

Role of methyl jasmonate and salicylic acid applications on bloom delay, flowering and fruiting of 'Elberta' Peach

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Abstract

Peach (*Prunus persica* L. Batsch.) is produced in most areas of Iran, where flowering is hindered by temperature. Using plant growth regulators to delay bloom is a suggestive measure to avoid frost damage. The objective of this study was to determine the effects of methyl jasmonate (MJ) and salicylic acid (SA) on peach bloom delay and yield. This study evaluated the applications of MJ 0 (control, water only), 100 and 200 mg L⁻¹ and SA at 150 and 300 mg L⁻¹ at swollen bud and green tip stages. In 'Elberta' peach, MJ at 200 mg L⁻¹, its combinations with SA at 150 mg L⁻¹ and MJ 200 mg L⁻¹ and SA300 mg L⁻¹ together at the two stages (green tip, swollen bud stages) delayed blooming for 6 and 8 days, respectively. The maturity and ripening of treated peach fruits were delayed for 8-12 days in green tip and swollen bud stage. Flowering percentage amount (57.83-61.80%), fruit set amount (22.59-23.53%) and yield (1.69-1.72 kg cm⁻² branch) were increased by MJ 200 mg L⁻¹ and SA300 mg L⁻¹ treatments compared to the control treatment (flowering percentage amount (39.31%), fruit set amount (6.25%) and yield (0.82 kg cm⁻² branch). The interactions of MJ and SA had more impacts on flowering (89.53%), fruit set (33.22%), fruit weight average (124.93 g), and yield (2.09 kg cm⁻² branch) compared to their individual application. The present study was the first evidence for the SA and MJ effect on bloom delay, flowering and fruiting of peach.

Keywords: Bloom delay, green tip stage, swollen bud stage, yield.

Abbreviations: ABA, Absciscic acid; AOS, allene oxide synthase; IAA, indoleacetic acid; JAs, jasmonates; JIPs, ja-induced proteins; JRGs; ja-responsive genes; MJ, methyl jasmonate; PDJ, propyl dihydro jasmonate; SA, salicylic acid; SAR, systemic acquired resistance; SSC, soluble solids concentration.

Introduction

Peach (*Prunus persica* L. Batsch.) trees are cultivated in large areas in temperate regions but in some places, flowers in different stages of development are damaged by low temperatures. Peach is a fruit with high nutritional value. Frost injury to peach buds, flowers, and small fruits is a major factor in determining commercial production in many regions of Iran. Blossoming occurs in the

first week of April when late spring frost is common in peach production regions of Iran. Cold injury is a major limitation to peach culture in the temperate zones because peach trees are not tolerant to the freeze injury, during late winter and early spring months (Brewer, 1981; Chaplin and Westwood, 1980; Durner and Gianfagna, 1989; Westwood, 1993). A number of criteria can impact the susceptibility of the developing peach flowers and fruits (Carlos, 1990; Westwood, 1993). Susceptibility varies

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within trees of an orchard, among orchards and cultivars (Durner, 1989). Peach bud or flower at a given floral development stage are prone to damage by early spring frosts at a given temperature (Probsting and Mills, 1973; Gianfagna *et al.*, 1986; Westwood, 1993). As buds begin to swell and mature into blossoms, they become less resistant to frost injury (Sloan and Matta, 1996). Fully dormant buds are most hardy while flowers in full petal fall are most susceptible (Webster, 1984; Grijalva-Contreras and Valenzuela-Ruiz, 1991). An artificial delay of flowering would be beneficial to avoid frost damage in cold conditions. Phytohormones can be used to delay bloom and is suggested as a measure to avoid frost damage. Fruit set and yield are increased in fruit trees treated with growth regulators to delay bloom (Crisosto *et al.*, 1990; Ebel *et al.*, 1999; Coneva and Cline, 2009). Fall application of ethephon and gibberellic acid delayed fruit maturity and increased winter hardiness in stone fruits (Buban and Turi, 1986; Coston *et al.*, 1986; Crisosto *et al.*, 1990). Bloom delay is due to an increase of endo-dormancy and inhibition of flower bud development, which occurred immediately after applying the hormones (Durner and Gianfagna, 1988; Coston *et al.*, 1986; Gianfagna *et al.*, 1986). Salicylic acid (SA) is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes in plants (Raskin, 1992). SA, for example, plays a role of natural inductor of thermogenesis in arum lily, induces flowering in a range of plants, controls ion uptake by roots and stomatal conductivity (Hayat and Ahmad, 2007; Raskin, 1992), inhibition of fruit ripening (Christelle *et al.*, 2001) and other processes. During the last 20

years this substance has drawn the attention of researchers due to its ability to induce systemic acquired resistance (SAR) in plants to different stresses (Korkmaz *et al.*, 2007).

