Effects of the Plant Defense Activators Actigard and Messenger on Phenolic Compounds and Antioxidant Capacity of Blueberry Fruit

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Hypothesis:

Spray applications of synthetic plant defense activators during the period of fruit ripening will stimulate the production of phenolic compounds, which, in turn, will increase antioxidant capacity of blueberry fruit at harvest.

Justification:

The health benefits of blueberries, attributed to their high antioxidant levels compared with other fruits and vegetables, have received considerable attention in the scientific and popular literature in recent years. Because of the significance of these health benefits, there is considerable interest in utilizing blueberries as a source for antioxidant-rich nutritional supplement formulations. For economical production of such 'nutraceuticals' from blueberries, the concentration of the phytochemicals responsible for the high antioxidant capacity in the fruit should be maximized.

Previous studies have shown that polyphenolic compounds, including various flavonoids and anthocyanins, are the major contributors to the antioxidant capacity in blueberry fruit. Similar phytochemicals are also critically involved in plant defense responses against certain pathogens. Since the late 1990s, there has been considerable interest in agrichemicals that induce resistance in plants against pathogens, and several such products have been labeled. These compounds have no fungicidal activity per se; instead, they act by activating the systemic acquired resistance (SAR) pathway *in planta*, which, in turn, activates several defense-related enzymes, including phenylalanine ammonia-lyase (PAL). PAL is a key enzyme in the biosynthesis of polyphenols possessing health-promoting antioxidant activity. Based on these considerations, we hypothesize that spray applications of plant defense activators to blueberry plants during fruit ripening will increase antioxidant capacity of the berries at harvest. This increase will occur via stimulation of the production of polyphenolic compounds. The experiments described below were designed to test this hypothesis.

Methodologies:

Two experiments were carried out, one in a mature rabbiteye blueberry planting near Alma (southern Georgia) and the other with 4-year-old potted rabbiteye blueberry plants kept outdoors near Athens (northern Georgia). In the field trial, treatments were arranged in a four-replicate split-plot design with plant defense activator (three commercial products or water control; Table 1) as the main plot factor and blueberry cultivar (Tifblue or Woodard) as the sub-plot factor. Individual sub-plots were six bushes long and were separated by untreated buffer rows. Plant defense activators were applied three times from the green fruit stage through the fruit maturation phase using a tractor-mounted airblast sprayer and spraying both sides of the plant rows at a volume of 50 gal/acre of each suspension in well water. Fully mature fruit were sampled 3 days after the final spray and stored at -20^{o}C until analyzed.

In the potted-plant experiment, which utilized cultivar Tifblue, the same plant defense activators were applied in a completely randomized design with four replicates and individual plants as experimental units. Sprays were made on 27 May, 10 June, 27 June, and 8 July 2003, and fully mature fruit were sampled on 30 June (3 days after the third spray) and again on 11 July

(3 days after the fourth spray). During each spray, a volume of 150 ml of suspension in distilled water was applied per plant using a hand-held spray bottle, whereby the concentration of active ingredient in the suspension was the same as in the field trial. Fruit were stored at −20°C until analyzed.

Fruit samples were analyzed for total polyphenols, total anthocyanins, and trolox-equivalent antioxidant capacity (TEAC) at Clemson University's Multi-User Analytical Laboratory. Sample extraction and analysis followed established protocols (*J. Agric. Food Chem.* 50:2432-2438, 2002). Treatment effects were analyzed using analysis of variance.

Results and Conclusions:

Across experiments, values of antioxidant-related variables ranged from 136.8 to 220.2 g/100 g fresh weight (FW) for total polyphenols, 39.0 to 77.0 g/100 g FW for total anthocyanins, and 5.38 to 9.70 μmol/g FW for TEAC (Tables 2 and 3). These values are at the low end of the range reported previously for fruit of rabbiteye blueberry.

