

YEA![®] Proprietary Elicitor Responses Influence Seed Germination and Delays Fruit Senescence

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ABSTRACT

The proprietary elicitor, YEA![®] Yield Enhancing Agent, improves seed germination rates with tomato, bean, corn, and many other vegetable and flower seeds. Compared to controls, treated plants emerge sooner and are more vigorous, which results in statistically increased crop performance and higher yields. This elicitor does not appear to be a systemic agent in plants, but to impact receptors on the cell surface and initiate molecular level signal transduction processes. For example, induction of β -1,3-glucanase in treated seeds is associated with improvement in germination rates. This elicitor does not utilize ethylene as a secondary messenger, but appears to reduce ethylene biosynthesis. Application of this elicitor either by foliar spray or root feeding prior to harvest delays fruit senescence by inhibiting ethylene biogenesis. Presently, ripening of harvested citrus is initiated with ethylene at the start of storage. Since fruit from YEA![®] treated trees exhibit better storage characteristics, it may be concluded that ethylene biosynthesis is reduced by application of the elicitor to citrus. This phenomenon is also studied using triple response assays on etiolated *Arabidopsis thaliana* seedlings. YEA![®] treated seeds show no stimulation of ethylene biosynthesis, as do some elicitors. Since this elicitor reduces ethylene formation, physiological factors controlling plant development are no longer negatively impacted by ethylene, resulting in improved growth at all stages. YEA![®] Yield Enhancing Agent (EPA reg. No. 83279-1) is manufactured by AgriHouse Inc., Berthoud, Colorado USA (US Patent No. 6,193,988).

KEYWORDS

elicitor, germination, β -1,3-glucanase, ethylene, senescence, triple response, signal transduction, tomato, adzuki bean, mung bean, maize, Arabidopsis, metabolites

REVIEWS

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INTRODUCTION

Plants produce various secondary metabolites that allow interaction with the environment. Elicitors can enhance secondary metabolite production and/or second messenger development, known as signal transduction¹. The interplay of these elicitors and secondary metabolites enables the now-alerted plant to better overcome biotic and abiotic (environmental) stresses. While some elicitors stimulate defense responses in the plant, others induce plant growth responses that result in increased dry weight biomass, root size and stem caliper, bloom and harvest².

As an organic patented material derived from exoskeletons of crustaceans, YEA![®] Yield Enhancing Agent is considered an elicitor³. Elicitors provide external stimuli that trigger the changes in the plant cells, which lead to cascades of reactions and production of secondary metabolites, ultimately helping the plant overcome stress factors⁴. Elicitors are stimuli of biotic and abiotic types. For example, the latter are represented by natural stresses to the plant from touch, shear forces (wind), temperature shocks and osmotic stresses. Biotic elicitors include glucan polymers, glycoproteins, low molecular weight organic acids, fungal xylanases and cell wall materials and segments of bacterial flagella. High affinity binding sites have been characterized for oligo- β -glucosides, such as oligochitins, oligochitosans, yeast N-glycan and β -1, 4-linked galacturonate oligomers⁵. The stimuli are perceived by receptors on the plant cells, which lead to activation of secondary messengers that transmit signals into the cell through signal transduction pathways that ultimately results in gene expression and the biochemical changes that benefit the plant. Interplay of the signaling molecules also regulates the entire pathway by factors, which influence signal transduction pathways. These factors include polyamines, calcium, jasmonates, salicylates, nitric oxide and ethylene⁵.

MATERIALS AND METHODS

Seed germination

Adzuki bean (*Phaseolus angularis*), mung bean (*Phaseolus aureus*) and tomato (*Lycopersicon esculentum*) seeds were germinated in trays containing fifty seeds each. Twelve layers of paper towels were placed in each tray, and the tray was covered with aluminum foil. Three identically treated trays represented replicates for each experiment that were repeated at least two times. These, along with one flask per tray containing 200 mL distilled water, were autoclaved for 20 min at 121°C. The dried seeds were surface sterilized in 1.0 (v/v) percent sodium hypochlorite and then thoroughly rinsed in sterile distilled water before being placed between the paper towels. For treatments the elicitor was added in a concentration of 1 mg/mL; controls were without elicitor. The solution was then poured over the paper towels, and the trays were re-covered with aluminum foil. The seeds were allowed to germinate at room temperature in a dark environment for indicated periods of time.

Alternatively, single seed germinations were conducted in 1.5 x 15 cm pyrex test tubes. Cylindrical rockwool plugs, 2.0 cm in length and 1.5 cm in diameter each with a hole 0.5 cm diameter and 1.0 cm in depth, served as the growing matrix for adzuki and mung beans. Aseptically, 1.0 mL of elicitor (at various concentrations from 0 to 2.0 mg/mL) was pipetted into the hole at the center of each plug. The rockwool plugs were then dried in a laminar flow hood.

After surface-sterilizing the beans in a 1.0 (v/v) percent bleach solution for 30 seconds, the beans were rinsed three times using the same elicitor concentration solution as added to the matrix. Finally, adzuki beans were loaded into the hole in the plugs, white crown up. The plugs were then placed aseptically into individual sterile test tubes and at the appropriate time, 1.5 mL of distilled water moistened the rockwool, which served to germinate the seed and to present the elicitor to the growing sprout. Beans generally germinated after 5 days of darkness at 22°C and allowed to grow for appropriate periods of time, as indicated.