Jasmonates (JAs) are signal molecules. Its biosynthesis starts from linolenic acid with allene oxide synthase (AOS) as a key enzyme. They elicit responses to biotic and abiotic stresses through up-regulation of the JA-responsive genes (JRGs) and the synthesis of JA-induced proteins (JIPs) (Wasternack, 2007; Rohwer and Erwin, 2008). As revealed by *Arabidopsis* mutants, JAs are also involved in developmental processes including male and female reproductive organs formation (Wasternack, 2007). Moreover, their biosynthesis are developmentally regulated during fruit growth and ripening in apple (Kondo *et al.*, 2000).

The objective of this research was to investigate the effects of MJ and SA on bloom delay and yield of 'Elberta' peach trees.

Materials and Methods

Orchard selection and management

Field studies were conducted in a commercial orchard located in Rayn (29° 21' 18" N, 57° 22' 30" E, 2300 m above sea level) Kerman, Iran. Ninety (90) trees (5 year-old trees) were selected for this experiment. The peach orchard, cv. 'Elberta' on peach rootstock, was planted at 4×4 m in sandy loam soil and all trees received similar cultural practices such as irrigation and fertilization. The experimental design was a factorial randomized complete-block with two factors, time and treatment. Parameters of the meteorological conditions during the experiment periods are presented in Table 1.

Table 1. The mean meteorological condition of Rayn, Kerman, Iran in 2012

	March	April	May	Jun	July	August	September
Temperature (°C)	1.1	10.02	17.14	21.01	28.7	29.10	24.02
Humidity (%)	65.17	53.10	48.11	48.04	42.32	42.09	48.10
Total rainfall (mm)	5	2	0	0	0	0	1.12

Treatments

The methyl jasmonate (MJ) and salicylic acid (SA) treatments were applied to delay bloom at swollen bud and green tip stages of peach buds. Treatments were applied on the same trees at swollen bud and green tip

stages. Four shoots 35 to 50 cm long were randomly sampled from each tree.

The selected trees were sprayed as follows:

Control (sprayed with water)	Combinations of MJ 100 mg L ⁻¹ + SA 150 mg L ⁻¹
MJ 100 mg L ⁻¹	Combinations of MJ 100 mg L ⁻¹ + SA 300 mg L ⁻¹
MJ200 mg L ⁻¹	Combinations of MJ 200 mg L ⁻¹ + SA 150 mg L ⁻¹
SA 150 mg L ⁻¹	Combinations of MJ200 mg L ⁻¹ + SA300 mg L ⁻¹
SA 300 mg L ⁻¹	

Measurements

Bloom delay was determined by subtracting the time of 80% anthesis of the control from the treatments and was expressed as number of delayed days. Flower number per node and fruit set were taken on four branches per tree. Yield per branch was also determined. Average fruit weight and maturity, expressed as soluble solids concentration (SSC), were taken on 25 fruits per tree at harvest using a hand-held refractometer (American Optical Co., Keene, N.H.).

Statistical analysis

The experimental design was a factorial randomized complete-block (with two factor time and plant growth regulators) with single tree and five replications. Data were analyzed by analysis of variance (ANOVA) and the means were compared ($P \leq 0.05$) by Duncan's multiple range test (DMRT). All analyses were performed using SAS software (Ver.9.1).

