Ursolic acid, Ascorbic acid, or Urea Together with Ethephon Accelerate Anthocyanin Production in Cranberry (*Vaccinium macrocarpon*) Fruit[1]

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Abstract. Ethephon has been used to accelerate anthocyanin production in cranberry fruit. However, field application of this chemical yielded inconsistent results which led the cranberry growers to discontinue its use. We investigated the possibility of accelerating anthocyanin production by the application of ursolic acid (125 mg liter^{-1}), urea (50 mM) and ascorbic acid (100 mM) with ethephon (1000 mg liter^{-1}). Field plots were sprayed with various combinations of these chemicals. The presence of these chemicals in ethephon solution consistently increased fruit anthocyanin content. Addition of ursolic acid, urea, or ascorbic acid to ethephon increased anthocyanin content by 16 to 36% compared to ethephon applied alone in two weeks after application. At the same time this increase in anthocyanin content was 24 to 49% over that found in untreated berries. In addition our new formulations of ethephon produced uniformly colored fruits. These treatments did not influence fruit size. There was no visible damage on the foliage in any of the treatments. Our results provide evidence that the addition of urea, ursolic acid, and ascorbic acid to ethephon can accelerate anthocyanin production in cranberry fruit.

Nomenclature: Ethephon [(2-chloroethyl) phosphonic acid], ursolic acid (triterpenoic acid), cranberry (*Vaccinium macrocarpon* Ait.)

Additional Index Words. ethylene, color development, fruit ripening, fruit size, cuticle permeability.

INTRODUCTION

Cranberry fruit is capable of producing intense color if harvest is delayed to late October in Wisconsin. However, risks associated with frost discourage this practice. Cranberry growers are trying to avoid the short season in Wisconsin by accelerating anthocyanin production. Experimental work with ethephon (an ethylene releasing compound) has been conducted to enhance ripening and increase anthocyanin content in cranberry fruit (Devlin and Demoranville, 1970; Rigby et al., 1972; Eck, 1969; Shawa and Ingalsbe, 1968). Rigby et al., (1972) found that drenching the cran-

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berry plant with ethephon in 3.74 k liter.ha^{-1} of water yielded enhanced color development. Ethephon was approved for commercial use on cranberries, however, this practice was not adopted by Wisconsin growers. This is perhaps due to inconsistent results from ethephon application to cranberry fields from season to season (Shawa, 1979).

Recently we have found that the poor efficacy of ethephon on cranberries is due to a fruit cuticle that has very poor surface binding and very low permeability to ethephon (Farag, 1989). Transport studies using cut and intact cranberry fruit showed that the addition of certain chemicals to the ethephon solution can increase the rate of ethylene production by the fruit (Farag and Palta, 1987a). We tested three types of natural compound additives. The first group included compounds such as ursolic acid (triterpenoic acid) which is known to be a chemical constituent of the cranberry epicuticular wax (Croteau and Fagerson, 1971). The second group included compounds which are lipophilic in nature such as ethanol and urea. These compounds, as apposed to salts, have a high degree of solubility in lipids. Thus these compounds can partition into the cranberry cuticle (Farag, 1989). The third group included compounds such as ascorbic acid which have been reported to increase ethylene action in some plants (Cooper et al., 1968). This paper is a report on three of these natural compounds which accelerate cranberry fruit ripening.

MATERIALS AND METHODS

Experiments were conducted during the 1987 and 1988 seasons in a commercial field of 'Searles' cranberry established near Stevens Point, Wisconsin.

Ethephon alone or in the presence of urea (50 mM), ursolic acid (125 mg liter^{-1}) or ascorbic acid (100 mM) was applied using a hand sprayer (Spray doc model 207 p) over 1 m^2 area. The final concentration of ethephon in the spray solution was 1000 mg liter^{-1}. Thus the dose of ethephon was 2.5 kg hectare. The nonionic surfactant Tergitol 15-S-9 was included in all the treatments at 0.3%, v/v. Each plot was sprayed with 250 ml of aqueous solution as a spray. Treatments were replicated four times in a completely randomized design. Applications were made on September 14 and September 12 for the 1987 and 1988 seasons, respectively. Fruits were harvested two weeks after application. Four samples were taken from each plot at harvest.

