J. BIOL. ENVIRON. SCI.,
2010, 4(12), 133-141

Influence of Preharvest Ethephon Spray on Fruit Quality and Chemical Attributes of 'Cigany' Sour Cherry Cultivar

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ABSTRACT

Influences of preharvest ethephon spray on fruit quality attributes and certain nutritional compounds of 'Cigany' sour cherry (*Prunus cerasus*) were investigated. Trees were sprayed with 250 ppm ethephon one week before anticipated commercial harvest. Fruits from ethephon-sprayed trees had significantly lower soluble solids concentration (SSC), anthocyanin content, antioxidant activity, and firmness than those from non-sprayed control. The ethephon spray did not affect total phenolic content, although its content tended to be higher in fruits from non-treated control. Titratable acidity (TA), pH and SSC/TA ratio were not affected by ethephon spray. There was a significantly positive correlation between anthocyanin content and SSC (r = 0.99).

Key Words: anthocyanins, antioxidant activity, phenolics, *Prunus cerasus*

INTRODUCTION

Sour cherry is one of the most benefit fruits containing phytonutrients such as anthocyanins, chlorogenic acid, gallic acid, *p*-coumaric acid, and quercetin which have antioxidant activity (Wang et al. 1999). Anthocyanins as one of the flavonoids belong to phenolic compounds which make a dark red color and attract pollinators. The combination of various aglycones, glycosylations, and acylations results in more than 635 anthocyanins in nature. Their aglycone structures undergo reversible transformation at different pHs (He and Monica Giusti 2010).

Ethephon (2-chloroethylphosphonic acid), an ethylene releasing compound, is usually used to reduce the fruit retention force of mature cherries to enable removal without stems so that harvesting with shakers or hands can be easily done in a shorter time. It also promotes early uniform fruit maturation while minimizing detachment damage (Peterson and Wolford 2001).

In preharvest application, ethephon, increases ripening but accelerates postharvest detrimental (Gerasopoulos and Stavroulakis 1999). Softening of ethephon-treated berries held at 0^0C was faster than their coloration (Gerasopoulos and Stavroulakis 1999). Eck (1970) showed that ethephon promoted the blueberry fruit maturity and the harvest period, however the treated berries had lower total soluble solids content (SSC) and acidity than control. Ban et al. (2007) showed that ethephon application stimulated the decrease in titratable acidity (TA), anthocyanin accumulation, and fruit softening of rabbiteye blueberry during the growth period.

Glozer et al. (2006) reported that application of ethephon in sweet cherry did not affect the fruit color. Also they demonstrated that after storage, firmness tended to be reduced by ethephon. Both Ethrel and silaid, applied at 200-300 ppm, facilitated mechanical harvesting of both 'Pándy 279' and 'Érdi Botermo' sour cherry cultivars by significantly reducing the fruit removal force, while soluble solids and flesh firmness of the fruits were not significantly affected (Kollár and Bukovac 1996). The 'Windsor' sweet cherry cultivar showed increasing weight and color when ethephon was applied at rates of 500 ppm or higher (Bukovac et al. 1971). The reaction appears to be cultivar-dependent, because subsequent research showed no significant changes in fruit quality (Bukovac 1979). Goodman (2007) reported that the anthocyanins in tart cherries effectively reduced painful inflammation relative to anti-inflammatory drug, indomethacin. This effect may arise from the ability of anthocyanins in reducing oxidative stress, which is a major cause of autoimmune disease. Because of having more anthocyanins, tart cherries award far more benefits than sweet cherries. The stability of anthocyanins is determined intrinsically by the types of glycosylation and acylation, which is affected externally by the pH, temperature, light intensity, enzyme, and the presence of other compounds interacting with anthocyanin molecules (He and Monica Giusti 2010).

Because of being non-climacteric, cherry fruits produced neither respiratory nor ethylene peaks near maturity (Li et al. 2003). More recent works have disclosed that some aspects of non-climacteric ripening may be associated with ethylene responses (Giovannoni 2001).

Effects of pre-harvest ethephon on inner compounds of sour cherry have not been completely investigated yet. In this study we evaluated whether ethephon treatment affects anthocyanin content, antioxidant activity, total phenolic content and other qualities.