The laboratory sweet corn (*Zea mays* var. *rugosa*) seed (Frontier Seed Sweetie 82, Glendale, AZ) were soaked for three minutes in YEA!® concentrate and subsequently sprayed with 1 percent solutions at seven day intervals. The testing consisted of seven day pre-chill followed by seven day standard germination using Association of Official Seed Analysts (AOSA) methods at STA Labs in Longmont, CO. The seedlings treated with YEA!® had slightly more vigor, longer root and shoot lengths and higher chlorophyll levels at the end of testing.

Laminarinase assay

The enzyme solution for the laminarinase assay was prepared by placing 0.1 g of adzuki bean root tissue in 10 ml of 0.05 M sodium phosphate buffer (pH 5.5) and homogenized for 30 seconds. Homogenization was conducted using a Kinematic Ag Model PT 1200 Polytron electronic homogenizer, set on power level 3 in 1.5 x 15 cm pyrex test tubes. The homogenate was centrifuged at 10,000 rpm for 10 min. The supernatant was then filtered using Sephadex G25 to remove low molecular weight sugars and peptides and allow the desired enzymes to pass through.

β-1,3-glucanase Assay

A colorimetric assay was used to determine enzyme levels in the solution. All assays were done in duplicate. The soluble β-1,3-glucan laminarin polysaccharide substrate is cleaved by laminarinase, leaving reducing ends. Dinitrosalicylic acid (DNSA) reagent reacts with these ends and leads to a visible color change that was quantified using a double beam spectrophotometer at 540 nm. The reaction tubes were prepared using 1.0 mL laminarin (10 mg/ml) solution and 1.0 mL enzyme solution, and incubated for 30 minutes at 37°C. DNSA reagent (3.0 ml) was then added to each tube and the tubes were placed in a boiling water bath for 10 min. The absorbance after the reaction was compared to a standard curve of known concentrations of glucose. Background reactions were calculated by preparing blanks with the sodium phosphate buffer replacing either the enzyme solution or the substrate. The absorbance of the blanks was subtracted from the absorbance of the reaction to get a corrected absorbance. The amount of laminarinase in the enzyme solution was calculated using the slope of glucose standard curves, prepared on the day of the assay.

Protein Content

Protein levels were calculated using Pierce bicinchoninic acid (BCA) protein assay. Bovine serum albumin concentrations from 0.1 to 0.4 mg/mL were used to generate a standard curve. Aliquots of 100 μL of each standard or unknown were placed in a test tube. 2.0 mL working reagent [50 parts reagent A (sodium carbonate, sodium bicarbonate, BCA, and sodium tartrate in 0.2N sodium hydroxide) to 1 part reagent B (4% cupric sulfate)] was added and the tubes were incubated in a water bath set at 37°C for 30 minutes. The absorbance readings of each tube taken at 562 nm were then used to determine the unknown protein levels.

Triple Response Assay

Using 47 mm Petri dishes, 15 to 30 seeds of *Arabidopsis thaliana* WT Col were placed in a row across agar (1.0%) containing 1X Murashige Skoog basal salts medium (no sucrose) and ½ X B5

vitamins. To the agar for treatments was added 1.0 mL / liter of YEA![®] controls were both with and without ethylene and without YEA![®]. The plates were held in a vertical position in a styrofoam box using double stick tape and kept at 4°C for 72 hours. The plates were then exposed to constant fluorescent lighting at room temperature for 18-20 hours. Two Petri dishes could then be placed vertically in 230 mL (pint) Mason jars, the lids for which had a 5 mm (¼ inch) hole drilled and fitted in place with a small serum cap glued in the center. Ethylene was serially diluted to 1000 ppm in a separate bottle and 0.23 mL was injected into 0.23 L into jars to yield 1.0 ppm ethylene during subsequent dark incubations. During the period of etiolated growth, the plates were kept in the dark at room temperature. After five days the jars were opened, the Petri dishes were placed face-down (without lids) on a scanner. The Adobe Photoshop images were analyzed by NIH image J software to obtain dimensions of epicotyl elongation, width and hook angle.

Treatment of Citrus Trees and Fruit Storage Evaluation

Sorenson¹¹ compared YEA![®] with a control by one 35 mL (total) application by spraying the leaves and two applications of 35 mL (total) each time to the soil around orange trees during a period several weeks preceding harvest. The subsequent post-harvest procedures that were used add more information to our understanding of the signal transduction process. The post-harvest gassing procedure with 10 ppm ethylene gas for four days at 68°F turns out to be important. This is applied to start the ripening process in harvested fruit; it is widely used for bananas and tomatoes.

RESULTS

Many germination experiments demonstrate improved responses in YEA![®] treatments. Such observations will be reported using seeds of tomatoes, mung beans, adzuki beans and sweet corn. The first instance of improved germination involves Leading Lady tomato seeds (Sunseeds, Morgan Hill, CA) in sterilized trays and toweling in the laboratory following germination in the presence of 5 mL of YEA![®] per liter, as shown below with data presented in Table 1. After 17 days of growth at room temperature in the dark, digital photographs were taken and the entirety of each treatment of seedlings was dried overnight at 50°C. The dried hypocotyls from the treated seeds weighed 72 percent more than did the controls. Image analysis of the hypocotyls revealed an 87 percent greater length for YEA![®] treated materials compared to the untreated controls.

