

Combined effects of ethephon and mepiquat chloride on late blooming, fruit set, and phytochemical characteristics of Black Diamond plum

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Abstract: This study was carried out in 2015 and 2016 to investigate the use of ethephon and mepiquat chloride for reduction of damage to flowering and fruit set caused by late spring frosts in Black Diamond plum. The single treatment of mepiquat chloride delayed full blooming by 5 days, while the ethephon treatment delayed blooming by 7 days. The interactive effect of the chemicals yielded more positive results in the delay of blooming, causing a delay of up to 11 days. An increase in fruit set was observed along with blooming. The fruit set percentage of the control was 4.18%, while it reached 21.28% with the combination of the chemicals. Combined or single applications of the chemicals resulted in a decrease in all phytochemical properties, except for vitamin C. In the control, total anthocyanin, total phenol, soluble solids content, and antioxidant capacity were 67.95 mg 100 mL⁻¹, 1056.00 mg L⁻¹, 19.68%, and 27.05 mmol L⁻¹, respectively. In the treatment groups these values were reduced to 39.02 mg 100 mL⁻¹, 854.90 mg L⁻¹, 16.98%, and 21.32 mmol L⁻¹, respectively. An ethephon treatment of 4000 ppm led to blighted branches and gummosis. The combined effect of higher mepiquat chloride (150 ppm) and lower ethephon (2000 ppm) doses are believed to contribute to reduction of damage caused by late spring frosts.

Key words: Frost damage, phenolic compounds, phenology, plant growth regulator

1. Introduction

Plums belong to the subgenus *Prunophora* within the genus *Prunus* and include species originating from Europe, Asia, the Far East (Japanese plums), and North America such as *Prunus cerasifera*, *Prunus domestica*, and *Prunus salicina*. Plum plants grow in the wide climatic conditions of the northern and southern hemispheres. Turkey is the gene center of some plum species and has an important place in plum growing (Celik et al., 2017).

Temperature increases due to climate change result in the early blooming of fruit trees (Funes et al., 2016; Szabo et al., 2017), while duration of the effects of late spring frosts and related damage increase (Rahimi et al., 2018). This threatens fruit trees, particularly stone fruit and nuts, which bloom early and have short blooming periods (Inouye, 2008; Mertoğlu and Evrenosoğlu, 2017). To help combat this issue, changes among different phenological phases in frost resistance were investigated. The sensitivity of current genetic resources to late spring frost were determined previously (Aygün and Şan, 2005; Gunes, 2006; Latocha, 2008; Salazar-Gutierrez et al., 2014; Matzneller et al., 2016; Sakar et al., 2017). Delaying the blooming of fruit trees until after the late spring frosts and

developing more cold-tolerant cultivars are reported to be the most effective methods to address these issues (Dicenta et al., 2017). Positive results were obtained in selection and breeding studies with this aim, and breeding of late-blooming cultivars with good fruit quality was reported to be the most efficient method in the long term (Akca and Ozongun, 2004; İmrak et al., 2017; Khadivi-Khub and Khalili, 2017; Prudencio et al., 2018). The identification of the genes controlling blooming and fruit traits and the use of parents that harbor positive traits are of great importance in obtaining cultivars that have the desired properties (Cai et al., 2018).

Air mixers and fogging units are not preferred for short-term elimination of the negative effects of late spring frosts because of their high installation costs. Instead, ethephon, abscisic acid, mepiquat chloride, antifreeze proteins, methyl jasmonate, nitric oxide, and certain herbal volatiles that prolong the duration of plastochron; delay blooming; increase the resistance of buds, flowers, and small fruits through differentiation; and reduce the rate of photosynthesis or the amount of catalyzed substrates are commonly used in plant growing (Krewer et al., 2005; Grijalva-Contreras et al., 2011; Qrunfleh and Read, 2013;

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Duman and Wisniewski, 2014; Mohammadi et al., 2015; Pakkish and Tabatabaenia, 2016). However, the excessive and untimely use of these chemicals results in negative outcomes such as the abscission of buds and flowers, gummosis, and irregular and reduced fruit set (Coneva and Cline, 2009; Mertoğlu and Evrenosoğlu, 2017). Thus, the timing and the dose of chemical treatments are of great importance. Furthermore, along with the physiological changes caused by the use of chemicals, changes are observed in the phytochemical properties of fruit (Saavedra et al., 2016; Korkmaz and Aşkın, 2017; Einhorn and Arrington, 2018).

The main aim of this study was to determine the effects of single or combined applications of mepiquat chloride and ethephon on late blooming in the Black Diamond plum cultivar in order to reduce the damage caused by late spring frosts. In addition, the effects of plant growth regulators on soluble solids content (SSC), vitamin C, total anthocyanin, total phenolics, and the antioxidant capacity of plum fruit at commercial harvest were determined.

2. Materials and methods

2.1. Plant materials and experimental treatments

The study was carried out in 2015 and 2016 with three replications (3 trees for each treatment) and designed in accordance with the randomized plot factorial experimental design by forming nine treatment combinations that comprised three different levels

[0/0 (control); 0/75; 0/150; 2000/0; 2000/75; 2000/150; 4000/0; 4000/75; and 4000/150] of each of the two factors [ethephon (0–2000 and 4000 ppm) and mepiquat chloride (0–75 and 150 ppm)].