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MATERIALS AND METHODS

Experiments were done on ‘Cigany’ sour cherry cultivar in a commercial orchard of Mashhad, Iran. Uniform 15-year-old trees were sprayed (1,250 $L \cdot ha^{-1}$ and about 5 L/tree) with Ethrel (48%, produced by Hockley Intl. Ltd., Cheshire, UK) at 250 ppm on whole tree and control trees receiving only water. Spraying was conducted 1 week before anticipated commercial harvest when a ting of red appeared on fruit. Aryanpooya et al. (2009) did similar experiment on ‘Érdi Jubileum’ sour cherry cultivar in the same period of time. They applied different concentrations and best results were obtained at 250 ppm. Childers (1983) has counseled 300 ppm ethephon for light color sweet cherries, 400 ppm for light ones and 200 ppm for sour cherries 7-14 days before harvest in Michigan condition. Nugent (2005) recommended the use of ethephon, 7 to 14 days before anticipated harvest. Fruits were harvested at commercial maturity from ten trees within a single plot at the end of June 2009 and sent in an ice flask to the laboratory.

Chemical Flavor Component Analysis

SSC was measured using a digital refractometer (model RFM340, Bellingham & Stanley, Kent, UK). TA was determined by titration with 0.1 N NaOH to reach to pH 8.1 and expressed as percent from malic acid in juice. The pH value was measured by using a digital pH meter. The flesh/pit ratio of fruits was obtained by the following equation: flesh/pit = $\frac{W_T - W_P}{W_P}$

Where W_T = total weight of fruit, W_P = pit weight.

The flesh firmness was measured by a firmness tester machine (Shinwa-MARUTO, Japan) and expressed as a rate of fruit deformation (mm) is caused by one minute impact of a weight (110 g) on fruit surface. This device consists of a 110 g weight and a graded ruler with 0.5 mm accuracy.
Measurements were conducted in three replications and each replication consisted of ten fruits. For measurement of anthocyanin content, antioxidant activity and total phenolic content, the samples were kept at -20°C until extraction.

Preparation and Chemical Analysis of Phenolics and Antioxidant Activity

Fruits were stoned and homogenized in a blender. Fifty grams of mixed cherry which had been covered with foil were macerated in 100 mL of 70% methanol containing 0.1% HCl and placed on the shaker for 24 h. Then the extract was filtered over Whatman No. 1 paper under vacuum, and the residue was again extracted with 100 mL of the same solvent until it became colorless. The extracted solutions were concentrated at 45°C under reduced pressure by a rotary evaporator and dried in an oven to have constant weight.

Total anthocyanins were determined by the pH differential method (Wrolstad, 1976). The resultant cyanidin-3-glucoside was expressed as milligram per 100 g of fruit. Total phenolic content was determined using the Folin-Ciocalteu colorimetric method. In this case, 0.1 mL of the methanolic extract solution was mixed with distilled water to reach 0.5 mL, then 0.25 mL Folin Ciocalteu reagent (1 N) was added and mixed well. After 3 min, 7.5% Na_2CO_3 was added and the mixture was vortexed. After remaining for 45 min at room temperature, the absorbance of the solution was read at 725 nm by using a UV spectrophotometer (CE2502, BioQuest & BioAquarius Series, Cecil Instr. Inc., Cambridge, UK). Tannic acid was used as a standard.

Determination of antioxidant activity was done by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity (Brand-Williams et al., 1995). To 3.9 mL methanolic DPPH solution (0.004%, w/v), 0.1 mL of methanolic extract at various concentrations was added, mixed thoroughly and left in a dark place. After 30 min the absorbance was read at 517 nm against control without the extract. DPPH radical scavenging activity was obtained with the following equation:

Radical scavenging activity (%) = $(A_0 - A)/A_0 \times 100$

Where A_0, control absorbance and A, sample absorbance.

Statistical Analysis

Results were analyzed according to a completely randomized experimental design. Statistical analysis was carried out using MSTAT-C software. The results were calculated by one-way analysis of variance (ANOVA). The Student’s *t* test was used for comparison between two treatments at $P < 0.01$.