Fruit size was estimated by counting the berries per 100 g fresh weight. Anthocyanin content was determined using a modified method of Fuleki and Francis (1968). Briefly, the procedure involved homogenizing 100 g of berries in a blender with 100 ml of a mixture of absolute ethanol and 1.5 N HCl (85:15 v/v). Duplicate 5.0 ml samples of this slurry were combined with an additional 25 ml of ethanol: HCl mixture and centrifuged at 16,000 rpm for 5 min (Beckman J2-21). The absorbance of the anthocyanin-containing supernatent was read at 535 nm using Beckman DU-50 spectrophotometer. The data were analyzed by analysis of variance and treatment means were compared using Fishers LSD test.

RESULTS AND DISCUSSION

The presence of ursolic acid (125 mg $liter^{-1}$), ascorbic acid (100 mM), or urea (50 mM), along with the surfactant and ethephon, significantly and consistently increased the amount of fruit anthocyanin content over the ethephon plus surfactant treatment (Table I). The magnitude of this increase ranged from 28 to 36% in the 1987 season and from 16 to 25% in the 1988 season (Table I). Compared to the water control, the new formulations of ethephon increased the anthocyanin content by 40 to 49% in 1987 season and by 24 to 35% in 1988 season. The fruit size was not affected by these formulations in either season (Table I). No visible adverse effects of our new ethephon formulations (reported in this paper) were observed either on the fruit or the foliage.

In addition to increased anthocyanin content, an advantage of the new ethephon formulations was color uniformity which could not be achieved consistently by the application of ethephon plus surfactant alone. Rigby et al. (1972) pointed out nonuniformity of color as a problem with commercial cranberry fruit production. The exposed surface gets dense color whereas the surface of the berry facing ground does not get fully colored. This nonuniformity problem can affect the marketability of the cranberry for fresh fruit consumption.

The added advantage of these chemicals to promote anthocyanin production can be explained by increased surface binding capacity and increased transport of ethephon in the presence of these chemicals. Urea is a somewhat lipophilic and was found to partition into the cranberry cuticle (Farag, 1989). This binding could alter the surface properties of the cuticle in such a way as to allow ethephon to penetrate the otherwise relatively impermeable cuticle. In support of this explanation, an increase in transport of Rb, Cl and Fe across cuticles in the presence of urea has been observed (Yamada et al., 1964; Wallace, 1962). We also found that urea was able to stimulate ethylene production in the excised fruit tissue of cranberry and apple (Farag and Palta, 1989). Thus, in addition to increasing transport of ethephon, urea may be stimulating ripening and ethylene production independent of ethephon. Ursolic acid is the main triterpenoic acid (about 50% of the

Table I. The effect of field applications on a limited scale of new ethephon formulations on anthocyanin content and fruit size of cranberry fruit cv. Searles during 1987 and 1988 seasons.

Treatments	Anthocyanin content[a] (mg/100 g fresh weight)		Fruit size[b] (# berries/100 g)	
	1987	1988	1987	1988
Water	17.3	21.7	81.1	100.1
Tergitol	...	21.9	...	99.1
Tergitol+Ethanol	19.3	...	81.8	...
Urea+Tergitol	...	20.6	...	104.0
Ethephon+Tergitol	19.1	23.3	80.8	102.3
Tergitol+Ursolic Acid	20.2	...	82.2	...
Ethephon+Tergitol+Ascorbic Acid	24.4	29.2	82.1	104.8
Ethephon+Tergitol+Ursolic Acid	24.4	27.0	78.0	100.0
Ethephon+Tergitol+Urea	26.0	28.8	79.4	106.9
LSD (P= 0.05)	3.1	3.5	5.1	8.3

[a]Mean of 4 replications (4 samples within each replication, 2 separate determinations from each sample).

[b]Mean of 4 replications (4 and 2 samples within each replication in 1987 and 1988 respectively).

acid fraction) in the epicuticular wax of cranberry fruit (Croteau and Fagerson, 1971). We found that ursolic acid also increased the surface binding capacity of ethephon (Farag, 1989). Ascorbic acid has been thought to induce ethylene production and has been used to promote abscission of fruit when applied together with auxins (Cooper et al., 1968).

The results presented here provide evidence that natural products such as urea, ursolic acid and ascorbic acid together with ethephon can be used to accelerate anthocyanin production in cranberry fruit.

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