non-systemic agent in plants YEA!<sup>®</sup> impacts receptors on the cell surface and initiates molecular level signal transduction processes.

YEA!<sup>®</sup> naturally activates the signal transduction pathways in a wide and diverse range of plant species and cultivars. During the past 13 years YEA!<sup>®</sup> has proven to significantly increase seed germination and sprouting under laboratory conditions and field conditions. This natural elicitor boosts  $\beta$ -1,3- glucanase, thus elevate the impact of physiological factors that enhance and control development in plants.

## Reference List

<sup>1</sup>Linden, J. C. and Stoner, R. J. (2005). Proprietary Elicitor Affects Seed Germination and Delays Fruit Senescence. *Journal of Food, Agriculture & Environment* **3**, 184-189.

<sup>2</sup>Brine, C. J., Sanford, P. A., and Zikakis, J. P. (1992). "Advances in Chitin and Chitosan." Elsevier Applied Science, London; New York.

<sup>3</sup>Linden, J. C. and Phisalaphong, M. (2000). Oligosaccharides potentiate methyl jasmonate-induced production of paclitaxel in *Taxus canadensis*. *Plant Science* **158**, 41-51.

### Related works on chitosan elicitors by the authors

Linden, J.C. and Stoner, R.J. 2007. *Pre-harvest application of proprietary elicitor delays fruit senescence*. A. Ramina et al. (eds.). *Advances in Plant Ethylene Research: Proceedings of the 7<sup>th</sup> International Symposium on the Plant Hormone Ethylene*. pp 301-302. Springer: Dordrecht, The Netherlands.

Linden, J.C. and Stoner R.. 2005. *Proprietary Elicitor Amends Potato Emergence and Yields*. Potato Grower. April. pp. 34-35.

Linden, J., Stoner, R., Knutson, K. Gardner-Hughes, C. "Organic Disease Control Elicitors". Agro Food Industry Hi-Te (p12-15 Oct 2000)

For more information visit <http://www.yeacrops.com/>

**YEA!**<sup>®</sup> Yield Enhancing Agent (Patented & Patent Pending) EPA reg. no. 83729-1

AgriHouse, Inc. copyright 2008

<http://www.agrihouse.com/>