None of the plant defense activators tested increased antioxidant-related variables above levels measured for the water-treated control ($P > 0.05$). This lack of a measurable effect could be due to two factors:

1) Blueberry may be among those plant species that are not or only weakly induced by plant defense activators. Previous studies on disease control with such materials reported relatively weak activity in some perennial crops such as peach. At this point, the number of published SAR studies in blueberry is still too limited to allow critical assessment of this idea.
2) Application of the plant defense activators may have "primed" the pathways involved in polyphenol synthesis, rather than actually inducing them. Priming, a common phenomenon following treatment with biological or synthetic resistance inducers, refers to the potentiation of defense responses, allowing plants to react faster and more effectively to a subsequently invading pathogen. Since the present study was carried out in the absence of fruit diseases, such a priming effect would not have resulted in increased induction of defense responses.

Further research with more detailed physiological measurements (e.g., of enzyme activity or associated transcript levels) is needed to shed more light on the response of blueberry to treatment with SAR inducers.

Table 1. Experimental treatments to determine the effect of plant defense activators on antioxidant capacity of blueberry fruit.

Treatment	Active ingredient	Rate per acre [a]
Untreated	Water	---
Messenger	Harpin protein	9 oz
Actigard	Benzothiadiazole	2 oz
AuxiGro	Gamma aminobutyric acid	4 oz + 3.5 oz Silwet

[a] Applications were made in 50 gal/acre of well water in the field trial and in 150 ml/plant of distilled water in the potted-plant trial.

Table 2. Effect of plant defense activators on antioxidant-related variables of ‘Tifblue’ and ‘Woodard’ rabbiteye blueberry fruit in the field trial [a].

Treatment	Tifblue			Woodard		
	Total polyphenols[b] (mg/100 g FW)	Total anthocyanins[b] (mg/100 g FW)	TEAC[b,c] (µmol/g FW)	Total polyphenols[b] (mg/100 g FW)	Total anthocyanins[b] (mg/100 g FW)	TEAC[b,c] (µmol/g FW)
Untreated	213.3 ± 31.2	72.2 ± 8.58	9.21 ± 0.79	190.0 ± 17.0	68.8 ± 6.25	8.45 ± 0.40
Messenger	198.5 ± 33.7	61.4 ± 5.11	7.58 ± 0.52	204.4 ± 16.9	77.0 ± 6.24	8.59 ± 0.44
Actigard	220.2 ± 30.8	70.0 ± 7.30	8.69 ± 0.50	207.1 ± 23.7	76.8 ± 8.34	9.70 ± 0.82
AuxiGro	212.3 ± 33.8	73.8 ± 8.71	9.27 ± 0.63	169.4 ± 12.5	72.8 ± 11.8	8.47 ± 0.47

[a] Three applications were made from the green fruit stage through the fruit maturation phase, with the final spray applied 3 days before harvest.
[b] Values are means ± standard errors based on four replicate plots. FW = fresh weight.
[c] TEAC = trolox-equivalent antioxidant capacity.

Table 3. Effect of plant defense activators on antioxidant-related variables of ‘Tifblue’ rabbiteye blueberry fruit in the potted-plant trial [a].

Treatment	30 June harvest			11 July harvest		
	Total polyphenols[b] (mg/100 g FW)	Total anthocyanins[b] (mg/100 g FW)	TEAC[b,c] (µmol/g FW)	Total polyphenols[b] (mg/100 g FW)	Total anthocyanins[b] (mg/100 g FW)	TEAC[b,c] (µmol/g FW)
Untreated	149.3 ± 19.3	42.3 ± 4.02	5.88 ± 0.70	156.6 ± 32.8	39.6 ± 10.3	5.46 ± 1.14
Messenger	156.7 ± 21.4	46.3 ± 11.3	6.32 ± 0.80	162.2 ± 34.9	49.1 ± 8.09	6.23 ± 0.95
Actigard	144.6 ± 26.0	41.7 ± 8.14	5.84 ± 1.01	139.4 ± 21.2	40.1 ± 3.89	5.47 ± 0.67
AuxiGro	169.1 ± 22.1	54.1 ± 10.0	7.45 ± 0.83	136.8 ± 42.8	39.0 ± 11.2	5.38 ± 1.18

[a] Four applications were made from the green fruit stage through the fruit maturation phase, with the third and fourth sprays applied 3 days before the first and second harvest, respectively.
[b] Values are means ± standard errors based on four replicate plants. FW = fresh weight.
[c] TEAC = trolox-equivalent antioxidant capacity.