Results and Discussion

The results of this study showed that bloom was delayed at the 2 stages for 8 and 6 days from MJ and SA applications on 'Elberta' trees. The treatments applied at swollen bud and green tip stage significantly delayed blooming in 'Elberta' peach (Table 2). Treatments at the earlier stage, delayed blooming more than the late application and the interaction of MJ and SA had significant effect on bloom delay in

both stages in comparison to their individual application (Table 2). MJ at 200 mg L⁻¹ and its combination with SA 150 mg L⁻¹ delayed blooming on 'Elberta' peach up to 8 days when applied at swollen bud stage, but only up to 6 days when applied at the green tip stage. When treatments were applied alone in both stages, their effects were lower (up to 2 days) (Table 2). Researchers reported that the exogenous application of MJ and SA altered the auxin, cytokinin and Abscisic acid (ABA) balances in plants, which cause delay in blooming (Raskin, 1992; Sekozawa *et al.*, 2003; Rohwer and Erwin, 2008). However, Sekozawa *et al.* (2003) observed, high ABA content in the flower buds treated with propyl dihydrojasmonate (PDJ) and SA treatment caused accumulation of both ABA and indole-acetic acid (IAA) in wheat seedlings. John *et al.* (2004) reported that jasmonates play an integral role in the intracellular signal transduction cascade, which acts as the inducible defense mechanism in plant stresses. SA treatment caused accumulation of both ABA and IAA in wheat seedlings (Raskin, 1992) where SA treatment reduced the damaging effects of salinity and water deficit on seedling growth and accelerated a restoration of growth processes. The SA treatment prevented the decrease in IAA and cytokinin content completely from shoots and roots which reduced stress-induced inhibition of plant growth (Ding *et al.*, 2001). Furthermore, high ABA levels were maintained in SA

treated plants such as wheat seedlings which provided the development of anti-stress reactions, for example, maintenance of proline accumulation in tissues under stress. Protective SA action includes the development of anti-stress reaction and accelerates the normal growth processes after the removal of stress factors (Gross and Parthier, 1994; Hayat and Ahmad, 2007; Yang *et al.*, 2008). However, in the present study with MJ and SA application, perhaps accumulation of ABA in peach, might have caused reduction of growth buds and created bloom delay (Raskin, 1992; Sekozawa *et al.*, 2003; Rohwer and Erwin, 2008). The results revealed, flowering, fruit set, fruit weight, and yield improved significantly with the applications of MJ and SA or their combinations at both stages compared to the control treatment. The interactions of MJ and SA had more impacts on flowering (89.53%), fruit set (33.22%), fruit weight (124.93 g), and yield (2.09 kg cm⁻² branch) compared to their individual application (Table 2). A greater increase in peach yield was observed when MJ and SA were applied separately and in combination at the green tip stage (Table 2). At the green tip stage of bud development, the MJ and SA applications decreased reduction of flower number, and increased fruit set and yield (Table 2). Although the higher flowering, higher fruit set, and higher yield in treated trees may probably be as a result of avoiding frost, because, after a frost (-2°C) during full bloom, this study observed damaged flowers on the control trees. The treated trees with MJ and SA escaped frost damages since they bloomed 2 to 9 days later. The MJ and SA-treated 'Elberta' peach trees had a significant increase in yield (Table 2). This increase may be as a result of the aftermath of cold hardiness increase of buds by MJ and SA. Results obtained after exogenous application of jasmonates is expected to be practical for preventing or alleviating frost damages to flowers and buds, because propyl

dihydrojasmonate application on 'Kousui' Japanese pear showed that PDJ enhanced the cold hardiness of the flowers and then alleviated cold injury in the flower organs; although sensitive to low temperature, it varies among different parts of the flowers of 'Kousui Japanese pear (Sekozawa *et al.*, 2003). Trees treated with MJ and SA in this research escaped frost damage, since they bloomed 2 to 8 days later. These may be because of the increase of flowering, fruit set and yield in the present research. These results are in accordance with the results of other researchers (Cleland and Ajami, 1974; Ding *et al.*, 2001; John *et al.*, 2004; Hayat and Ahmad, 2007; Karlidag *et al.*, 2009; Eetezaz *et al.*, 2011) who showed increase of flowering, fruit set and yield by treatment with MJ and SA. The results showed, that MJ and SA treatments decreased flower and fruit drop and increased yield (Table 2). Fruit set per tree improved significantly with the application of MJ and SA or their combinations at the two stages. The highest amount of fruit set was recorded in the trees sprayed with MJ at 200 mg L⁻¹ and its combination with SA 150 mg L⁻¹, at the swollen bud stage followed by MJ 200 mg L⁻¹ and SA 300 mg L⁻¹ together at the swollen bud stage and they were significantly different when compared to other treatments (Table 2). The sprayed trees with MJ and SA showed significant increase in fruit set (Table 2). The application of MJ and SA and their combination reduced the flower and fruit drop at all selected trees (Figure 1). Flower (60%) and fruit (95%) drop was the highest in trees which were sprayed with distilled water (control) (Table 2). The lowest flower and fruit drop percentage was recorded in trees treated with MJ at 200 mg L⁻¹ and combined with SA 150 mg L⁻¹ and by MJ 200 mg L⁻¹ and SA 300 mg L⁻¹ together at the swollen bud stage. The flower and fruit drop in fruit trees are problems which may be due to different reasons such as nutrients and growth regulator deficiencies. Different types of