Dry Weight Analysis of Tomato Seed Treated with YEA![®]



Control prior to drying



YEA![®] prior to drying

<u>Treatment</u>	<u>Dry weight</u>	<u>Percent increase</u>
Control	0.40 g	
YEA! [®]	0.45 g	13%

COMPARISON OF HYPOCOTYL SIZE FROM GERMINATING TOMATOES FOLLOWING SEED TREATMENT WITH YEA![®] AND WATER

Tomato Variety:	0.5g Leading Lady tomato seeds per treatment	Application Rate:	5.0 mL / L solution of YEA!
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Treatments:	<u>quantity</u>	<u>dry wt.</u>	<u>Average length</u>	<u>std dev</u>	<u>% Dry wt. Increase</u>
	mL	g	mm	mm	
YEA! [®]	6 of solution	0.19	16.3	4.5	73%
Control	6 of water only	0.11	8.7	3.3	na

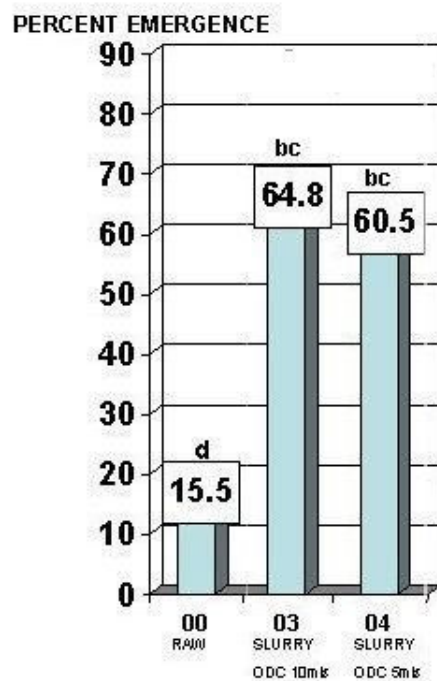
Table 1. Dry weight and image analyses of hypocotyls from germinated tomato seeds treated with YEA![®] and water after 17 days of growth in the dark at room temperature. Approximately 180 seeds represent 0.5 g of these seeds. Image analysis of approximately 20 representative seedlings were conducted to obtain the data shown. The experiment was repeated twice.

The second instance of enhanced seed germination is noted using sweet corn. Two independent organizations, STA Labs (Longmont, Colorado) reported 14 percent increased seed germination viability of sweet corn under laboratory conditions. The germination rates of the control (water) and the YEA![®] treated seeds were 65 percent and 71 percent, respectively. Also an organization associated with Bayer Crop Science, Celpril of Manteca, CA (www.celpril.com) conducted a sweet corn seed germination trial in the field in the fall of 2003. These data, presented in Figure 1, show effects of treatments using 1.25 mL and 2.50 mL of YEA![®] per liter compared to control treatments that received only water. Emergence rates, 21 days after sowing, were approximately 60, 65 and 15 percent, respectively. The extent of the differences between treatments and controls points out the difficulty

growers experience with germinating sweet corn, because of high sugar content (Lynn Loken, Loken Associates, Loveland Colorado; private communication).

The third instance of germination enhancement is with adzuki beans grown in sterilized trays and toweling in the laboratory. Growth rates were again measured at room temperature during the first seven days in darkness following wetting of 10 gram of seeds with either 200 mL of water or the same quantity of 0.75 mL of YEA![®] per liter water. The mass of dried hypocotyls originating from the respective treatments was as follows:

SWEET CORN SEED APPLIED BIOLOGICALS FIELD TRIAL FALL 2003



PLANTED-10-29-03 ORDER #5074 COUNTED DAY-21

Alpha level-0.05 CV-20.3%

BEYOND all natural Plant Amendment - test data provided by www.celpril.com (BAYER Crop Science)

Figure 1. Sweet corn germination study comparing treatment with YEA![®] at two application rate (5 and 10 mL per gal = 1.25 and 2.50 mL / liter) with water controls. Data with different letters represent statistical significance at 0.05 level.

NASA

In preparation for tests that were conducted aboard the NASA space shuttle ATLANTIS and the MIR space station in late 1997 and early 1998⁶, another parameter was measured in adzuki bean and mung bean seedlings. The enzyme β -1,3-glucanase specific activity was assayed using laminarin (a soluble β -1,3-glucan) as substrate. Crude homogenates of the seedlings yielded the data in Figures 2 and 3. An increase of the β -1,3-glucanase activity was obtained in the YEA![®] treatments between seven and twelve days following germination of both bean types (Figure 2 for adzuki beans and Figure

3 for mung beans). The data in Figure 3 also indicate advantageous differences between the treatments of mung beans seeds with YEA![®] over those treated with various concentrations of purified colloidal chitin and the derived chitin oligosaccharide containing six glycan moieties, N-acetylchitohexaose. The concentration of YEA![®] used in this study is greater than the concentrations of chitin and oligosaccharide used. However, any indication of dose response relationship to the oligosaccharide concentration is negative; i.e. lower doses after ten days resulted in greater specific enzyme activities.