The Black Diamond plum cultivar (*Prunus salicina* Lindell), grown in the experimental field of the Eskişehir Osmangazi University Faculty of Agriculture, was used as the material. The cultivars were planted in 2011 after grafting onto seedling rootstocks. Table 1 shows the climatic data of the experimental area. The phytochemical analyses were performed in the pomology laboratory of the Agricultural Sciences and Technology Department of Isparta Applied Sciences University. Two different plant growth regulators were applied both to the underground and upper organs of the plants. The method is based on the fact that ethephon (AGROBEST, Efhun; 480 g L⁻¹ ethephon) applied to the aerial parts of plants is rapidly absorbed through stomas, lenticels, and hydathodes, while mepiquat chloride (BASE, Pix; 50 g L⁻¹ mepiquat chloride) is absorbed through the roots at a slower pace (Mertoğlu and Evrenosoğlu, 2017). To allow the access of mepiquat chloride to the effective root depth, the plants were irrigated until they reached field capacity, and the next day (20 February), 15 L of mepiquat chloride–water solution was given to the plants using the root irrigation method. The ethephon–water solution was sprayed onto the upper parts of the plants using a knapsack pulverizator on 1 March when the daylight temperatures reached

Table 1. Mean weather data for the related months in Eskişehir in 2015 and 2016.

	Monthly number of frost days	Lowest temperature	Mean temperature	Mean monthly 50-cm soil temperature	Total rainfall (mm)	Mean humidity (%)
2015						
March	10	-7.2	5.7	7.3	38.9	78.6
April	13	-4.7	7.8	9.8	26.6	68.2
May	0	3.3	15.8	17.1	47.8	68.1
June	0	5.8	17.1	18.2	151.1	81.0
July	0	10.1	22.0	21.3	0	63.3
August	0	9.6	22.8	22.9	37.2	66.1
September	0	8.9	21.0	22.6	3.1	64.9
2016						
March	9	-6.7	7.5	8.6	41.2	70.3
April	2	-1.9	12.9	12.4	36.7	64.5
May	0	2.8	14.1	15.6	44.7	74.2
June	0	4.8	21.0	21.0	6.3	62.1
July	0	10.4	22.8	24.5	14.5	58.3
August	0	9.6	22.8	24.2	27.7	66.0
September	0	2.3	17.8	21.1	31.7	64.9

about 10 °C. The chemicals were applied according to the instructions provided by the producers and the relevant literature (Wang et al., 2014; Mertoğlu and Evrenosoğlu, 2017).

After the bud swell period, daily observations were carried out in the field to record the blooming dates of the plum trees. The first group to enter the full blooming period was chosen as the control. The blooming dates of other plants were recorded as + days with respect to the control.

For the determination of fruit set percentage (%), a minimum of 500 bloomed or blooming flowers in four different directions on the plants were counted and labeled as parallel for each replication. On the 40th day after enumeration, the number of fruits was divided by the number of flowers and multiplied by 100 to calculate the fruit set percentage (Mertoğlu et al., 2018).

For phytochemical analyses, the fruits of each tree belonging to each treatment were harvested without mixing with fruits from other trees and divided into three parallels, each consisting of 50 fruits (in total: 3 trees, 9 parallels, and 150 fruits for each treatment). Fruits were squeezed with a juice extractor to obtain their juices. The fruits were harvested at consumption maturity in August according to sense of taste (Arion et al., 2014). A digital refractometer (Atago PR-32, Japan) was used to determine total SSC and results were expressed as a percentage (%) (Ercisli et al., 2011).

First, juices were centrifuged, and the upper parts of the solutions were used directly for phytochemical analyses. Total polyphenol content was determined using the colorimetric reaction with Folin–Ciocalteu reagent (Selçuk and Erkan, 2016). Gallic acid was used as an external standard for the calibration curve, and the results were expressed as milligrams of gallic acid equivalent (mg GAE L⁻¹) of fruit juice. The antioxidant activity of juices was determined using the ABTS + radical cation decolorization assay, as described by Gündoğdu (2019). Antioxidant activity was calculated using the ratio of the regression coefficient of the dose response curve and expressed in terms of millimoles of trolox equivalents per liter of fruit juice. Total monomeric anthocyanin content was determined using the pH differential method as described by Selçuk and Erkan (2016). Total anthocyanins were calculated as cyanidin-3-glucoside and expressed in terms of milligrams of cyanidin-3-glucoside per 100 mL of fruit juice. Ascorbic acid content in fruit juices was estimated using the volumetric method. For this purpose, juices were titrated with potassium iodate, in which starch was used as an indicator. Results were given in mg 100 mL⁻¹ (Spinola et al., 2013). Phytochemical analyses were conducted when the last treatment group was harvested each year. The juices of the previously harvested groups were stored at -20 °C.

2.2. Statistical analysis

In the statistical model of the study years, ethephon and mepiquat chloride were regarded as independent factors. SPSS 18 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses of the effects of the treatments on the investigated properties. Before the application of repeated ANOVA, transformation was applied to the total SSC, total phenol, anthocyanin, vitamin C, and antioxidant activity properties, while the delayed blooming property was

transformed with $\sqrt{X_i + \frac{3}{8}}$ and fruit set was transformed

using the reverse angle transformation (Zar, 2013). The differences between the levels and interactions of the factors were revealed using the Tukey HSD multiple comparison test at a significance level of 5%.

3. Results and discussion

The effects of ethephon, mepiquat chloride, and year on the properties investigated in this study were determined to be significant, except for vitamin C. The binary and ternary interactions of these factors were also significant for certain properties.

Ethephon was more effective for delay of blooming when compared with mepiquat chloride. The mean values obtained in 2015 and 2016 showed that in the single applications of mepiquat chloride, blooming was delayed by between 4.0 and 5.3 days, while it was delayed between 6.3 and 7.7 days in the single applications of ethephon. However, the combined effects of the chemicals yielded better results, and blooming was delayed between 8.3 and 10.7 days in the combined applications of the chemicals (Table 2).