treatments have been suggested by Chaplin and Westwood (1980), Westwood (1993) and Rasori *et al.* (2002). Applications of MJ and SA are effective in the retention of fruit up to the final harvest, resulting to increase in fruit yield. Sekozawa *et al.* (2003) reported that proline and sorbitol contents were increased by PDJ (propyl dihydrojasmonate) treatment, it was suggested that intracellular freezing can be avoided by supercooling of flowers and protection of the fruitlets from freezing and dehydration (Sekozawa *et al.*, 2003). Improving flowering percentage and density could also be interpreted as the role of SA as an endogenous growth regulator which plays an important role in increasing antioxidant content. This interpretation is confirmed by Karlid *et al.* (2009) who noticed that SA treatment decreased catalase and peroxidase with concomitant increase in glutathione reductase which play a role as an antioxidant. The mechanism of SA and jasmonates were reported by Charles and Tanaka (1979), Cleland *et al.* (1982), Eetezaz *et al.* (2011), Handro *et al.* (1997), Heitholt *et al.* (2001), John *et al.* (2004) and Jabbarzadeh *et al.* (2009), who concluded that jasmonates and SA induced flowering by acting as a chelating agent. In this study, decreased flower and fruit drop and increased fruit set with application of SA and MJ and the result of this study are similar to the findings of Cleland *et al.* (1982), Handro *et al.* (1997), Heitholt *et al.* (2001), John *et al.* (2004), and Eetezaz *et al.* (2011). The highest average fruit weight (115-120 g) were found in trees sprayed with MJ at 200 mg L⁻¹ and its combination with SA 150 mg L⁻¹, at swollen bud stage and was significantly different at MJ 200 mg L⁻¹ and SA 300 mg L⁻¹ together at the swollen bud stage. The findings of the present study show that to obtain higher peach fruit yield, application of MJ+SA is useful. This view is supported by Raskin (1992) who confirmed that SA functioned as

and florigenic effects. The literature indicates that nutrients and the management of plant regulators can increase fruit yield by enhancing fruit number, retention and reducing fruit drop (Raskin, 1992). It was reported that SA application promotes cell division, cell enlargement and application of SA increased the leaf area of treated plants (Hayat and Ahmad, 2007). According to the study of John *et al.* (2004) and Raskin (1992), the positive effects of jasmonates and SA on growth and yield can be due to its interaction on other plant hormones. These may be because of increased flowering, fruit set and yield in the present research in which MJ and SA altered the auxin, cytokinin and ABA balances in treated trees, thereby increasing growth and yield (Gross and Parthier, 1994; Hayat and Ahmad, 2007; Yang *et al.*, 2008). Perhaps, the increase in yield of the present research under foliar application of MJ and SA, could be ascribed to the well-known roles of MJ and SA on photosynthetic parameters and plant water relations (Yang *et al.*, 2008). The previous results show that exogenous application of MJ and SA enhanced the net photosynthetic rate, internal CO₂ concentration and water use efficiency in muskmelon seedlings and tomato (Korkmaz *et al.*, 2007; Rohwer *et al.*, 2008).

Therefore, yield increase in treated peach with MJ and SA could be ascribed to these reasons. Fruit maturity of peach fruits, as determined by SSC, was delayed by the MJ and SA treatment (Fig. 1-4). The 'Elberta' peach harvests were delayed by 4 and 12 days in both stages (swollen bud and green tip stages). But, treatments at earlier timing, delayed ripening more than the late applications (8 days). The interaction of MJ and SA showed significant effects on maturity delay in both stages in comparison to their individual application (Fig. 1-4).