SPECIFIC ENZYME ACTIVITY IN GERMINATING ADZUKI BEANS

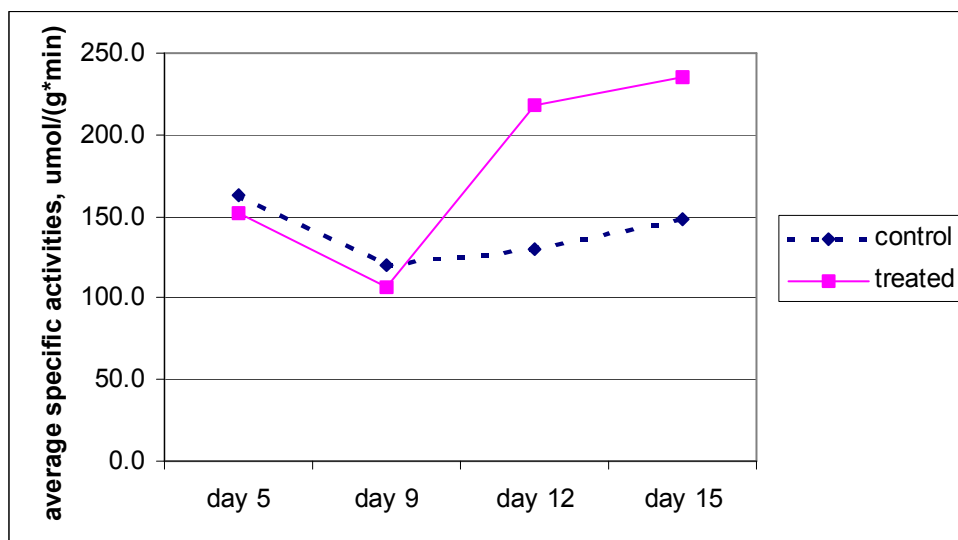
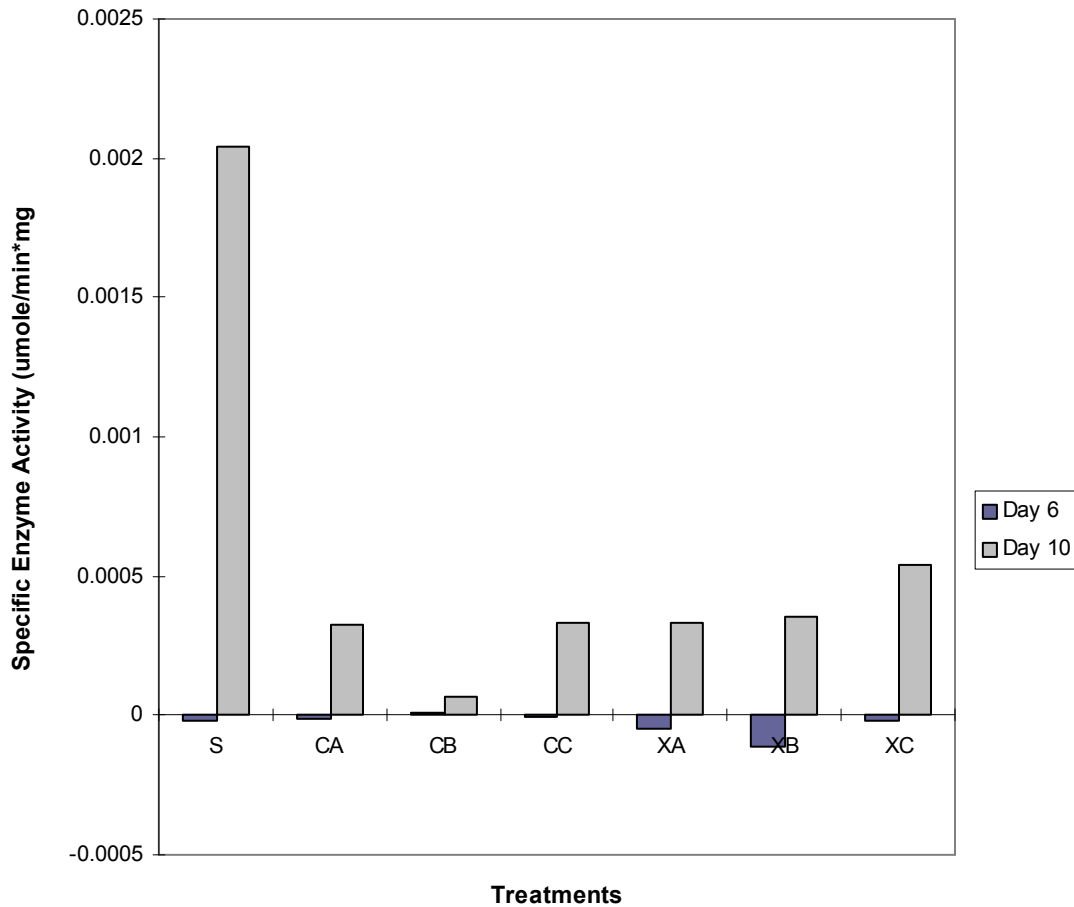


Figure 2. Kinetics of β -1, 3- glucanase formation in homogenates of adzuki bean seedlings between five and fifteen days after germination in test tubes in the presence of 1 mg YEA![®] per seed.

A dose response for YEA![®] in induction of elevated β -1,3- glucanase activity in adzuki beans is demonstrated by data in Figure 4. Induction of this enzymatic activity increases with quantity of YEA![®] applied to the seeds and with time. Twelve days after germination there was no differences between treatments and controls in specific enzyme activity in both hypocotyls and epicotyl tissues. However, 21 days after germination the specific enzyme activity in both hypocotyls and epicotyl tissues increase with dosage, becoming significant at 2.0 mg per seed. Both with adzuki and mung beans, a delay between germination and elevation of specific enzyme activity is noted.