Compared with 2016, blooming was relatively more delayed in 2015, during which cooler climatic conditions were observed (Table 1; Figure 1). Indeed, blooming was delayed for an average of 8.59 days in 2015 and 7.41 days in 2016 (Table 2). Mertoğlu et al. (2018) reported that the blooming period was shorter when temperatures were higher, and vice versa.

Ethephon and mepiquat chloride inhibit enzyme activation by inhibiting the production and function of gibberellins and gibberellin derivatives, especially during the early phenological period (Wang et al., 2014). Mepiquat chloride reduces the activity of ribulose biphosphate (RuBP), which is a CO₂ acceptor. The reduction in RuBP activity results in decreased catalyzed substrate amounts and thus decreased assimilation (Reddy et al., 1996). These effects of the chemicals lead to limited energy production and transfer (Polat et al., 2017). This affects the duration of plastochron and prolongs the time needed by flower buds to open (Krewer et al., 2005; Grijalva-Contreras et al., 2011; Wang et al., 2014). In the current study, the single

Table 2. Bloom delay, fruit set, and phytochemical characteristics of treatment combinations.

Treatment combinations		Delay in blooming (+ days)	Fruit set (%)	Total anthocyanin (mg 100 mL ⁻¹)	Total phenol (mg L ⁻¹)	Vitamin C (mg 100 mL ⁻¹)	SSC (%)	Antioxidant activity (mmol L ⁻¹)
M. chloride	Ethephon							
0	0	0.0 ^e	4.18 ± 0.81 ^f	67.95 ± 4.75 ^a	1056.00 ± 101.00 ^a	4.97 ± 0.29	19.68 ± 1.12	27.05 ± 1.10 ^a
75	0	5.3 ± 0.43 ^{cd}	15.98 ± 0.78 ^{bc}	39.02 ± 4.46 ^d	855.80 ± 82.20 ^b	5.20 ± 0.23	17.12 ± 0.99	21.32 ± 1.35 ^f
150	0	4.0 ± 0.54 ^d	13.73 ± 0.45 ^d	44.85 ± 3.81 ^{cd}	921.40 ± 61.10 ^{ab}	5.17 ± 0.26	17.45 ± 0.87	24.33 ± 0.43 ^c
0	2000	6.3 ± 0.43 ^c	14.58 ± 1.46 ^{cd}	52.58 ± 6.10 ^{bcd}	873.70 ± 21.80 ^{ab}	5.90 ± 0.43	17.23 ± 0.40	22.02 ± 0.19 ^e
0	4000	7.7 ± 0.48 ^b	7.74 ± 0.47 ^e	41.08 ± 3.92 ^{cd}	854.90 ± 37.30 ^b	5.23 ± 0.29	16.98 ± 0.91	21.63 ± 0.56 ^{ef}
75	2000	8.3 ± 0.34 ^b	17.71 ± 1.32 ^b	54.57 ± 3.15 ^{abc}	945.90 ± 35.60 ^{ab}	5.10 ± 0.13	17.37 ± 0.26	24.50 ± 0.33 ^c
150	2000	9.5 ± 0.33 ^a	21.28 ± 0.75 ^a	52.73 ± 7.23 ^{bcd}	985.30 ± 83.50 ^{ab}	4.85 ± 0.20	16.23 ± 0.54	23.07 ± 1.36 ^d
75	4000	9.7 ± 0.48 ^a	9.42 ± 0.60 ^c	65.42 ± 3.94 ^{ab}	1054.00 ± 102.00 ^a	5.53 ± 0.25	18.32 ± 0.88	25.23 ± 1.13 ^b
150	4000	10.7 ± 0.31 ^a	8.26 ± 0.32 ^c	55.85 ± 3.87 ^{abc}	987.80 ± 90.40 ^{ab}	4.82 ± 0.23	17.15 ± 0.70	24.03 ± 1.37 ^c
ANOVA significance levels								
Between subjects								
Ethephon	***	***	ns	ns	ns	ns	ns	***
M. chloride	***	***	ns	ns	ns	ns	ns	ns
E × M	***	***	***	**	ns	ns	ns	***
Within subject								
Year	***	**	***	***	ns	***	***	***
Year × E	ns	ns	ns	ns	ns	ns	ns	***
Year × M	ns	ns	ns	ns	ns	ns	ns	***
Year × E × M	ns	ns	ns	ns	ns	ns	ns	***

*: P < 0.05, **: P < 0.01, ***: P < 0.001, ns: nonsignificant. Values sharing the same superscripted letter are not significantly different.