Table 2. Effects of methyl jasmonate (MJ) and salicylic acid (SA) treatments on bloom delay (day), flowering (%), primary fruit set (%), final fruit set (%), average fruit weight (g), and yield (kg cm⁻² branch) of ‘Elberta’ peach trees. MJ1: MJ 100 mg L⁻¹, MJ2: MJ 200 mg L⁻¹, SA1: SA 150 mg L⁻¹, SA2: salicylic acid 300 mg L⁻¹

Spraying time	Treatments	Bloom delay (days)	Flowering (%)	Primary fruit set (%)	Final fruit set (%)	Average Fruit weight (g)	Yield (kg cm ⁻² branch)
Swollen bud	Control	0 ^{*j}	39.31 ^m	24.84 ^m	6.25 ^l	91.26 ^k	0.82 ^j
	MJ1	2.66 ^{gh}	52.68 ^{ij}	53.94 ^{fg}	20.56 ^{de}	110.32 ^j	1.53 ^h
	MJ2	3.66 ^{efg}	61.80 ^e	56.54 ^{de}	23.53 ^{bc}	115.31 ^{gh}	1.72 ^{de}
	SA1	3.00 ^{fgh}	51.13 ^{jk}	49.53 ^{ij}	19.75 ^e	110.15 ^j	1.47 ⁱ
	SA2	4.66 ^{cde}	57.83 ^{gh}	52.63 ^g	22.59 ^{cd}	115.04 ^h	1.69 ^{ef}
	MJ1+SA1	5.00 ^{cd}	64.79 ^d	58.34 ^d	25.58 ^b	115.36 ^{gh}	1.76 ^{cd}
	MJ1+SA2	8.00 ^a	68.14 ^c	62.29 ^c	25.55 ^b	115.41 ^g	1.75 ^{cde}
	MJ2+SA1	8.66 ^a	89.53 ^a	75.58 ^a	33.22 ^a	120.29 ^c	2.09 ^a
	MJ2+SA2	6.66 ^b	79.83 ^b	69.57 ^b	34.15 ^a	116.34 ^f	1.79 ^{bc}
	Control	0.00 ^j	38.81 ^m	25.82 ^m	8.47 ^k	91.31 ^k	0.85 ^j
Green tip	MJ1	1.33 ⁱ	49.60 ^{kl}	44.72 ^{kl}	10.66 ^{ijk}	113.24 ⁱ	1.50 ^{hi}
	MJ2	2.33 ^{hi}	53.53 ⁱ	47.86 ^j	13.26 ^{fgh}	117.77 ^d	1.66 ^{fg}
	SA1	3.66 ^{efg}	47.28 ^l	43.62 ^l	10.07 ^{jk}	113.16 ⁱ	1.49 ^{hi}
	SA2	4.00 ^{def}	50.21 ^k	45.82 ^k	11.54 ^{hij}	117.54 ^{de}	1.63 ^g
	MJ1+SA1	4.33 ^{de}	56.14 ^h	50.54 ^{hi}	12.23 ^{ghij}	117.57 ^{de}	1.72 ^{de}
	MJ1+SA2	4.66 ^{cde}	58.60 ^{fg}	52.16 ^{gh}	12.91 ^{ghi}	117.39 ^e	1.74 ^{cde}
	MJ2+SA1	5.66 ^{bc}	59.96 ^{ef}	55.80 ^{ef}	15.25 ^f	124.93 ^a	2.13 ^a
	MJ2+SA2	6.33 ^b	59.26 ^{fg}	53.11 ^g	14.49 ^{fg}	120.73 ^b	1.82 ^b

*Means in each column followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