In summary, β -1,3-glucanase enzyme activity aids germination of many types of seeds. According to Leubner-Metzger from the University of Freiburg⁷, one form of β -1,3-glucanase (class I), which has been studied extensively in several types of seeds, including tobacco, tomato and other solanaceous seeds, is induced in the endosperm of the seeds just prior to penetration of the radicle, and is believed to help weaken the

SPECIFIC ENZYME ACTIVITY IN GERMINATING MUNG BEANS FOLLOWING SEED TREATMENT WITH YEA![®] AND OTHER ELICITORS



<u>Name</u>	<u>Treatments:</u>	<u>Rate:</u>
S:	YEA! [®]	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 4. Differences between controls and seed treatments with YEA![®] chitin/ chitosan and N-acetylchitohexose oligosaccharide on specific activities of β -1,3- glucanase in homogenates of mung bean seedlings ten days after germination in test tubes.

endosperm wall⁸. A thick β -1,3-glucan layer, which imparts limited permeability to the seed envelope of cucurbitaceous species, is degraded during germination⁹. The difference between stimulated germination in some cases (tomato; Table 1, sweet corn; Figure 1 and adzuki beans) and improved seedling vigor in other cases may be related to differences in seed anatomy, the composition of the endosperm barrier represented by a β -1,3-glucan layer, and other unknown factors.

**SPECIFIC ENZYME ACTIVITY IN GERMINATING ADZUKI BEANS
FOLLOWING SEED TREATMENT WITH VARIOUS CONCENTRATIONS OF YEA![®] (test
name: Beyond)**

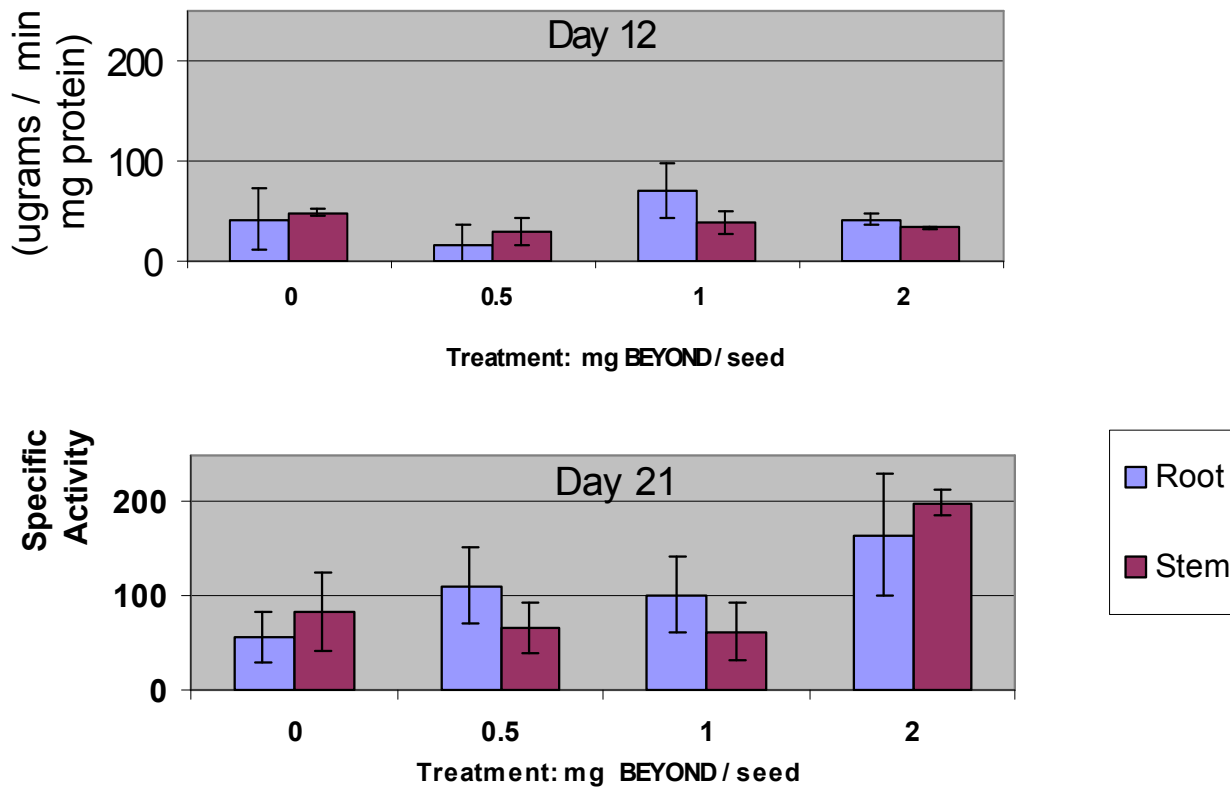
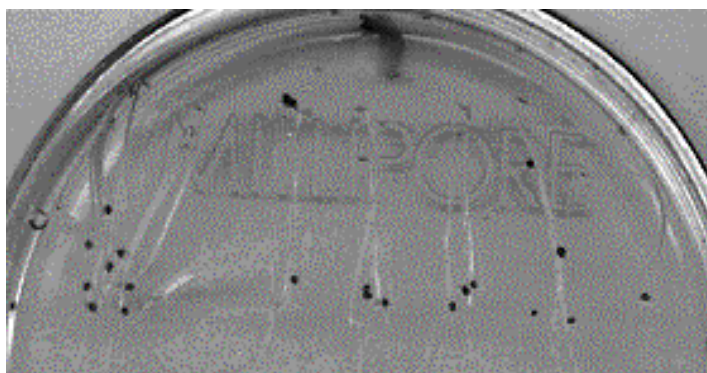


Figure 4. Specific activities of β -1,3- glucanase of treatments and those of water controls in homogenates of adzuki bean epicotyl tissue (blue) and hypocotyls tissue (red) twelve and twenty-one days after germination in test tubes in the presence of various concentrations of YEA![®], 0, 0.5, 1.0 and 2.0 mg /seed.