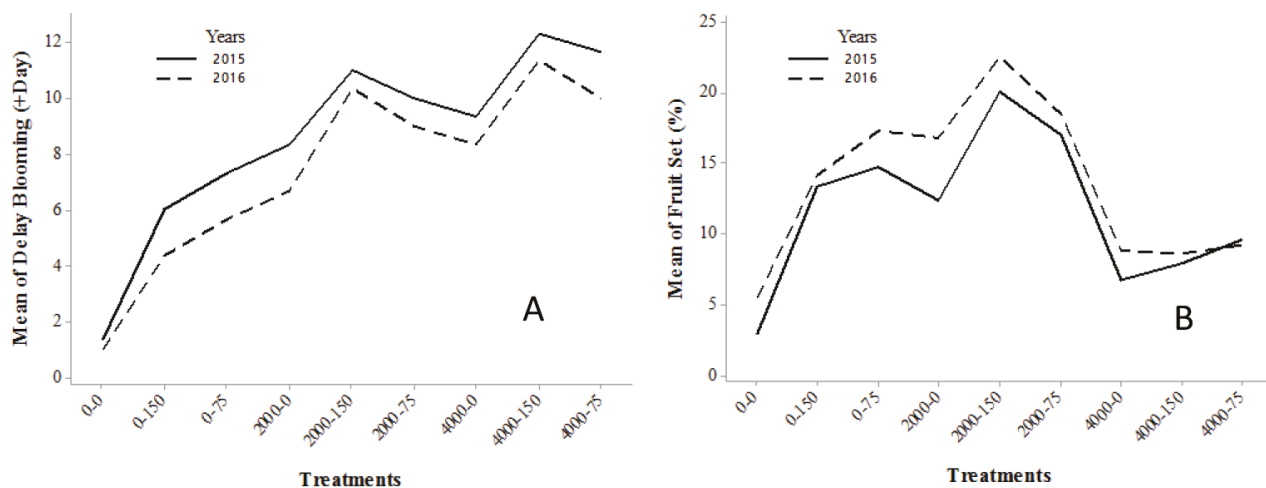


Figure 1. The differences between years in delayed blooming (A) and fruit set (B) with respect to treatment combinations.

application of mepiquat chloride during the dormant period delayed blooming up to 8 days, while the single ethephon treatment yielded a delay in blooming up to 10 days (Figure 1).

The studies on delay of blooming using ethephon have shown that blooming was delayed by 5 to 9 days in peach trees depending on the year (Crisosto et al., 1989), 1 to 3 days in cherry trees depending on the cultivar (Engin et al., 2004), 4 to 11 days in Japanese apricot (Paksasorn et al., 1995), 12 to 15 days in pistachio (Askari et al., 2011), 5 to 7 days in cranberry (Krewer et al., 2005), 17 to 30 days in *Physalis* (Yadava, 2012), and 2 to 9 days in almond (Grijalva-Contreras et al., 2011).

Combined applications of chemicals that have the same purpose but different mechanisms of action obtain better results (Jain et al., 2011). The results of this study showed that the interaction between ethephon and mepiquat chloride positively affected the delay in blooming. In the combined application of the chemicals, blooming was delayed by up to 12 days in 2015 (Figure 1). Mertoğlu and Evrenosoğlu (2017) reported that the mepiquat chloride treatment of the roots and ethephon treatment of the upper parts of the Angeleno plum variety in the dormant period resulted in a delay of 11 days. In a similar manner, single applications of salicylic acid and methyl jasmonate caused a delay of 3–4 days in the Elberta peach cultivar, while the combined application of these chemicals delayed blooming by up to 8 days (Mohammadi et al., 2015). The blooming and small-fruit periods are when fruit trees are the most susceptible to cold, so delaying full blooming is important in order to recover from these periods with minimal damage. Depending on their water content, losses were observed in up to 90% of the buds, flowers, and small fruit of plums. This was thought to be caused by frost damage due to a drop in temperature to $-5\text{ }^{\circ}\text{C}$ for more than 90 min (Palonen and Buszard, 1997). The results of the present study revealed an increase in the fruit set with delayed blooming compared to the control. The fruit set in the control was 4.18%, while it reached 21.28% in 150 ppm Mep-Ch + 2000 ppm ethephon treatment (Table 2). In plums it was reported that a fruit set of 20% in blooming flowers is sufficient to obtain a high yield (Neumüller, 2011). Increase in fruit set is also attributable to the increased resistance of buds, flowers, and small fruit due to the morphological and biochemical changes caused by ethephon and mepiquat chloride. According to the results obtained by Ye et al. (2016) and Kamran et al. (2018), mepiquat chloride and ethephon increased resistance by increasing the lignin and cellulose amounts in the plants. Different studies have reported that ethephon-treated flower pistils showed dehydration and reduced their moisture content and sorbitol and saccharose contents by increasing sucrose synthase activity (Durner and

Gianfagna, 1991; Wang et al., 2013). The reduction of cell size and the consequent increase in cell concentration led to decreased freezing degrees and thus increased resistance to cold (Angelcheva et al., 2014).

Harsh climatic conditions were observed in 2015, and frost incidences occurred on a total of 23 days in the study area in March and April, whereas in 2016 frost occurred in the study area on a total of 11 days (Table 1). Brown necrosis and darkening were observed in the flowers of the frost-exposed plum trees of the control, whereas the distinctive color and appearance of the flowers of the treated plum trees indicated that the flowers of treated trees were healthy (Figure 2).

In this study, the most successful results for the delay of blooming were obtained with the interaction groups that received 4000 ppm of ethephon (Table 2; Figure 1). However, an ethephon level up to 4000 ppm caused blight, especially in the spurs, i.e. primary fruit organs of the plants and young branches (Figure 3). No yield was collected from the blighted fruit branches. Blight reached 40% in some trees, and irregular fruit set was observed in these trees in the following period. Hence, the application of 4000 ppm ethephon for delayed blooming is undesirable.



Figure 2. Blooming conditions after frost in the control and chemical-treated plum trees (treated trees are located in the front; control trees are located in the back).



Figure 3. Physical damage in plum tree treated with 4000 ppm ethephon.

Despite its positive effects, the excessive and untimely use of ethephon can result in negative outcomes such as the abscission of buds and flowers, gummosis, and irregular and reduced fruit set (Coneva and Cline, 2009; Mertoğlu and Evrenosoğlu, 2017). The adverse effects that were observed with the application of 4000 ppm ethephon were not observed in 2000 ppm ethylene groups.