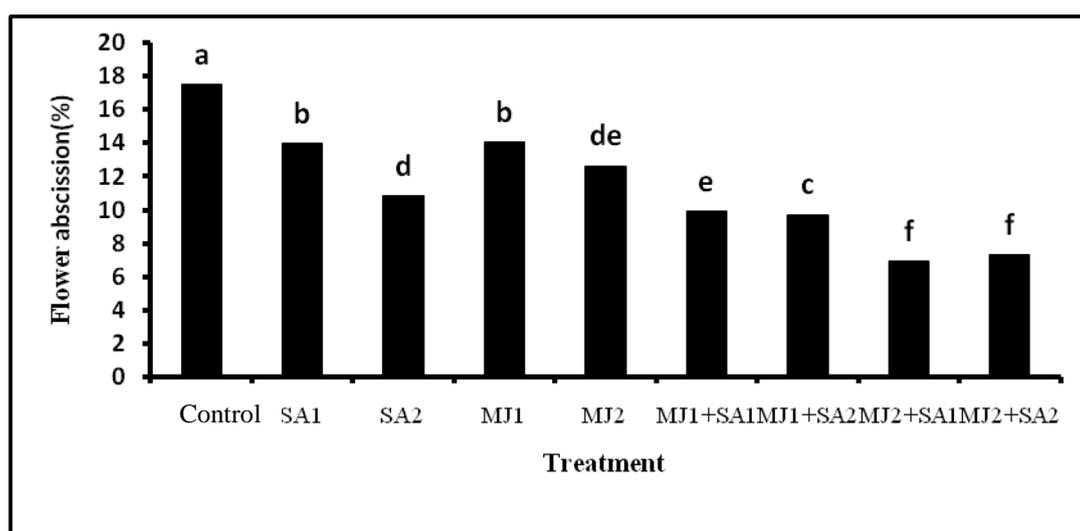


Fig. 1. Effects of MJ and SA treatments on flower abscission of ‘Elberta’ peach trees in swollen bud and green tip stage. MJ1: MJ 100 mg L⁻¹ and MJ2: MJ 200 mg L⁻¹, SA1: SA150 mg L⁻¹, SA2: SA300 mg L⁻¹. Different letters mean significant differences ($P \leq 0.05$) for each column. Comparisons are between the control and each treatment.

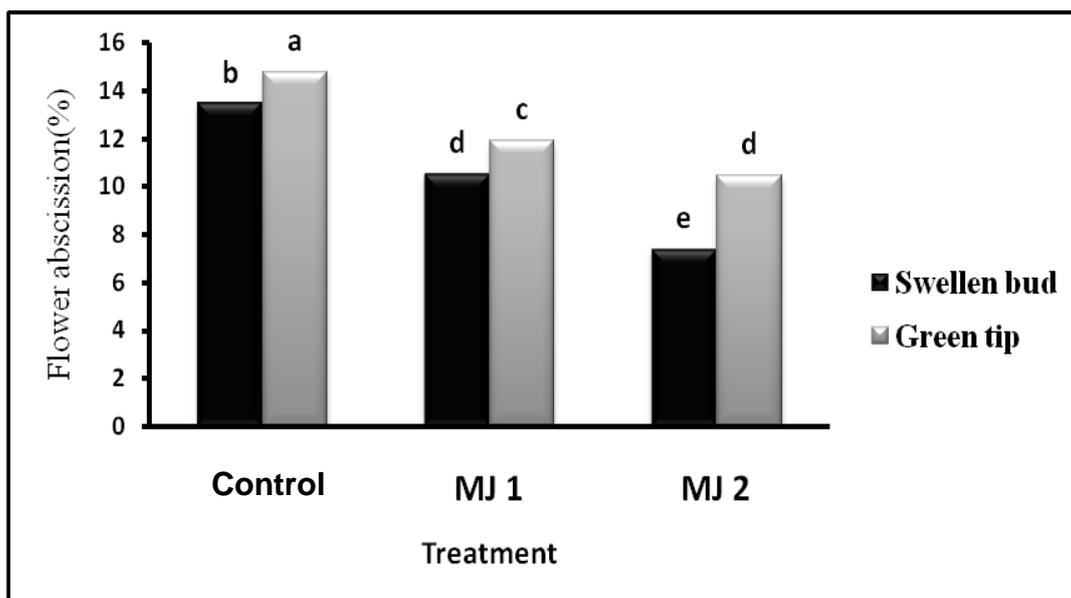


Fig. 2. Effects of MJ spraying time (swollen bud and green tip stage) on flower abscission of 'Elberta' peach trees. MJ1: MJ100 mg L⁻¹ and MJ2: MJ200 mg L⁻¹. Different letters mean significant differences ($P \leq 0.05$) for each column. Comparisons are between the control and each treatment.