RESULTS AND DISCUSSION

Chemical Attributes

Total SSC was significantly lower in ethephon-treated sour cherry. Ethephon spray did not have any significant effect on pH, TA, and SSC/TA ratio (Table 1). Szyjewicz et al. (1984) mentioned that the effect of ethephon on fruit composition varied with cultivars, and timing, concentration, and method of application, so contradictory results have been noted about its effects on SSC, TA, and pH. The pH value of the treated ‘Cigany’ sour cherry juice was not significantly different from control. Aryanpooya and Davarynejad (2009) reached the same result about pH, TA, and SSC/TA ratio in ‘Érdi Jubileum’ sour cherry treated with various concentrations of ethephon which was not different from control. Micke et al. (1973) demonstrated that the SSC of treated ‘Royal Ann’ sweet cherry decreased as the concentration of ethephon increased. As reported by Delgado et al. (2004), ethephon application decreased the SSC of ‘Tempranillo’ grapes at harvest time in relation to the control. Lombard et al. (2004) reported that SSC and TA levels of ‘Flame Seedless’ grape decreased or tended to decrease by increasing ethephon dosages above 100 $mg \cdot L^{-1}$. The higher dosages having significantly lower SSC and TA levels than the control. Singh and Shafiq (2008) resulted that there were no significant differences in fruit firmness, SSC, or TA of the fruits harvested from Ethrel -treated and control ‘Pink Lady’ apple trees, at commercial harvest. Similarly, no significant differences in pH and SSC were reported in relation to ethephon treated rabbiteye blueberries (*Vaccinium ashei*) (Dekazos and Russell 1976). According to Miller and McDonald (1997), ethylene-treated carambola fruits had lower total SSC, higher TA and pH, and a less preferred flavor and texture than control fruits.

Table 1. Effect of preharvest ethephon spray on chemical attributes of ‘Cigany’ sour cherry cultivar.

Treatment	SSC (^{o}Brix)	pH	TA(g malic acid/100 mL)	SSC/TA
Control	18.1 a^{z}	3.09 a	2.38 a	7.60 a
Ethephon sprayed	15.87 b	3.08 a	2.04 a	7.84 a

^{z}Mean separation within columns by Student’s *t*-test at $P = 0.01$.

Physical Properties

As shown in Table 2, ethephon treatment significantly influenced cherry weight, flesh/pit ratio, and fruit firmness ($P < 0.01$). The harvested fruits that receiving ethephon were heavier and softer than the control. Smith and Whiting (2010) indicated that ethephon-treated ‘Chelan’ sweet cherry was significantly heavier and darker than non-treated fruit. Bukovac et al. (1971) showed increased fresh weight and increased pigment formation in ‘Windsor’ sweet cherry cultivar at a rate of 500 ppm. Average berry mass was influenced by dosage and tended to reach a maximum at 200 ppm (Lombard et al. 2004). El-Zeftawi (2003) reported ethephon at 250 ppm produced the largest and heaviest ‘Imperial’ mandarin.

Table 2. Effect of preharvest ethephon spray on physical properties of ‘Cigany’ sour cherry cultivar.

Treatment	Cherry weight (g/fruit)	Flesh/pit	Deformation of fruit (mm)
Control	2.81 b^{z}	8.92 b	5.02 b
Ethephon sprayed	3.08 a	9.88 a	6.54 a

^{z}Mean separation within columns by Student’s *t*-test at $P = 0.01$.

Elfving et al. (2003) showed that spraying with 1-methylcyclopropene improved the ‘Bing’ sweet cherry flesh firmness in ethephon-treated fruits at harvest (which undergo flesh softening) and reversed the negative effect of ethephon on firmness. Firmness of ‘Chelan’ sweet cherry cultivar was reduced by ethephon applications (Smith and Whiting, 2010). The softening of fruits occurs due to deterioration of membrane integrity and its functionality (Sankhla et al. 2004).

Total Anthocyanin, Phenolic Content, and DPPH Radical Scavenging Activity

Influences of ethephon on total anthocyanin content, total phenolic content, and DPPH radical scavenging activity were shown in Table 3. Anthocyanin content was lower in treated fruits than in control. Wicks and Kliewer (1983) found variable responses for two table grapes cultivars 'Ribier' and 'Emperor' in response to ethephon application. In 'Emperor', ethephon increased anthocyanin concentration in sun exposed fruit skin; conversely the same treatment in 'Ribier' had negligible effect on anthocyanin concentration. Glozer et al. (2006) mentioned that ethephon-treated fruit was not significantly different than the untreated control with respect to color at harvest in 'Bing' sweet cherry. Ban et al. (2007) found that ethephon application accelerated the anthocyanin accumulation and greatly increased its final concentration compared to the control in rabbiteye blueberry (*V. ashei*). In 'Jonagold' apple, ethephon application stimulated the anthocyanin accumulation in the skin, but did not affect the total SSC, TA, and fruit firmness (Awad and Jager 2002). The significantly high positive correlation between SSC and anthocyanin content (Figure 1) was specified with a correlation coefficient (r= 0.99, P <0.01), while a high negative correlation existed between anthocyanin content and fruit weight (r= -0.95, P <0.01) (Figure 2). Gholami (2004) showed that changes in total SSC closely accompanied changes in color or anthocyanin levels in the skin of Shiraz grape berries, but Wicks and Kliewer (1983) found contrary result about grape berry.