All phytochemical properties were higher in 2016, when budding occurred earlier, and ripening of fruit took place during a longer vegetation period (Figure 4). Plant biochemistry varies depending on changing environmental conditions (Johansen et al., 2017; Molmann et al., 2018). As the time spent under optimum incubation conditions increases, the amounts of anthocyanin, saccharose, and phenolic acid, which are produced until a certain stage of ripening, increase as well (Riedel et al., 2012; Saw et al., 2012; Wang et al., 2013).

Single and combined applications of the chemicals resulted in decreases in the amounts of phytochemical properties, except for vitamin C. Total anthocyanin, total phenol, SSC, and antioxidant activity of the control were 67.95 mg 100 mL⁻¹, 1056.00 mg L⁻¹, 19.68%, and 27.05

mmol L⁻¹, respectively, while in the treatment groups, they were reduced to 39.02 mg 100 mL⁻¹ (75/0), 854.90 mg L⁻¹ (0/4000), 16.98% (0/4000), and 21.32 mmol L⁻¹ (75/0), respectively (Table 2). However, the decreases in the phytochemical properties were not associated with the chemicals; they were attributed to increased conversion of the assimilation products into fruit. Accordingly, in the control, the mean fruit set between the two years was 4.18%, while it reached 21.28% in the treatment groups (Table 2). Decreases in chemical properties of the fruits as fruit load per plant increases have been reported in many fruit species, including peach (Meitei et al., 2013), jujube (Galindo et al., 2015), and plum (Rajput and Bhatia, 2017).

Previous studies have shown that ethylene increased the amounts of phenolic compounds and anthocyanin by increasing the activity of phenylalanine ammonia lyase and tyrosine aminotransferase in *Vitis* (Saw et al., 2012; Liang et al., 2013); increased sugar accumulation by increasing the activity of sucrose synthase, sucrose phosphate synthase, and acid invertase in *Saccharum* (Jain et al., 2011); and increased the rate of photosynthesis by increasing the efficiency of photosynthetic nitrogen use in *Brassica* (Khan and Khan, 2014). Mepiquat chloride increases photosynthetic activity by regulating the *nif* genes in *Bradyrhizobium* (Chen et al., 2015), promotes the production of phenolic compounds in *Gossypium* (Jafari et al., 2018), and increases protein synthesis by increasing the activity of proline oxidase in *Xerophyta* (Mundree et al., 2002). The increased need for assimilation products due to increased fruit load is thought to be met through these pathways.

In conclusion, in recent years, both the frequency and the severity of late spring frosts have increased due to global climate change. Late spring frosts affect fruit trees when they are most susceptible to cold and severely damage buds, flowers, small fruit, and fresh leaves, eventually leading to decreased yield. The delaying of blooming in early-blooming fruit species and fruit species with short blooming periods minimizes the damage caused by late spring frosts. The present study showed that ethephon and mepiquat chloride successfully delayed the blooming period in plums, and their cumulative effects yielded better results. With delayed blooming, the damage caused by late spring frosts was reduced and fruit set was increased. Use of the chemicals resulted in reduced phytochemical properties in fruit; however, these decreases were attributed to the increased yield per plant that comes with increased fruit set, not from the negative effects of the chemicals. An ethephon dose of 4000 ppm caused blight and gummosis in plants. The best results were obtained with the use of 2000 ppm ethephon and 150 ppm mepiquat chloride.