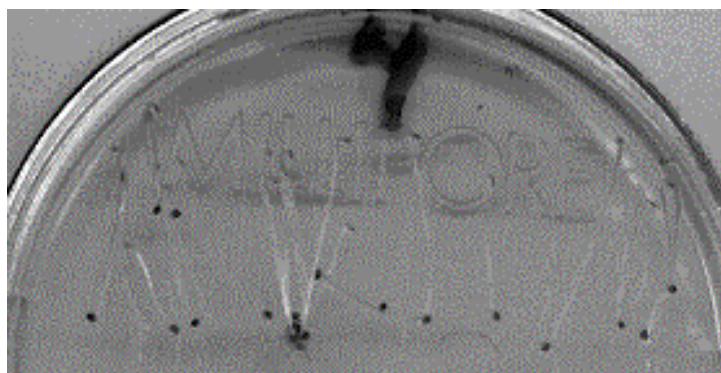
YEA![®] is not considered a systemic agent in plants, because it does not result in hydrogen peroxide production (data not shown). Instead it causes receptors on the cell surface to initiate molecular level processes called signal transduction¹⁰. YEA![®] does not stimulate ethylene biosynthesis, as do some elicitors. By conducting a test using *Arabidopsis thaliana* seeds that is widely known as the "triple response"¹⁰, seeds are germinated in the dark, a condition that produces elongated epicotyls (stems). One of the triple responses, epicotyl elongation, is easily measured and is seen in Figure 5 to be severely reduced by concentrations as low as 1 ppm ethylene. When seeds are germinated

EFFECT OF ETHYLENE ON ETIOLATED EPICOTYL DEVELOPMENT

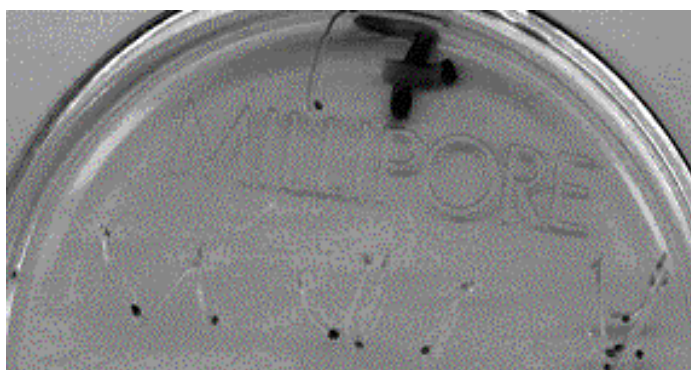
WITH & WITHOUT YEA![®]



0 ppm C₂H₄ without YEA![®]



0 ppm C₂H₄ + YEA![®]



1 ppm C₂H₄ without YEA![®]



1 ppm C₂H₄ + YEA![®]

Figure 5. Etiolated seedlings of *Arabidopsis thaliana* Col exposed for five days to ethylene (C₂H₄) as follows:

<u>Ethylene</u>	<u>Elicitor</u>	<u>Result</u>
0 ppm C ₂ H ₄	without YEA! [®]	(Petri dish labeled 1);
0 ppm C ₂ H ₄	+ YEA! [®]	(Petri dish labeled 4);
1 ppm C ₂ H ₄	+ YEA! [®]	(Petri dish labeled 5);
1 ppm C ₂ H ₄	without YEA! [®]	(Petri dish labeled 7)

on agar medium containing 0.1 mg / mL YEA![®] the results of this very sensitive test indicate ethylene is not produced by the seedlings (Figure 6). These data can be summarized to say YEA![®] did not increase ethylene formation by the seedlings either in the presence or absence of exogenous 1 ppm ethylene.

Ethylene is a plant hormone commonly associated with senescence (aging) and abscission¹⁰. The shedding of leaves, flowers, and fruits from the living plant is known as abscission. These parts abscise in a region called the abscission zone (AZ), which is located near the base of the respective petioles. In most plants, leaf abscission is preceded by the differentiation of a distinct layer of cells, the abscission

layer, within the AZ. During senescence, the walls of the cells in the abscission layer are digested by cellulase enzymes, which cause them to become soft and weak. As a result of stress on the weakened cell walls, the leaves, flowers and fruit eventually break off at the abscission layer⁵.

Ethylene stimulates abscission by causing cellulase formation, which breaks down the cellulose in cell walls in the AZ. Recent citrus field tests conducted by Sorenson¹¹ verify our laboratory results with *A. thaliana*, and reinforce our original hypothesis concerning