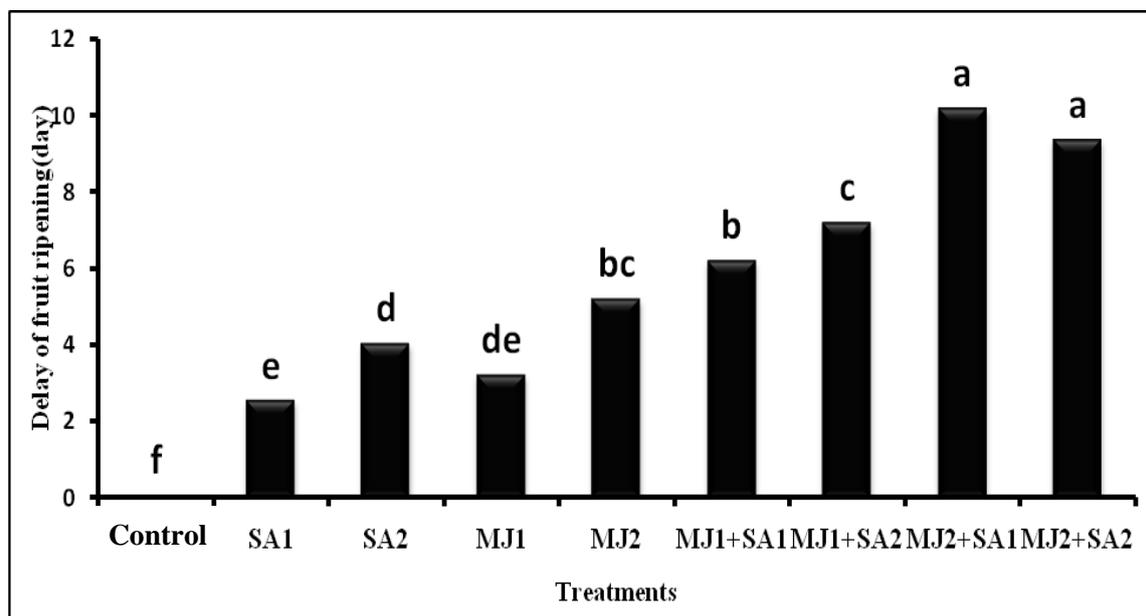


Fig. 3. Interactions of MJ and SA on delay of fruit ripening of 'Elberta' peach trees. MJ1: MJ100 mg L⁻¹, MJ2: MJ200 mg L⁻¹, SA1: SA150 mg L⁻¹, SA2: SA 300 mg L⁻¹. Different letters mean significant differences ($P \leq 0.05$) for each column. Comparisons are between the control and each treatment.