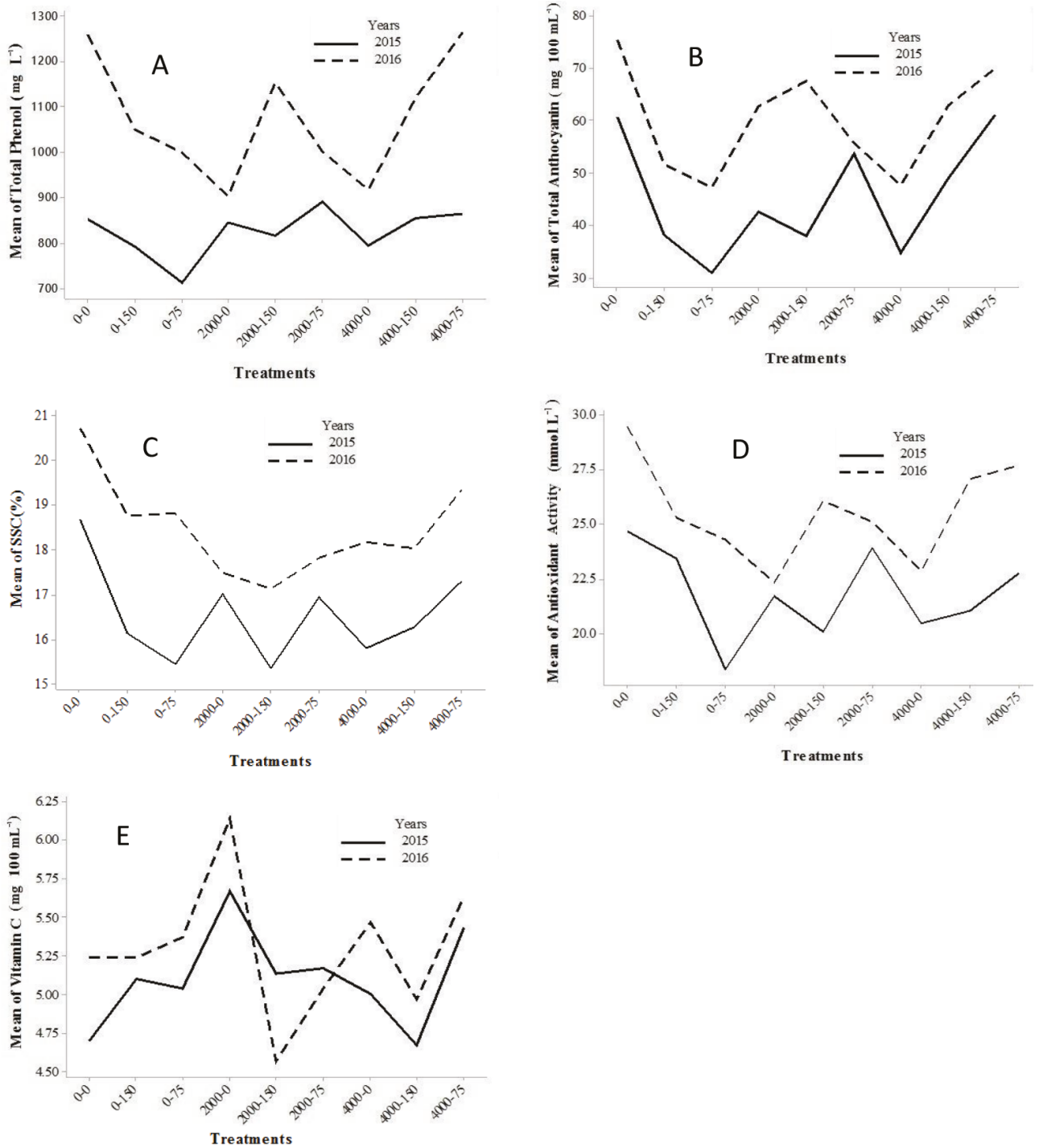


Figure 4. The changes between years in total phenol (A), total anthocyanin (B), soluble solids content (C), antioxidant capacity (D), and vitamin C (E) with respect to treatment combinations.

References

- Akca Y, Ozongun S (2004). Selection of late leafing, late flowering, laterally fruitful walnut (*Juglans regia*) types in Turkey. *New Zealand Journal of Crop and Horticultural Science* 32: 337-342.
- Angelcheva L, Mishra Y, Antti H, Kjellsen TD, Funk C et al. (2014). Metabolomic analysis of extreme freezing tolerance in Siberian spruce (*Picea obovata*). *New Phytologist* 204: 545-555.
- Arion CM, Tabart J, Kevers C, Niculaua M, Filimon R et al. (2014). Antioxidant potential of different plum cultivars during storage. *Food Chemistry* 146: 485-491.
- Askari E, Irani S, Razmjoo K (2011). Bloom, maturity, and fruit set of pistachio in response to early season application of ethephon. *Horticulture, Environment, and Biotechnology* 52: 29.
- Aygün A, Şan B (2005). The late spring frost hardiness of some apple varieties at various stages of flower buds. *Tarım Bilimleri Dergisi* 11: 283-285.
- Cai L, Stegmeir T, Sebolt A, Zheng C, Bink MC et al. (2018). Identification of bloom date QTLs and haplotype analysis in tetraploid sour cherry (*Prunus cerasus*). *Tree Genetics & Genomes* 14: 22.
- Celik F, Gundogdu M, Alp S, Muradoglu F, Ercisli S et al. (2017). Determination of phenolic compounds, antioxidant capacity and organic acids contents of *Prunus domestica* L., *Prunus cerasifera* Ehrh. and *Prunus spinosa* L. fruits by HPLC. *Acta Chromatographica* 29: 507-510.
- Chen W, Zheng D, Feng N, Liu T, Liu Y et al. (2015). The effects of gibberellins and mepiquat chloride on nitrogenase activity in *Bradyrhizobium japonicum*. *Acta Physiologiae Plantarum* 37: 1723.
- Coneva E, Cline J (2009). Ethrel Delays Blossoming, Reduces Fruit Set, and Increases Fruit Size of 'Baby Gold 5' Peaches. Guelph, Canada: University of Guelph.
- Crisosto CH, Lombard PB, Fuchigami LH (1989). Fall ethephon delays blooming 'Redhaven' peach by delaying flower differentiation and development during dormancy. *Journal of the American Society for Horticultural Science* 114: 881-884.
- Dicenta F, Sanchez-Perez R, Batlle I, Martinez-Gomez P (2017). 7 Late-Blooming Cultivar Development. In: Gradziel TM (editor). *Almonds: Botany, Production, and Uses*. Wallingford, UK: CAB, pp. 168-187.
- Duman, JG, Wisniewski MJ (2014). The use of antifreeze proteins for frost protection in sensitive crop plants. *Environmental and Experimental Botany* 106: 60-69.
- Durner EF, Gianfagna TJ (1991). Peach pistil carbohydrate and moisture contents and growth during controlled deacclimation following ethephon application. *Journal of the American Society for Horticultural Science* 116: 507-511.
- Einhorn TC, Arrington M (2018). ABA and shading induce 'Bartlett' pear abscission and inhibit photosynthesis but are not additive. *Journal of Plant Growth Regulation* 37: 300-308.
- Engin H, Ünal A, Gür E (2004). The effects of CCC, PP333, GA₃, hydrogen cyanamide and ethephon on flowering in some cherry cultivars. *Ege Üniversitesi Ziraat Fakültesi Dergisi* 41: 35-43.
- Ercisli S, Yilmaz, SO, Gadze J, Dzubur A, Hadziabulic S et al. (2011). Some fruit characteristics of Cornelian cherries (*Cornus mas* L.). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 39: 255-259.
- Funes I, Aranda X, Biel C, Carbo J, Camps F et al. (2016). Future climate change impacts on apple flowering date in a Mediterranean subbasin. *Agricultural Water Management* 164: 19-27.
- Galindo A, Noguera-Artiaga L, Cruz ZN, Burlo F, Hernandez F et al. (2015). Sensory and physico-chemical quality attributes of jujube fruit as affected by crop load. *Food Science and Technology (LEB)* 63: 899-905.
- Grijalva-Contreras RL, Martinez-Diaz G, Macias-Duarte R, Robles-Contreras F (2011). Effect of ethephon on almond bloom delay, yield, and nut quality under warm climate conditions in Northwestern Mexico. *Chilean Journal of Agricultural Research* 71: 34.
- Gündoğdu M (2019). Effect of rootstocks on phytochemical properties of apricot fruit. *Turkish Journal of Agriculture and Forestry* 43: 1-10.
- Gunes NT (2006). Frost hardiness of some Turkish apricot cultivars during the bloom period. *HortScience* 41: 310-312.
- İmrak B, Küden A, Yurtkulu V, Kafkas E, Ercişli S et al. (2017). Evaluation of some phenological and biochemical characteristics of selected new late flowering dried apricot cultivars. *Biochemical Genetics* 55: 234-243.
- Inouye DW (2008). Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology* 89: 353-362.
- Jafari M, Saadatmand S, Zangi MR, Iranbakhsh A (2018). Effect of pix (mepiquat chloride) on the salinity resistance of new cultivars of cotton (*Gossypium hirsutum* L.) in the seedling stage. *Applied Ecology and Environmental Research* 16: 4097-4114.
- Jain R, Solomon S, Shrivastava AK, Chandra A (2011). Effect of ethephon and calcium chloride on growth and biochemical attributes of sugarcane bud chips. *Acta Physiologiae Plantarum* 33: 905-910.
- Johansen TJ, Molmann JA, Bengtsson GB, Schreiner M, Velasco P et al. (2017). Temperature and light conditions at different latitudes affect sensory quality of broccoli florets (*Brassica oleracea* L. var. *italica*). *Journal of the Science of Food and Agriculture* 97: 3500-3508.
- Kamran M, Ahmad I, Wang H, Wu X, Xu J et al. (2018). Mepiquat chloride application increases lodging resistance of maize by enhancing stem physical strength and lignin biosynthesis. *Field Crops Research* 224: 148-159.