EFFECT ON LENGTH OF EPICOTYLS SHOWING YEA![®] (test name: Beyond) DOES NOT INDUCE ETHYLENE FORMATION

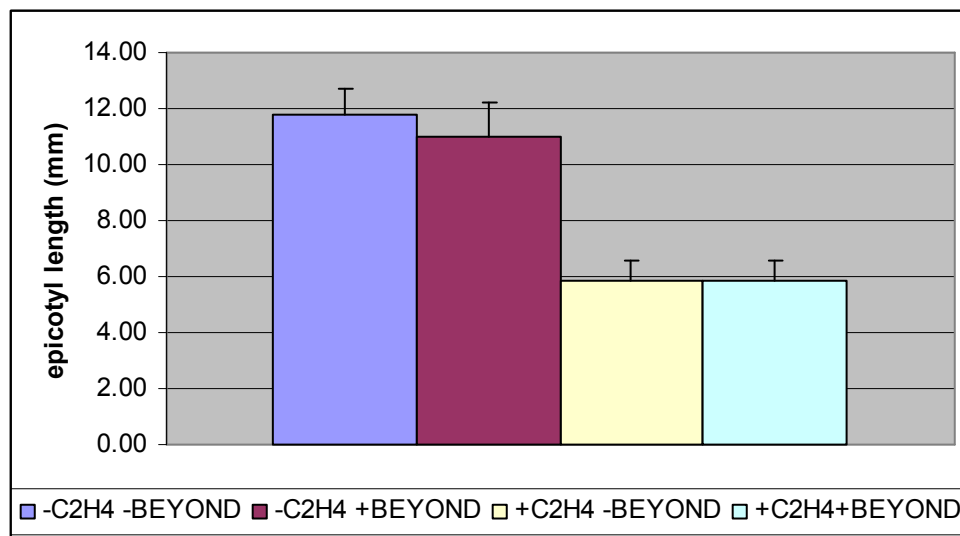


Figure 6. Average epicotyl elongations of etiolated seedlings of *Arabidopsis thaliana* exposed to YEA![®] (test name: Beyond) and C₂H₄ in four combinations from image analysis of scanned material represented in Figure 5. The error bars represent standard deviations from two independent studies of each treatment consisting of 15-20 analyses each.

the induction of signal transduction processes by YEA!^{®2}. YEA![®] treated fruit exhibit improved fruit quality, which apparently results from shutdown of the ethylene biosynthesis process in the pre-harvested fruit and consequent delayed senescence. Data in Figure 7 represent citrus treated with YEA![®] in comparison with fruit picked from trees treated in a manner identical, except with water¹¹. Application of YEA![®] results in the delay of fruit senescence, and gassed fruit does not exhibit expected signs of aging, as demonstrated by lower quantity of ethanol produced during a three month storage period¹¹. Because the fruit treated with YEA![®] requires longer than normal gassing with ethylene to initiate ripening post-harvest, it may be concluded that ethylene biosynthesis is reduced by YEA![®] application in citrus. Controlled harvesting is a benefit of the YEA![®] application. More uniform fruit retention on the tree until picking translates to higher field yields. The use of YEA![®] also allows for a greater degree of control in the ripening process and longer shelf life. Other fruit, called preclimacteric fruit that are similarly affected by ethylene include melons, pears, kiwi fruit, apples, nectarines, and avocados¹⁰. Cut flowers also benefit from blockage of ethylene synthesis and the delay of senescence.

Broader experience by Sorenson¹¹ as well as other commercial producers with multiple applications of YEA![®] following planting, has demonstrated improved biological responses compared to a single treatment. Hence, the question arises: If YEA![®] reduces

YEA![®] (test name: Beyond) IMPROVES THE STORAGE STABILITY OF CITRUS FRUITS

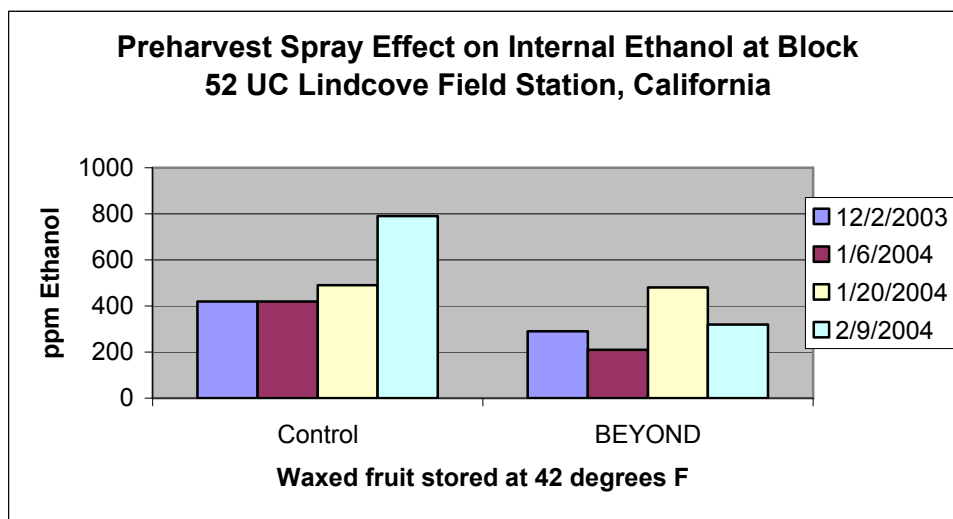


Figure 7. Ethanol production internalized in citrus fruit at four sampling dates during three months of storage following pre-harvest treatment of trees with YEA![®] (test name: Beyond) is lower than in fruit from water-treated control trees and indicates lower rates of fruit senescence and better fruit quality with YEA![®] (test name: Beyond) treatments. Data kindly provided by Sorenson¹¹.

ethylene formation by signal transduction processes at fruit senescence, are there physiological factors in the young or developing plant that are negatively impacted by ethylene? Review of the literature indicates physiological effects on plant seed germination, root and shoot growth, flower development, senescence and abscission of flowers and leaves, ripening of fruit and modulation of responses to biotic and abiotic stresses¹⁰. The endogenous ethylene biosynthesis pathway is fairly well understood¹⁰, but how YEA![®] impacts any one of the enzymatic steps in the pathway needs to be investigated. Similarly, the observed up-regulation of β -1,3-glucanase by treatment with YEA![®] requires further investigation.