Table 3. Total anthocyanin, phenolic content and free radical scavenging activity by DPPH assay

Treatment	Total anthocyanin content (mg cyanidin3-glucoside equivalent/100 g fresh wt)	Total phenolic content (mg tannic acid equivalent/100 g fresh wt)	Free radical scavenging activity (%)
Control	49.02 a^{z}	392.56 a	42.38 a
Ethephon sprayed	35.89 b	348.69 a	35.42 b

^{z}Mean separation within columns by Student's t-test at P = 0.01.

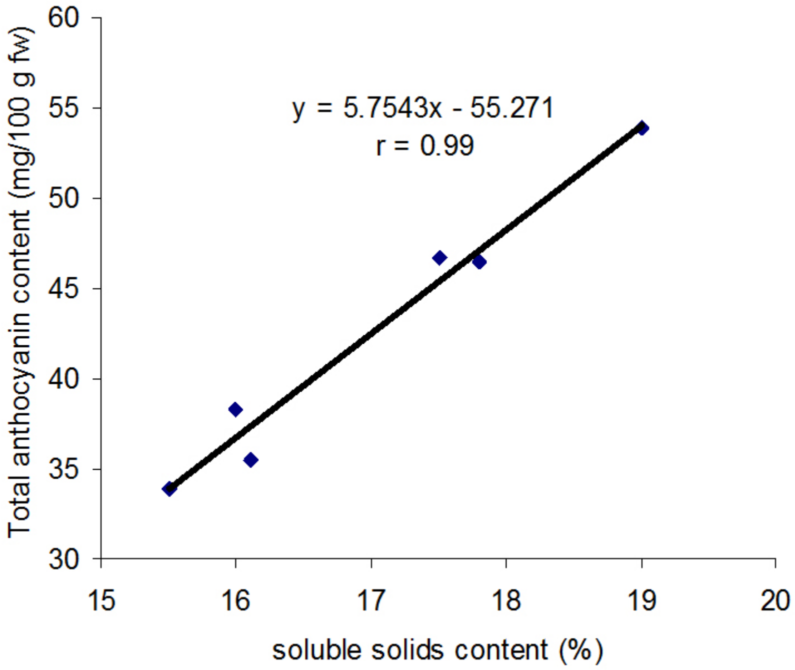

Figure 1. Correlation between total anthocyanin and SSC of 'Cigany' sour cherry (r = 0.99).

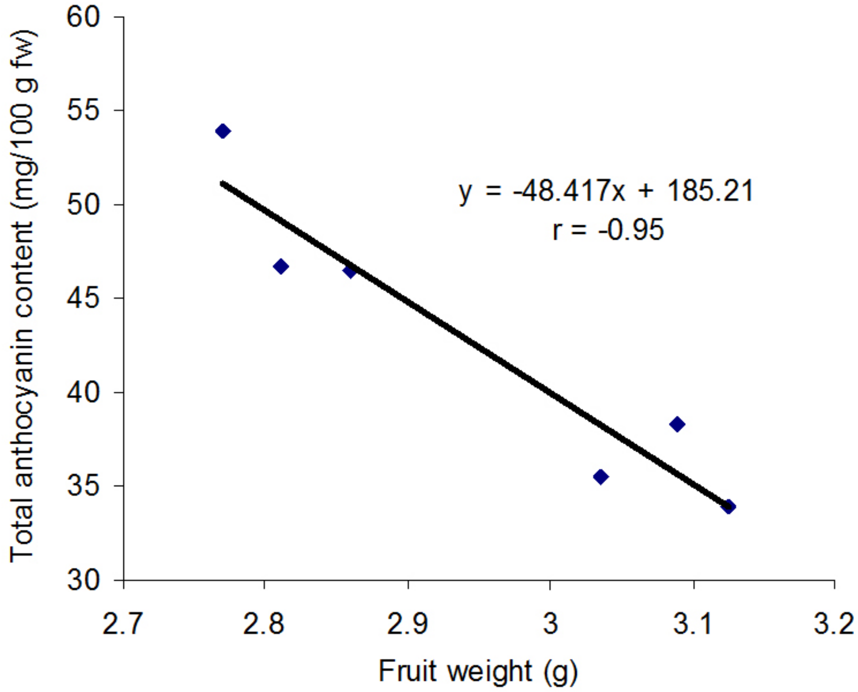

Figure 2. Correlation between total anthocyanin and fruit weight of 'Cigany' sour cherry (r = -0.95).