- Khadivi-Khub A, Khalili Z (2017). A breeding project: the selection of promising apricot (*Prunus armeniaca* L.) genotypes with late blooming time and high fruit quality. *Scientia Horticulturae* 216: 93-102.
- Khan MIR, Khan NA (2014). Ethylene reverses photosynthetic inhibition by nickel and zinc in mustard through changes in PS II activity, photosynthetic nitrogen use efficiency, and antioxidant metabolism. *Protoplasma* 251: 1007-1019.
- Korkmaz N, Aşkın MA (2017). Effects of GA₃, calcium and boron applications to seasonal changes of leaf, peel and aril mineral nutritions on Hicaznar pomegranate (*Punica granatum* L.). *International Journal of Agriculture, Forestry and Life Sciences* 1: 27-51.
- Krewer G, Nesmith DS, Williamson J, Maus B, Mullinix B (2005). Ethephon for bloom delay of rabbiteye and southern highbush blueberries. *Small Fruits Review* 4: 43-57.
- Latocha P (2008). Frost resistance and spring frost sensibility of a few cultivars of *Actinidia* grown in Central Poland. *Annals of Warsaw University of Life Sciences - SGGW* 29: 111-120.
- Liang Z, Ma Y, Xu T, Cui B, Liu Y et al. (2013). Effects of abscisic acid, gibberellin, ethylene and their interactions on production of phenolic acids in *Salvia miltiorrhiza* Bunge hairy roots. *PLoS One* 8: e72806.
- Matzneller P, Götz KP, Chmielewski FM (2016). Spring frost vulnerability of sweet cherries under controlled conditions. *International Journal of Biometeorology* 60: 123-130.
- Meitei SB, Patel RK, Deka BC, Deshmukh NA, Singh A (2013). Effect of chemical thinning on yield and quality of peach cv. 'Flordasun.' *African Journal of Agricultural Research* 8: 3558-3565.
- Mertoğlu K, Evrenosoğlu Y (2017). Effects of ethephon and mepiquat chloride on late blooming of 'Angeleno' plum. *Mediterranean Agricultural Sciences* 30: 79-84.
- Mertoğlu K, Evrenosoğlu Y, Altay Y (2018). Determination of suitable pollinators for 0900 Ziraat cherry cultivar in Eskişehir ecology. *Süleyman Demirel University Journal of Natural and Applied Sciences* 22: 89-97 (in Turkish with an abstract in English).
- Mohammadi H, Pakkish Z, Saffari V (2015). Role of methyl jasmonate and salicylic acid applications on bloom delay, flowering and fruiting of 'Elberta' peach. *International Journal of Horticultural Science and Technology* 2: 75-85.
- Molmann JA, Hagen SF, Bengtsson GB, Johansen TJ (2018). Influence of high latitude light conditions on sensory quality and contents of health and sensory-related compounds in swede roots (*Brassica napus* L. ssp. *rapifera* Metzg.). *Journal of the Science of Food and Agriculture* 98: 1117-1123.
- Mundree SG, Baker B, Mowla S, Peters S, Marais S et al. (2002). Physiological and molecular insights into drought tolerance. *African Journal of Biotechnology* 1: 28-38.
- Neumüller M (2011). Fundamental and applied aspects of plum (*Prunus domestica*) breeding. *Fruit, Vegetable and Cereal Science and Biotechnology* 5: 139-156.
- Pakkish Z, Tabatabaenia MS (2016). The use and mechanism of NO to prevent frost damage to flower of apricot. *Scientia Horticulturae* 198: 318-325.
- Paksasorn A, Masuda M, Matsui H, Ohara H, Hirata N (1995). Effect of fall ethephon application on bloom delay and fruit set in Japanese apricot (*Prunus mume* Sieb. et Zucc.). *Acta Horticulturae* 395: 193-200.
- Palonen P, Buszard D (1997). Current state of cold hardiness research on fruit crops. *Canadian Journal of Plant Science* 77: 399-420.
- Polat T, Özer H, Öztürk E, Sefaoğlu F (2017). Effects of mepiquat chloride applications on non-oilseed sunflower. *Turkish Journal of Agriculture and Forestry* 41: 472-479.
- Prudencio AS, Martinez-Gomez P, Dicenta F (2018). Evaluation of breaking dormancy, flowering and productivity of extra-late and ultra-late flowering almond cultivars during cold and warm seasons in South-East of Spain. *Scientia Horticulturae* 235: 39-46.
- Qrunfleh IM, Read PE (2013). Delaying bud break in 'Edelweiss' grapevines to avoid spring frost injury by NAA and vegetable oil applications. *Advances in Horticultural Science* 27: 18-24.
- Rahimi J, Khalili A, Bazrafshan J (2018). Modeling uncertainty of statistical downscaling methods in quantifying the climate change impacts on late spring frost risk over Iran. *Modeling Earth Systems and Environment* 4: 1-15.
- Rajput V, Bhatia SK (2017). Changes in fruit quality parameters in Japanese plum cv. 'Kala Amritsari' with chemical flower thinning. *Journal of Pharmacognosy and Phytochemistry* 6: 2220-2223.
- Reddy RA, Reddy KR, Hodges HF (1996). Mepiquat chloride (PIX)-induced changes in photosynthesis and growth of cotton. *Plant Growth Regulation* 20: 179-183.
- Riedel H, Akumo DN, Saw NMMT, Smetanska I, Neubauer P (2012). Investigation of phenolic acids in suspension cultures of *Vitis vinifera* stimulated with indanoyl-isoleucine, N-linolenoyl-L-glutamine, malonyl coenzyme A and insect saliva. *Metabolites* 2: 165-177.
- Saavedra GM, Figueroa NE, Poblete LA, Cherian S, Figueroa CR (2016). Effects of preharvest applications of methyl jasmonate and chitosan on postharvest decay, quality and chemical attributes of *Fragaria chiloensis* fruit. *Food Chemistry* 190: 448-453.
- Sakar EH, El Yamani M, Rharrabti Y (2017). Frost susceptibility of five almond [*Prunus dulcis* (Mill.) D.A. Webb] cultivars grown in North-Eastern Morocco as revealed by chlorophyll fluorescence. *International Journal of Fruit Science* 17: 415-422.
- Salazar-Gutierrez MR, Chaves B, Anothai J, Whiting M, Hoogenboom G (2014). Variation in cold hardiness of sweet cherry flower buds through different phenological stages. *Scientia Horticulturae* 172: 161-167.
- Saw NMMT, Riedel H, Cai Z, Küttik O, Smetanska I (2012). Stimulation of anthocyanin synthesis in grape (*Vitis vinifera*) cell cultures by pulsed electric fields and ethephon. *Plant Cell, Tissue and Organ Culture* 108: 47-54.