CONCLUSIONS

YEA![®] treated fruit apparently resulted from shutdown of the ethylene biosynthesis process in the pre-harvested fruit, which delayed senescence and improved the fruit quality.

Application of YEA![®] resulted in the delay of fruit senescence, and gassed fruit did not exhibit expected signs (9). Because fruit ripening is initiated post-harvest by ethylene, it concluded that ethylene biosynthesis is reduced by YEA![®] application in citrus as well as in *Arabidopsis*. The delayed



of aging longer may be

senescence enables later harvest of greater harvestable yield and becomes advantageous to citrus producers and other fruit and vegetable processors by resulting in:

- a. greater yield,
- b. later harvest,
- c. larger sweeter tasting fruit (more number one grade), and
- d. longer shelf life (less spoilage) and extended shipping destinations.

By controlled harvesting, a benefit of the YEA![®] signal transduction process, the grower will have more uniform fruit retention which translates to higher field pack out. The YEA![®] use allows for a greater degree of control in the ripening process with the benefit being longer shelf life. Other fruit, such as melons, pears, kiwi fruit, apples, nectarines, avacados and tomatoes, as well as flowers and cut blossoms, may benefit from delay of the senescence (8).

The beneficial use of YEA![®] on seedlings of many plants has also been documented (3, 7). Hence, the question arises: If YEA![®] reduces ethylene formation by signal transduction processes in the young plant, are there physiological factors in the developing plant that are negatively impacted by ethylene. Review of the literature indicates physiological effects on plant seed germination, root and shoot growth, flower development, senescence and abscission of flowers and leaves, ripening of fruit and modulation of responses to biotic and abiotic stresses (8). The endogenous ethylene biosynthesis pathway is fairly well understood; how YEA![®] might affect any one of the enzymatic steps in the pathway needs to be investigated.

The ability of the proprietary elicitor to amend plant growth characteristics by means of signal transduction processes makes YEA![®] a unique plant amendment that differs from other types of elicitors and treatments, including chitin and chitosan. As a non-systemic agent in plants YEA![®] impacts receptors on the cell surface and initiates molecular level signal transduction processes. YEA![®] Yield Enhancing Agent naturally activates the signal transduction pathways in a wide and diverse range of plant species and cultivars. During the past 15 years YEA![®] has proven to significantly increase seed germination and sprouting under laboratory conditions and field conditions. This natural elicitor reduces ethylene formation, thus reducing the impact of physiological factors that negatively control development in plants. Scientists and growers across North America, Mexico, Europe, Asia and India attest that by incorporating YEA![®] into seed treatments and field applications by side dressing, drip irrigation systems, flood irrigation and overhead sprays, resulted in better crop yields of higher quality and improved shelf life of produce.

BIBLIOGRAPHY

For more information regarding YEA![®] Yield Enhancing Agent (EPA reg. No. 83729-1) and its benefits to agriculture visit <http://www.yeacrop.com> & <http://www.agrihouse.com/>.

¹ Linden, J.C. Phisalaphong, M. Oligosaccharides potentiate methyl jasmonate-induced production of paclitaxel in *Taxus canadensis*, Plant Science, 2000. 158 (1/2): 41-51.

² Stoner, R. J. II, Stoner, R. J. Sr., Linden, J. C., Knutson, K. W., Kreisher, J. H. 2001. Tuber Planting System Comprising Chitin or Chitosan. US Patent 6,193,988.

³ YEA![®] is manufactured by AgriHouse, Inc. of Berthoud, Colorado and distributed by Loken-Flack LLC of Loveland, Colorado.

- ⁴ Linden, J., Stoner, R., Knutson, K. Gardner-Hughes, C. Organic Disease Control Elicitors, Agro Food Industry Hi-Tech Oct 2000, p12-15.
- ⁵ Taiz, L. Plant Physiology. Sunderland, MA: Sinauer Associates, Inc., 2002.
- ⁶ Coleman, C. Seed Of An Idea - Berthoud firm's experiment now aboard Mir. The Denver Post Business Section pp.1D & 7D, Oct 11, 1997.
- ⁷ Leubner-Metzger, G. and Meins, F. Antisense-transformation reveals novel roles for class I beta-1,3-glucanase in tobacco seed after-ripening and photodormancy. *Journal of Experimental Botany* 2001. 52: 1753-1759.
- ⁸ Leubner-Metzger, G. Functions and regulation of beta-1,3-glucanases during seed germination, dormancy release and after-ripening. *Seed Science Research* 2003. 13: 17-34.
- ⁹ Petruzzelli, L., Muller, K., Hermann, K., and Leubner-Metzger, G. Distinct expression patterns of beta-1,3-glucanases and chitinases during the germination of solanaceous seeds. *Seed Science Research* 2003. 13: 139-153.
- ¹⁰ Buchanan, B.B., Gruissem, W. and Jones, R.L. Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists, Rockville, MD: 2000.
- ¹¹ Sorenson, D., Smilanick J. The Use of Preharvest Plant Elicitors to Improve Post Harvest Quality of Fruit, FGS Packing Services for Sunkist September 16, 2004.

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