The increase in fruit size was due to increase in water content (lower SSC), so dilution effect might explain the inverse relationship between fruit size and anthocyanin content. Because ethephon treatment caused a significant increase in fruit weight but surface area increases less rapidly than the total volume of the fruit thus anthocyanins are diluted.

While ethephon was generally found to increase the accumulation of highly methoxylated monoglucoside of peonidin and malvidin in the berry skin during ripening, so increasing in red color without any effect on total anthocyanin content in the must of 'Tempranillo' grapevines might have been associated with a higher level of methylation in the anthocyanin molecules (Delgado et al. 2004).

Total phenolic content was not affected by ethephon, however control fruits tended to be higher and antioxidant capacity was significantly higher in control fruits (Table 3). Antioxidant capacity was assayed by DPPH method which is based on the reduction of methanolic solution of DPPH in presence of hydrogen donating molecules. The activity to scavenge DPPH radical increased significantly with increasing extract concentration in both groups (Figure 3). There is little information about effects of ethylene on antioxidant activity and phenolic content.

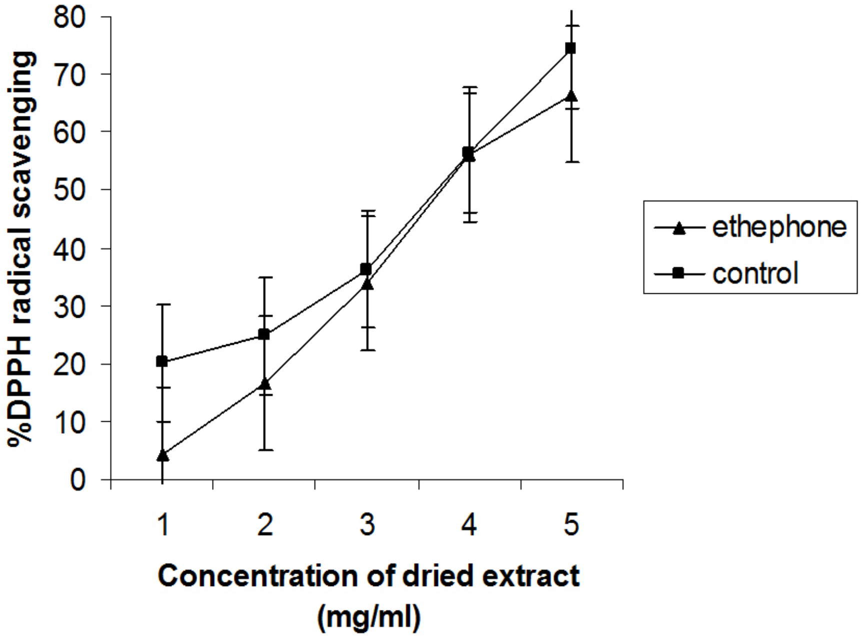

Figure 3. Comparison of DPPH radical scavenging activities of ethephon treated and untreated 'Cigany' sour cherry.

Delgado et al. (2004) demonstrated that the combined application of 700 ppm ethephon and 800 ppm of ABA at veraison increased the total polyphenol content of 'Tempranillo' grapevines must up to 16% in relation to the control. Park et al. (2006) used ethylene on 'Hayward' kiwifruits after harvest then the ethylene treated and untreated kiwifruits were ripened separately under the same conditions at 20°C in a growth chamber for 10 days. The content of total polyphenols and the antioxidant activity were significantly increased only in ethylene treated kiwifruits at the period of 4–6 days after the beginning of the ripening process. There was also a positive correlation between total phenolic content and antioxidant capacity (Figure 4) in all fruits (treated and untreated) ($r = 0.73$). Pedisic et al. (2007) indicated that there was no correlation between antioxidant activity and total phenolic content of sour cherry cv. Marasca. Li et al. (2005) showed that there was no correlation between total phenolic contents and antioxidant activities in jujube fruits. Papp et al. (2008) showed that there was a close correlation between ferric reducing ability of plasma (FRAP) and total phenolic content in sour cherry. Karlidag et al. (2009) found that total phenolic content and antioxidant activity of wild sweet cherries showed positive relationship ($r = 0.76$).