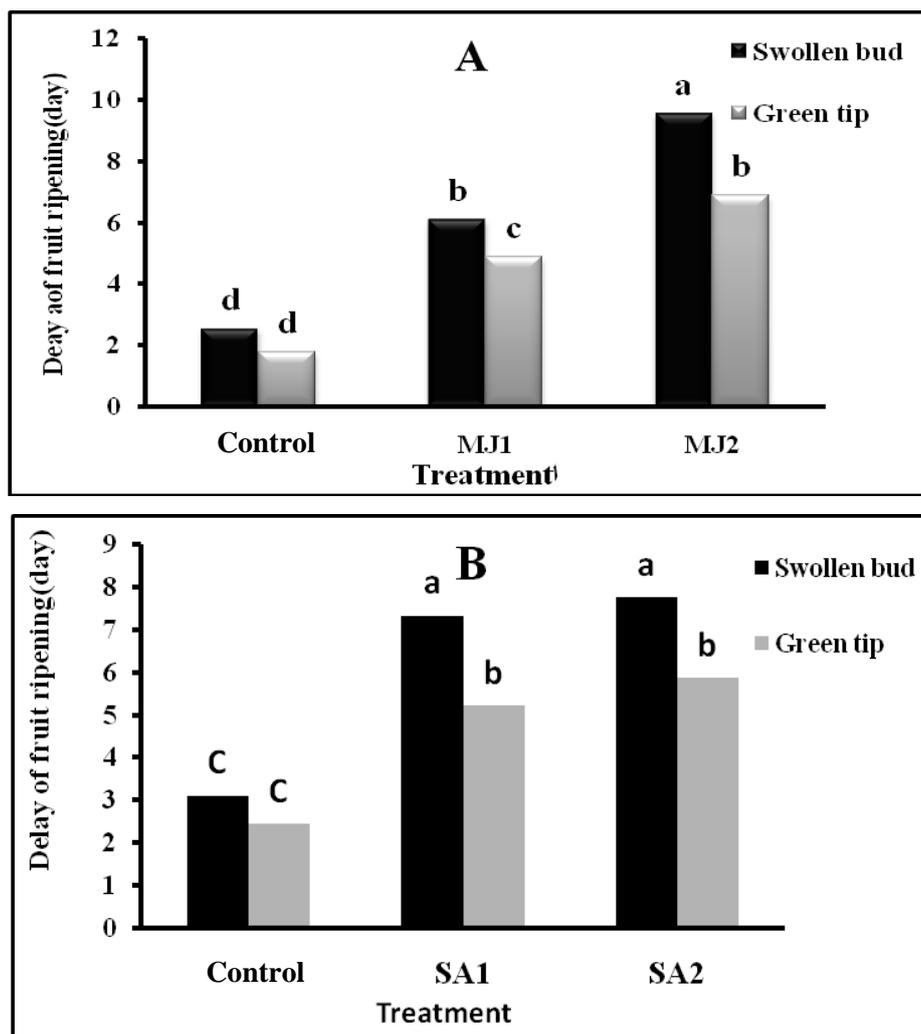


Fig. 4. Interactions of A: MJ, B: SA and spraying time (swollen bud and green tip stage) on delay of fruit ripening of 'Elberta' peach trees. MJ1: MJ100 mg L⁻¹ and MJ2: MJ 200 mg L⁻¹, SA1: SA150 mg L⁻¹, SA2: SA 300 mg L⁻¹. Different letters mean significant differences ($P \leq 0.05$) for each column. Comparisons are between the control and each treatment.

MJ and SA are plant hormones that inhibit ethylene biosynthesis and delay senescence (Christelle *et al.*, 2001; Gonzalez-Aguilar *et al.*, 2000). SA has been shown to inhibit the conversion of ACC (1-aminocyclopropane-1-carboxylic acid) into ethylene by suppressing the activity of ACC oxidase (Serek, 2010). Exogenous applications of MJ and SA have been reported to delay the ripening of climacteric fruits (Serek, 2010; Ziosi *et al.*, 2006; Ziosi *et al.*, 2008). Both SA and its derivative acetyl SA (ASA) have been shown to inhibit ethylene production in cultured pear cells (Raskin, 1992). However, the relationship between

jasmonates and ethylene during ripening is not well established (Miyamoto *et al.*, 1997; Kondo *et al.*, 2007). Ripening is a complex and genetically regulated phenomenon in which numerous endogenous and environmental factors must be correctly balanced, in order to obtain the best fruit quality (Bonghi *et al.*, 1998; Bregoli *et al.*, 2002; Torrigiani *et al.*, 2008). In climacteric fruits, ripening is mainly controlled by ethylene and interfering with ethylene biosynthesis, which has been well documented in peach by field and detached fruit experiments (Torrighiani *et al.*, 2003; Ziosi *et al.*, 2006; Torrigiani *et al.*, 2008).

The natural derivative of jasmonic acid, MJ, and its synthetic analog n-ropyl dihydrojasmonate (PDJ) and salicylic acid (SA) were applied to peach fruit at different developmental stages under field conditions and their effects were evaluated at harvest on ethylene production and some fruit quality traits, and ripening fruit (Rohwer *et al.*, 2008). The results show

that MJ and SA caused fruit maturity delay (4-12 days) (Raskin, 1992; Rohwer *et al.*, 2008). So, in accordance with the results, MJ and SA can be recommended to delay bloom in peach and may be other stone fruits growing in cold climates. However, the present study is the first evidence of the effect of MJ and SA on bloom delay, flowering and fruiting of peach.

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