- Selçuk N, Erkan M (2016). Impact of passive modified atmosphere packaging on physicochemical properties, bioactive compounds, and quality attributes of sweet pomegranates. *Turkish Journal of Agriculture and Forestry* 40: 475-488.
- Spinola V, Mendes B, Camara JS, Castilho PC (2013). Effect of time and temperature on vitamin C stability in horticultural extracts. UHPLC-PDA vs iodometric titration as analytical methods. *Food Science and Technology (LEB)* 50: 489-495.
- Szabo T, Lakatos LT, Vaszily B, Lakatos L (2017). Climate change effects on apple and sour cherry phenology in a gene bank plantation of Hungary. *Aerul și Apa Componente ale Mediului* 2017: 251-258.
- Wang AQ, Huang WJ, Niu JQ, Liu M, Yang LT et al. (2013). Effects of ethephon on key enzymes of sucrose metabolism in relation to sucrose accumulation in sugarcane. *Sugar Tech* 15: 177-186.
- Wang L, Mu C, Du M, Chen Y, Tian X et al. (2014). The effect of mepiquat chloride on elongation of cotton (*Gossypium hirsutum* L.) internode is associated with low concentration of gibberellic acid. *Plant Science* 225: 15-23.
- Yadava LP (2012). Effect of growth retardants on floral biology, fruit set and fruit quality of cape gooseberry (*Physalis peruviana* L.). *American Journal of Plant Physiology* 7: 143-148.
- Ye DL, Zhang YS, Al-Kaisi MM, Duan LS, Zhang MC et al. (2016). Ethephon improved stalk strength associated with summer maize adaptations to environments differing in nitrogen availability in the North China Plain. *Journal of Agricultural Science* 154: 960-977.
- Zar JH (2013). *Biostatistical Analysis*. London, UK: Pearson Higher Education.