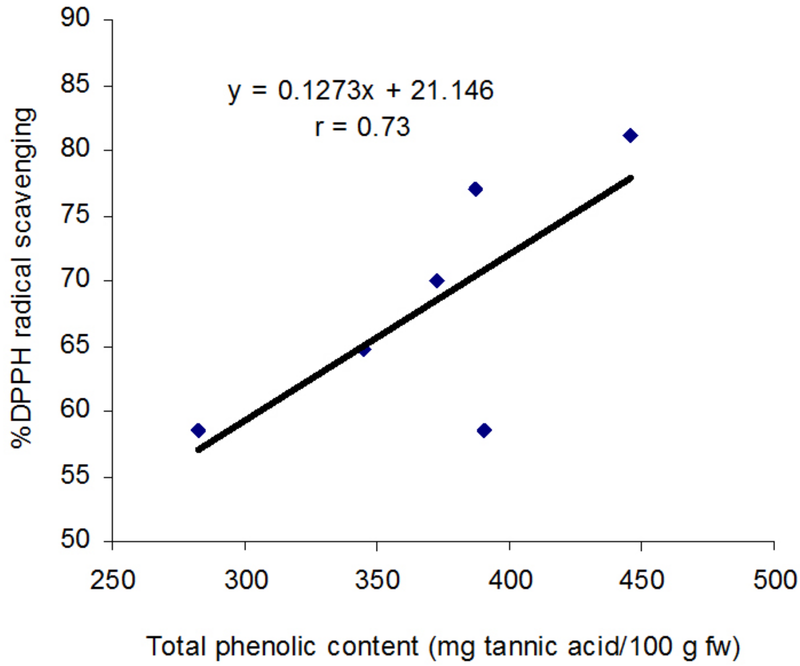

Figure 4. Correlation between antioxidant activity and phenolic content of 'Cigany' sour cherry (r = 0.73).

Gil et al. (2002) reported a strong correlation (r = 0.93-0.96) between total phenolics and antioxidant activity in stone fruits included nectarine, peach and plum cultivars. Similar results obtained by other researchers in black berries and raspberries (Wang and Lin, 2000). In this study the correlation between antioxidant activity by DPPH and total anthocyanin content was r = 0.64. Chaovanalikit and Wrolstad (2004) found a good correlation between total phenol and total antioxidant activity in different fruit portions of four sweet cherry cultivars, although a poor correlation was obtained with anthocyanins. Radical scavenging activity might be related to other phenolic compounds such as neochlorogenic acid and 3-*p*-coumaroylquinic acid. Laranjinha et al. (1995) reported that 3-*p*-coumaroylquinic acid inhibit the generation of free radicals, have a chain-breaking activity and be the main phenolic compound in sweet cherries (Mozetiĉ et al. 2002).

CONCLUSIONS

The regulation of ripening processes in non-climacteric fruit is still under question and not so detailed compared to climacteric fruit. The role of ethylene, plant triggering growth hormone, in non-climacteric fruit is not very known (Hartmann et al. 1987). Post harvest metabolic changes of sweet cherry polyphenols are not regulated by ethylene (Mozetič et al. 2006, Hartmann 1992).Ethylene has no role in ripening processes of sweet cherries at all (Gong et al. 2002, Hartmann 1992). Ban et al. (2007) concluded that ethephon application in fruits promoted ripening, but the stimulatory effect of ethephon on fruit ripening differed in degree for each fruit ripening character.

It cannot be excluded that the low ethylene production may have a role in the ripening process of some but not all non-climacteric fruits. Ripening-related chlorophyll breakdown in non-climacteric fruits is therefore assumed to be either ethylene dependent or independent based on the type of fruit (Goldschmidt 1997). More recent works have revealed that some aspects of non-climacteric fruit ripening may be associated with ethylene responses (Giovannoni 2001). We concluded that ethephon application did not affect anthocyanin accumulation of 'Cigany' sour cherry, but increased fruit weight, flesh/pit ratio, and fruit softening.

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